

142

ANTILYMPHOCYTE GLOBULINS— CLINICAL USE

Thomas E. Starzl and K. A. Porter

ANTILYMPHOCYTE GLOBULINS— CLINICAL USE

Thomas E. Starzl and K. A. Porter

During the past eight months, more than twenty patients at the University of Colorado have been treated with heterologous antilymphocyte globulin (ALG), prepared from the serum of horses which had been immunized against lymphocytes from the thymus, lymph nodes, and spleens of fresh human cadavers. Although the clinical experience with this immunosuppressive agent has admittedly been limited, the observations made so far provide hope that ALG can be used with benefit and with relative safety after homotransplantation of kidneys or other organs in man.

As reviewed by Medawar in Chapter 32, there has been intermittent interest in heterologous antilymphocyte sera (ALS) for almost seventy years. However, the notion that ALS or its derivatives might be used for the treatment of patients stemmed from the historic report of Woodruff²⁴ in 1963. Since that time, numerous other important publications have appeared dealing with various features of the preparation, mechanism of action, testing, and purification of antilymphocyte products for use in mice,^{4,5,8,12,13,15-17} rats,^{10,25} guinea pigs,^{12,23} dogs^{1-3,7,9,11,14,18,20} and humans.^{9,21,22}

Therapeutic Protocol

The regimen used was based upon guidelines provided by animal experimentation and included the following considerations. First, although ALS or ALG have powerful immunosuppressive qualities,

these do not invariably prevent homograft rejection, a point particularly well demonstrated with renal or liver homotransplantations in the outbred canine population. Secondly, they have a variable additive effect when combined with various anti-metabolites,^{8,21} steroids,¹³ or, under carefully defined conditions, with total body irradiation¹³; by far the most striking synergism in animals has been seen with the combination of steroids and ALS.

Third, ALS therapy in animals has a significant risk from anaphylaxis and from foreign protein nephritis,⁹ although this is apparently less than with the use of normal horse serum. Fourth, the most effective program of therapy includes treatment before as well as after transplantation.^{16,21} Finally, an immunosuppressive effect is not dependent upon the production of lymphopenia.^{1,7,13,21}

For these reasons, ALG was used clinically only as an adjuvant agent added to therapy with azathioprine and prednisone. For twenty newly operated cases, it was usually started five to six days in advance of surgery, continued daily for ten to fourteen days afterward, then every other day for two weeks, two times a week for two months, and once a week for a final month (Fig. 1).

The ALG used was an ammonium sulphate precipitate consisting principally of gamma G globulin, but with components of T-equine and beta globulin. Various batches had leuko-agglutinating titers of 1:4000 to 1:32000 and a protein concentration of 4.6 to 9.3 Gm. per cent. Doses were 1 to 5 ml.,

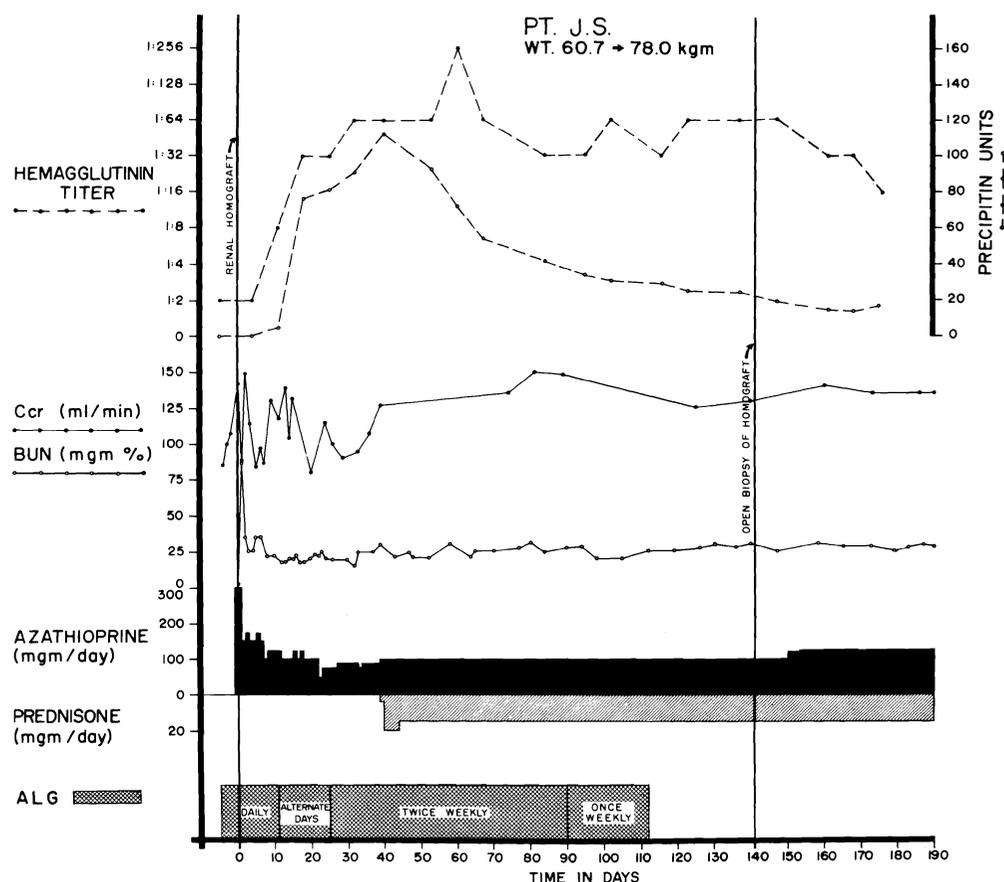


Fig. 1.—Course of a patient who received ALG before and after renal homotransplantation. The homograft was provided by a brother. There has never been a rejection. The prednisone was started after forty days because of the increases in hemagglutinin and precipitin titers. Note the subsequent decline in titers despite continuation of the globulin injections. (Courtesy J. and A. Churchill, Ltd., London).

depending upon the titer of the material and the weight of the patients. The intramuscular route was used. During the course of globulin administration, the doses per week ranged from 14 to 50 mg./Kg. at the beginning and 2 to 6 mg./Kg. at the end.

The effect of ALG therapy upon the peripheral white cells was unpredictable during the period of preoperative treatment when only this immunosuppressive agent was being used. The per cent of lymphocytes was decreased (Fig. 2). There was, however, an accompanying leukocytosis, with the result that the peripheral lymphocyte count was not usually significantly reduced (Fig. 2).

Nevertheless, a profound effect upon the immunologic reactivity of the host was evident during this period. A number of patients were studied by Drs. Frank Brunstetter and Henry N. Claman before the onset of therapy for cutaneous hypersensitivity to

Candida albicans, tuberculin, mumps, histoplasmin, and *Trichophyton*. In five instances, one or the other of these was positive, and in each, the skin test had become negative when redetermined after three or four days of ALG therapy. There were no examples of failure of a skin reaction to disappear. These results indicate that the ALG being used prevented the expression of pre-existing delayed hypersensitivity reactions.

In all freshly operated cases, azathioprine was started the day before transplantation and continued indefinitely thereafter. Prednisone treatment was instituted either in the event of rejection, or because of the development of high precipitin titers against horse protein, or in a number of later cases prophylactically to forestall either of the above complications. In all instances, it was attempted to use the smallest possible quantities of steroids. Actinomycin

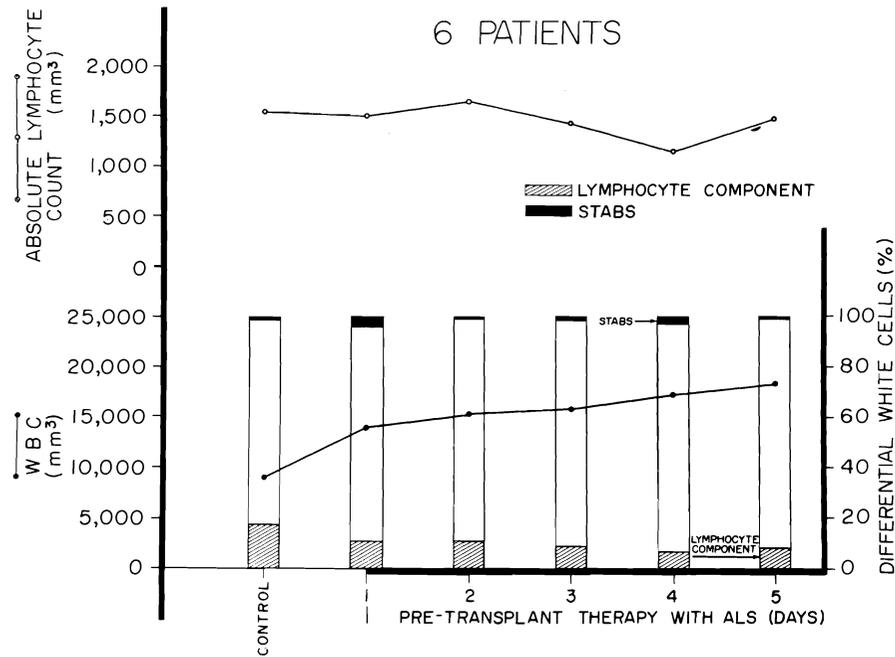


Fig. 2.—The effect of antihuman-lymphoid globulin in six patients treated daily for five days before renal homotransplantation. "Stabs" refer to nonsegmented neutrophils. (Courtesy Surg. Gynec. Obstet.)

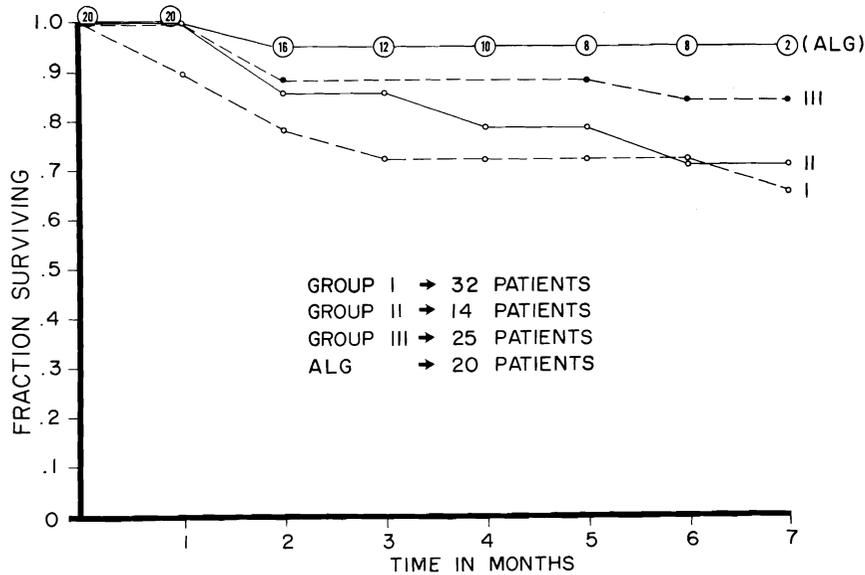


Fig. 3.—Mortality in patients treated with adjuvant ALG compared to that in three preceding series of consanguineous transplantations carried out in Denver. The numbers in the ALG series refer to the patients at risk at the indicated times. (Courtesy J. and A. Churchill, Ltd., London.)

C and local homograft irradiation were used irregularly.

In four other cases, a similar course of ALG was added from five to eleven months after operation for the treatment of patients with late failing homografts. In these cases, deterioration of renal function had not been preventable when prednisone doses smaller than 0.5 to 1.7 mg./Kg. per day had been used. After beginning globulin therapy, steroid dosages were then rapidly attenuated.

New Cases

MORTALITY

There has only been one death in these twenty cases (Fig. 3), resulting from a technical accident. The first eight patients have now been followed for six and a half to seven and a half months, the next eight for three to five months, and the last four for six to eight weeks. In nineteen of the twenty cases, the homografts were provided by blood relatives; parents for five, siblings for thirteen, and a maternal uncle for the other. The twentieth patient received a cadaver graft. Analysis of lymphocyte antigens was performed by Dr. Paul Terasaki in these donor-recipient pairs, but no attempt was made to obtain a good antigen match; the pairings were therefore on an essentially random basis. The mortality of the test series was compared to that obtained in the past with three successive groups of patients treated at the University of Colorado Medical Center with homografts from consanguineous donors. The death rate was higher in each of these retrospective control series during the early postoperative months (Fig. 3).

IMMUNOSUPPRESSION

The probable reason for the improvement in survival was the reduction which was possible in the quantity of the standard immunosuppressive drugs azathioprine and prednisone. During the period when the original retrospective control series were being compiled, there had been an increasing conservatism with use of azathioprine in an attempt to avoid the bone marrow depression which in earlier days had been the chief cause of death. This trend continued into the ALG cases (Fig. 4). This information is shown graphically as the average daily dose for the first 105 postoperative days for patients of the three retrospective groups who lived this long, compared to the first ten ALG patients. Inclusion in the study was contingent upon survival for the period of study; thereby fourteen cases were elim-

inated from the retrospective series but none among the first ten ALG patients.

The ability to treat with reduced doses of steroids was even more significant than the decreased quantities of azathioprine. In the three preceding series, the average steroid doses per day were the same for Groups 1 and 2, and in Group 3 there had been an increase. In contrast, the ALG patients required less than half the daily prednisone doses (Fig. 4).

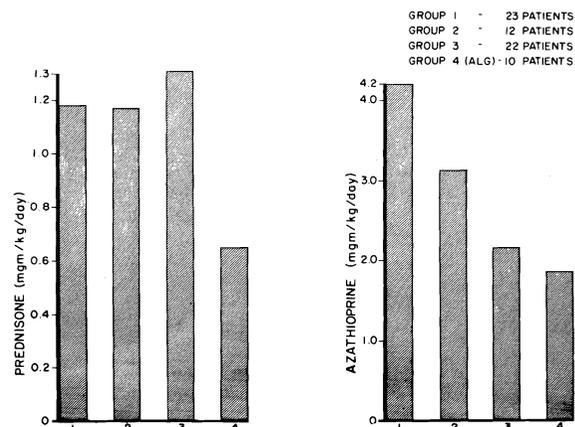


Fig. 4.—Average daily doses of azathioprine and prednisone for the first fifteen postoperative weeks in three preceding series of consanguineous homo-transplantations done in Denver, compared to the doses used for the first ten consecutive patients treated with adjuvant ALG. Since inclusion in the study was contingent upon survival for this period, nine, two, and three other patients from the retrospective control series were eliminated by their early death; such a correction was unnecessary in the ALG cases. Note that the ALG-treated patients received reduced quantities of the “standard” immunosuppressive agents. (Courtesy J. and A. Churchill Ltd., London.)

FUNCTION

The reduction in doses of standard immunosuppressive agents was not at the sacrifice of renal function. The average BUN's and creatinine clearances in the ten ALG-treated patients were better in all but one of the retrospective control groups. It will be recalled that more than a dozen patients who died during this 105 day period of analysis and who generally had the poorest function were excluded, thus insuring the retention of only the best cases; all of the first ten cases in the ALG series were included. The excellent showing of the test cases was therefore in spite of this bias.

By pooling the functions as described above, it would be possible to conceal trends in function for either the control or test series. To obtain a more

dynamic view of the postoperative events, creatinine clearances were obtained on a weekly basis for the patients in each of the control series who lived at least twenty-two weeks, and these were compared to the first eight consecutive patients in the ALG series. The creatinine clearances in all groups were initially variable. After two months, however, the ALG-treated patients showed an improvement which distinguished them from the members of the other series (Fig. 5), and which was sustained for at least the first months after discontinuance of the ALG course. Thus, as ALG therapy was being reduced and finally stopped, the continuing high quality of renal function made it unnecessary to make upward adjustments in the other drugs.

Late Cases

These four patients had poor homograft function when ALG therapy was started. In two cases, it was possible to reduce prednisone from 1.3 and 1.0 mg./Kg./day, respectively, to 0.4 and 0.3 mg. without deterioration in renal function. The course of one of these patients is shown in Figure 6. A series of disasters had followed transplantation including pancreatitis, disruption and repair of a ureteroureterostomy, a left paravertebral gutter abscess, massive wound hemorrhage, and complete heart block which had necessitated transvenous insertion of a cardiac pacemaker. The prognosis was worsened by the fact that all attempts to reduce prednisone below 40

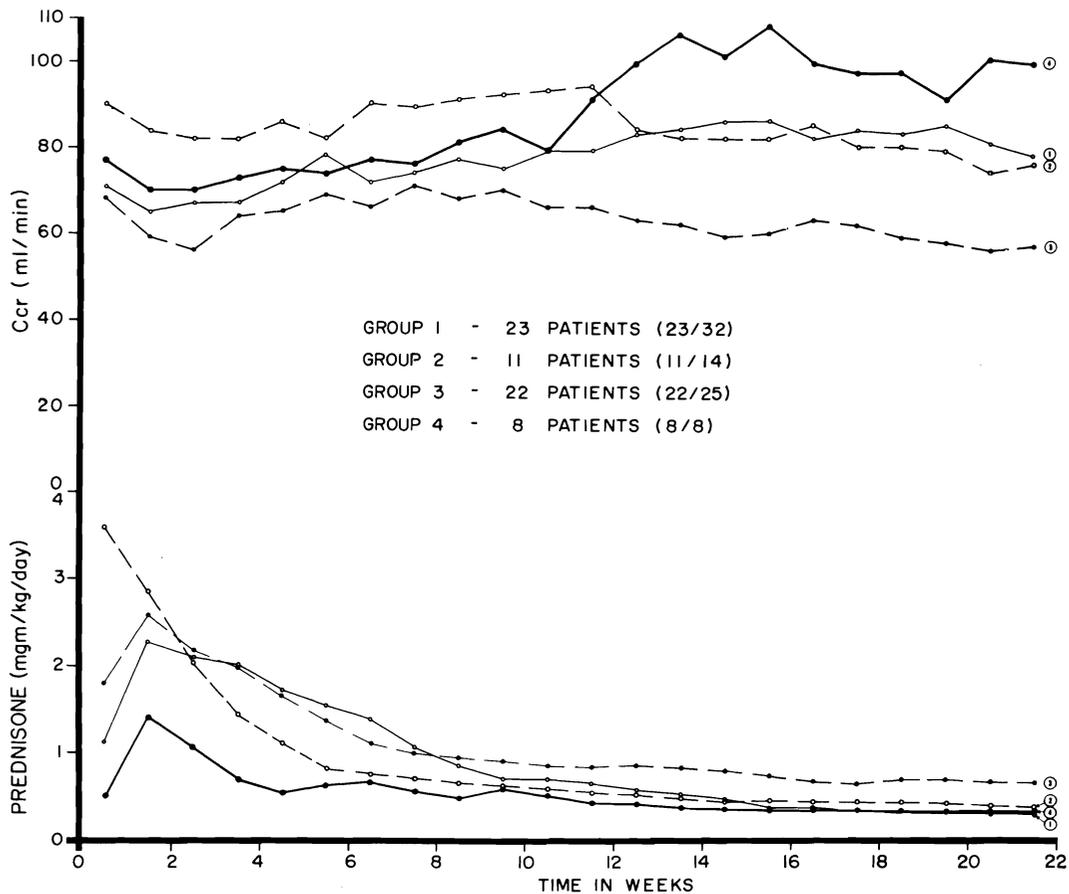


Fig. 5.—Relation of average prednisone doses to creatinine clearances during the first twenty-two weeks for the first eight ALG-treated patients, compared to comparable data in the three retrospective control series. Note that the patients in the ALG group received the smallest quantities of steroids, and that they ultimately had the highest average clearances. (Courtesy J. and A. Churchill, Ltd., London.)

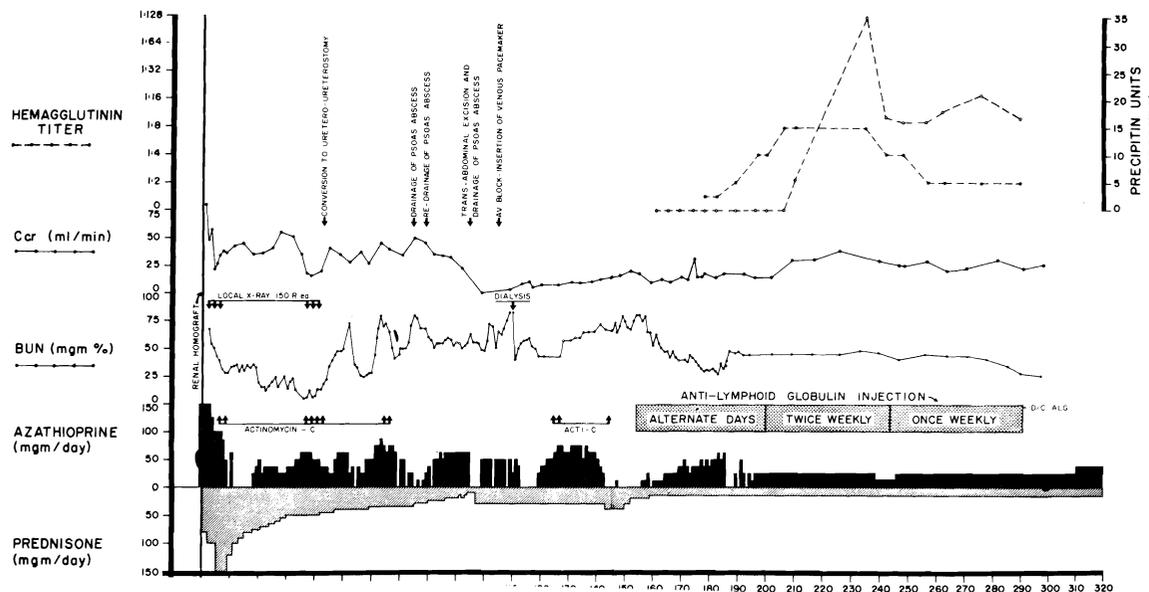


Fig. 6.—Course of patient with a failing renal homograft who was treated late with antilymphoid globulin. During the first four postoperative months, there were several potentially lethal postoperative complications which were made more grave by the need for heavy steroid therapy. Efforts to reduce the prednisone dosage resulted in deterioration of renal function. After institution of antilymphoid globulin therapy, renal functions slightly improved despite a rapid decrease of the steroid doses, and this has been sustained since completion of the ALG course. (Courtesy J. and A. Churchill, Ltd., London.)

60 mg./day caused deterioration of graft function. After starting ALG, it was possible to reduce prednisone to 15 mg. per day. The BUN fell and the creatinine clearance rose slightly. The other successfully treated case was similar.

One of the other patients, whose creatinine clearance was 3 to 5 ml./min. at the time ALG was started, died of viral pneumonitis and heart failure. All therapy was eventually stopped for the fourth patient who ultimately had a second transplantation several months later during retreatment with ALG. The second course of globulin injections was well tolerated. Further details of these cases are published elsewhere.²²

Toxicity of ALG

PAIN

All patients had pain at the site of injection, which usually reached a peak in two to six hours. It was particularly intense with the first few injections, sometimes requiring narcotics, but later it was better tolerated. Local swelling, edema, and erythema were often noted.

FEVER

Hyperpyrexia occurred regularly. Usually this was low grade, but a few patients had temperature rises to more than 40° C.

ANAPHYLAXIS

Four of the twenty-four patients had reactions which were diagnosed as anaphylaxis. These occurred within a few minutes after injection and lasted 1 to 30 min. The patients had air hunger. Three became mildly hypotensive and the fourth was transiently hypertensive. Symptomatic treatment was given. In three of these four cases, additional subsequent injections were used for several weeks or months.

Every patient studied had increases in precipitin titers against horse serum. These developed from two to seven weeks after beginning ALG therapy (Fig. 7) and were apparently influenced by the way in which steroids were used. The greatest titer increases were in patients who did not receive prednisone until late in their course; here, the addition of prednisone was followed by a secondary fall in the

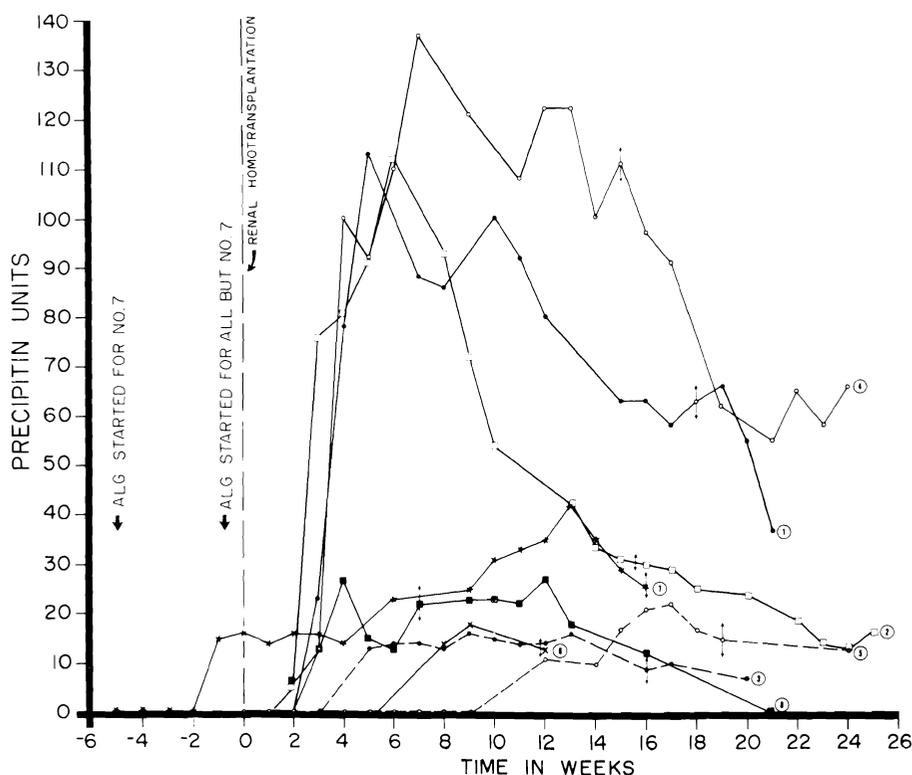


Fig. 7.—Rises in precipitin titers in the first eight consecutive patients treated with antilymphocyte globulin. The vertical arrows indicate the last dose of ALG given in each case; note that the titers had often begun to decline before this time. (Courtesy J. and A. Churchill, Ltd., London.)

precipitin levels. If steroids were started early, the titer rises were later and were sluggish.

It has been demonstrated by Dr. Noburu Kashiwagi in our laboratories that most of the precipitins in these patients were directed against alpha and beta classes of the immunoglobulins. Little activity was found against the horse gamma G-globulin in which an estimated 80 per cent of the desired antibodies are located. Hemagglutinin titers against sheep red blood cells, which are a measure of host response to Forssman-like antigens in the horse serum, followed roughly the same pattern as the precipitin titers.

HISTOPATHOLOGIC STUDIES

The most encouraging findings came from examination of the homograft biopsies which were obtained in the first eight cases from 108 to 145 days after transplantation. There was no evidence whatsoever of horse protein in the homografts. Subendothelial accumulations of amorphous material were present in the glomerular capillary walls of six of the

eight renal homografts biopsied 108 to 145 days after homotransplantation (Table 1), but only in four of the kidneys could any thickening of the basement membranes be detected by light microscopy. The change was focal, and in every glomerulus normal loops were present. The density and compactness of the deposits varied. In one kidney, it was as though the lamina densa had been split and a broad band of loosely arranged material inserted between the two layers. Mesangial cell processes extended into a few of the thickened basement membranes. There were no subepithelial deposits.

Immunofluorescence of the six affected kidneys demonstrated glomerular capillary basement membrane localization of IgM generally in a linear pattern; one homograft also had glomerular basement membrane deposits of B1A and B1C globulin and fibrinogen. No localization of IgG or horse globulin was detected.

In all the kidneys with glomerular capillary basement membrane changes, there was some increase in the number of mesangial cells and the amount of

TABLE 1.—Fluorescent and Electron Microscopic Observations on the Glomeruli of eight Renal Homografts in Patients Treated with Horse Antihuman-lymphocyte Globulin

Patient	Electron Microscopy			Fluorescent Microscopy						
	Thickening of Glomerular Capillary Basement Membranes		Fusion of Epithelial Foot Processes	Deposits Demonstrated by Fluorescent Antibody Technique						
	Subendothelial	Subepithelial		IgM	IgG	Horse Globulin	Complement B1A	B1C	Fibrinogen	
LD 107	±	0	±	+	0	0	0	0	0	0
LD 108	0	0	±	0	0	0	0	0	0	0
LD 109	±	0	+	+	0	0	0	0	0	0
LD 110	+	0	++	+	0	0	++	++	+	+
LD 111	++	0	±	+	0	0	0	0	0	0
LD 112	0	0	±	0	0	0	0	0	0	0
LD 113	+	0	++	±	0	0	0	0	0	0
LD 114	+	0	±	+	0	0	0	0	0	0

0 = negative; ± = slight in amount; + = moderate in amount; ++ = marked in amount.

mesangial matrix; the epithelial, endothelial, and mesangial cells possessed increased numbers of free ribosomes, cytosomes, and cytoseresomes, and larger amounts of rough endoplasmic reticulum. In the four most severely damaged homografts, some of the capillary lumina were narrowed by swollen endothelial cells. Neutrophile polymorphonuclear leukocytes were present in the glomerular capillary loops of three of the latter transplants. In each of the eight kidneys, there were areas in which the epithelial foot processes were fused, but this change was only pronounced in two of the grafts.

Although the majority of these renal homografts showed some vascular damage in the form of subendothelial collections of hyaline in arteriolar walls and fibrous intimal thickening of interlobular arteries, in four of the kidneys these changes were minor and affected only a few vessels. In only two were they at all marked.

Dense focal collections of mononuclear cells were present in seven of the homografts and were frequent in four of these. Up to 40 per cent of the cells had pyroninophilic cytoplasm. In two of the grafts, the cytoplasm of some of the infiltrating cells stained positively for IgG while others were positive for IgM; in three other grafts, only cells that stained for IgM were found. Ultrastructurally, some of the cells were plasma cells with elaborate rough endoplasmic reticulum and others were lymphoid with abundant free ribosomes in the cytoplasm but no rough endoplasmic reticulum.

Future Use of ALG

It is, of course, too early to predict what effect antilymphocyte globulin will have on the long-term course after renal homotransplantation or to what

extent it will influence the development of this field. However, two features of the clinical experience reported above are distinctly encouraging. First, the early mortality which has plagued efforts at clinical renal homotransplantation has been sharply reduced if not virtually eliminated. Secondly, this apparently beneficial effect has been possible without prohibitive toxicity. Anaphylactic reactions were seen, but these were surprisingly minor and did not necessitate cessation of therapy in most cases.

Two possible complications of serum nephrotoxicity which could have defeated the purpose for which treatment was intended were proven not to have occurred in any of the first eight cases. These were direct binding of the horse antibodies with the grafted kidney (Masugi nephritis), or secondary injury to the kidney with entrapped soluble antigen-antibody complexes (serum sickness nephritis). The absence of horse protein in these biopsies exclude both possibilities at least in these cases since the heterologous protein in both kinds of experimental nephritis is known to remain in renal tissue for many months after infliction of the injury and would surely have been detectable had it been present.

Furthermore, the continued good function in the first eight cases from two to three months after completion of the course of antilymphocyte globulin is an additional positive notation, particularly since there is reason to believe that any therapy which is of value during the early post-transplant period may confer a long-term benefit. This is not because of a continuing immunosuppressive effect. Rather, it appears to be due to an alteration which leads to a state of relative host-graft nonreactivity. This poorly understood change, termed "adaptation" by Woodruff and Woodruff,²⁶ who were the first to show its development, is the cornerstone upon which many of

the advances in clinical homotransplantation are based, since it implies that the maximum need for immunosuppression is soon after operation. Later it has often been possible to maintain patients for years on drug doses no greater or even less than those which at an earlier time had been quite inadequate for control of rejection.¹⁹ Thus, although it could be reasoned that upward adjustments of azathioprine and especially prednisone dosage might be required at the termination of the four month course of ALG, there was good reason to believe that this might not be the case. In point of fact, none of the eight patients who have been followed for two to four months since their last ALG injection have required such adjustments.

It would be myopic, however, to think that improvements cannot be made with ALG therapy. The product now being used is a relatively crude extract from the serum of horses which conceivably could be immunized in a more effective way. It has been remarked that the principal precipitin response in the patients was to alpha and beta fractions which contain minor quantities of anti-white-blood-cell antibodies. Presumably, if these portions were removed there would be less antigenicity with only slight loss of potency.

In the cases treated until now, the ALG has primarily been used as a steroid-sparing device. That this objective appears to have been met is a significant gain since the steroids have contributed heavily to post-transplantation morbidity and mortality.⁶ Nevertheless, should efforts be successful either to decrease the antigenicity of the foreign protein or to increase its potency, there should be no reason why ALG could not be used as a primary rather than an adjuvant immunosuppressive agent.

Addendum

The foregoing account was written in February 1967. On August 24, 1967, the results remained essentially the same. All patients then alive were still surviving with function of the originally transplanted kidney.

References

1. Abaza, H., Nolan, B., Watt, J., and Woodruff, M. F. A.: The effect of antilymphocytic serum on the survival of renal homotransplants in dogs. *Transplantation*. 4:618, 1966.
2. Abbott, W. M., Otherson, H. B., Monaco, A. P., Simmons, R. L., Wood, M. L., and Russell, P. S.: Prolonged survival of renal allografts in dogs treated with antilymphocyte serum. *Surg. Forum*. 17:228, 1966.
3. Atai, M., and Kelly, W. D.: Preparation of an effective heterologous antilymphocyte serum for use in the dog. *Surg. Forum*. 17:249, 1966.
4. Gray, J. G., Monaco, A. P., and Russell, P. S.: Heterologous mouse antilymphocyte serum to prolong skin homografts. *Surg. Forum*. 15:142, 1964.
5. Gray, J. G., Monaco, A. P., Wood, M. L., and Russell, P. S.: Studies on heterologous antilymphocyte serum in mice. I. *In vitro* and *in vivo* properties. *J. Immunol.* 96:217, 1966.
6. Hill, R. B., Jr., Dahrling, B. E., Starzl, T. E., and Rifkind, D.: Death after transplantation: An analysis of 60 cases. *Amer. J. Med.* 42:327, 1967.
7. Huntley, R. T., Taylor, P. D., Iwasaki, Y., Marchioro, T. L., Jeejeebhoy, H., and Starzl, T. E.: The use of antilymphocyte serum to potentiate canine homograft survival. *Surg. Forum*. 17:230, 1966.
8. Hoehn, R., and Simmons, R. L.: Immunosuppressive drugs combined with heterologous antilymphocyte serum: A clinical regimen for homograft prolongation. *Surg. Forum*. 17:251, 1966.
9. Iwasaki, Y., Porter, K. A., Amend, J., 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. Gynec. Obstet.* 124:1, 1967.
10. Jeejeebhoy, H. F.: Immunological studies on the rat thymectomized in adult life. *Immunology*. 9:417, 1965.
11. Lawson, R. K., Ellis, L. R., and Hodges, C. V.: Antilymphocyte serum in prolongation of dog homografts. *Surg. Forum*. 17:515, 1966.
12. Levey, R. H., and Medawar, P. B.: Some experiments on the action of antilymphoid antisera. *Ann. N. Y. Acad. Sci.* 129:164, 1966.
13. Levey, R. H., and Medawar, P. B.: Nature and mode of action of antilymphocyte antiserum. *Proc. Nat. Acad. Sci.* 129:164, 1966.
14. Mitchell, R. M., Sheil, A. G. R., Slafsky, S. F., and Murray, J. E.: The effect of heterologous immune serum on canine renal homografts. *Transplantation*. 4:323, 1966.
15. Monaco, A. P., Wood, M. L., and Russell, P. S.: Effect of adult thymectomy on the recovery from immunologic depression induced by heterologous antilymphocyte serum. *Science*. 149:423, 1965.
16. Monaco, A. P., Wood, M. L., Gray, J. G., and Russell, P. S.: Studies on heterologous antilymphocyte serum in mice. II. Effect on the immune response. *J. Immunol.* 96:229, 1966.
17. Nagaya, H., and Sieker, H. O.: Allograft survival: Effect of antiserum to thymus glands and lymphocytes. *Science*. 150:1181, 1965.
18. Pichlmayr, R.: Wirkung eines heterologen antilymphocytenserums auf die transplantatabstossung beim hund. *Klin. Wschr.* 44:594, 1966.
19. Starzl, T. E., Marchioro, T. L., and Waddell, W. R.: The reversal of rejection in human renal homografts with subsequent development of homograft tolerance. *Surg. Gynec. Obstet.* 117:385, 1963.

20. Starzl, T. E., Marchioro, T. L., Faris, T. D., McCardle, R. J., and Iwasaki, Y.: Avenues of future research in homotransplantation of the liver: With particular reference to hepatic supportive procedures, antilymphocyte serum and tissue typing. *Amer. J. Surg.* 112:391, 1966.
21. 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. Gynec. Obstet.* 124:301, 1967.
22. Starzl, T. E., Porter, K. A., Iwasaki, Y., Marchioro, T. L., and Kashiwagi, N.: The use of antilymphocyte globulin in human renal homotransplantation. *In* Wolskuholme, G. E. W. and O'Connor, M. (Eds.): *Antilymphocytic Serum*. London, J. and A. Churchill Ltd., 1967, pp. 4-34.
23. Waksman, B. H., Arbouys, S., and Arnason, B. G.: The use of specific "lymphocyte antisera" to inhibit hypersensitive reactions of the delayed type. *J. Exp. Med.* 114:997, 1961.
24. Woodruff, M. F. A., and Anderson, N. F.: Effect of lymphocyte depletion by thoracic duct fistula and administration of antilymphocyte serum on the survival of skin homografts in rats. *Nature (London)*. 200:702, 1963.
25. Woodruff, M. F. A., and Anderson, N. F.: The effect of lymphocyte depletion by thoracic duct fistula and administration of antilymphocyte serum on the survival of skin homografts in rats. *Ann. N. Y. Acad. Sci.* 120:119, 1964.
26. Woodruff, M. F. A., and Woodruff, H. G.: The transplantation of normal tissue: with special reference to auto- and homotransplants of thyroid and spleen in the anterior chamber of the eye, and subcutaneously, in guinea pigs. *Phil. Trans.* 234:559, 1950.