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# THE USE OF HETEROLOGOUS ANTILYMPHOCYTE GLOBULIN (ALG) IN HUMAN RENAL AND LIVER TRANSPLANTATION*

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Early in 1967, preliminary reports were made from our institutions (Starzl, Marchioro *et al.* 1967; Starzl, Porter *et al.*, 1967) of the first clinical use of heterologous antilymphocyte globulin (ALG). This immunosuppressive agent was prepared from the serum of horses that had been immunized against cadaveric lymphoid tissue, especially spleens (Iwasaki *et al.*, 1967). It was added to therapy with standard anti-rejection drugs, azathioprine and prednisone, and its use was restricted to the first 4 postoperative months. The evidence then was that ALG was of value at least in avoiding some of the problems which were present early after renal homotransplantation.

Because ALG was used only during the first months after transplantation, an important unanswered question was whether late rejection would be a serious problem after the foreign protein administration was stopped. Evidence is now available on this point since the survivors amongst the first 20 patients who received consanguineous homografts are 11 to 17 months postoperative; their last ALG injections were 7 to 13 months ago.

The present report will be concerned with a summary follow-up of these early cases; a more detailed analysis is being published elsewhere (Starzl, Groth, Terasaki *et al.*, 1968). In addition a brief description will be given of 4 patients who received orthotopic transplantation of cadaveric livers while under therapy with the same immunosuppressive regimen.

## RENAL HOMOTRANSPLANTATION

### *Management*

Intramuscular globulin injections were given daily for 5 days before and for 2 weeks after transplantation, then every other day for 2 weeks, twice a week for 2 months, and once a week for a final month (Fig. 1). Azathioprine was administered indefinitely. Prednisone was added if rejection developed despite prior therapy with azathioprine and ALG, in some cases because of serologic evidence of a host antibody response to the horse protein (Fig. 1), or in a few instances as a prophylactic immunosuppressive measure from the time of operation.

After the 4 month course of ALG therapy had ended, the patients were watched closely for the appearance of delayed rejection. This complication, which was observed in only 2 of the first 20 cases, was treated by upward adjustments of the prednisone dosage (Fig. 1).

### *Mortality*

The first 20 ALG-treated recipients of consanguineous homografts were operated upon 11 to 17 months ago. Nineteen (95%) are still alive with function of their original homografts. All are in excellent condition. The only death was the consequence of a surgical error (Starzl, Porter *et al.*, 1967).

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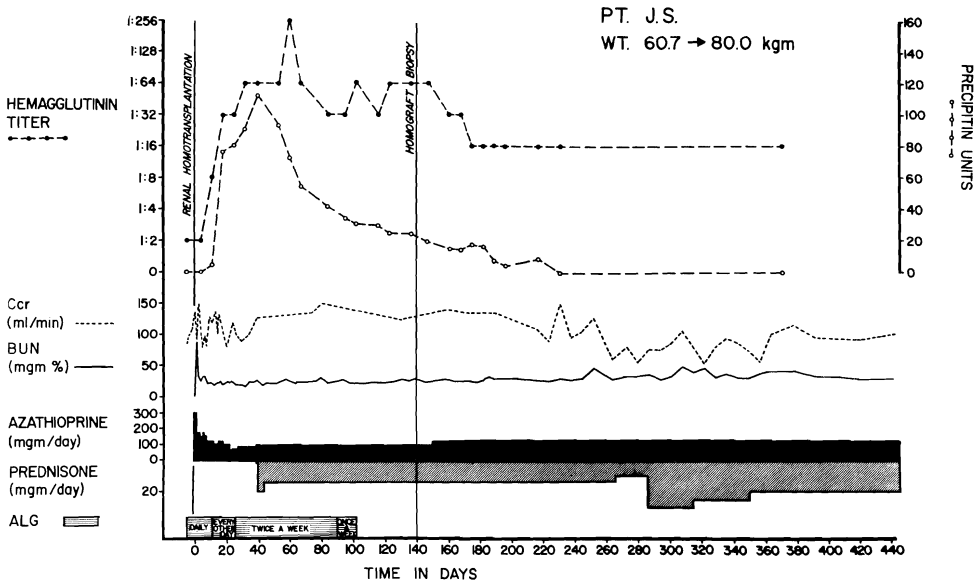


*Fig. 1.* The course of a patient who received antilymphocyte globulin (ALG) before and for the first 4 months after renal homotransplantation. The donor was an elder brother. There was a good histocompatibility match between donor and recipient. There was no early rejection. Prednisone therapy was started 40 days postoperatively because of the high rises in the serologic titers which warned against a possible anaphylactic reaction. Note the insidious onset of late rejection after cessation of globulin therapy. This was treated by increasing the maintenance dose of steroids. This delayed complication was seen in only 2 of the original 20 patients. (By permission of *Surgery, Gynecology and Obstetrics*, 1968, *126*.)

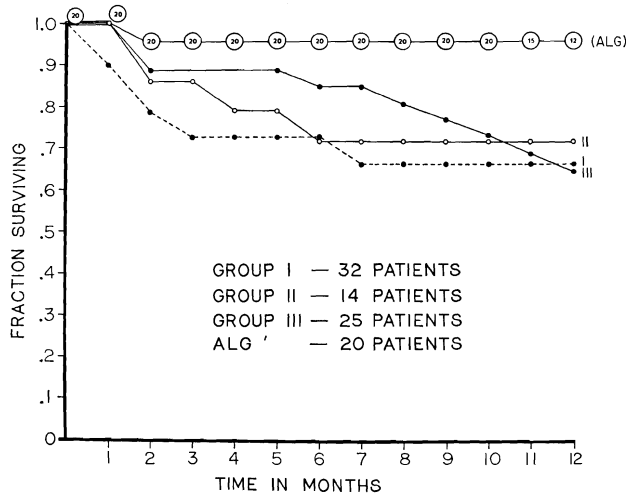


*Fig. 2.* Survival curve of the first 20 recipients of renal homografts who were treated with antilymphocyte globulin (ALG) compared to the survival of those patients in 3 previous series of consanguineous transplantations at our institutions. Follow-ups in the globulin-treated group are 11 to 17 months. (By permission of *Surgery, Gynecology and Obstetrics*, 1968, *126*.)

In Figure 2, these results are contrasted with those obtained in 3 previous consecutive series of 32, 14, and 25 similar patients treated with kidneys from related donors at our institutions. In the past cases, there was a mortality during the same interval of follow-up of 28 to 31%.

*Drug therapy and function*

The improved survival in the ALG-treated patients can most readily be explained by the fact that rejection could be controlled despite the use of reduced doses of the standard immunosuppressive agents. As the earlier series were being compiled there was a progressive tendency to use smaller quantities of azathioprine in an effort to avoid bone marrow depression. This trend continued into the globulin-treated series (Fig. 3).

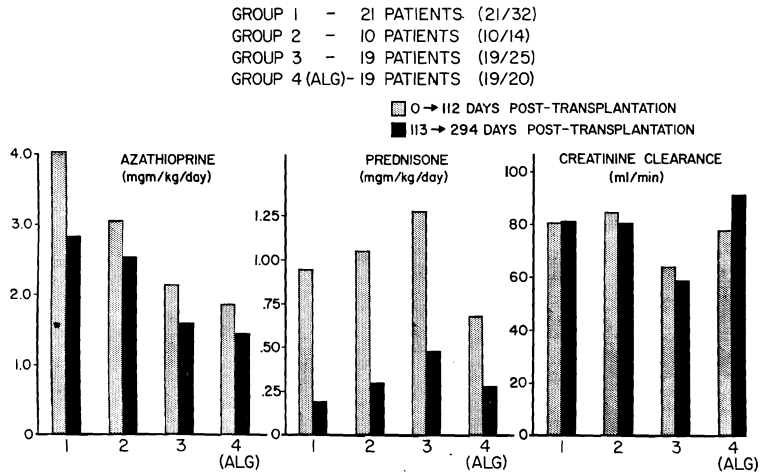


*Fig. 3.* Intrafamilial renal homotransplantation. The average azathioprine and prednisone doses per kg/day and the creatinine clearances for the first 16 postoperative weeks (shaded) and for the subsequent 6 months (solid). Shown are the retrospective control series 1-3, and the ALG series (Group 4). Inclusion in the analysis was contingent upon survival for 294 days, a condition which was met with the highest regularity in the ALG patients. (By permission of *Surgery, Gynecology and Obstetrics*, 1968, *126*.)

In the 3 consecutive retrospective series, the more cautious use of azathioprine had necessitated progressively increasing average doses of prednisone in order to prevent rejection of the homografts. The latter trend was reversed in the ALG series (Fig. 3). The steroid doses in the first 4 months were the smallest of any of the groups, and in the ensuing 6 months these remained at a low level. The latter was a particularly encouraging notation since ALG therapy had then been discontinued.

The gain of treating these patients with smaller doses of standard immunosuppressive agents was not paid for with a loss of homograft function. The creatinine clearances (Fig. 3) and other measures of renal function in the ALG-treated patients compared favorably with those in the retrospective series during the first 4 months post-transplantation, and in the ensuing 6 months they were distinctly better (Fig. 3). The comparative data with prednisone dosages and creatinine clearances are presented on a week to week basis in Figure 4.

In all the foregoing studies, inclusion of any patient in the statistical analysis was contin-

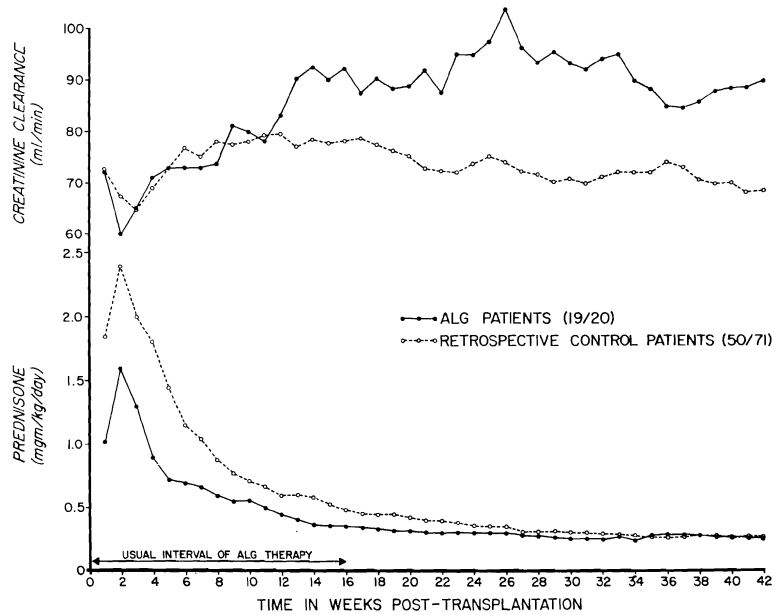


*Fig. 4.* Intrafamilial renal homotransplantation. Average creatinine clearances and daily prednisone doses for the 19 of 20 globulin-treated patients who lived for at least 10 months after receipt of homografts from blood relatives. These results are compared with pooled data from the 50 of 71 patients previously observed who had lived for this long after similar intrafamilial homotransplantation. Note the superior function and the smaller steroid doses of the globulin-treated patients who, because of their greater incidence of survival, were a less highly selected group than the included recipients in the retrospective control series. (By permission of *Surgery Gynecology and Obstetrics*, 1968, *126*.)

gent upon survival for at least 10 months. This requisite was met with a 95% frequency in the ALG series but far less uniformly in the other groups. The favorable showing of the globulin-treated patients was thus in spite of a bias which excluded a number of unfavorable cases from each of the retrospective control series.

*Histocompatibility*

From the survival data and functional analyses presented thus far, it could be concluded that considerable histoincompatibility can be overridden in intrafamilial homotransplantation with the described therapeutic regimen since these cases were not selected because of highly compatible donor-recipient antigen matches as determined by Terasaki *et al.* (1967). Does this suggest that histocompatibility matching techniques are going to become less important?

That question was reviewed by examining the courses during the first 63 postoperative days of the 19 recipients in the above described series plus those of 19 more patients subsequently treated with homografts from blood relatives. The 38 globulin-treated patients were divided into those who had excellent, fair, and poor histocompatibility matches with their donors. The results, which are described fully elsewhere (Starzl, Groth, Terasaki *et al.*, 1968), showed that the best renal function was in the cases with the best matches and that, conversely, the smallest average steroid doses were required in these cases. Thus, the penalties of breeching histocompatibility barriers, even with the improved treatment program, were the need for more stringent therapy and the necessity of accepting somewhat poorer function.

*Cadaveric renal transplantation*

The foregoing results all concern intrafamilial homotransplantation. They indicate that the use of ALG has permitted a reduction in early mortality, that it has contributed to a very considerable overriding of histocompatibility barriers, and that the benefits conferred by having an improved early course outlast the period of globulin therapy. What effect will these developments have upon the field of cadaveric transplantation and upon the transplantation of other organs? Our experience is too small to permit decisive conclusions, but there seems little reason to doubt that improvements will be possible here also.

We have done 6 cadaveric renal homotransplantations using globulin therapy, with follow-ups of 4 to 12 months. Two patients have died, both after receipt of kidneys from a common cadaveric donor, both as a result of multiple pulmonary emboli and both within one day of each other after 3½ months. There was little or no evidence of rejection in either homograft despite the presence of very poor histocompatibility matches in both. Three of the other 4 patients have from good to excellent function of their original cadaveric homografts. The fourth patient, who is 1 year postoperative, has a creatinine clearance of 10-20 ml/min. and a BUN of 60-80 mg%.

*Toxicity*

Pain at the site of ALG injection was almost invariable. A variety of systemic reactions were seen including fever, hives, and generalized skin rashes. Anaphylactic reactions have occurred in 20% of the patients, but these were managed without difficulty. An impending anaphylactic reaction could often be predicted by rises in the titers of host precipitins or hemagglutinins or by the development of positive skin tests to intradermal horse protein.

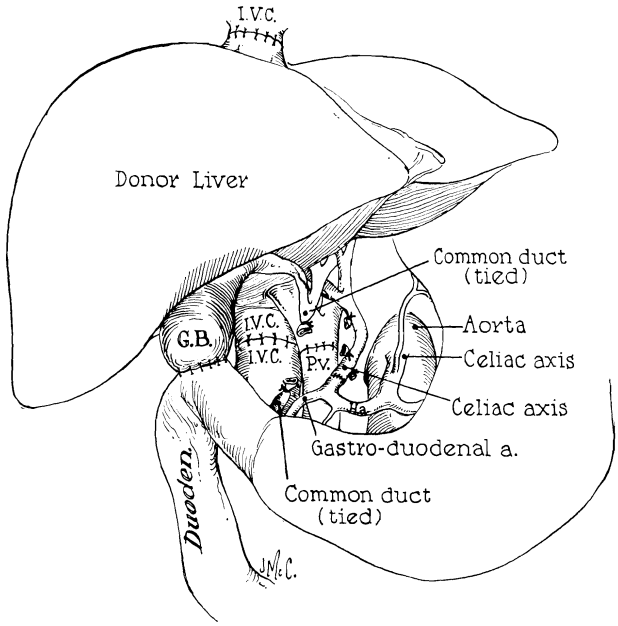


*Fig. 5.* Technique of orthotopic liver transplantation. Note that the celiac axis (or alternatively the common hepatic artery) of the homograft is anastomosed to the proper hepatic artery of the recipient.

The toxicity encountered with more than 2,000 injections of ALG in the first 53 cases has been reviewed elsewhere (Kashiwagi *et al.*, 1968). There have been no deaths caused by this agent. Biopsies of the homografts after 4 months in the first 8 cases revealed no evidence of either Masugi or serum sickness nephritis when examined with immunofluorescence and ferritin-labeling antibody techniques (Starzl, Porter *et al.*, 1967).

## LIVER HOMOTRANSPLANTATION

From 1963 to the spring of 1967, 7 attempts were made at orthotopic liver transplantation at our institutions. All of the patients died in 23 days or less. The many reasons for these failures have been cited elsewhere and suggestions made for their correction (Starzl, Brettschneider and Groth, 1967). Amongst the most hopeful signs was the possibility that ALG administration might contribute to more incisive and less toxic immunosuppression.

In July 1967, 2 children were treated with orthotopic liver homotransplantation (Fig. 5); the first had a hepatoma and the second had extrahepatic biliary atresia. On September 5 and October 8, 2 more girls with biliary atresia received the same procedure. All 4 patients are still alive from 1 to 3½ months postoperatively. A detailed account of this recent series is being published (Starzl, Groth, Brettschneider *et al.*, 1968) but some editorial comments may be in order.

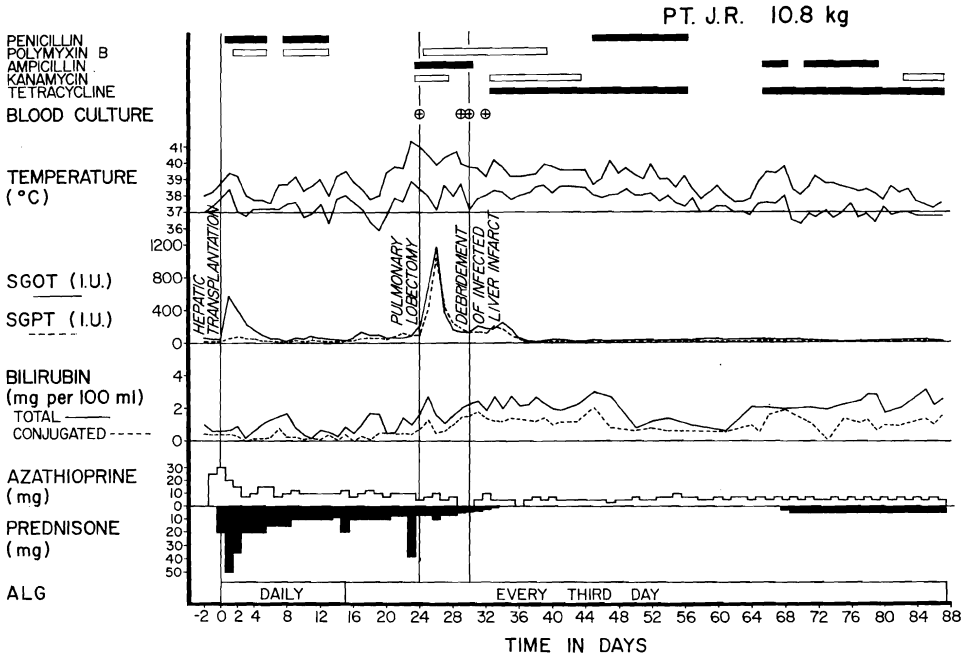


*Fig. 6.* Course after orthotopic liver transplantation in a 19 month old patient whose liver disease was hepatoma. The initial rises in SGOT and SGPT were due to ischemic damage. The high secondary increases occurred at the time a septic liver infarction was diagnosed. The septicemia, indicated by encircled crosses, was with E.coli or Aerobacter-Klebsiella. Her present condition is excellent. (By permission of *Surgery*, 1968, *63*.)

Other improvements than those of immunosuppression were brought to these cases. Every effort was made to minimize ischemic injury to the livers during the terminal course of the donors and during the normothermic postmortem period. Moreover, the extirpated organs were subsequently kept in an extremely effective preservation unit (Brettschneider *et al.*, 1967) until the recipients were prepared. In every instance, good immediate function of the transplanted livers was obtained.

Subsequently, adequate to excellent liver function (Fig. 6) was retained in 3 of the 4 patients. Relatively small doses of azathioprine and prednisone were used. ALG was given every postoperative day for 2 weeks and every third day thereafter (Fig. 6).

The fourth patient had ALG stopped after 7 weeks because of the fear that an anaphylactic reaction was impending; the child had developed high titers of precipitating antibodies against horse protein and had periorbital edema with each injection. Within 2 weeks, severe rejection developed which could be controlled only by increasing the daily prednisone dose to 20 to 60 mg.

A number of serious complications have been seen in one or more of these patients including paralysis of the right diaphragm, septicemia, the development of septic liver infarctions (Fig. 6), atelectasis, and pneumonitis. The fact that none proved lethal may in part be attributable to the fact that heavy immunosuppression with the standard immunosuppressive agents could be avoided. This was possible even though a good donor-recipient antigen match was present in only one case; in the other 3 there was at least 1 breech of the major human histocompatibility antigens which were recently summarized by Terasaki *et al.* (1967).

It is, of course, too early to speculate upon the ultimate fate of these patients. Even now, however, it is evident that their lives have been prolonged. They are the first examples of extended survival after clinical homotransplantation of the liver.

*Summary*

Horse antihuman-lymphocyte globulin (ALG) has been used as an adjuvant immunosuppressive agent for all recipients of renal homografts treated at the University of Colorado in the last 17 months. There is evidence that the addition of ALG has permitted adequate control of rejection when used in combination with reduced quantities of azathioprine and prednisone, that the postoperative mortality has thereby been reduced, and that the risks of the repeated foreign protein therapy are not prohibitive.

Four recipients of orthotopic liver homografts were recently treated with the same therapeutic regimen. They are alive 3½, 3¼, 2, and 1 months postoperatively.

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