HISTOCOMPATIBILITY STUDIES IN A CLOSELY BRED COLONY OF DOGS

II. Influence of the DL-A System of Canine Histocompatibility Upon the Survival of Cardiac Allografts*

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Modern surgical technology has solved most, if not all, technical problems of cardiac transplantation in experimental animals (1) and in human subjects (2). Successful application of this new procedure to the treatment of end-stage cardiac disease has been hampered, however, by the host's recognition of a cardiac transplant as foreign tissue and his attempts to destroy the intruder through immunological responses normally used as defenses against infectious microorganisms (3).

Experimental heart transplantation began almost 65 years ago with the studies of Carrel and Guthrie (4, 5). A satisfactory experimental model for the investigation of host responses to cardiac allografts was not available, however, until 1933, when Mann et al. (6) transplanted canine hearts to the necks of the recipients, with reestablishment of the coronary circulation by anastomosis of the host carotid artery to the donor aorta. This work, and a number of other studies (7–9) provided early evidence of the immunogenicity of cardiac allografts, which were usually rejected by the recipients within 4–8 days. The microscopic features of the rejected organs were characterized by the same type of mononuclear cell infiltrate and vascular change which has been associated with the acute rejection of other organ allografts (7, 10, 11).

The potential usefulness of cardiac allografts in supporting the circulation of the host was considered "a matter of fantastic speculation for the future" as late as 1951 (12). The advent of the heart–lung machine, and the studies of Lower and Shumway (13) made this fantastic speculation a reality by 1960. Through the combined use of topical hypothermia for preservation of the anoxic myocardium, anastomosis of the donor heart atrial walls and atrial septum to the posterior atrial wall and septum of the recipient, and joining of the aortic and pulmonary

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arteries of donor and recipient, Lower and Shumway completed the first series of technically successful orthotopic canine cardiac allografts at that time, with a survival range of 6–21 days in five recipients (13). The method of Lower and Shumway has resulted in cardiac transplants fully capable of maintaining the host's circulation (14), leaving the immunological barrier as the last serious obstacle to the continued functioning of such allografts for the normal life span of the recipient (15). As a result of immunological rejection responses, the average survival time of cardiac allografts performed in randomly selected unmodified mongrel dogs has been reported as 7 days, with a range of 4–21 days (16). Treatment of recipients with immunosuppressive drugs, such as azathioprine and methyl prednisolone, has prolonged survival to a range of 2–18 months (1, 16, 17). More recently, the use of anti-lymphocyte globulins has, on occasion, resulted in even longer survivals, as reported by Cachera, Dubost, Halpern, and associates (18, 19).

These studies have provided a valuable background of information on the criteria for the diagnosis of impending rejection crises, and on the pathological changes associated with cardiac allograft rejection. It is generally agreed that serial electrocardiographic study, with particular regard to voltage drops and conduction abnormalities, may provide the most reliable early sign of impending rejection, and that the prompt and judicious use of immunosuppressive agents may reverse such crises (20–22). The resulting long-term survivals have also facilitated a more precise analysis of the different pathological parameters of cardiac allograft rejection. Acute cardiac allograft rejections which usually occur during the first two postoperative weeks are characterized by mononuclear cell infiltration of the myocardium, with capillary and venular damage (including endothelial swelling and necrosis, vascular engorgement, multiple thrombi, and disruption of some vessels) with exudation of fibrin and erythrocytes, and with multiple foci of myocytolysis and myocyte necrosis. In contrast, the chronic type of rejection observed in allografts accorded longer survival times consists mainly of a diffuse destructive arteritis, intimal fibrosis with luminal narrowing, and resulting myocardial ischemia (23, 24). The acute and chronic types of cardiac allograft rejection would therefore appear to be very similar to the changes described for renal allografts by Porter and his associates (25–28).

The careful experimental work in canine cardiac transplantation culminated in the first technically successful human cardiac transplant, performed by Barnard in 1967 (2). This event stimulated a world-wide effort to treat a variety of end-stage cardiac disease entities by transplantation. This effort, which rapidly gained epidemic proportions, resulted in the performance of 158 human cardiac allografts by 59 different surgical teams by April 1970 (29). It was also frequently associated with allograft failure and death of the patient (29). As had been shown previously in the canine species, modern surgery had conquered the technical aspects of cardiac surgery, but further progress was dependent upon the acquisition of a better understanding of the immunological processes responsible for cardiac allograft failure (30–35).

These considerations suggest a need for further investigations of the host's immunological responses to cardiac allografts, with particular regard to a search for new methods to attenuate the tempo and intensity of the rejection response. The colony of closely bred beagles maintained in Cooperstown, New York, by Ferrebee et al. (36) appeared to provide an unusually suitable source of experimental animals for this purpose. Previous studies of the Cooperstown beagles have demonstrated that the DL-A system of canine histocompatibility defined in this colony (37) may play an important role in conditioning the survival of skin (38) and kidney allografts (37). The present study describes the responses of 30 littermate (i.e. siblings) and nonlittermate beagles with known DL-A (37)

and Swisher erythrocyte-group antigens (39) to orthotopic cardiac allografts obtained from donors selected on the basis of leukocyte and erythrocyte group compatibility with the recipients. The survival of such allografts is compared with the behavior of 28 orthotopic cardiac allografts performed in randomly selected mongrel dogs. The results indicate that DL-A compatibility exerts a potent influence upon the survival of cardiac allografts in the unmodified canine host (i.e., a recipient whose immunological responsiveness has not been altered by preexisting disease or immunosuppressive therapy). This influence is exerted more profoundly in littermates than in recipients of nonlittermate transplants. Comparison of cardiac and renal (37) allografts under similar conditions of donor–recipient DL-A compatibility suggests that the heart may be less immunogenic than the kidney. Cardiac allografts appear, however, to be more vulnerable to allograft rejection than renal transplants.

Materials and Methods

Criteria for Selection of Experimental Animals.—A closely bred colony of beagles of known leukocyte (DL-A) groups (37) and randomly selected mongrel dogs were used throughout this study. Male and female dogs weighing 18–25 lb. were maintained on a standard diet. Cooperstown beagle donor–recipient pairs were selected for cardiac transplantation on the basis of (*a*) coefficients of relationship (siblings and nonlittermates), (*b*) Swisher erythrocyte group antigens A, C, and D, and (*c*) leukocyte group (DL-A) compatibility.

The erythrocyte antigens were detected with the typing sera and techniques of Swisher (39). A battery of antisera prepared by reciprocal exchanges of skin allografts and subcutaneous inoculations of blood leukocytes in five pairs of beagle littermates and in two nonlittermate pairs (38) was employed to identify 11 currently known DL-A leukocyte group antigens, b, c, d, e, f, g, h, j, k, l, and m, in the Cooperstown beagles. The technique of lymphocytotoxicity described by Epstein et al. (40) was employed, but with removal of erythrocytes by sedimentation before nylon filtration for the separation of lymphocytes from polymorphonuclear eukocytes. This technique yielded a 90% lymphocyte population in the final cell suspension. A lmodification of this technique has been instituted recently, whereby this cell preparation is re suspended in veronal-buffered saline solution before performance of the lymphocytotoxicity test. This modification has significantly enhanced the sensitivity of the typing procedure, and has permitted identification of a number of DL-A phenotypes in which antigens f and m, and g and l do not occur together. These antigens are therefore no longer expressed in paired fashion (37) in the beagle phenotypes listed in the present study.

Previous results of a limited number of skin (38) and kidney (37) allografts in the Cooperstown beagles have suggested that DL-A antigens e, f, g, l and m may be strong histocompatibility antigens, and constitute major barriers to transplantation, while DL-A antigens b, c, d, h, j, and k behave as weak antigens, and may therefore constitute a major threat to allograft survival. The present study extends the same criteria of donor–recipient compatibility to an assessment of cardiac allograft responses in this colony of beagles.

Method of Cardiac Transplantation and Determination of Allograft Rejection.—The technique of orthotopic canine cardiac allotransplantation of Lower and Shumway (13, 14) was used, with some modifications, throughout this study. Donors and recipients were anesthetized with a 30 mg/kg dose of pentobarbital (Abbott Laboratories, North Chicago, Ill.) and received 3 mg/kg of heparin sodium (Organon, Inc., West Orange, N. J.). The operative fields were exposed through a median sternotomy incision. Cardiopulmonary bypass was instituted in the recipient

utilizing the femoral artery for arterial return, with venous drainage into a standard pump oxygenator which was primed with fresh blood obtained from five mongrel dogs and was connected with cannulas placed through the right atrium and into the superior and inferior vena cavae of the recipient. The oxygenated blood flow rate was 100 cc/kg throughout the period of bypass. The recipient's aorta and pulmonary artery were occluded with a clamp placed through the transverse sinus, and the heart was excised, leaving the host's posterior atrial walls and septum in place.

The donor heart was excised in similar fashion; it was immediately placed for 20 min in psysiological salt solution maintained at a temperature of 4°C. Sutures approximating the superior and inferior edges of the interatrial septa of donor and recipient were inserted; the septa were anastomosed, followed by anastomosis of the right atria. The aortic anastomosis was then performed and a venting catheter was inserted into the left ventricle through the left atrial appendage and mitral valve. The coronary circulation was restored by unclamping the aorta, the left atrium was closed, and the heart was defibrillated by electric shock. The pulmonary anastomosis was completed at this time and the cardiopulmonary bypass was discontinued after an additional 15 min of support. After careful hemostasis, a chest tube was inserted into the left thoracic cavity and was connected for the next 24 hr to external underwater drainage. The median sternotomy was then closed, and routine postoperative care for cardiac operations was given. The latter included antibiotic therapy for 2 wk, consisting of intramuscular injections of 500 mg per day of Polycillin (ampicillin trihydrate, Bristol-Myers Co., Syracuse, N. Y.), and 1 g per day of Staphcillin (sodium methicillin, Bristol-Myers Co.).

The recipient's arterial and central venous pressures, hematocrits, PO_2, pCO_2, and pH levels were monitored at regular intervals during the immediate postoperative period (41, 42). The cardiac and respiratory rate, temperature, hematocrit, white blood count level, and electrocardiogram were monitored daily until the time of allograft rejection. The onset of rejection was regularly heralded by changes in voltage of the electrocardiogram, particularly in the R-wave (1). The diagnosis of rejection was confirmed in each instance by death of the animal and pathological examination of the transplanted heart.

RESULTS

The results of 56 cardiac allografts performed during this study are summarized in Table I. The first group of animals included six beagles grafted with major DL-A antigen-compatible transplants obtained from littermates. The allografts were rejected in 22, 24, 31, 72, 80, and 90 days respectively, with a mean survival time (MST)[1] of 53.2 days. The MST of seven hearts transplanted to littermates from major DL-A antigen-incompatible donors was 7.3 days, with a span of 5–10 days. The next two groups of animals included recipients of hearts obtained from nonlittermate donors. Nine recipients of major DL-A antigen-compatible donors had an MST of 26.3 days, with a survival span of 10–55 days; six dogs survived for 21 days or more. In contrast, the MST of six transplants obtained from major DL-A antigen-incompatible donors was 6.3 days, with a span of 4–9 days. The last group of animals included 28 transplants performed in randomly selected outbred mongrel dogs. The MST of these allografts was 10.0 days, with a span of 4–19 days; one additional allograft survived for 30 days.

[1] *Abbreviation used in this paper:* MST, mean survival time.

TABLE I

Influence of Major DL-A Antigen Compatibility Upon Cardiac Allograft Survival in Unmodified Canine Recipients

Method of selection of allograft donors and recipients	No. of dogs	Number of recipients rejecting cardiac allografts on postoperative day																											Mean survival time
		4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	28	30	31	44	55	72	80	90	(*Days*)
DL-A*-compatible littermates	6																			1		1			1			1	1	1	53.2
DL-A*-incompatible littermates	7		1	2	1	1	1	1																							7.3
DL-A*-compatible nonlittermates	9							1				1			1				1		2			1		1	1				26.3
DL-A*-incompatible nonlittermates	6	1		3	1		1																								6.3
Outbred mongrel dogs‡	28	1	5	3	2	5	1	1	2	1		1	2	1	1		1							1							10.0

* Animals selected from a closed colony of inbred beagles.

† Randomly selected outbred dogs.

The DL-A and erythrocyte group antigens of 11 littermate and nonlittermate recipients of DL-A–compatible allografts are listed in descending order of histocompatibility (as gauged by survival times) in Table II. The MST of five

TABLE II

Relationship Between DL-A Compatibility and Cardiac Allograft Survival in a Closely Bred Colony of Beagles. (a) Transplants Obtained From Compatible Donors

Donor–recipient relationships	No. of animals	Recipient No.	Donor No.	DL-A antigens in		Erythrocyte antigens in		Cardiac allograft survival time
				Recipient	Donor	Recipient	Donor	
								(*Days*)
Littermates	5	20–66	20–67	g, l	g, l	C	C	90
		21–33	21–28	b, c, d, e, f, k, m	b, c, f, k, m	A	C*	80
		21-13	21–12	e, f, g, l, m	e, f, g, l, m	AC	AC	72
		21–20	21–21	b, c, d, e, f, k, m	b, c, d, e, f, k, m	A	A	31
		20–33	20–38	b, f, g, k, l, m	g, l	AC	AC	22
								MST: 59.0
Nonlittermates	6	20–84	20–31	g, l	g, l	AC	AC	44
		21–23	21–35	b, c, d, f, k, m	b, c, d, f, k, m	C	AC*	30
		20–95	20–80	b, f, k, m	b, f, k, m	AC	AC	23
		17–57	16–89	g, l	g, l	None	ACD*	23
		21–98	21–07	b, f, k, m	b, f, k, m	C	AC*	17
		20–76	21–22	b, f, k, m	b, f, k, m	AC	C	10
								MST: 24.5

* Presence in the donor of an erythrocyte antigen (Swisher) absent in the recipient.

TABLE III

*Relationships Between DL-A Compatibility and Cardiac Allograft Survival in a Closely Bred Colony of Beagles. (b) Transplants Obtained from Donors Differing from the Recipients by Weak DL-A Antigens**

Donor–recipient relationship	Recipient No.	Donor No.	DL-A antigens in		Erythrocyte antigens in		DL-A antigen incompatibility	Erythrocyte antigen incompatibility	Cardiac allograft survival time
			Recipient	Donor	Recipient	Donor			
									(*Days*)
Littermates	20–51	20–52	g, l	b, c, g, k, l	C	None	b, c, k	—	24
Nonlittermates	20–99	21–01	b, e, f, g, k, l, m	b, c, f, k, m	—	A	c	A	55
	21–45	21–27	b, f, g, k, l, m	b, c, d, f, k, m	—	AC	c, d	A, C	21
	21–44	21–19	b, f, g, k, l, m	b, c, d, g, k, l	AC	C	c, d	—	14

* DL-A Antigens b, c, d, and k.

DL-A–compatible transplants obtained from littermate donors was 59.0 days (span 22–90 days). In contrast, the MST of six DL-A–compatible transplants obtained from nonlittermate donors was 24.5 days, with a span of 10–44 days. Table III summarizes the results observed when cardiac allografts were per-

formed across weak DL-A barriers (i.e., incompatibilities for antigens b, c, d, or k). One littermate transplant performed under such conditions survived for 24 days, and three other allografts obtained from nonlittermate donors were accorded survival times of 14, 21, and 55 days respectively. The longest survival

TABLE IV

*Relationship Between DL-A Compatibility and Cardiac Allograft Survival in a Closely Bred Colony of Beagles. (c) Transplants Obtained from Donors Differing from the Recipients by Strong DL-A Antigens**

Donor–recipient relationship	Recipient No.	Donor No.	DL-A antigens in		Erythrocyte antigens in		DL-A antigen incompatibility	Erythrocyte antigen incompatibility	Cardiac allograft survival time
			Recipient	Donor	Recipient	Donor			
									(*Days*)
Littermates (7 Dogs)	21–10	21–11	e, g, l, m	f, g, l, m	A, C, D	A, C	f	—	10
	17–07	17–11	e, g, l	e, f, h, k, m	A, C	A, C	f, h, k, m	—	9
	20–68	20–69	g, l	f, g, l	C	C	f	—	8
	21–36	21–38	g, l	f, g, l	C	C	f	—	7
	21–17	21–18	g, l	b, e, g, k, l	AC	A	b, e, k	—	6
	20–91	20–90	b, f, g, k, l, m	b, e, f, g, k, l, m	AC	AC	e	—	6
	20–97	21–09	b, c, e, f, g, k, l	f, g, l, m	A	A, C, D	m	C, D	5
									MST: 7.3
Nonlittermates (6 Dogs)	17–79	16–93	b, e, g, k, l	b, f, g, h, j, k, l, m	None	None	b, f, h, j, k, m	—	9
	20–73	21–05	g, l	b, f, k, m	C	C	b, f, k, m	—	7
	21–15	21–29	g, l	b, c, d, f, k, m	A	C	b, c, d, f, k, m	C	6
	20–72	20–23	g, l	f, g, l, m	C	A, C, D	f, m	A, D	6
	20–96	21–08	b, c, e, f, g, k, l	g, l, m	A	A, C, D	m	C, D	6
	16–80	17–75	b, e, g, l	b, f, g, h, j, k, l, m	A	A, C	f, h, j, k, m	C	4
									MST: 6.3

* Antigens e, f, g, l, and m.

(55 days) occurred in the face of a donor–recipient incompatibility for antigen c only.

5 of the 15 cardiac allografts listed in Tables II and III were performed across donor–recipient incompatibilities for Swisher erythrocyte antigens A, C, and/or D. The results show no direct evidence that such incompatibilities had an adverse influence upon the duration of cardiac allograft survival.

The effects of donor–recipient incompatibilities for DL-A antigens e, f, g, l, and m are illustrated in Table IV, which summarizes the results of 13 cardiac allografts performed under such conditions. The MST of seven cardiac allo-

TABLE V

Comparative Survival of Cardiac and Renal Allografts in Randomly Selected Mongrel Dogs

Type of transplant performed	No. of dogs	Number of recipients rejecting the allografts on postoperative day																											Mean survival time
		4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
																													(*Days*)
Cardiac	21	1			3	6	3	2	2		1	1	1	1															9.5
Renal	28	1	5	3	2	5	1	1	2	1		1	2	1	1		1											1	10.0

grafts obtained from DL-A–incompatible littermate donors was 7.3 days, with a span of 5–10 days. Six transplants performed under similar conditions in nonlittermates had an MST of 6.3 days, with a span of 4–9 days. The five donor–recipient erythrocyte antigen incompatibilities observed in these 13 animals were associated with the shortest cardiac allograft survival times (4–6 days). An interpretation of this result is hampered, however, by the documented effects of donor–recipient incompatibilities for known and/or as yet undetectable major DL-A antigens.

Two other cardiac allografts obtained from nonlittermate donors who had no serologically detectable DL-A incompatibilities with the recipients were rejected in 8 and 5 days respectively. In contrast, when donor and recipient were genotype as well as phenotype identical in the DL-A system, the longest cardiac allograft survival in the present series (90 days) was observed. These results

TABLE VI

Comparative Survival of Cardiac and Renal Allografts in Beagles of Known DL-A Phenotypes

Genetic status of donor and recipient	Organ transplanted	No. of transplants	Mean survival time In		Survival range	
			DL-A–compatible transplants	DL-A-in-compatible transplants	DL-A–compatible transplants	DL-A-in-compatible transplants
			(*Days*	(*Days*)	(*Days*)	(*Days*)
Littermates	Heart	13	53.2	7.3	22–90	5–10
	Kidney	21	28.6	14.8	13–38	11–20
Nonlittermates	Heart	15	26.3	6.3	10–55	4–9
	Kidney	28	28.3	12.4	16–45	10–18

highlight the need for further genetic data in order to facilitate interpretation of the results of currently available serological techniques in the DL-A system.

The results of cardiac and renal allografts performed in the same population of randomly selected mongrel dogs are compared in Table V. The MST of 28 cardiac allografts was 10.0 days, with a span of 4–31 days, and the MST of 21 renal allografts performed under similar conditions was 9.5 days, with a span of 4–16 days. Table VI lists a similar comparison in beagle donor–recipient combinations selected on the basis of DL-A compatibility. It is of interest that the MST of DL-A–compatible cardiac allografts obtained from littermate donors (53.2 days) was significantly greater than the MST of renal allografts performed under similar conditions (28.6 days). Conversely, the MST of cardiac allografts performed in DL-A–incompatible littermates (7.3 days) was significantly shorter than the survival of renal transplants done under such circumstances (14.8 days). There were no great differences, however, between cardiac (MST = 26.3 days) and renal (MST = 28.3 days) allograft survivals in DL-A

compatible nonlittermate beagles. Cardiac allografts performed in DL-A incompatible nonlittermates survived for shorter periods (MST = 6.3 days), however, than the corresponding renal allografts (MST = 12.4 days). Sex differences or coefficients in relationship in the nonlittermate beagles did not appear to play a significant role in these results.

DISCUSSION

The elegant studies of Epstein et al. (40) have demonstrated that cross-immunization of canine littermates with buffy coat cells can stimulate the formation of leukocyte group-specific antisera, and that the latter may be of value in the selection of compatible donor–recipient combinations for bone marrow transplantation in littermates (4) and in unrelated dogs (43). Mollen et al. (38) and Ferrebee et al. (36) have recently isolated 11 different leukocyte group specificities in the Cooperstown colony of closely bred beagles (44). The observation that such antigens behave as Mendelian autosomal dominants in this colony of dogs (36), taken together with their role in conditioning host responses to skin (38) and kidney (37) allografts, have suggested that the same general rules of histocompatibility encountered in the murine H-2 system (45) and in the human HL-A system (46) may also be operative in the canine species, and have led to the suggestion that the term DL-A be adopted for this system in dogs (37).

The results of the present study indicate that the major leukocyte group antigens detectable by Mollen et al.'s (38) battery of typing antisera play an important role in conditioning the survival of cardiac allografts in the Cooperstown colony of beagles. As has been noted previously for bone marrow (44), skin (38), and kidney (37) transplants, donor–recipient coefficients of correlation in nonlittermates or erythrocyte-group incompatibility did not appear to play decisive roles as determinants of cardiac allograft survival. There were significant differences, however, between the results of cardiac transplantation in littermate and nonlittermate dogs.

The results of cardiac allografts performed under conditions of donor–recipient DL-A compatibility were uniformly better than those observed in randomly selected mongrel dogs (MST = 10.0 days). DL-A–compatible transplants performed in littermates were accorded far longer survival times, however, than the corresponding allografts performed in nonlittermate beagles (MST = 53.2 days in littermates and 26.3 days in nonlittermates). This observation provides a possible explanation for an earlier report by Leandri-Cesari et al. (47) that the longest survival times of cardiac allografts are observed in littermate transplants. Cardiac allografts performed across major DL-A incompatibilities exhibited significant decreases in survival in littermate as well as nonlittermate donor–recipient combinations (MST = 7.3 and 6.3 days, respectively). These findings are consistent with the interpretation that, as has

been suggested for the HL-A system in man (32), currently available DL-A–typing sera may have a greater ability to select optimally compatible donor–recipient combinations in littermates (i.e. siblings) than in nonlittermates. It is of interest, however, that transplants performed across major DL-A incompatibilities are accorded comparable decreases in survival time in both groups. The latter results were less favorable than those noted in randomly selected mongrel dogs, possibly because prospective DL-A typing succeeded in eliminating from the DL-A–incompatible group the chance-compatible donor–recipient combinations which may occur when transplants are performed in a randomly selected population.

The present study extends to heart transplantation previous suggestions, based upon results of skin (38) and renal (37) allografts, that antigens e, f, g, l, and m may be strong histocompatibility antigens, constituting major barriers to allotransplantation, while antigens b, c, d, and k may be less effective in this regard. A cardiac allograft obtained from a nonlittermate donor whose only incompatibility with the recipient was antigen c survived for 55 days. Three other donor–recipient combinations with incompatibilities for antigens b, c, d, and/or k resulted in cardiac allograft survival times of 14, 21, and 24 days respectively. Although these data are insufficient to warrant any definitive conclusions, they would appear to suggest that, as has been noted for the murine species (48), incompatibilities for weak histocompatibility antigens may exert an additive effect.

Performance of cardiac and renal allografts in the same colony of beagles, under controlled conditions of donor–recipient DL-A–compatibility, has provided a useful opportunity for a biological assessment of the comparative immunogenicity of these two organs. The significantly longer survival of cardiac as compared with renal allografts in DL-A–compatible littermate animals is in agreement with previous observations suggesting that cardiac tissue may be less immunogenic than renal tissue (49, 50). The very short survival of cardiac allografts performed in major DL-A–incompatible donor–recipient combinations provides convincing evidence however that, as has been suggested by van Bekkum and his associates (51, 52), the heart may be more vulnerable to allograft rejection than the kidney. Such a difference in the ability of the heart and kidney to withstand the effects of the rejection response may be a reflection of actual differences in the concentration of histocompatibility antigens (49, 50). It may also be a consequence of physiological differences in the susceptibility of these two organs to allograft rejection. In this regard, it may be pertinent to note that the pathological lesions of allograft rejection have been found to be particularly prominent within cardiac conducting tissue. Such lesions include endothelial edema and perivascular mononuclear cell infiltrates in the atrioventricular node, conduction bundles, and bundle branches (35, 53, 54). A possible explanation for this finding is suggested by Clarke's demonstration of the

particularly rich vascularity of the sinus node, atrioventicular node, and main conducting bundles (55).

It is of interest that two cardiac allografts performed in nonlittermate dogs in the absence of serologically detectable donor–recipient incompatibilities were accorded unusually brief survival times (8 and 5 days respectively). In contrast, a cardiac allograft performed between DL-A–identical littermate dogs survived for 90 days. This observation highlights the concept that DL-A phenotype identity, at the present level of understanding of DL-A serology, may not necessarily signify the absence of incompatibility between donor and recipient in all instances. The fact that genetic DL-A identity is associated with prolonged allograft survival serves, on the other hand, to demonstrate the predominant role of the DL-A system, or of a gene closely linked to it, in canine transplantation.

The results provide a further illustration of the vicissitudes of tissue typing for organ transplantation, which appear to be common to most species studied. They highlight the need for a further serologic definition of the known and the yet undetected antigenic components of the DL-A system. The evidence also supports the contention that use of the DL-A system on a prospective basis for the selection of those donor–recipient combinations which may result in long-term survival of the major transplantable organs is not only dependent upon currently available serological criteria of compatibility, but also upon a genetic analysis (i.e. a family study) of the transmission of DL-A antigens to donor and recipient by their respective forebears. Such genetic studies may be of particular value for the establishment of guidelines for further experimental approaches to the long-term facilitation of major organ transplant survival in the mammalian host.

SUMMARY

The DL-A system of histocompatibility plays an important role in conditioning the survival of cardiac allografts in the unmodified canine host. The mean survival time of six cardiac allografts performed in DL-A–compatible littermate dogs obtained from a closely bred colony of beagles was 53.2 days, while the MST of transplants performed in seven DL-A–incompatible animals was 7.3 days. The MST of cardiac allografts performed in nine DL-A–compatible nonlittermate beagles was 26.3 days, as compared with 6.3 days in six DL-A–incompatible nonlittermate transplants. The results did not appear to be affected by Swisher erythrocyte-group incompatibilities. The MST of 28 cardiac allografts performed in randomly selected mongrel dogs was 10.0 days.

Incompatibilities for DL-A antigens e, f, g, l, and m may constitute major barriers to transplantation, but antigens b, c, d, and k appeared to act as weak histocompatibility antigens.

Under controlled conditions of donor–recipient DL-A compatibility, cardiac allografts may be less immunogenic than renal transplants. Heart transplants performed across major donor–recipient DL-A incompatibilities appeared, however, to be more vulnerable to the events of allograft rejection than renal allografts performed under similar conditions.

The selection of optimally compatible donor–recipient combinations for organ transplantation may be aided materially by genetic studies of the transmission of DL-A antigens to the animals under consideration.

BIBLIOGRAPHY

1. Lower, R. R., E. Dong, and N. E. Shumway. 1965. Long-term survival of cardiac homografts. *Surgery.* **58:**110.
2. Barnard, C. N. 1967. A human cardiac transplant. *S. Afr. Med. J.* **41:**1271.
3. Lawrence, H. S. 1959. Homograft sensitivity—An expression of the immunological origins and consequences of individuality. *Physiol. Rev.* **39:**811.
4. Carrel, A., and C. C. Guthrie. 1905. The transplantation of veins and organs. *Amer. Med.* **10:**1101.
5. Carrel, A. 1907. The surgery of blood vessels. *Bull Johns Hopkins Hosp.* **18:**18.
6. Mann, F. C., J. T. Priestley, J. Markowitz, and W. M. Yater. 1933. Transplantation of the intact mammalian heart. *Arch. Surg.* **26:**219.
7. Downie, H. G. 1953. Homotransplantation of the dog heart. *Arch. Surg.* **66:**624.
8. Wesolowski, S. A., and J. F. Fennessey. 1953. Pattern of failure of the homografted canine heart. *Circulation.* **8:**750.
9. Sayegh, S. F., and O. Creech. 1957. Transplantation of the homologous canine heart. *J. Thorac. Surg.* **34:**692.
10. Chiba, C., P. L. Wolf, S. Gudbjarnason, A. Chrysohou, H. Ramos, B. Pearson, and R. J. Bing. 1962. Studies on the transplanted heart—its metabolism and histology. *J. Exp. Med.* **115:**853.
11. Rowlands, D. T., R. B. Vanderbeek, H. F. Seigler, and P. A. Ebert. 1968. Rejection of canine cardiac allografts. *Amer. J. Pathol.* **53:**617.
12. Marcus, E., S. N. T. Wong, and A. A. Luisada. 1951. Homologous heart grafts: transplantation of the heart in dogs. Surg. For. **2:**212.
13. Lower, R. R., and N. E. Shumway. 1960. Studies on orthotopic homotransplantation of the canine heart. *Surg. Forum* **11:**18.
14. Lower, R. R., R. C. Stofer, and N. E. Shumway. 1961. Homovital transplantation of the heart. *J. Thorac. Cardiovasc. Surg.* **41:**196.
15. Shumway, N. E., and R. R. Lower. 1964. Special problems in transplantation of the heart. *Ann. N.Y. Acad. Sci.* **120:**773.
16. Lower, R. R. 1969. Rejection of the transplanted heart. *Transplant Proc.* **1:**733.
17. Blumenstock, D. A., H. B. Hechtman, J. A. Collins, A. Jaretzki, J. D. Hosbein, W. Zingg, and J. H. Powers. 1963. Prolonged survival of orthotopic homotransplants of the heart in animals treated with methotrexate. *J. Thorac. Cardiovasc. Surg.* **46:**616.
18. Cachera, J. P., M. Lacombe, J. P. Bui-Mong-Hung, Y. Lacassagne, A. Creptin, B. Hattaway, B. Halpern, and Ch. Dubost. 1968. Greffe allogenique du coeur

chez le chien traité par un serum ou une blobuline antilymphocytaire. *Presse Med.* **76**:1557.

19. Halpern, M. B., M. J. P. Cachera, Y. Crepin, M. M. Mojovic, M. Lacomb, J. P. Bui-Mong-Hung, and M. Mendoca. 1969. Tolerance permanente de l'allogreffe cardiaque chez des chiens traités par les gammaglobulines antilymphocytaires de brebis. *C. R. H. Acad. Sci. Ser. D.* **269**:2274.
20. Lower, R. R., H. A. Kontos, J. C. Kosek, D. H. Sewell, and W. H. Graham. 1968. Experiences in heart transplantation. *Amer. J. Cardiol.* **22**:766.
21. Lower, R. R., E. Dong, and F. S. Glazener. 1966. Electrocardiograms of dogs with heart homografts. *Circulation.* **33**:455.
22. Lower, R. R., and R. J. Cleveland. 1967. The current status of heart transplantation. *In* Proceedings 1st International Congress Transplantation Society. J. Dausset, J. Hamburger, and G. Mathé, editors. A/S Munksgaard, Copenhagen, Denmark. 657.
23. Kosek, J. C., E. J. Hurley, and R. R. Lower. 1968. Histopathology of orthotopic canine cardiac homografts. *Lab. Invest.* **19**:97.
24. Kosek, J. C., E. J. Hurley, D. H. Sewell, and R. R. Lower. 1969. Histopathology of orthotopic canine cardiac homografts and its clinical correlation. *Transplant Proc.* **1**:311.
25. Porter, K. A., R. Y. Calne, and C. F. Zukoski. 1964. Vascular and other changes in 200 canine renal homotransplants treated with immunosuppressive drugs. *Lab. Invest.* **13**:809.
26. Porter, K. A., W. S. Peart, J. R. Kenyon, N. H. Joseph, R. J. Hoehn, and R. Y. Calne. 1964. Rejection of kidney homotransplants. *Ann. N.Y. Acad. Sci.* **120**:472.
27. Porter, K. A. 1965. Morphological aspects of renal homograft rejection. *Brit. Med. Bull.* **21**:171.
28. Porter, K. A., J. B. Dossetor, T. L. Marchioro, W. S. Peart, J. M. Rendall, T. E. Starzl, and P. I. Terasaki. 1967. Human renal transplants. I. Glomerular Changes. *Lab. Invest.* **16**:153.
29. Bergan, J. J. 1970. Spring Newsletter. American College of Surgeons, National Institutes of Health Organ Transplant Registry. Chicago, Ill. 1.
30. Cooley, D. A., R. D. Bloodwell, G. L. Hallman, and J. J. Nora. 1968. Transplantation of the heart. A report of four cases. *J. Amer. Med. Ass.* **205**:479.
31. Barnard, C. N. 1968. Human heart transplantation. The Diagnosis of Rejection. *Amer. J. Cardiol.* **22**:811.
32. Botha, M. C., and E. D. Du Toit. 1969. Human leucocyte typing in clinical transplantation. *S. Afr. Med. J.* **43**:1423.
33. Botha, M. C. 1969. Leucocyte-antigen matching in donor selection for heart transplantation. *Lancet.* **2**:508.
34. Cooley, D. A., G. L. Hallman, R. D. Bloodwell, J. J. Nora, and R. D. Leachman. 1969. Cardiac transplantation as palliation of advanced heart disease. *Arch. Surg.* **98**:619.
35. Bieber, C. P., E. B. Stinson, and N. E. Shumway. 1969. Pathology of the conduction system in cardiac rejection. *Circulation.* **39**:567.
36. Ferrebee, J. W., F. D. Cannon, N. Mollen, and D. St. John. 1970. Beagles for study of histocompatibility and organ transplantation. *Transplantation.* **9**:68.

37. Rapaport, F. T., T. Hanaoka, T. Shimada, F. D. Cannon, and J. W. Ferrebee. 1970. Histocompatibility studies in a closely bred colony of dogs. I. Influence of leukocyte group antigens upon renal allograft survival in the unmodified host. J. Exp. Med. **131**:881.
38. Mollen, J., D. St. John, F. D. Cannon, and J. W. Ferrebee. 1968. Lymphocyte typing in allografted beagles. *Transplantation.* **6**:939.
39. Swisher, S. N., and L. E. Young. 1961. The blood grouping system of dogs. *Physiol. Rev.* **41**:495.
40. Epstein, R. B., R. Storb, H. Ragde, and E. D. Thomas. 1968. Cytotoxic typing of antisera for marrow grafting in littermate dogs. *Transplantation.* **6**:45.
41. Clark, L. C., Jr. 1956. Monitor and control of blood and tissue oxygen tension. *Trans. Amer. Soc. Artif. Intern. Organs.* **2**:41.
42. Severinghaus, J. W. 1962. Electrodes for blood and gas pCO_2, pO_2 and blood pH. *Acta Anaesthesiol. Scand. Suppl.* **2**:207.
43. Storb, R., R. B. Epstein, J. Bryant, H. Ragde, and E. D. Thomas. 1968. Marrow grafts by combined marrow and leukocyte infusions in unrelated dogs selected by histocompatibility typing. *Transplantation.* **6**:587.
44. Thomas, E. D., and J. W. Ferrebee. 1962. Transplantation of marrow and whole organs: experience and comments. *Can. Med. Ass. J.* **86**:435.
45. Snell, G. D., and J. H. Stimpfling. 1966. Genetics of tissue transplantation. *In* Biology of the Laboratory Mouse. E. L. Green, editor. McGraw-Hill, New York. 457.
46. Dausset, J., and F. T. Rapaport. 1968. Blood group determinants of human histocompatibility. *In* Human Transplantation. F. T. Rapaport and J. Dausset, editors. Grune and Stratton, New York. 383.
47. Leandri-Cesari, J., Y. Crepin, J. P. Bui-Mong-Hung, M. Lacombe, and J. P. Cachera. 1969. Histologie des transplants cardiaques expérimentaux. *Ann. Anat. Pathol.* **14**:129.
48. Eichwald, E. J., and I. L. Weissman. 1966. Weak Histocompatibility Loci. *Ann. N. Y. Acad. Sci.* **129**:94.
49. Basch, R. S., and C. A. Stetson. 1962. The relationship between hemagglutinogins and histocompatibility antigens in the mouse. *Ann. N. Y. Acad. Sci.* **97**:83.
50. Berah, M., J. Hors, and J. Dausset. 1970. A study of HL-A antigens in human organs. *Transplantation.* **9**:185.
51. Van Bekkum, D. W., G. A. Heystek, R. L. Marquet, R. W. de Bruin, and W. T. Tinbergen. 1969. Comparative studies on immunosuppression of heart and kidney allograft rejection in rats. *Excerpta Med. Int. Congr. Ser.* 197. 14.
52. Van Bekkum, D. W., G. A. Heystek, and R. L. Marquet. 1969. Effects of immunosuppressive treatment on rejection of heart allografts in rats. *Transplantations* **8**:678.
53. Lower, R. R. 1969. Rejection of the transplanted heart. *Transplant. Proc.* **1**:733.
54. Bieber, C. P., E. B. Stinson, N. E. Shumway, R. Payne, J. Kosek. 1970. Cardiac transplantation in man. VII. Cardiac allograft pathology. *Circulation.* **41**:753.
55. Clarke, J. A. 1965. X-Ray microscopic study of the arterial supply to the conducting system of the heart. *Brit. Heart. J.* **27**:879.