# Immunologic Responses of Graft Recipients to Antilymphocyte Globulin: Effect of Prior Treatment with Aggregate-Free Gamma Globulin

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ABSTRACT The immunologic response of graft recipients to antilymphocyte globulin has been studied. The clearance from the serum of $^{125}$I-labeled antilymphocyte globulin was studied in 15 graft recipients previously treated with antilymphocyte globulin and in 4 control patients not previously treated with antilymphocyte globulin. The mean serum half-life of antilymphocyte globulin was 7.2 days in control patients, 3.8 days in 13 renal graft recipients, and 22 hr in 2 heart graft recipients. All but one of the antilymphocyte globulin-treated patients had rapid clearance. Patients treated with equine antilymphocyte globulin had rapid clearance of rabbit and goat antilymphocyte globulin as well as horse antilymphocyte globulin. All patients with rapid clearance of antilymphocyte globulin had circulating antibodies to xenogeneic gamma globulin. Two patients with rapid clearance of antilymphocyte globulin had circulating complexes of antilymphocyte globulin.

Five renal graft recipients were treated with aggregate-free equine gamma globulin before antilymphocyte globulin therapy in an attempt to induce tolerance to xenogeneic gamma globulin. In these five patients neither rapid clearance of antilymphocyte globulin nor significant titers of circulating antibody to xenogeneic gamma globulin developed. The induction of tolerance to xenogeneic gamma globulin may benefit patients treated with antilymphocyte globulin.

## INTRODUCTION

Antilymphocyte globulin (ALG) is an extremely potent immunosuppressive agent in animals (1). Recent evidence suggests that ALG is also effective in man and may improve the survival of human renal transplants (2). Najarian, Simmons, Gewurz, Moberg, Merkel, and Moore (3) have reported that ALG used as the sole immunosuppressant prolongs the survival of skin grafts in man. The use of ALG in man, however, is complicated by immune reactions to the xenogeneic gamma globulin. A large proportion of patients treated with ALG become sensitized to xenogeneic protein and acute anaphylaxis and serum sickness have occurred (2, 4, 5). Butler et al. (6) reported that in cardiac transplant patients treated with ALG immune clearance of $^{125}$I-labeled ALG rapidly ensues. Studies recently published by Taub, Brown, Kochwa, Rubin, and Dameshek (7) and by Butler et al. (6) suggest that tolerance to xenogeneic gamma globulin may be achieved in man by treatment with aggregate-free xenogeneic gamma globulin. We have studied the immune response of ALG-treated graft recipients to xenogeneic ALG and the effect of treatment with aggregate-free xenogeneic gamma globulin upon the subsequent immune response to ALG.

## METHODS

*Antilymphocyte globulin preparation.* Horses, goats, and rabbits were immunized with human lymphoid cells (purified blood lymphocytes, splenic cells, and thymic cells). Two immunization schedules were followed: (*a*) a two-pulse intravenous course (8) and (*b*) a course of multiple subcutaneous inoculations with Freund's adjuvant (9). Anti-lymphocyte serum was absorbed with one-tenth its volume of human plasma and three times with human erythrocytes representing 30, 20, and 10% of its volume. ALG was prepared from absorbed whole serum by precipitation with 50% cold saturated ammonium sulfate followed by batch DEAE-cellulose chromatography. The isolated gamma globulin was centrifuged for 60 min at 100,000 *g*, passed through a 0.22 $\mu$m millipore membrane,

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and stored at 4°C before use. Tests for sterility and pyrogenicity were performed on each batch of ALG. ALG so prepared formed a single precipitin arc on immunoelectrophoresis with rabbit anti-horse serum having the migration characteristic of gamma globulin. The cytotoxicity titer, as measured by trypan blue dye exclusion, ranged from 1:64 to 1:512. Several batches of ALG had mitogenic activity when added to human lymphocytes in culture.

*Preparation of aggregate-free equine gamma globulin.* Commercial equine gamma globulin (Pentex Cohn fraction II) was purified by batch elution from DEAE-cellulose and lyophilized. The purified equine gamma globulin was dissolved in physiologic saline immediately before use and aggregated protein removed by centrifugation for 60 min in a Beckman Ti 50 rotor at 45,000 rpm ($g_{av} = 133{,}500$). Before treatment patients were evaluated for anaphylactic sensitivity to equine gamma globulin by intradermal skin tests. Patients having negative skin tests were given 50 mg/kg aggregate-free equine gamma globulin.

*Iodination of gamma globulin.* ALG from a single batch of horse, rabbit, or goat ALG and autologous human gamma globulin were labeled with $^{125}I$ by the chloramine T method as described by McConahey and Dixon (10). The iodine product was dialyzed against 0.15 M sodium chloride containing 0.01 M sodium phosphate buffer, pH 7.2. After dialysis more than 98% of the radioactivity was precipitated with 10% trichloroacetic acid. The iodinated protein retained its characteristic immunoelectrophoretic and gel filtration properties. Iodinated rabbit ALG injected intravenously into two rabbits was found to have a serum half-life of 5.3 and 6.1 days. This disappearance rate is compatible with data in the literature (11), and suggested that only minimal denaturation of the gamma globulin had occurred as a result of the iodination procedure. Before administration to patients the iodinated ALG was sterilized and centrifuged as described above.

*Immune clearance of ALG-$^{125}I$.* 0.1 μCi of ALG-$^{125}I$ per kg was administered intravenously (10–50 μg of protein per μCi) to patients. $^{125}I$ uptake by the thyroid was blocked by the administration of a saturated solution of potassium iodine, 10 drops twice daily. Daily clotted blood samples were obtained for the following 14 days. A 2 ml serum sample from each of the 14 days of study was counted in a sodium iodide crystal well counter. After the initial 48 hr, the decline in serum radioactivity followed first order kinetics. The half-life of iodinated ALG was calculated from the decline in serum radioactivity after the first 48 hr. 1 ml of serum obtained 1 hr after intravenous administration of ALG-$^{125}I$ was analyzed by gel filtration through a 2.5 × 40 cm column of Sephadex G 200 using 0.15 M sodium chloride as eluant.

*Circulating antibody to xenogeneic gamma globulin in ALG-treated patients.* Serum samples were examined for the presence of antibody to xenogeneic gamma globulin. Precipitating antibody was assayed by immunodiffusion in gel (12); complement fixing antibody was assayed by the micromethod of Wasserman and Levine (13); and hemagglutinating antibody was assayed by the passive hemagglutinating method of Stavitsky (14) using tanned human type O red cells to which gamma globulin was absorbed.

*Immunosuppressive therapy in graft recipients.* Graft recipients were initially treated after transplantation with azathioprine (2–4 mg/kg), corticosteroid (1–2 mg/kg of prednisolone), and ALG. The subsequent dosage of azathioprine and corticosteroid was determined by the clinical state of graft function. Most patients were given 20–200 mg of ALG per day during the first 3–4 wk after transplantation and the dose was subsequently tapered so that most of the outpatients were receiving 20 mg/wk after 3 months. Several patients were studied more than 3 months after transplantation and had received more than one preparation of ALG. Patients E.T., P.S., J.K., and C.H. as well as the five patients pretreated with aggregate-free gamma globulin received the same preparation of ALG. Initially most of the ALG was administered subcutaneously but more recently as larger doses of ALG have been used the intravenous route has been employed.

## RESULTS

*Survival of ALG-$^{125}I$ in patients before and after ALG therapy.* The first immunological response studied in graft recipients was the clearance of ALG from the serum. The survival of ALG-$I^{125}$ in serum was studied in four patients not previously treated with ALG. Two patients (M.P. and J.V.) were studied before transplantation and were not receiving immunosuppressive therapy. Two patients (W.C. and W.Ch.) were studied immediately after transplantation and were receiving azathioprine and corticosteroids and ALG for a part of the study period. The serum half-lives of these four patients ranged from 6.0 to 8.3 days with no significant difference attributable to immunosuppressive therapy. Clearance of ALG-$^{125}I$ was then studied in 15 graft recipients treated with ALG for a minimum of 4 wk. Of these 15 patients, 13 patients were renal graft recipients and 2 were cardiac graft recipients. Fig. 1 graphically illustrates the survival of ALG-$^{125}I$ in the serum of the 4 patients not previously treated with ALG and the 15

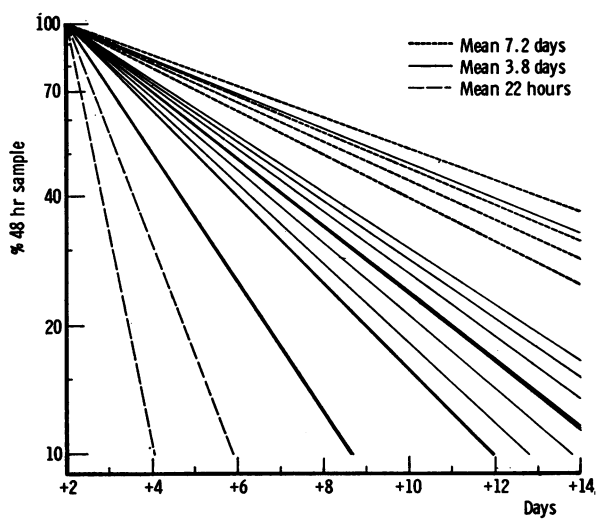


FIGURE 1 Disappearance of ALG-$^{125}I$ from the serum of 4 patients not previously treated with ALG (- - - - -), 13 renal transplant recipients previously treated with ALG (———), and 2 cardiac transplant recipients previously treated with ALG (— — —). The results are expressed as the percentage of radioactivity 48 hr after administration of ALG-$^{125}I$ found on the subsequent 12 days.

patients who had received over 4 wk of ALG therapy before clearance studies. The calculated serum half-lives in each of these patients and the cumulative dose of ALG before survival study are given in Table I. All but one (G.M.) of these patients on ALG treatment had rapid clearance of ALG-$^{125}$I from the serum. This unique patient had received small doses (20 mg/wk) of ALG for 9 months before the clearance study. His cumulative dose of ALG before the survival study, however, was comparable to several other graft recipients studied. No known immunologic deficiency was recognized in this patient. A highly significant difference ($P < 0.01$) in the mean serum survival of ALG-$^{125}$I was found between the patients studied before ($t_{\frac{1}{2}} = 7.2$ days) and after 4 wk of ALG therapy ($t_{\frac{1}{2}} = 3.8$ days). The two heart graft recipients had the most rapid clearance of ALG-$^{125}$I ($t_{\frac{1}{2}} = 15$ and 29 hr). These patients were treated more intensively with ALG than were the renal graft recipients. One patient (W.Ch.) was studied both at the inception of ALG therapy and after 4 months of ALG treatment. His serum half-life of ALG-$^{125}$I was 6.8 days initially and decreased to 3.6 days 4 months later.

TABLE I

*Serum Half-Life of ALG-$^{125}$I in Man*

| | Cumulative dose of ALG before study | $t_{\frac{1}{2}}$ |
|---|---|---|
| | *mg* | *days* |
| I Patients not treated with ALG before survival study | | |
| J. V. | — | 6.0 |
| W. Ch. | — | 6.8 |
| M. P. | — | 7.6 |
| W. C. | — | 8.3 |
| | | Mean = 7.2 |
| II Renal graft recipients treated with ALG before survival study | | |
| W. P. | 780 | 2.0 |
| E. M. | 1240 | 2.0 |
| E. S. | 1110 | 3.0 |
| E. T. | 880 | 3.2 |
| P. S. | 3600 | 3.6 |
| W. Ch. | 560 | 3.6 |
| D. A. | 1720 | 4.0 |
| S. B. | 720 | 4.0 |
| J. G. | 580 | 4.0 |
| J. K. | 1800 | 4.2 |
| C. H. | 1000 | 4.4 |
| H. L. | 540 | 4.6 |
| G. M. | 760 | 7.9 |
| | | Mean = 3.8 |
| | | *hr* |
| III Heart graft recipients treated with ALG before survival study | | |
| G. B. | 4000 | 15 |
| P. M. | 3000 | 29 |

TABLE II

*Serum Half-Life of Globulin-$^{125}$I in Patients with Rapid Clearance of Equine ALG*

| Patient | Horse ALG | Goat ALG | Rabbit ALG | Autologous globulin |
|---|---|---|---|---|
| | *hr* | *hr* | *hr* | *days* |
| G. B. | 15 | 48 | 26 | — |
| P. M. | 29 | 24 | — | — |
| W. P. | 48 | 22 | 39 | 15.2 |
| J. G. | 96 | — | 86 | 16.8 |

*Survival of rabbit ALG, goat ALG, and human gamma globulin in patients with rapid clearance of equine ALG-$^{125}$I.* Several patients in whom rapid clearance of equine ALG had been documented were switched to ALG raised in other species. In order to evaluate the success of this means to circumvent the rapid clearance of ALG, the survival of goat and rabbit ALG in patients treated only with equine ALG was studied. Two patients not previously treated with ALG who received iodinated rabbit ALG had half-lives of 8.6 and 10.4 days. In another similar patient iodinated goat ALG had a half-life of 6.8 days. In two patients rendered tolerant to equine gamma globulin the half-life of goat and rabbit ALG was 6.4 and 6.2 days, respectively. Thus the half-life of goat and rabbit ALG does not appear to be shorter than the half-life of equine ALG in man. The clearance of ALG-$^{125}$I raised in goats and rabbits was studied in four patients who showed rapid clearance of equine ALG. The results are displayed in Table II. The survival of goat and rabbit ALG in one patient (G.B.) was greater than that of equine ALG although shorter than the survival of ALG in the four patients not previously treated with ALG (Table I). In three other patients studied the survival of goat and rabbit ALG was the same as that of equine ALG despite the fact that the patients had not received any rabbit or goat globulin before testing. It was not possible to circumvent the rapid clearance of ALG once established by changing the species source of ALG. This accelerated clearance of xenogeneic gamma globulin does not appear to be due to a generalized increase in protein catabolism since the serum half-life of $^{125}$I-labeled autologous gamma globulin measured in two patients (Table II, patients W.P. and J.G.) was in the normal range. Thus, the immune response assayed by the rate of clearance of ALG shows broad cross-reactivity among xenogeneic gamma globulins.

*Distribution of ALG-$^{125}$I in serum of ALG-treated graft recipients.* The occurrence of serum sickness in patients treated with ALG results from the circulation of immune complexes, presumably of ALG and antibody to it. We have examined the distribution of radioactive ALG in the serum of patients treated with ALG as an

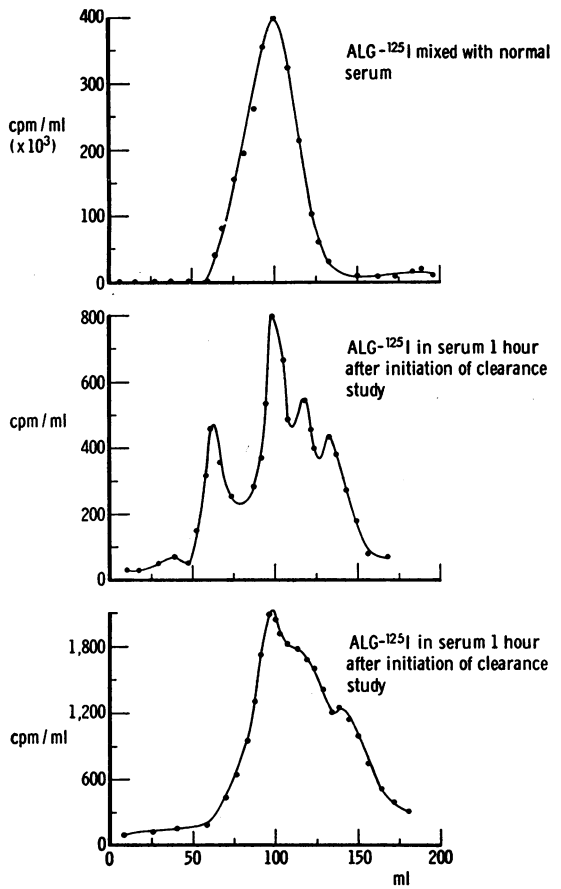


FIGURE 2 Elution of ALG-$^{125}$I from (*a*) whole serum mixed in vitro with ALG-$^{125}$I (top panel), (*b*) whole serum obtained from patient sensitized to ALG 1 hr after intravenous administration of ALG-$^{125}$I (middle panel), and (*c*) whole serum obtained from patient not sensitized to ALG 1 hr after intravenous administration of ALG-$^{125}$I (bottom) after gel filtration through Sephadex G 200.

index of the presence of immune complexes. The size distribution of radioactivity in blood 1 hr after intravenous administration of ALG-$^{125}$I was studied by gel filtration through Sepadex G 200. The vast bulk of radioactivity in whole blood was found in the plasma. Less than 1% of the whole blood radioactivity was associated with the washed cell pellet. The distribution of radioactivity among the serum proteins was examined by Sephadex G 200 gel filtration of whole serum. Studies were carried out on (*a*) normal serum mixed in vitro with iodinated ALG, (*b*) serum obtained 1 hr after intravenous administration of ALG-$^{125}$I to the patient (G.B.) with the most rapid serum clearance of ALG, and (*c*) serum obtained 1 hr after ALG-$^{125}$I administration to the patient (G.M.) with the least rapid serum clearance of ALG. The elution of radioactivity after gel filtration in these three situations is shown in Fig. 2. The radioactivity after in vitro mixture of normal serum and ALG-$^{125}$I has the elution characteristic of 7S gamma globulin, $K_{av} = 0.26$, (15). In contrast serum from patient G.B. had a significant portion of radioactivity present in the void volume. Slower peaks of radioactivity with $K_{av}$ values greater than the starting material may represent degradation products of infused ALG. A significant fraction of radioactivity was found in the void volume in two patients (G.B. and W.P.) both of whom had a very rapid clearance of ALG-$^{125}$I. Serum from several remaining patients showed a peak of radioactivity with shouldering toward the late fractions.

Aggregation of ALG by serum from G.B. and W.P. could be demonstrated by gel filtration after in vitro incubation of ALG-$^{125}$I and serum. The elution of radioactivity after gel filtration of such an in vitro mixture is shown in Fig. 3. The amount of radioactivity that appeared in the void volume seemed to be limited by the amount of serum and not the amount of ALG-$^{125}$I. A 10-fold increase in ALG-$^{125}$I mixed with serum did not increase the amount of radioactivity appearing in the void volume. These studies indicate that in certain patients treated with ALG aggregates of ALG form and

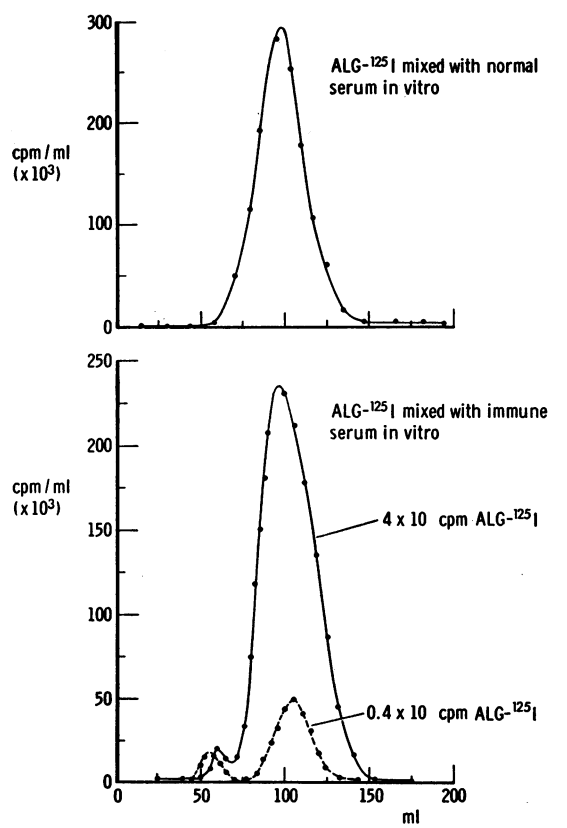


FIGURE 3 Elution of ALG-$^{125}$I after in vitro mixture with normal serum (top panel) and serum obtained from a patient with the most rapid clearance of ALG (bottom panel).

these most probably represent complexes of ALG and human antibody to it.

*Circulating antibody to xenogeneic gamma globulin in ALG-treated graft recipients.* It is clear that sensitization to xenogeneic gamma globulin occurs during the treatment of graft recipients with ALG. The presence of circulating antibody was tested by the following three techniques: precipitation in gel, complement fixation, and passive hemagglutination. Precipitating antibody could not be detected by double diffusion in gel. Complement fixing antibody was looked for in 14 of the ALG-treated graft recipients. In only three patients (G.B., S.B., and W.P.) was complement fixing antibody demonstrated. It was possible to demonstrate hemaggutinating antibody activity in some patients with rapid clearance of ALG-$^{125}$I (Table III). The hemagglutination titer in four patients not treated with ALG and eight normal volunteers is shown in Table IV. The occasional occurrence of low titers in these individuals may be a nonspecific reaction or alternatively indicate that low titers of circulating antibody in certain patients may not result from specific immunization to ALG. A correlation between the titer of hemagglutinating antibody activity and the rate of clearance of ALG-$^{125}$I can be seen from the data. In general, the higher titers of antibody were found in patients with the most rapid clearance of ALG. The serum from four patients (G.B., W.P., S.B., and P.S.) were tested for hemagglutinating activity against rabbit, goat, and horse gamma globulin. The results are shown in Table IV.

TABLE III

*Circulating Antibody Activity in ALG-Treated Patients*

| | Precipitating | Complement fixing | Hemagglutinating (titer) |
|---|---|---|---|
| I ALG-treated renal transplants | | | |
| W. P. | 0 | + | 512 |
| E. M. | 0 | 0 | 64 |
| E. S. | 0 | 0 | 128 |
| E. T. | 0 | 0 | 8 |
| P. S. | 0 | N.T. | 512 |
| W. Ch. | 0 | 0 | 2 |
| D. A. | 0 | 0 | 4 |
| S. B. | 0 | + | 128 |
| J. G. | 0 | 0 | 1 |
| J. K. | 0 | 0 | 2 |
| C. H. | 0 | 0 | 2 |
| H. L. | 0 | 0 | 2 |
| G. M. | 0 | 0 | 0 |
| II ALG-treated heart transplants | | | |
| G. B. | 0 | + | 512 |
| P. M. | 0 | 0 | 2 |
| III ALG-treated renal transplants, pretreated with equine gamma globulin | | | |
| P. F. | 0 | N.T. | 0 |
| D. Y. | 0 | N.T. | 0 |
| V. R. | 0 | N.T. | 1 |
| J. B. | 0 | N.T. | 1 |
| C. N. | 0 | N.T. | 1 |

TABLE IV

*Hemagglutination Titers to Goat, Rabbit, and Horse Gamma Globulin in Serum of Normal Volunteers and Patients Treated with and without Equine ALG*

| Patients | Horse | Goat | Rabbit |
|---|---|---|---|
| Volunteers | | | |
| M. W. | 1 | 0 | 0 |
| B. W. | 0 | 0 | 0 |
| L. H. | 1 | 0 | 1 |
| C. B. | 0 | 0 | 1 |
| M. L. | 0 | 0 | 0 |
| R. R. | 0 | 0 | 0 |
| W. L. | 1 | 0 | 0 |
| R. P. | 0 | 0 | 0 |
| Patients not previously treated with ALG | | | |
| J. V. | 2 | 1 | 0 |
| W. Ch. | 0 | 0 | 0 |
| W. C. | 1 | 0 | 0 |
| M. P. | 0 | 0 | 0 |
| Patients with circulating antibody to equine gamma globulin | | | |
| W. P. | 512 | 128 | 128 |
| E. S. | 128 | 16 | 32 |
| P. S. | 512 | 16 | 16 |
| S. B. | 128 | 128 | 32 |
| G. B. | 512 | 512 | 256 |

*Influence of pretreatment with aggregate-free equine gamma globulin on the survival of ALG-$^{125}$I and antibody development after ALG treatment in graft recipients.* The demonstration that rapid sensitization to xenogeneic gamma globulin follows ALG therapy implies that the induction of tolerance to ALG may have clinical benefits. Five renal graft recipients have been pretreated with aggregate-free equine gamma globulin before transplantation and the initiation of ALG therapy. These patients received 50 mg/kg aggregate-free equine gamma globulin by intravenous infusion 1–4 days before transplantation and 3–7 days before the initiation of ALG therapy. Clearance of ALG-$^{125}$I was studied 3–8 wk after transplantation. The cumulative dose of ALG before survival study and the calculated serum half-life of ALG-$^{125}$I are given in Table V. The mean survival of

TABLE V

*Serum Half-Life of ALG-$^{125}I$ in Patients Pretreated with Aggregate-free Equine Gamma Globulin*

| Patient | Cumulative dose of ALG before survival study | $t_{\frac{1}{2}}$ |
|---|---|---|
| | | *days* |
| V. R. | 1400 | 5.5 |
| C. N. | 2400 | 5.8 |
| P. F. | 1820 | 5.9 |
| J. B. | 2100 | 6.1 |
| D. Y. | 920 | 6.2 |
| | | Mean 5.9 |

ALG-$^{125}I$ in this group of patients ($t_{\frac{1}{2}} = 5.9$ days) is significantly ($P < 0.02$) longer than that seen in the group of ALG-treated renal graft recipients ($t_{\frac{1}{2}} = 3.8$ days) described in Table I. The mean serum survival appears to be shorter than that found in the patients not previously treated with ALG; however, the difference is not statistically significant ($P > 0.10$). Serum obtained from three patients 1 hr after ALG-$^{125}I$ administration did not show any radioactivity in the void volume when subjected to gel filtration on Sephadex G 200. Except for the occurrence of fever 4 hr after infusion of aggregate-free gamma globulin in one patient, no untoward effects of aggregate-free gamma globulin administration were noted. One patient (P.F.) had an open renal biopsy 5 wk after the aggregate-free equine gamma globulin infusion and ALG therapy. No deposits of gamma globulin or complement were found in the glomeruli by immunofluorescence staining techniques. Serum from the five pretreated patients showed insignificant titers of hemagglutinating activity (Table III). In two cases no antibody activity was detectable and in three hemagglutinating activity was found only in undiluted serum specimen.

## DISCUSSION

Since the introduction of antiserum therapy of human disease at the turn of the century, investigators have repeatedly documented the dangers of serum therapy in man (16). The administration of large doses of xenogeneic protein may lead to a rather typical clinical syndrome, serum sickness, in both man (17) and experimental animals (18). We have studied the immunologic reactions of renal and cardiac allograft recipients to ALG. Despite immunosuppressive therapy, 14 of 15 graft recipients treated with ALG developed rapid immune clearance of ALG and produced circulating antibodies to xenogeneic gamma globulin. This antibody response in immunosuppressed patients is not altogether surprising for Rowley, MacKay, and McKenzie (19) have demonstrated a normal primary response to *Salmonella* flagellin antigen in renal allograft recipients receiving similar immunosuppressive therapy, and Lance and Dresser (20) and Clark, James, and Woodruff (21) have shown that ALG is highly immunogenic.

Butler et al. (6) first reported that ALG-treated cardiac graft recipients rapidly cleared radioactive ALG from the serum, as early as 10–14 days after the initiation of ALG therapy. We have confirmed the rapid clearance of ALG in 2 cardiac recipients and found 12 of 13 ALG-treated renal graft recipients had rapid clearance of ALG. A number of graft recipients with rapid clearance of ALG had significantly elevated titers of circulating hemagglutinating antibodies. The antibody titer was highest in those patients with the most rapid clearance of ALG. Despite laboratory evidence of sensitization in nearly all the ALG treated graft recipients, only three patients exhibited allergic phenomena.

One of the most serious side effects of chronic ALG administration is the development of serum sickness. The role of circulating complexes of antigen and antibody in the pathogenesis of serum sickness has been clearly demonstrated (18). Circulating immune complexes were demonstrated in the serum of two patients. In addition one cardiac transplant recipient showed evidence of deteriorating renal function during ALG therapy. In this patient creatinine clearance fell markedly during ALG therapy, rose when ALG was discontinued, and fell again when ALG therapy was restarted. No immunofluorescent study was made at postmortem examination in this case and the pathogenesis of the renal failure remains unproven. Immune complex nephritis, however, has been documented during ALG therapy in man (5) and experimental animals (22).

The effect of circulating antibody to ALG and the resulting rapid clearance of ALG from the serum on the immunosuppressive potency of ALG is uncertain. Some investigators suggest that sensitization to ALG is associated with a loss of immunosuppressive potency (23–25). On the other hand some workers (26, 27) have found no loss in immunosuppressive potency of ALG despite actively induced anti-ALG antibody. Nonetheless, the induction of tolerance to xenogeneic gamma globulin before ALG therapy is acknowledged to potentiate its immunosuppressive potency in animals. Tolerance to ALG has been produced in animals by the transfer of biofiltered gamma globulin (26), administration of normal xenogeneic serum in the neonatal period (27, 28), and the administration of aggregate-free (ultracentrifuged) gamma globulin (21, 29). It has not been possible to induce tolerance to ALG in man with ultracentrifuged ALG (6) although both Taub et al. (7) and Butler et al. (6) have offered evidence that the administration of aggregate-free gamma globulin be-

fore ALG prevents immunologic reactivity of patients to ALG.

The present study demonstrates that treatment of graft recipients with aggregate-free gamma globulin prevents subsequent rapid immune clearance of ALG and the development of significant titers of antibody to ALG after the initiation of ALG therapy. No untoward effects of treatment with aggregate-free gamma globulin were found. The induction of tolerance to xenogeneic gamma globulin should prevent the occurrence of serum sickness during ALG therapy and may potentially augment its immunosuppressive potency.

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