Superiority of Antithymus Serum to Antilymphocyte Serum in Prolonging Cardiac Homograft Survival (35530)

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(Introduced by R. C. Wiliams, Jr.)

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The rejection phenomenon remains a major obstacle in organ transplantation. Radiation, immunosuppressive agents, steroids, and more recently antilymphocyte serum (ALS) have been employed to retard the rejection process. While ALS has been shown to be an effective new agent, its immunosuppressive activity cannot be quantitatively measured because *in vitro* tests are often unreliable in assessing *in vivo* activity (1, 2). Furthermore, the activity of ALS may vary according to cell type used for immunization and the injection schedule used in its production (1–3).

Some investigators have suggested that antithymus serum may be more effective than other forms of ALS in prolonging skin allograft survival (4–6). The purpose of this study was to evaluate the relative effectiveness of antithymus (ATS) serum as opposed to antilymphocyte serum in whole organ (cardiac) transplantation.

Materials and Methods. Cardiac ectopic allografts. Ectopic cardiac allotransplantation was performed by implanting hearts from Wistar-Furth rats intra-abdominally into Fisher (CDF) rats. Using the microsurgical technique of Ono and Lindsey (7), anastomoses were performed between descending aorta of the recipient and ascending aorta of the donor; between pulmonary artery of the donor and inferior vena cava of the recipient. Such transplanted hearts continued to beat until rejection occurred. Rejection was monitored by cessation of a palpable beat and loss of electrical activity from the transplanted heart as measured by the electrocardiogram (Fig. 1).

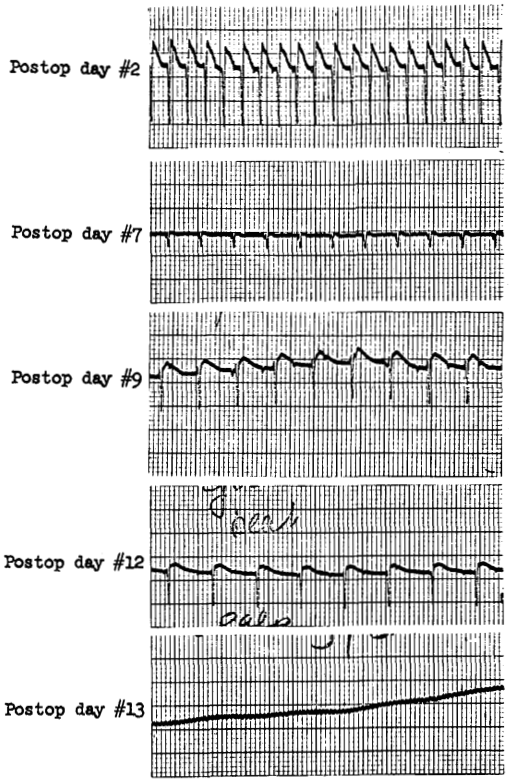

Fig. 1. Serial ECG's obtained from ectopically transplanted rat heart: Rat C5, electrocardiograms show persistent electrical activity in the transplanted heart until postoperative day 13. On day 12, electrical activity was measured and a strong heart beat was palpable.

Antiserum production. Antithymus serum was produced by immunizing 7-kg New Zealand white rabbits with thymus cells obtained from Fisher (CDF) rats. Antilymphocyte serum was produced in an identical manner using equal numbers of spleen cells from the same Fisher strain.

TABLE I. Relative Effectiveness of Antisera.

Group	Animal no.	Duration homograft survival (days)	Mean survival ± SEM (days)	p
Control	C1	12		
	C2	13		
	C3	13		
	C4	13	13 ± 1	
	C5	14		
	C6	14		
	C8	12		
	C10	14		
ALS	S4	24		
	S7	22		
	S9	24		
	S10	25	23.9 ± 1.1	<.001
	S11	25		
	S12	25		
	S1	22		
ATS	T1	69		
	T6	54		
	T9	51	50.8 ± 12.6	<.001
	T10	51		
	T7	37		
	T4	43		

Cells were obtained by pressing thymus (or spleen) through a micromesh screen. Cells were washed, then resuspended in Earle's solution at a pH of 7.2–7.4 by the addition of 7.5% $NaHCO_3$. The cells were washed and resuspended twice; rabbits received immunizing injections of 2×10^8 spleen or thymus cells at a weekly interval for 4 weeks. Initial injection was intraperitoneal and subsequent injections were subcutaneous. One ml of a water–oil emulsion composed of equal parts of Freund's complete adjuvant and buffered saline was injected subcutaneously at the time of the first injection of cells but was not used again. After a rest period of 6 weeks, the animals received a booster injection, and the antiserum was harvested by sterile cardiac puncture 10 days later. Antiserum was pooled and leuko-agglutinin titers were performed. Pooled leuko-agglutinin titers of both antithymus serum and antilymphocyte serum were in excess of 1:320. Antiserum was frozen at —20° and stored until time of administration.

Treatment groups. Group I, controls.

Group II received antilymphocyte serum, 0.5 ml ip, 1, 3, 5, and 7 days after transplantation.

Group III received antithymus serum, 0.5 ml ip, 1, 3, 5, and 7 days after transplantation.

Results. In 8 control animals, cardiac rejection occured at 13 ± 1 day. (Table I). The end point of rejection was clearly identified by cessation of palpable beat and loss of electrical activity on electrocardiogram. (Fig. 1). In 7 animals treated with antilymphocyte serum, rejection occurred at 23.9 ± 1.1 day, $p<.001$. In 7 animals treated with antithymus serum, rejection occurred at 50.8 ± 12.6 days, $p<.001$. Cardiac allografts in animals treated with antithymus serum survived significantly longer than did animals treated with antilymphocyte serum, $.005> p<.001$.

Discussion. Although the skin graft preparation has been used as a classical model for studying transplantation biology, it is well known that skin graft rejection may occur more rapidly and be more pronounced than whole organ allografts in similar animal systems (8, 9). Furthermore, the skin graft is susceptible to a variety of adverse conditions in addition to the rejection process.

Recent developments in microsurgical techniques have made it possible to achieve whole organ allografts between inbred animal strains. Such whole organ transplants with vascular anastomosis between donor and recipient may offer certain advantages over the traditional skin graft: (i) the pathophysiology and histopathology of the rejection process is more closely related to clinical situations; (ii) the establishment of new endothelial lining of the allograft derived from host may mask foreign surface antigens (10); (iii) the slow continuous release of antigen may favor desensitization and thereby prolong allograft survival (11).

Ectopic cardiac allotransplantation in rats, initially devised by Abbot and associates (12) and modified by Ono and Lindsey (7), affords the opportunity to study transplantation phenomena in inbred animal strains. The reliability and uniformity of such a system offers greater reproducibility than is present

in noninbred systems. Thus, such a system is ideally suited for evaluation of factors which may affect the rejection process.

Although antilymphocyte serum has been shown to be effective in prolonging survival of allografts from various organs, its effectiveness may depend ultimately upon the source of cells employed in the production of antiserum (1–3). Our studies would suggest that ATS is more effective in prolonging cardiac homograft survival than is ALS, and would confirm the earlier reports of enhanced effectiveness using ATS in the skin graft model.

Ono and co-workers (13) showed no difference between ATS and ALS in prolonging cardiac allograft survival. The difference in the present results from those previously reported may be due in part to the fact that heat was not employed to decomplement antiserum in our study. We have observed that heating antiserum may result in a loss of immunosuppressive activity. Perhaps of greater importance is the fact that the potency of antisera may depend upon the number and timing of immunizing injections. In this study, an injection schedule was employed in which there was a long rest period (6 weeks) between the initial series of injections and the final booster injection. Previous studies in our laboratory have shown this to be an effective method of producing highly active antisera. Indeed, Good (14) noted that 100 times more antibody to BSA was produced in rabbits when the interval between first and second injection of antigen was prolonged from 10 to 30 days. We have found, by the immunization schedule noted above, that it may be possible to obtain antiserum with enhanced effectiveness, although small amounts of antigen are employed.

Cardiac transplantation is technically feasible and the transplanted heart is capable of adequate hemodynamic function to sustain life (15, 16). The rejection phenomenon however remains the limiting factor which prevents this form of therapy from being clinically feasible. If cardiac transplantation is to be more widely utilized, it may be necessary to develop more effective immunosuppressive antisera and to attempt to produce partial acquired tolerance by immunizations with donor antigen (16).

Summary. Cardiac allografts between inbred rat strains provide a unique and valuable way of studying various aspects of transplantation biology. It has been stated that immunosuppressive antiserum varies in its effectiveness depending upon the cell type used in its preparation. Cardiac allografts in rats survived significantly longer when graft recipients were treated with antithymus serum than with antilymphocyte serum.

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