Canadian clinical trial of antilymphocyte globulin in human cadaver renal transplantation

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A multicentre, randomized clinical trial of antilymphocyte globulin (ALG) was conducted among patients who had undergone cadaver kidney transplantation; follow-up was continued for a minimum of 1 year. Of the 179 patients 92 were given conventional treatment only, while 87 were given in addition ALG (from a standardized, highly immunosuppressive, common pool of equine ALG), 20 mg/kg•d intravenously for 10 days after transplantation. The ALG-treated group had better accumulated graft survival, fewer nephrectomies, better graft function, less than half the number of acute rejection episodes and less prednisone use. There was a beneficial drug (ALG)-related effect in both the graft and the host during the first 3 months after transplantation.

Une étude clinique multicentres, avec randomisation des sujets, de la globuline antilymphocytaire (GAL), a été réalisée chez des patients qui ont reçu une greffe de rein provenant d'un cadavre; la surveillance s'est poursuivie sur une période d'au moins 1 an. Sur 179 patients 92 ont reçu le traitement classique seul, alors que 87 ont reçu de la globuline antilymphocytaire (provenant d'une réserve commune de GAL de cheval, standardisée et fortement immunodépresseur), à raison de 20 mg/kg par jour administré par voie intraveineuse jusqu'à 10 jours après la transplantation. Le groupe qui a reçu la GAL a présenté une survie accumulée améliorée du greffon, un nombre inférieur de néphrectomies, un meilleur fonctionnement de la greffe, moins de la moitié du nombre d'épisodes aigus de rejet et une moins grande utilisation de prednisone. On a constaté un effet bénéfique de la GAL aussi bien pour le greffon que pour le receveur durant les 3 premiers mois qui ont suivi la transplantation.

Antilymphocyte globulins (ALG) have been used as an adjunct to immunosuppressive therapy in organ transplantation for the past 10 years. Their usefulness has been difficult to evaluate because of many variables, including different manufacturing methods, standardization, dose schedules, administration routes and criteria for patient selection, the bias of individual centres and even the use of different lots of serum in the same patient.[1]

For this reason the Medical Research Council of Canada established a cooperative, multicentre, randomized clinical trial with a common pool of ALG shown to be immunosuppressive. In this trial, carried out in 12 Canadian renal transplant centres (Table I), patients treated by conventional methods (control group) were compared with patients given conventional therapy plus ALG (ALG group). This paper reports the findings in these patients during the first 12 months after transplantation.

Methods

Production of ALG

Preparation of the antigen (the membrane- and microsome-rich fraction of human thymocytes), immunization and purification of the horse antihuman thymocyte ALG used in the trial have

Table I—Participants in trial of antilymphocyte globulin (ALG) as part of therapy after renal transplantation

Transplant centre	No. of patients: ALG group	Control group	Total	Director
Dalhousie University (Victoria General Hospital)	17	17	34	A. S. Macdonald
University of Montreal (Hôpital Maisonneuve)	13	14	27	Claude Beaudry
McGill University (Montreal General Hospital)	11	11	22	C.F.D. Ackman
McGill University (Royal Victoria Hospital)	4	4	8	R.D. Guttmann
Queen's University (Kingston General Hospital)	1	1	2	P.A.F. Morrin
University of Toronto (Toronto General Hospital)	6	10	16	D.R. Wilson
University of Toronto (Toronto Western Hospital)	5	4	9	G.A. de Veber
McMaster University (St. Joseph's Hospital)	3	4	7	P.R. Knight
University of Manitoba (Health Sciences Centre)	1	3	4	A.E. Thomson
University of Saskatchewan (University Hospital)	7	7	14	M.A. Baltzan
University of Alberta (University of Alberta Hospital)	11	9	20	J.B. Dossetor
University of British Columbia (Vancouver General Hospital)	8	8	16	C.E. Reeve
Totals:	87	92	179	

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been described in detail by Wilson, Laurie and Logan.[2]

The common pool (168 *l*) of crude antilymphocyte serum (ALS) was derived from blood collections on days 56, 63 and 70 after the start of immunization. The serum was adsorbed with human erythrocyte stroma wet pack and fresh human plasma negative for hepatitis B antigen, then was purified by treatment with QAE-Sephadex A-50 (Pharmacia, Sweden) and ammonium sulfate. The resulting ALG was diluted to 5.0% protein to yield 30 *l* of ALG with a gamma globulin content of more than 99%. It contained no antibodies to human protein or glomerular basement membrane.[3]

Immunosuppressive activity of ALG

The final purified product had a lymphocytotoxic titre of 1:8000, a hemagglutination titre of 1:64, a platelet complement fixation titre of 1:128 and rosette inhibition titres of 1:16 000 and 1:32 000 (tested twice) (J. Dormont: personal communication, 1971).

In vivo tests for immunosuppressive activity were conducted. Mean survival of 12 skin grafts in three cynomolgus monkeys given 120 mg of ALG per kilogram of body weight three times weekly for a total of 10 injections was 32.7 ± 6.4 days; two skin grafts in a chimpanzee survived for 49 and more than 66 days (H. Balner: personal communication, 1971). One human skin graft in a patient with multiple sclerosis given the clinical trial dosage of ALG survived 28 days. Tuberculin skin tests converted from positive to negative in three patients given the clinical trial dosage of ALG.

Criteria for admission to the trial

The following criteria had to be met before a patient was admitted to the trial:

1. ABO blood group compatibility between recipient and donor.
2. Negative direct crossmatch between recipient and donor for lymphocytotoxic antibodies.
3. No previous ALG therapy in the recipient.
4. Negative skin test for sensitivity to horse serum protein, with equine tetanus antitoxin as the antigen, at a 1:10 dilution.
5. No previous transplantation.
6. Receipt of a cadaver renal transplant by the patient.

Allocation to groups

Patients at each participating centre were allocated by means of a table of random numbers to one of the two treatment groups being compared. A sealed envelope system was used, the envelope being opened during the operation, after the decision to transplant had been made and the graft was in position.

A total of 179 patients were admitted to the trial, 87 to the ALG group and 92 to the control group. There were no major differences between the two groups with regard to age, sex, underlying renal disease, frequency of previous bilateral nephrectomy and tissue matching patterns, which indicated that the randomization had produced comparable groups.

Treatment schedule

Based on the report of Najarian and Simmons,[4] a dose of 20 mg of ALG per kilogram of body weight was selected, to be given intravenously in saline over 8 hours each day for 10 days after transplantation. The initial dose was given only after the graft was vascularized. Some centres used central venous pressure catheters to administer the ALG but with experience the use of the hemodialysis arteriovenous fistula was adopted by most.

It was agreed that "conventional therapy" could include administration of azathioprine, hydrocortisone and actinomycin D, and radiation therapy directed to the transplant, but it was left to the treating physician to make a choice and to adjust dosage according to the progress and response of the patient.

Evaluation of effect of treatment

The effect of ALG treatment on the survival of the transplant and on the quality of survival of the recipient was evaluated from the following measures:

1. Accumulated graft survival.[5]
2. Creatinine clearance and serum creatinine concentration.
3. The numbers of major and minor rejection episodes.
4. Prednisone consumption.
5. Occurrence of complications.

Renal biopsy was performed at the discretion of the physician.

Results

Accumulated graft survival

Accumulated graft survival by treat-

Table II—Accumulated survival of grafts and patients

	No. (and %) of patients; time after transplant (mo)							
	ALG group (n = 87)				Control group (n = 92)			
Status	3	6	9	12	3	6	9	12
Graft survived	66 (75.9)	58 (66.7)	55 (63.2)	54 (62.1)	60 (65.2)	55 (59.8)	53 (57.6)	51 (55.4)
Graft failed	21 (24.1)	29 (33.3)	32 (36.8)	33 (37.9)	32 (34.8)	37 (40.2)	39 (42.4)	41 (44.6)
Nephrectomy	14 (16.1)	16 (18.4)	17 (19.5)	18 (20.7)	23 (25.0)	24 (26.1)	25 (27.2)	27 (29.3)
Patient died	7 (8.0)	13 (14.9)	15 (17.2)	15 (17.2)	9 (9.8)	13 (14.1)	14 (15.2)	14 (15.2)

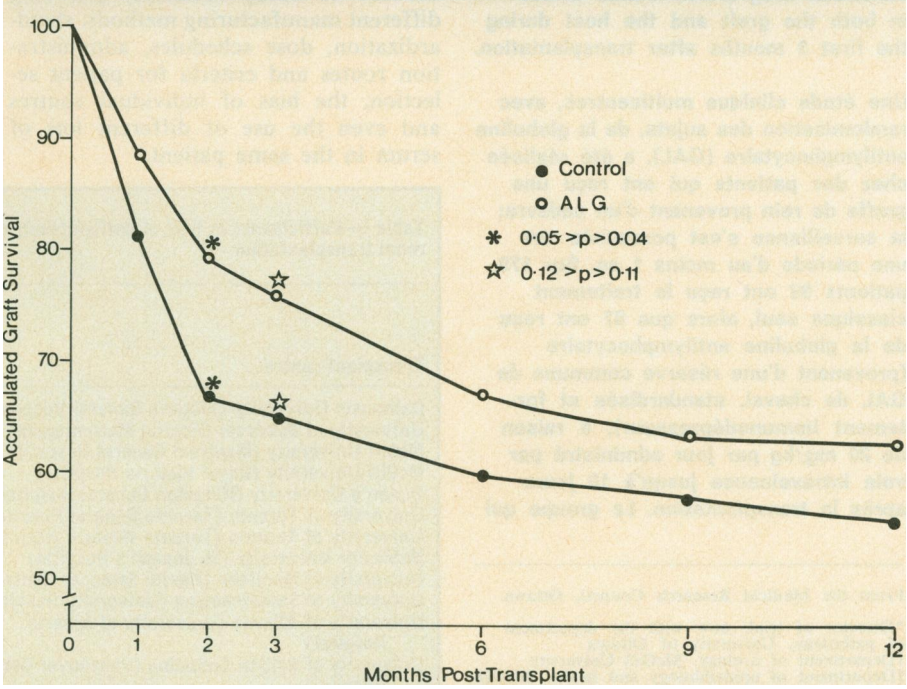

FIG. 1—Accumulated graft survival (%) in patients treated with conventional therapy alone (control group) and patients treated with conventional therapy plus antilymphocyte globulin (ALG group).

ment group for the first 12 months after transplantation is shown in Table II and Fig. 1. At the end of each 3-month period the accumulated graft survival was better in the ALG group than in the control group and there were fewer nephrectomies in the ALG group. The difference was most pronounced at the end of the first 3-month period, when accumulated graft survival was 75.9% for the ALG group and 65.2% for the control group ($Z = 1.57$; $0.12 > P > 0.11$). At 2 months after transplantation, graft survival was significantly greater in the ALG group than in the control group (79.3% v. 66.3%; $Z = 1.980$; $0.05 > P > 0.04$).

Twenty patients of the 87 in the ALG group failed to get the full course of ALG therapy. In most this was because of thrombocytopenia. These patients, however, were retained in the ALG group for the purposes of this report.

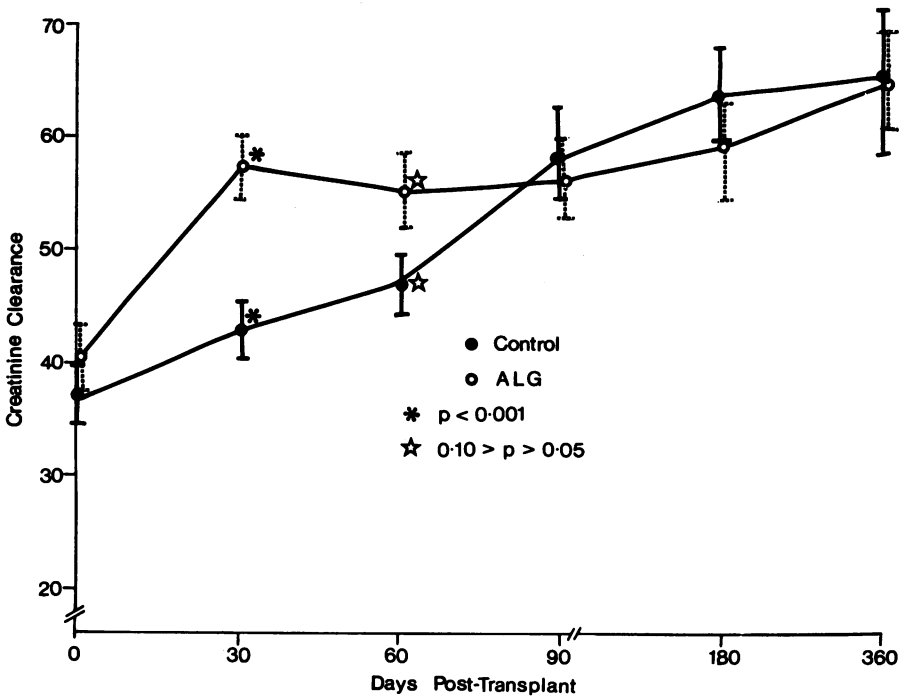

FIG. 2—Creatinine clearance values (ml/min; means ± SE) in the two groups.

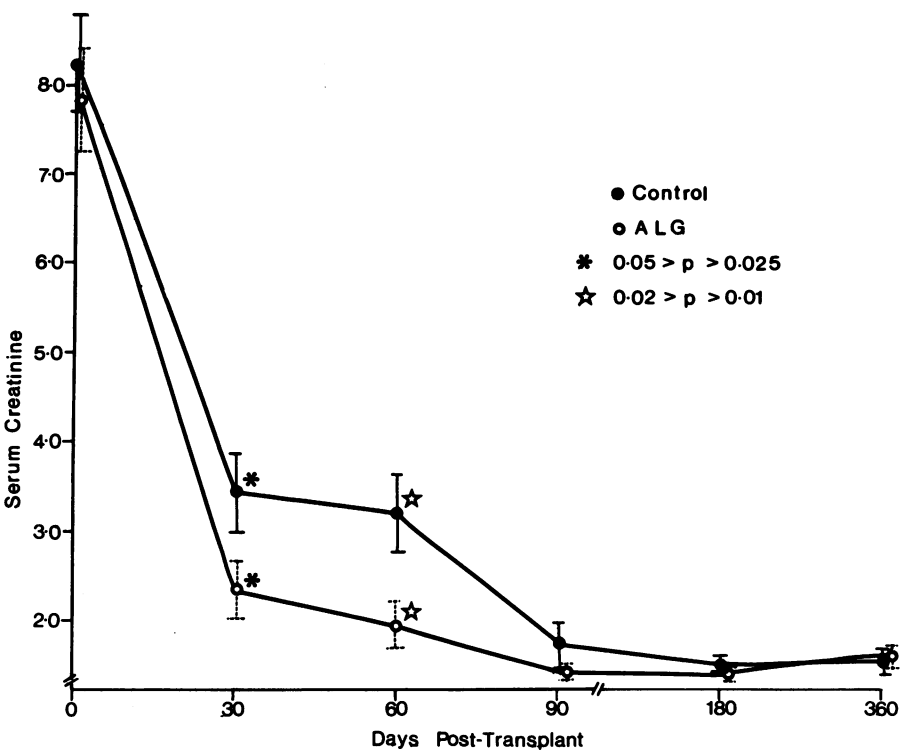

FIG. 3—Serum creatinine values (mg/dl; means ± SE) in the two groups.

Creatinine clearance and serum creatinine concentration

Renal function was assessed from these two measures. The creatinine clearance values, plotted in Fig. 2, were higher in the ALG group at both 30 days (56.7 v. 43.3 ml/min; $t = 3.418$; $P < 0.001$) and 60 days (55.2 v. 46.9 ml/min; $t = 1.939$; $0.10 > P > 0.05$) after transplantation. The results at 90, 180 and 360 days were similar in the two groups.

The serum creatinine values, plotted in Fig. 3, were significantly lower in the ALG group at both 30 days (2.4 v. 3.4 mg/dl; $t = 2.047$; $0.05 > P > 0.025$) and 60 days (2.0 v. 3.2 mg/dl; $t = 2.517$; $0.02 > P > 0.01$) after transplantation. The results at 90, 180 and 360 days were similar in the two groups.

Numbers of rejection episodes

The numbers of rejection episodes (diagnosed according to criteria detailed in the protocol) reported from the various centres are shown in Table III. In the first 3 months after transplantation there were 38 major rejection episodes in 37 patients in the control group, compared with 15 such episodes in 14 patients given ALG ($X^2 = 12.771$; $P < 0.0005$). After 3 months the incidence was the same in each group. For minor and chronic rejection episodes the incidence was the same in each group throughout the 12 months after transplantation.

Table III—Numbers of rejection episodes

	ALG group	Control group
Instant	2	1
Major		
< 3 months	15	38
> 3 months	8	6
Minor	24	22
Chronic	7	5

Prednisone consumption

The average daily prednisone dose (excluding boluses for acute rejection) over the first 90 days after transplantation was 116 mg in the control group and 84 mg in the ALG group. The average daily maintenance doses for both groups at 180 and 360 days were 25 mg and 20 mg, respectively.

Acute rejection episodes were treated by administering boluses of prednisone. Patients in the ALG group received one third the number of boluses of prednisone as patients in the control group in the first 3 months after transplantation.

Occurrence of complications

The accumulated patient survival was practically identical in the two groups, indicating that the ALG therapy was not responsible for any increase in mortality. Immediate fever or urticaria or both developed in 22 of the 87 patients and 10 others had delayed fever and were reported to have had some degree of serum sickness.

Because increased rates of infection and unusual pathogens have been reported in ALG-treated patients in some trials,[6] detailed reports of infections were obtained every 3 months on all patients in this trial. A total of 52 infections occurred in 44 patients in the control group, compared with 65 infections in 57 patients in the ALG group ($X^2 = 5.66$; $0.025 > P > 0.01$). The ratio of number of infections to number of patients affected was the same in the two groups. No unusual pathogens were reported.

Noninfectious operative, gastrointestinal, cardiovascular, pulmonary and transplant wound complications were also recorded for each 3-month period. There was no difference in the incidence of any of these complications in the two groups.

Clinically significant transient proteinuria was noted in five patients given ALG, three of whom (all at one regional centre) were considered to have nephrotic syndrome.[7] No adequate explanation could be offered. There were no horse protein precipitates in the glomeruli.

There was a clinically significant decrease in platelet count in 30% of the ALG-treated patients. Not infrequently the count decreased to between 30 and 40 x $10^9/l$, and in the early stages of the trial this was the major reason of some physicians for discontinuing ALG therapy. However, there were no reports of clinically significant bleeding and the platelet counts promptly returned to normal when the ALG therapy was stopped.

Discussion

One of the most important features of this multicentre, national clinical trial was the use of a standardized common pool of equine antihuman thymocyte-membrane ALG that was shown to be highly immunosuppressive by the usual in vitro and in vivo tests. The most troublesome side effect was the occurrence of thrombocytopenia of various degrees in many patients, probably the result of an antiplatelet antibody in the pool of serum. Although this did not cause any serious problem it was the reason ALG treatment was stopped in some patients in the early days of the trial. Subsequently the clinicians became familiar with this form of treatment, and practically all patients later in the trial received the full schedule of ALG therapy.

The most important criterion of the effectiveness of ALG treatment is transplant survival. In the ALG group transplant survival was better throughout the 1st year, the difference being most pronounced at 2 and 3 months after transplantation.

The differences in graft survival between the two groups were minimized because 20 of the 87 patients in the ALG group did not receive the full course of ALG therapy and hence could not have been expected to benefit fully from it. These 20 patients did not differ from the rest of the ALG group in terms of age, sex, underlying renal disease or any other baseline variable. If they are removed from the analysis, the difference in graft survival between patients given full-dose ALG treatment and the control group becomes more significant — for example, at 3 months the accumulated graft survival for the former was 80.6%, and for the latter, 65.2% ($Z = 2.22$; $0.03 > P > 0.02$).

Function of the grafted kidney, as demonstrated by results of creatinine studies, was better in the ALG group than the control group at 30 and 60 days after transplantation. Thereafter the function was similar in the two groups. The number of major rejection episodes in the control group was more than double the number in the ALG group in the first 90 days after transplantation. Furthermore, the total amount of prednisone used in the first 90 days was much less in the ALG group than in the control group. These facts suggest that during administration of the drug, and for a lag period of about another 2 months, there was a drug (ALG)-related beneficial effect on both the graft and the host.

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