Effect of Rabbit ALG on Cadaver Kidney Transplant Survival

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THE CURRENT SURVIVAL RATE of renal transplants from related living donors is reported to be about 80% at 1 year, although the survival of transplants from unrelated cadaver donors is far less satisfactory and averages, world wide, about 50% at one year.[12] The poorer results achieved with cadaver transplants undoubtedly reflect the greater genetic and, therefore, antigenic disparity that exists between cadaver donors and recipients. However, the correlation between the degree of donor and recipient compatibility, as revealed by typing their respective lymphocytes for HL-A antigens, and the clinical results of cadaver kidney transplantation has been disappointing.[14] Furthermore, the goal of obtaining a well-matched organ for each recipient of a cadaver kidney remains impractical at present.

Since a major cause of the lower survival rate of cadaver kidney transplants is uncontrollable rejection in the first few weeks after transplantation,[3,11,12] it appeared logical to attempt to increase transplant survival by improving immunosuppresive therapy. Therefore, we began, 3 years ago, a program of early, low dose treatment of cadaver kidney recipients with rabbit antihuman thymocyte globulin (ALG). We hoped by this brief, early ALG treatment to prevent acute transplant rejection without significantly increasing the risk of conventional immunosuppressive therapy which each patient also received. We were quickly impressed with the efficacy of this therapeutic regimen and we,[9] as well as others,[14] have previously reported a high, 1-year transplant survival rate in a small group of cadaver kidney recipients treated with ALG. It is the purpose of the present report to record a sizable series of cadaver kidney transplants in which the 1-year transplant survival compares favorably with that ordinarily achieved with related, living donor transplants.

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Patient Population

Thirty-nine consecutive recipients of cadaver kidney transplants at University Hospital, the Boston University Services at Boston City Hospital and the Boston Veterans Administration Hospital, have received a course of five subcutaneous injections of rabbit antihuman thymocyte globulin (ALG) on alternate days beginning at the time of transplantation. The 39 patients, 31 men and eight women, ranged in age from 18 to 51 years. Each had reached terminal renal failure. The original kidney disease was glomerulonephritis in 27, pyelonephritis in four, polycystic kidney disease in three, amyloidosis in two, and hereditary nephritis, diabetic nephropathy and nephrosclerosis in one each. Each patient had undergone bilateral nephrectomy prior to transplantation and was maintained on hemodialysis for a period of several weeks to several months before transplantation.

Each recipient received a kidney from a cadaver donor of compatible erythrocyte group. Before transplantation the recipient's serum was tested against the donor's lymphocytes for preformed, cytotoxic antibodies and the transplant was not performed if this crossmatch was positive. In addition, the lymphocytes from each donor and recipient were typed for HL-A antigens by the Typing Laboratory of the New England Interhospital Organ Bank. The degree of donor-recipient

compatibility was graded by the criteria of Terasaki.[8] There were 4 B, 13 C, and 22 D transplant matches. The results of HL-A typing were not used in donor selection.

Kidneys were removed aseptically from the cadaver donor as soon after death as possible and were then perfused with cold Ringer's solution containing heparin and albumin, and stored in iced Ringer's solution until transplantation. The warm ischemia time varied from 5 to 60 minutes and the cold ischemia time from 1 to 7 hours. Kidneys were transplanted to the iliac vessels in the pelvis by standard technics. The urinary tract was reconstituted by ureteroneocystostomy.

In addition to ALG, each recipient was treated with conventional immunosuppressive therapy in the form of azathioprine beginning at a dose of 3 mg./Kg. of body weight daily and prednisone beginning at a dose of 1 mg./Kg. daily. Each recipient also received local irridation to the cadaver kidney transplant delivered with a cobalt (Co^{60}) radiotherapy unit. Doses were 150 rads on days 1, 3 and 5 after transplantation.

Acute transplant rejection was diagnosed by a fall in urinary output, a rise in serum creatinine and a fall in creatinine clearance. These signs were often accompanied by fever, proteinuria, an active urinary sediment, leukocytosis and hypertension. Acute rejection was treated by temporary increase in the daily prednisone dose to 100–200 mg./day.

Preparation and Evaluation of ALG

Antihuman ALG was raised in rabbits by a method described previously.[9] The thymocytes used for immunization were obtained from pieces of fresh thymus gland excised to facilitate surgical exposure during pediatric open-heart operations. The final globulin preparation obtained after ammonium sulfate precipitation, was stored in sterile, single dose bottles. Merthiolate was added to a concentration of 0.01% as a preservative. By conventional electrophoresis the preparations used clinically were approximately 90% gamma globulin and 10% beta and alpha globulin. The thymocyte agglutinin titres were 1:512 or higher. Rosette inhibition titres, determined by the procedure of Bach *et al.*,[1] were greater than 1:64,000. Hemagglutinination titres were 1:128 or lower.

The rabbit antihuman ALG used clinically in this study was independently evaluated under the supervision of Dr. Donald Kayhoe of the National Institute of Allergy and Infectious Diseases. Administration of the ALG to Rhesus monkeys according to a standard protocol resulted in prolongation of skin allograft survival to an average of 29 days, considered a marked increase in graft survival. The ALG was also found to be non-toxic to formed elements in the blood, with the exception of lymphocytes.

The ALG was tested for antiglomerular basement membrane antibodies by Dr. Edmund J. Lewis, Thorndike Memorial Laboratory, Boston City Hospital, Harvard Medical School, and was found to be free of such antibodies.

An individual dose of ALG for clinical use was selected that compared, on the basis of body surface area, to a mid-range immunosuppressive dose of identically prepared ALG in laboratory animals. This human dose of 75–90 mg., dissolved in 3 ml. of phosphate buffered saline was administered subcutaneously in the anterior thighs.

Histologic Studies

Each transplanted kidney was biopsied at the time of transplantation and at 1 year and 2 years after transplantation. Several kidneys were also biopsied at intermediate times. All were open biopsies performed under local anesthesia.

All biopsies were studied by light microscopy and most biopsies were also studied by fluorescence microscopy and by electron microscopy, utilizing technics described previously.[9]

TABLE 1. *Cadaver Transplant Survival*

	No. Transplants Entering Interval	No. Rejections In Interval	No. Patient Deaths in Interval
0–6 months	39	3*	3
6–12 months	33	1	1
12–24 months	31	1	2
24–36 months	16	0	0

* all hyperacute rejections

Results

The clinical results of the 39 cadaver kidney transplants in this series followed for more than 1 year are recorded in life table fashion in Table 1. Three patients lost their kidneys from hyperacute rejection[16] as indicated by the immediate onset of rejection and the typical histologic appearance of fibrin accumulation in the cortical microvasculature. Each of these patients was known to have preformed antibodies against at least 20% of a normal lymphocyte donor panel, preoperatively. Each had a negative crossmatch with his kidney donor's lymphocytes prior to transplantation. Six other presensitized patients in this series were transplanted without evidence of early rejection.

Four patients died in the first year following transplantation. One of these patients died of an apparently unrelated cause, rupture of a congenital intracerebral aneurysm. The other three patients died of sepsis, one

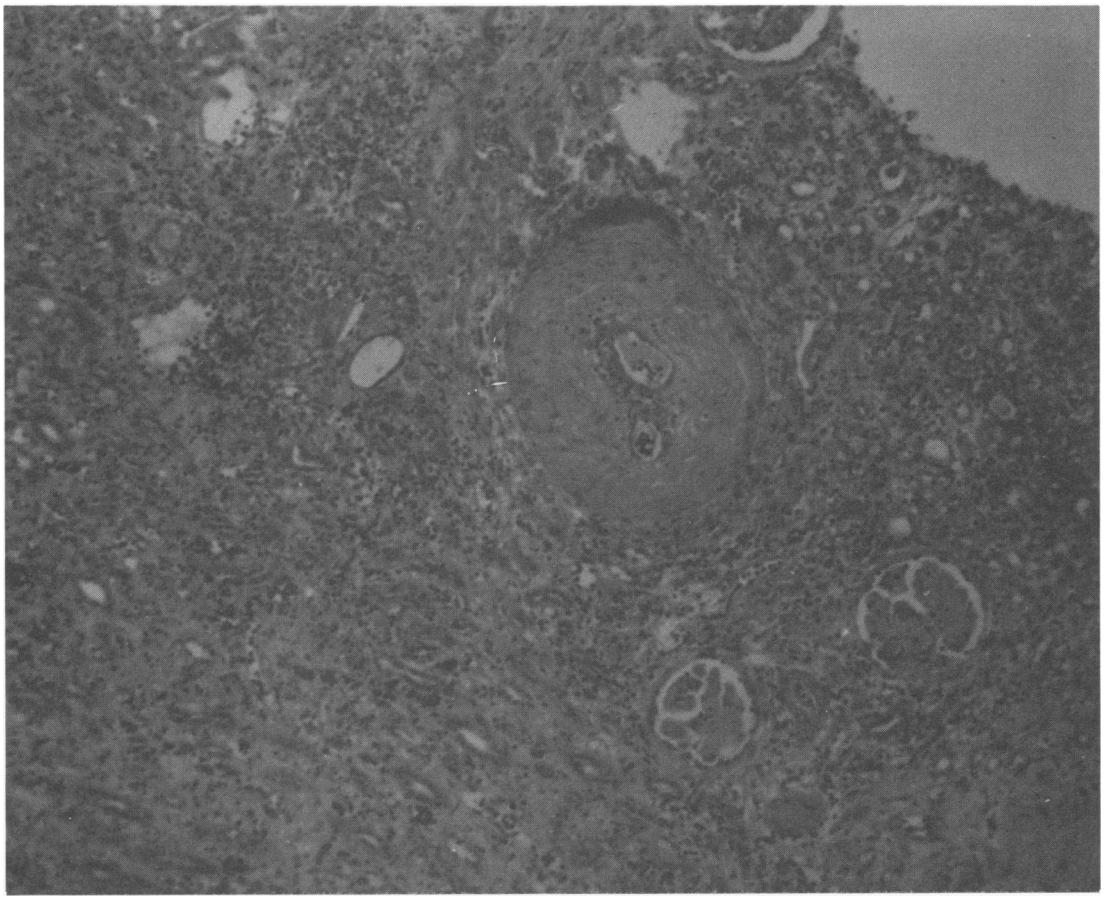

FIG. 1. Histologic appearance of kidney transplant 7 months after transplantation removed from 48-year-old man following three episodes of acute rejection. There are many hyalinized glomeruli, moderate interstitial infiltrate and fibrosis and marked intemal hyperplasia of the arteries. (H & E ×100)

of an abscess surrounding a previous gastroenterostomy, one of empyema following drainage of a spontaneous pneumothorax and one of miliary tuberculosis. Only one patient in the group of 30 lost his kidney from conventional acute rejection in the first year following transplantation. This 48-year-old man had three episodes of acute rejection during the first 6 months following transplantation of a C match cadaver kidney. The kidney was removed in the 7th month when it failed to maintain life sustaining function. The histologic appearance of this transplant is illustrated in Figure 1. In the second year after transplantation two more patients died of sepsis, one following urethral instrumentation and the second following cholecystectomy for acute cholecystitis. One further patient lost his kidney in the second year after transplantation from typical chronic rejection.[4]

Thirty-one of the 39 transplants were functioning at 1 year for a crude 1-year transplant survival rate of 80%. The 1-year patient survival rate was 90%. Sixteen of 24 transplants were functioning at the 2-year interval for a crude 2-year transplant survival rate of 67%. The 2-year patient survival rate was 75%. If the three hyperacute transplant rejections are excluded from consideration, since no known immunosuppressive therapy could be expected to alter the outcome in these individuals, the 1-year transplant survival rate is 86% and the 2-year survival rate is 76%.

One of the most striking features of this series of 39 cadaver transplants was the paucity of acute rejection episodes. Excluding the three hyperacute rejections noted above, there were 16 episodes of acute rejection in 13 patients. Only two patients had more than one rejection episode. The first of these patients, as discussed above, lost his transplant in the 7th month. The second patient had two well documented rejection episodes and has diminished, but life sustaining, renal function at the 1 year interval. The earliest acute rejection episode in the entire group of patients occurred on the forty-second day after transplantation.

As a correlary to the relative lack of acute rejection episodes in this series of patients, the average daily steroid dose was considerably less than we had previously observed in cadaver transplant patients and lower than that reported by others.[3,7,10,11] The average steroid dose for the first 15 patients in this series is illustrated in Figure 2. The curve is remarkably similar to that reported by Starzl and associates [13] for a series of related living donor transplants treated with ALG.

Twenty-nine of the 31 transplants functioning at 1 year were classified as having good renal function,

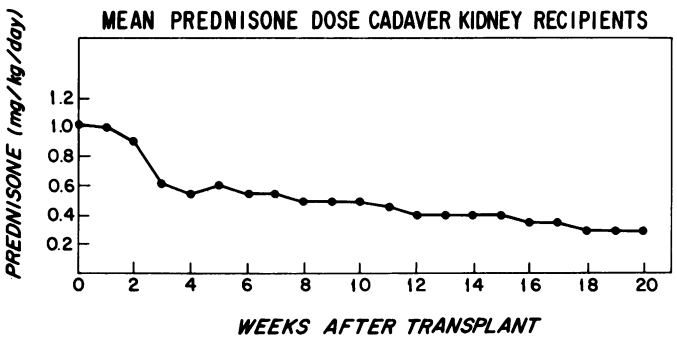

FIG. 2. The mean prednisone dose for the first 15 patients in this series is illustrated. The use of ALG appeared to permit early reduction of the prednisone dosage.

serum creatinine 2.0 mg./100 ml. or less, creatinine clearance 50 ml./minute or more. Two transplants had diminished renal function with creatinine clearances in the 15–20 ml./minute range. One of the latter two patients had two clear cut episodes of acute rejection as noted above. The second had a kidney damaged by misdiagnosed ureteral obstruction. The renal function of all 16 transplants reaching the 2-year interval remains good. However, two of the transplants surviving 2 years have appreciable proteinuria (24-hour protein excretion greater than 500 mg.).

TABLE 2. *Thirty-nine Cadaver Transplants*

Deaths	Non-fatal Complications	
Sepsis—3 1st year	Sepsis	5
—2 2nd year	Complicated peptic ulcer	3
Rupture of congenital	Pulmonary embolus	2
intracerebral aneurysm—1	Parathyroidectomy	2
	Aseptic necrosis of hip	2
	Ureteral obstruction	2
	Urinary leak	1
	Small bowel obstruction	1
	Hepatitis	1

Non-fatal complications in the 39 transplant recipients are listed in Table 2. Five patients had significant bacterial or fungal abscesses in the postoperative period. One of these patients required enucleation for nocardial infection of the orbit. Three patients required operation for complications of peptic ulcer. Two patients had well documented pulmonary emboli in the post-transplant period and two patients ultimately required parathyroidectomy for marked hyperparathyroidism persistent beyond the 6-month post-transplant interval. Two patients

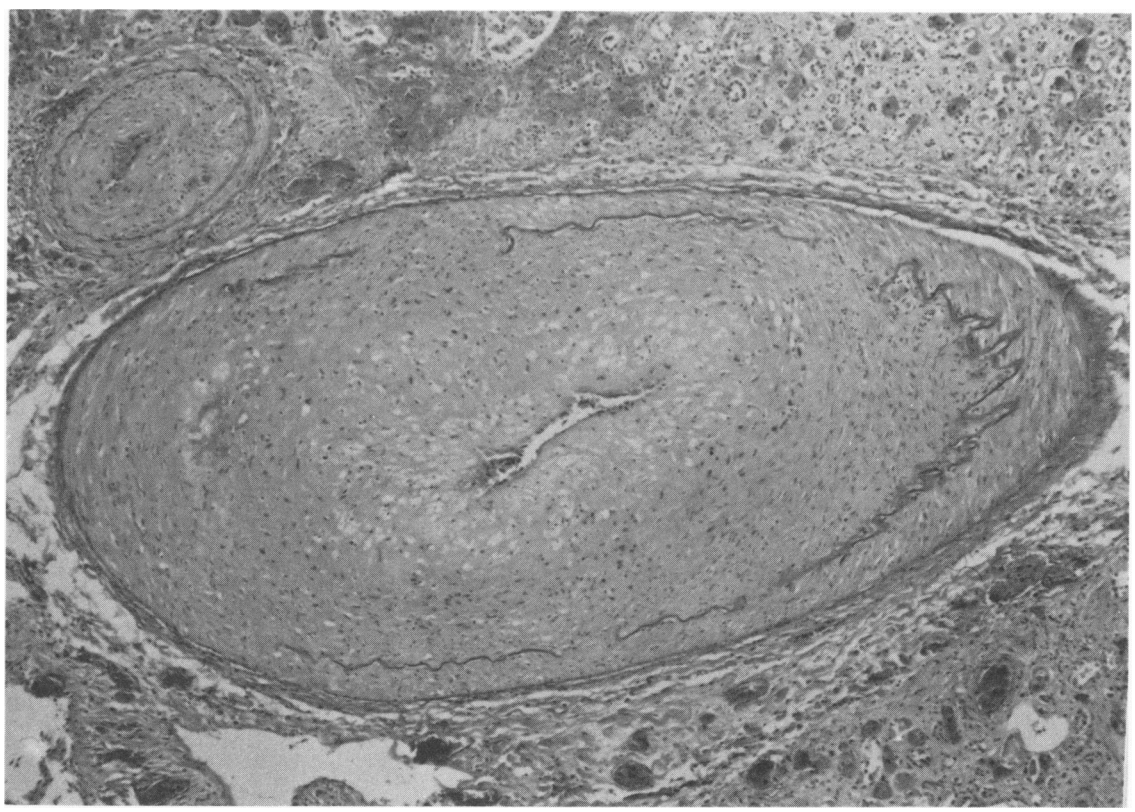

FIG. 3. Histologic appearance of biopsy of renal transplant 1 year after transplantation in a 41-year-old man in this series. Marked intimal hyperplasia of the arteries and interstitial fibrosis is noted suggesting marked changes of chronic rejection. (H & E ×60)

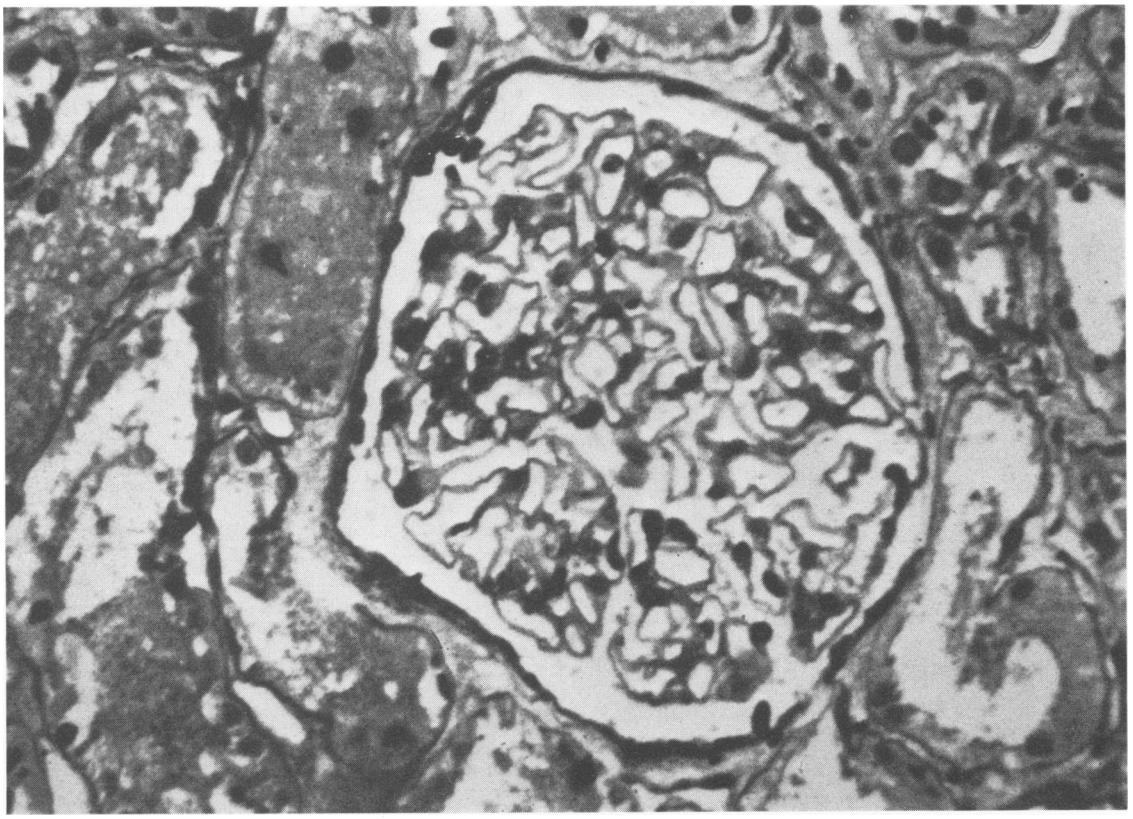

FIG. 4. Histologic appearance of 1 year transplant biopsy in 29-year-old man in this series. There is mild focal mesangial condensation and mild interstitial edema. (PAS stain ×250)

required operation for aseptic necrosis of the hip, presumably secondary to steroid therapy. Two patients developed ureteral obstruction, one from fibrous stenosis of the distal transplant ureter and the other from lymphocele. Both were corrected surgically. One patient developed a urinary leak from a small necrotic area in the renal pelvis which closed after nephrostomy drainage. One patient developed small bowel obstruction presumably secondary to prior abdominal surgery and one contracted infectious hepatitis.

The subcutaneously administered ALG was well tolerated by almost all patients in this series. Fifteen of the 39 patients had clear cut local inflammatory reactions at the injection sites and suffered mild discomfort. However, no patient had symptoms of sufficient severity to warrant consideration of cessation of ALG therapy. A febrile response in the early postoperative period was common in this group of patients. However, it was impossible to decide in how many instances this was related to the ALG therapy. There was no evidence of systemic toxicity, rash, urticaria or serum sickness-like syndrome. The one consistant hematologic effect of ALG administration appeared to be profound lymphopenia which was noted immediately after the first injection of ALG and persisted for from 4 to 6 weeks thereafter when lymphocytes again gradually reappeared in the peripheral blood. While lymphopenia is certainly seen after conventional immunosuppressive therapy, we have not encountered lymphopenia of this degree or duration previously. There was no evidence of red cell or platelet destruction in any of the patients treated in this series.

Histologic examination of the 1-year renal biopsies in this group of patients revealed evidence of severe chronic rejection in only three (Fig. 3). Two of these patients had evidence of diminished renal function at the 1-year interval. The third had normal function but went on to lose his kidney from progressive chronic rejection at the 22nd month. The remaining patients had evidence of mild or minimal changes of chronic rejection consisting of mild focal mesangial condensation in the glomeruli, mild interstitial fibrosis, occasional edema and patchy interstitial round cell infiltration (Fig. 4). Electron microscopy confirmed these findings and showed focal fusion of glomerular foot processes, irregular basement membrane thickening and occasional subendothelial amorphous deposits and focal increase in mesangial matrix.

Fluorescence microscopy studies indicated significant deposition of immunoglobulin in only one patient at the 1-year interval and deposition of fibrin in the glomeruli of three patients. Rabbit gamma globulin was not demonstrated in any kidney biopsy. The 2-year kidney biopsies have not indicated a significant progression of chronic rejection in the patients examined (Fig. 5).

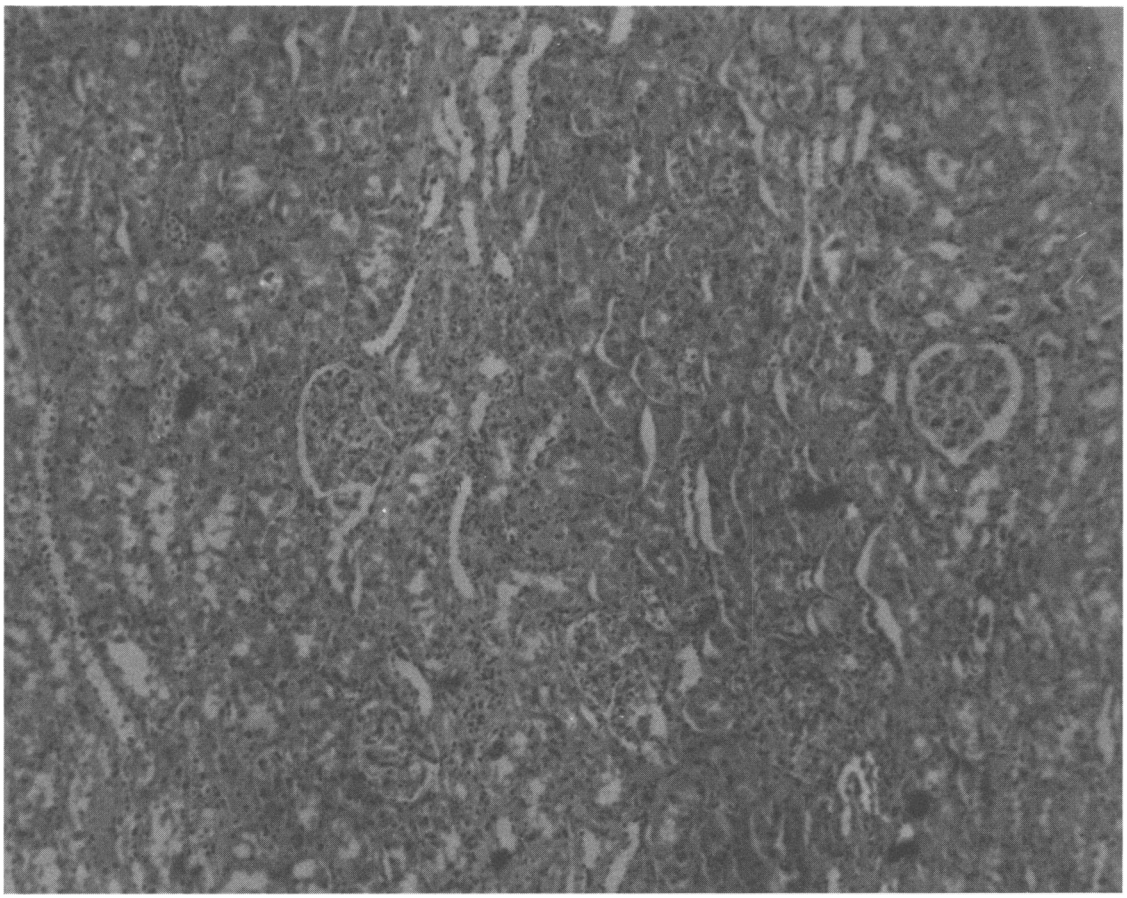

FIG. 5. Histologic appearance of 2-year renal kidney transplant biopsy in 26-year-old patient in this series. Renal architecture is well preserved. There are minor interstitial infiltrate and mild focal mesangial condensation. (H & E ×100).

Discussion

Previous work in the experimental laboratory convinced us that the injection of rabbits with foreign thymocytes in Freund's complete adjuvant followed in 4 weeks by an intravenous booster of the same cells would yield antilymphocyte globulin of consistently high potency.[2] Three years clinical experience with rabbit anti-human ALG prepared in an identical manner has suggested that this conclusion was correct. The early administration of low doses of rabbit ALG to a series of 39 consecutive cadaver kidney recipients has, we believe, altered the clinical course of these patients significantly from what we and others have previously experienced with cadaver transplant recipients treated with conventional immunosuppressive therapy.

First, all acute rejection episodes were postponed to the sixth week after transplantation or beyond, when the patients had recovered from operation, their incisions were healed, and the danger of septic complications of antirejection treatment were reduced. Second, only one-third of the patients experienced any detectable acute rejection episodes and those that occurred were easily controlled in the vast majority of instances. Third, the average prednisone dose could be kept lower than in our previous experience with cadaver kidney transplants. The average dose at 6 months after transplantation in this series of patients was 20 mg. per day and at 1 year 10 mg. per day. Finally, the 1-year transplant survival rate for this group of patients is comparable to that generally reported for related living donor transplants, 80%.

These results were achieved without an unacceptably high number of infectious complications which, perhaps, reflects the fact that the ALG was administered only in an initial brief course and, therefore, did not contribute to the suppression of host defense mechanisms beyond the first few postoperative weeks.

The older literature on the serum therapy of pneumococcal pneumonia suggests that rabbit serum was more easily tolerated by patients than horse serum and produced less sensitivity reactions.[5] Certainly, it has been possible to administer rabbit ALG to all the patients in the present series without significant side effects. Since we had convincing evidence that the potency of this ALG was high, we felt it reasonable to keep the individual dose low which permitted us to utilize the subcutaneous route of administration. This avoids potential dangers of intravenous administration which include im-

mediate systemic hypersensitivity reactions and possible vascular damage within the transplanted kidney and elsewhere.[6]

While the 1-year transplant survival is satisfactorily high in this series of patients, the 2-year transplant survival is reduced to 67% in the 24 patients who could have reached the 2-year interval at the time of the present writing. It is also true that three patients in this series showed marked changes of chronic rejection in their 1-year transplant biopsies and that one of these patients has lost his transplant from chronic rejection. However, 1 year biopsies on the remaining patients have shown very little evidence of chronic rejection and the renal function of those patients who have reached the 2-year interval has been uniformly good. It has also been our experience, and that of others,[11,12] that transplants which are functioning normally at the 2-year interval will most commonly go on functioning for several years thereafter. It therefore would appear that the immunosuppressive therapy received by the patients in the present series has not only diminished early transplant loss but may very well have favorably influenced the late rejection of these transplants as well.

While the transplant survival rates in this series are among the highest reported for cadaver kidney transplants, it is not entirely certain that these results are attributable to the use of rabbit ALG. A randomized or double blind study has not been performed. However, a randomized study of the effect of goat ALG on cadaver kidney transplants has recently been reported by Sheil and associates [15] who have concluded that the use of ALG has resulted in a significant improvement in cadaver transplant survival.

Summary

1. Thirty-nine consecutive recipients of cadaver kidney transplants were treated with a short, early course of rabbit antihuman ALG in addition to conventional immunosuppressive therapy. The 1-year transplant survival in this group of patients is 80%.

2. Hyperacute rejection, apparently caused by preformed antibodies against donor tissue, and deaths from infection accounted for the majority of transplant losses. Only one patient lost his transplant because of conventional acute rejection. Only one-third of the patients had any clinically detectable acute rejection episodes.

3. Marked histologic and clinical evidence of chronic rejection was seen in the transplants of three patients in the present series. Sixteen patients have reached the 2-year interval with good renal function. The 2-year transplant survival rate in those patients followed for that period of time is 67%.

4. The subcutaneously administered rabbit ALG was well tolerated by the patients in this series and caused no significant complications.

Acknowledgments

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References

1. Bach, J. F., Dormont, J., Dardenne, M. and Balner, H.: In Vitro Rosette Inhibition by Antihuman Antilymphocyte Serum: Correlation with Skin Graft Prolongation in Subhuman Primates. Transplantation, **8**:265, 1969.
2. Davis, R. C., Cooperband, S. R. and Mannick, J. A.: Preparation and In Vitro Assay of Effective and Ineffective Antilymphocyte Sera. Surgery, **66**:58, 1969.
3. Gault, M. H., Ackman, D., Morehouse, D. and Dossetor, J. B.: Kidney Transplants: Computer Display of Clinical and Laboratory Parameters: The First 100 Days. Can. Med. Assoc. J., **100**:359, 1969.
4. Harlan, W. R., Jr., Holden, K. R., Williams, G. M. and Hume, D. M.: Proteinuria and Nephrotic Syndrome Associated with Chronic Rejection of Kidney Transplants. N. Engl. J. Med., **277**:769, 1967.
5. Horsfall, F. L., Jr., Goodner, K., MacLeod, C. M. and Harris, A. H.: Antipneumococcus Rabbit Serum as a Therapeutic Agent in Lobar Pneumonia. JAMA, **108**:1483, 1937.
6. Iwasaki, Y., Porter, K. A., Amend, J. R., Marchioro, T. L., Zuhlke, 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.
7. Khastagir, B., Montandon, A., Nakamoto, S. and Kloff, W. J.: Early and Late Failures of Human Cadaveric Renal Allografts. Arch. Intern. Med., **123**:8, 1969.
8. Lee, H. M., Hume, D. M., Vredevoe, D. L., Mickey, M. R. and Terasaki, P. I.: Serotyping for Homotransplantation. IX. Evaluation of Leukocyte Antigen Matching with the Clinical Course and Rejection Types. Transplantation, **5**: 1040, 1967.
9. Mannick, J. A., Davis, R. C., Cooperband, S. R., Glasgow, 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.
10. Marshall, V., Kincaid-Smith, P., Morris, P. J., Saker, B. and Eremin, J.: Cadaveric Renal Transplantation. Lancet, **2**: 927, 1968.
11. Moore, T. C. and Hume, D. M.: The Period and Nature of Hazard in Clinical Renal Transplantation. III. The Hazard to Transplant Kidney Survival. Ann. Surg., **170**:25, 1969.
12. Murray, J. E., Barnes, B. A. and Atkinson, J. C.: Eighth Report of the Human Kidney Transplant Registry. Transplantation, **11**:328, 1971.
13. 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.
14. Starzl, T. E., Porter, K. A., Andres, G., Halgrimson, C. G., Hurwitz, R., Giles, G., Terasaki, P. I., Penn, I., Schroter, G. T., Lilly, J., Starkie, S. J. and Putman, C. W.: Long-term Survival after Renal Transplantaion in Humans: With Spe-

cial Reference to Histocompatibility Matching, Thymectomy, Homograft Glomerulonephritis, Heterologous ALG, and Recipient Malignancy. Ann. Surg., 172:437, 1970.

15. Sheil, A. G. R., Kelley, 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.
16. Williams, G. M., Hume, D. M., Hudson, R. P., Jr., Morris, P. J., Kano, K. and Milgrom, F.: "Hyperacute" Renal-Homograft Rejection in Man. N. Engl. J. Med., 279:611, 1968.

Discussion

President Rhoads: I would like to ask Dr. Mannick about the ratio of rabbits to patients which is needed for this program. I recall when rabbits were breeded to produce pneumococcus type specific sera, just before the advent of penicillin and the sulfonamides, that it was a very expensive operation and very many animals were involved.

Dr. John Sarkis Najarian (Minneapolis): I congratulate Dr. Mannick and his group on the work they have done with rabbit ALG. I am pleased that his work is confirmatory to others who have been working in this field. ALG, when added to an immunosuppressive regimen, has made a great deal of difference, in kidney survival, and I think the studies by the Boston University group further clarify this issue.

I think if one is in the transplant business, he must have several types of ALG. We particularly use horse ALG, but in addition, if one is going to do a second transplant and the patient is sensitized—about 50% of patients become sensitized to the ALG in our experience—then one has to have some other source of ALG. Our second choice currently happens to be goat, and approximately 6 months ago we also started raising ALG in rabbits.

They are all good and are all needed in an active program. Dr. Kountz stressed the ability to proceed and retransplant as soon as the patients appear to be losing their kidney, this is important and requires other back-up ALG's.

I ask Dr. Mannick: What is your other source of ALG, other than the rabbit? What do you use for retransplantation?

The other question relates to dosage, which is a point of contention that we have had in the past. We have stressed a very high dose, based on our series of experiments with some 176 skin grafts in man, using our own ALG, which is prepared in horses. We found a very nice dose response curve with a maximum effect at about 30mg./Kg.

The dosage you are using, of course, is far less than this, and it obviously is working; your results are very good. The question I would have is: In the testing of ALG on monkey skin grafts, was the dose of ALG adjusted in the monkeys to the kind of dose that you are giving to humans, or was it the same standard dose that Dr. Kayhoe gives to each one of the animals when they are tested with horse, goat and other types of ALG?

Dr. John A. Mannick (Closing): In answer to Dr. Rhoads' question, it is about five patients per rabbit in the dose we are using.

In response to Dr. Najarian's questions, in Dr. Kayhoe's comparative testing series all ALG doses are the same, as I am sure Dr. Najarian is aware, and ours is no exception. This is the only way, of course, he could get comparative data.

As his testing goes on, it is quite apparent that a number of the ALG preparations in clinical use in this country have no effect in his system. Therefore the pronounced effect that rabbit ALG has had there speaks for its potency. I believe that this kind of comparative study gives us assurance that we are dealing with very potent antiserum, and that is why I believe the clinically acceptable dose is relatively low.

Back-up ALG, I think, will someday be a problem for us. About 20% of our patients are sensitized to their ALG, and we so far have not had to use back-up ALG. If we must use ALG from another species, I am afraid we will have to go to friends who produce it in goats, horses, et cetera.