HISTOCOMPATIBILITY STUDIES IN A CLOSELY BRED COLONY OF DOGS

I. Influence of Leukocyte Group Antigens upon Renal Allograft Survival in the Unmodified Host*

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It is generally agreed that the principal obstacle to successful organ transplantation today is of an immunological nature, as a consequence of the host's recognition of the presence of foreign cells, and of his response to this invasion through cellular and/or humoral pathways ordinarily triggered by infectious microorganisms (1). The tempo and intensity of this response parallel the degree of genetic or antigenic disparity between donor and recipient; the closer the similarity between the two subjects, the more favorable will be the recipient's response to a transplant from that particular donor (2, 3). This fact has stimulated a search for techniques capable of providing a guide to the selection of compatible subjects for organ transplantation. Such methods have included in vivo tests, such as the third man test (4–6), the normal lymphocyte transfer test (7, 8), and the irradiated hamster test (9), and in vitro tests such as the mixed lymphocyte culture test (10, 11) and leukocyte grouping techniques (12). In terms of their practicality and rapidity of performance, the methods of leukocyte typing, which are based upon reactions of blood leukocytes with antisera obtained from multiparous women and polytransfused patients have been shown to have significant advantages over other techniques (13).

Dausset's description of the first human leukocyte group (Mac) in 1958 (14) marked the beginning of a phase of intensive studies of other group antigens detectable on the surface of human leukocytes. Such antigens have been shown to be present not only on leukocytes, but also on platelets, and in most other human tissues (15), and it is now generally accepted that the HL-A system of leukocyte groups is the main system of histocompatibility in man (16).

Recent genetic studies of the HL-A system have resulted in the isolation of two

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loci in the HL-A chromosomal region, determining respectively antigens HL-A1, HL-A2, HL-A3, Da 15, Da 16 (Lc 11) and Da 17 at the first locus, and antigens Da 4, HL-A5, HL-A7, HL-A8, Da 6 and Da 9 at the second locus (17). The antigens determined at each locus have been shown to occur as alternative alleles.

An overwhelming mass of evidence has accumulated during the past few years to demonstrate that the HL-A antigens are, in fact, transplantation antigens (18, 19), and that there is a significant correlation between the ratio of serologically detectable HL-A identities to incompatibilities at each locus and the duration of skin allograft survival in man (20). In spite of the recognized deficiencies of typing methods, it has also been demonstrated that the long-term duration and quality of renal transplant survival in man are a function of the number of donor–recipient antigenic identities at the two known HL-A loci (20, 21). It must be noted, however, that serological tissue typing is still at a comparatively early stage of development, and that many important questions with regard to its exact role in human organ transplantation still remain unanswered. Progress in the elucidation of such problems has been hampered significantly by the fact that the clinical organ transplant situation includes a number of variables which inevitably obscure the interpretation of the observed results. Progress in studies of the comparative immunogenicity of the individual HL-A antigens in organ transplantation, and of the possible usefulness of various different regimens of immunosuppressive drug therapy under specific donor-recipient histocompatibility situations has been particularly difficult. Sakai, Simonsen, and Jensen (22) have recently highlighted the need to extend studies of renal transplantation to related large animals, such as canine siblings. Indeed, these authors indicate that it is only through this type of investigative effort that progress applicable to the clinical situation may be made. For example, such studies may provide an answer to the question of whether long-term immunosuppressive therapy, and the hazards which it entails, is actually necessary in leukocyte group-identical siblings.

The colony of closely bred beagles recently described by Ferrebee, Cannon, Mollen, and St. John (23) appeared to provide a particularly useful source of experimental material for further studies of the role of donor–recipient histocompatibility as a determinant of the fate of renal allografts in the unmodified recipient (i.e., a recipient whose immunological responsiveness has not been altered by preexisting disease or immunosuppressive therapy). The present study describes the response of 49 littermate (i.e., siblings) and nonlittermate beagles of known leukocyte (24) and erythrocyte group phenotypes (25) to renal 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 21 renal allografts performed in randomly selected mongrel dogs. The results indicate that leukocyte group compatibility exerts a potent influence upon renal allograft survival in the unmodified canine host. It must be noted, however, that all allografts were eventually rejected, including those performed in siblings with no detectable leukocyte group incompatibilities. Selection of donors and recipients on the basis of available leukocyte grouping antisera in the colony of beagles under

study may provide a useful approach to the search for more satisfactory immunosuppressive and/or tolerantogenic techniques for the facilitation of renal allograft survival. It may also permit further studies of the comparative immunogenicity of the major transplantable organs.

Materials and Methods

Selection of Experimental Animals.—A closely bred colony of beagles of known leukocyte group phenotypes (24) served as the source of animals. Male and female dogs, weighing 18–25 lb, were maintained on a standard diet. Donor–recipient pairs for kidney transplantation were selected on the basis of (*a*) coefficients of relationship (siblings and nonlittermates); (*b*) Swisher erythrocyte group antigens A, C, and D; and (*c*) leukocyte group antigen compatibility.

The erythrocyte antigens were detected with the typing sera and techniques of Swisher (25). A battery of 12 lymphocytotoxic antisera prepared by reciprocal exchanges of skin allografts and subcutaneous inoculations of blood leukocytes in 5 pairs of beagle littermates and in 2 nonlittermates (24) was employed to identify 10 different leukocyte group antigens, including b, c, d, h, k, fm, gl, and e (antigens f and m, and g and l are joined to indicate that these antigens occur together in this dog colony). The technique of lymphocytotoxicity employed was a modification of the method of Epstein, Storb, Ragde, and Thomas (26), with removal of erythrocytes by sedimentation prior to nylon filtration for the separation of lymphocytes from polymorphonuclear leukocytes. This technique yielded a 90% lymphocyte population in the final cell suspension.

Method of Grafting and Criteria for the Determination of Allograft Rejection.—Each recipient underwent bilateral nephrectomy under general halothane anesthesia, followed immediately by transplantation of a kidney obtained from the selected donor. All kidneys were perfused with 120 ml of phosphate-buffered saline solution at room temperature, and 120 ml of the same solution at 4°C prior to transplantation. After perfusion, the kidneys were implanted into the iliac fossa of the recipients, using standard surgical techniques (27). The renal vessels were anastomosed end-to-end to the common iliac vessels of the recipient, and the stump of the ureter was implanted into the urinary bladder through a submucosal channel (28). The average ischemia time of the transplanted kidneys was 40 min. Complete urinalyses and blood urea nitrogen (BUN) determinations were performed daily. Criteria of allograft rejection included: (*a*) cessation of urinary output; (*b*) marked BUN elevations (180 mg/100 ml or above); and (*c*) at least 2 days of anorexia, nausea, and vomiting. Dogs fulfilling these criteria were sacrificed, and the transplants were examined histologically to confirm the diagnosis of rejection.

RESULTS

The results of 70 renal allografts performed during this study are summarized in Table I. The first group of animals included 13 beagles grafted with leukocyte group-compatible transplants obtained from littermates; 12 of the transplanted kidneys survived for 18 or more days, with two allografts continuing to function for 38 days; one allograft was rejected in 13 days (mean survival time: 28.6 days). The mean survival time (MST) of eight kidneys transplanted to littermates from leukocyte group-incompatible donors was 14.8 days, with a span of 11 to 20 days. The next group of animals tested included nine recipients of kidneys obtained from nonlittermate, leukocyte antigen-compatible donors. Eight of the transplants survived for 20 or more days, with one allo-

graft still functioning at 45 days; one allograft was rejected in 16 days (MST = 28.3 days). In contrast, none of 19 transplants obtained from leukocyte group-incompatible nonlittermate donors survived for more than 18 days, with a mean survival time of 12.4 days. The last group of animals included 21 transplants performed in randomly selected outbred mongrel dogs. None of these allografts survived beyond 16 days; the MST was 9.5 days.

TABLE I

Influence of Leukocyte Group Compatibility upon Kidney Allograft Survival in Unmodified Canine Recipients

Method of selection of allograft donors and recipients	Number of dogs	Number of recipients rejecting kidney allografts on postoperative day																							Mean survival time
		4	7	8	9	10	11	12	13	14	15	16	18	20	21	23	27	28	31	32	34	35	37	38	45	
																										days
Leukocyte group-compatible littermates*	13								1				1	1	1		1	1		1	3	1		2		28.6
Leukocyte group-incompatible littermates*	8						1	1	1	1	2		1	1												14.8
Leukocyte group-compatible nonlittermates*	9											1		1		1	1	2	1				1		1	28.3
Leukocyte group-incompatible nonlittermates*	19					2	6	4	4		1	1	1													12.4
Outbred mongrel dogs‡	21	1	3	6	3	2	2		1	1	1	1														9.5

* Animals selected from a closed colony of inbred beagles.

‡ Randomly selected outbred dogs.

The leukocyte and erythrocyte group phenotypes of 22 recipients of leukocyte group-compatible littermate and nonlittermate transplants are listed in descending order of histocompatibility (as gauged by renal allograft survival times) in Table II. Nine of the transplants were performed across incompatibilities for erythrocyte antigens A, C, or D; seven of these allografts survived for 31 or more days. The blood group phenotypes observed in recipients of leukocyte group-incompatible littermate and nonlittermate transplants are listed in similar fashion in Table III. Comparison of the observed phenotypes with allograft survival again showed no direct evidence that erythrocyte group incompatibilities had an adverse effect upon the observed results. Possible

effects of such incompatibilities may, however, have been obscured by the leukocyte antigen incompatibilities, and the associated decreases in renal allograft survival times. In general, there appeared to be a rough relationship between the number of donor-recipient leukocyte group incompatibilities and the duration of allograft survival. This relationship was not constant, however,

TABLE II

Relationship between Leukocyte Antigen Compatibility and Kidney Allograft Survival in a Closely Bred Colony of Beagles. A. Transplants Obtained from Compatible Donors

Donor–recipient relationship	Recipient number	Donor number	Leukocyte antigens detected in		Erythrocyte antigens detected in		Kidney allograft survival time
			Recipient	Donor	Recipient	Donor	
							days
Littermates	19-14	19-16	b, h, k, fm,* gl*	b, h, k, fm, gl	C	A‡, C	38
	18-81	18-80	gl	gl	A	A, C‡	38
	19 17	19-25	b, e, h, k, fm	b, e, h, k, fm	A, C	A	35
	18-45	18-47	gl	gl	A, C, D	A, C	34
	19-35	19-33	b, h, k, fm, gl	gl	D	A‡, C‡, D	34
	19-25	19-17	b, e, h, k, fm	b, e, h, k, fm	A	A, C‡	34
	18-85	18-84	gl	gl	C	C	32
	18-56	18-57	b, h, k, fm, gl	b, h, k, fm, gl	A	A	28
	19-16	19-14	b, h, k, fm, gl	b, h, k, fm, gl	A, C	C	27
	19-09	19-07	b, e, h, k, fm	b, e, h, k, fm	C	C	21
	18-84	18-85	gl	gl	C	C	20
	18-80	18-81	gl	gl	A, C	A	18
	19-07	19-09	b, e, h, k, fm	b, e, h, k, fm	C	C	13
Nonlittermates	18-55	18-46	b, h, k, fm, gl	b, h, k, fm, gl	A	A, C‡	45
	19-15	19-26	b, h, k, fm	b, h, k, fm	C	A‡, C	37
	19-29	19-20	b, h, k, fm, gl	b, h, k, fm, gl	None	A‡, C‡	31
	18-42	18-32	b, h, k, fm, gl	b, h, k, fm, gl	A, C	C	28
	19-20	19-29	b, h, k, fm, gl	b, h, k, fm, gl	A, C	None	28
	19-26	19-15	b, h, k, fm	b, h, k, fm	A, C	C	27
	18-32	18-42	b, h, k, fm, gl	b, h, k, fm, gl	C	A‡, C	23
	18-21	18-28	e, gl	e, gl	A, C	C	20
	19-23	19-38	b, e, h, k, fm, gl	b, e, h, k, fm, gl	A, C	A, D‡	16
Mean survival time							25.5

* fm and gl are so written to indicate that these antigens occur together in the colony of beagles under study.
‡ Presence in the donor of an erythrocyte antigen (Swisher) absent in the recipient.

and some of the results, and in particular, incompatibilities of gl did not appear to follow this trend.

34 of the 70 transplants reported in this study were performed as exchanges within 17 pairs of littermate and nonlittermate beagles. As noted in Table IV, 16 allografts were exchanged in eight pairs of dogs (five littermate and three nonlittermate pairs) with no detectable leukocyte antigen incompatibilities. In six of the eight pairs, the survival time of one of the two transplants exceeded that of the other by 5 or more days, reaching a maximum of 20 days in one instance. The differences in survival time noted in nine pairs of beagles

with known leukocyte group incompatibilities were less pronounced, with only one pair differing by 5 days, and none of the other pairs differing by more than 2 or 3 days. In 15 instances, transplants were performed with kidneys obtained from donors which were only incompatible with the recipients for one detectable

TABLE III

Relationship between Leukocyte Antigen Compatibility and Kidney Allograft Survival in a Closely Bred Colony of Beagles. B. Transplants Obtained from Incompatible Donors

Donor-recipient relationship	Recipient number	Donor number	Leukocyte antigens detected in		Erythrocyte antigens detected in		Leukocyte antigen incompatibility	Erythrocyte antigen incompatibility	Kidney allograft survival time
			Recipient	Donor	Recipient	Donor			
									days
Littermates	19-00	18-99	b, h, k, fm, gl	b, c, h, k, fm	A	A, C	c	C	20
	18-31	18-30	b, e, h, k, fm	b, e, h, k, gl	A, C	A, C	gl	—	18
	19-46	19-45	b, c, d, h, k, fm	gl	A, C	A, C	gl	—	15
	18-29	18-26	b, e, j, k, gl	h, k, fm, gl	A, C	A, C	h, fm	—	15
	19-13	19-10	b, h, k, fm	b, h, k, fm, gl	None	C	gl	C	14
	19-45	19-46	gl	b, c, d, h, k, fm	A, C	A, C	b, c, d, h, k, fm	—	13
	19-03	19-04	b, c, h, k, fm	e, gl	C	C	e, gl	—	12
	19-04	19-03	e, gl	b, c, h, k, fm	C	C	b, c, h, k, fm	—	11
Nonlittermates	19-30	19-44	b, e, k, gl	b, c, k, gl	None	A, C	c	A, C	18
	19-38	19-23	b, h, k, fm, gl	b, e, h, k, fm, gl	A, D	A, C	e	C	16
	19-11	19-06	b, h, k, fm	b, e, h, k, fm	C	C	e	—	15
	19-44	19-30	b, c, k, gl	b, e, k, gl	A, C	None	e	—	13
	18-37	18-27	h, k, fm, gl	e, h, k, fm	None	A, C	e	A, C	13
	19-32	19-36	b, e, h, k, fm	gl	C	None	gl	—	13
	18-38	18-25	b, k, gl	b, h, k, fm	None	A, C	h, fm	A, C	13
	19-12	18-53	b, h, k, fm	gl	A	A, C	gl	C	12
	16-72	16-45	b, e, j, gl	b, h, j, k, fm, gl	A, C	C	b, k, fm	A	12
	19-37	19-34	gl	b, h, k, fm, gl	A, D	A, C	b, h, k, fm	C	12
	19-39	18-60	gl	b, c, h, k, fm	A, D	A, C	b, c, h, k, fm	C	12
	16-45	16-72	b, h, j, k, fm, gl	b, e, j, gl	A, C	C	e	—	11
	19-27	19-05	b, e, h, k, fm	b, e, k, gl	C	C	gl	—	11
	18-27	18-37	e, h, k, fm	h, k, fm, gl	A, C	None	gl	—	11
	19-05	19-27	b, e, k, gl	b, e, h, k, fm	C	C	h, fm	—	11
	18-53	19-12	gl	b, h, k, fm	A, C	A	b, h, k, fm	—	11
	19-50	19-52	gl	b, h, k, fm, gl	A	A, C	b, h, k, fm	C	11
	18-25	18-38	b, h, k, fm	b, k, gl	A, C	None	gl	—	10
	19-36	19-32	gl	b, e, h, k, fm	None	C	b, e, h, k, fm	C	10
Mean survival time									13.1

leukocyte antigen or pair of associated antigens. As noted in Table V, donors and recipients were incompatible for antigen c in two instances; the transplant survival times were 20 and 18 days, respectively. Five donors and recipients were incompatible for antigen e, with renal allograft survivals of 16, 15, 13, 13, and 11 days, respectively. The survival times of 8 kidneys transplanted across a gl incompatibility were 18, 15, and 14 days in littermates, and 13, 12, 11, 11, and 10 days in nonlittermates.

TABLE IV

Results of Kidney Allograft Exchanges between Sibling and Nonlittermate Pairs of Beagles

Pair number	Genetic status	Recipient number	Donor number	Recipient's leukocyte group phenotype	Donor's leukocyte group phenotype	Resulting leukocyte group incompatibility	Detectable erythrocyte group incompatibility	Survival of renal allografts	Differences in survival time of crossed renal allografts
								days	
1	LM*	19-14	19-16	b, h, k, fm, gl	b, h, k, fm, gl	—	A	38	11
		19-16	19-14	b, h, k, fm, gl	b, h, k, fm, gl		—	27	
2	"	18-81	18-80	gl	gl	—	C	38	20
		18-80	18-81	gl	gl		—	18	
3	"	19-17	19-25	b, e, h, k, fm	b, e, h, k, fm	—	—	35	1
		19-25	19-17	b, e, h, k, fm	b, e, h, k, fm		C	34	
4	"	18-85	18-84	gl	gl	—	—	32	12
		18-84	18-85	gl	gl		—	20	
5	"	19-09	19-07	b, e, h, k, fm	b, e, h, k, fm	—	—	21	8
		19-07	19-09	b, e, h, k, fm	b, e, h, k, fm		—	13	
6	Non-LM‡	19-15	19-26	b, h, k, fm	b, h, k, fm	—	A	37	10
		19-26	19-15	b, h, k, fm	b, h, k, fm		—	27	
7	"	19-29	19-20	b, h, k, fm, gl	b, h, k, fm, gl	—	A, C	31	3
		19-20	19-29	b, h, k, fm, gl	b, h, k, fm, gl		—	28	
8	"	18-42	18-32	b, h, k, fm, gl	b, h, k, fm, gl	—	—	28	5
		18-32	18-42	b, h, k, fm, gl	b, h, k, fm, gl		A	23	
9	LM	19-46	19-45	b, c, d, h, k, fm	gl	gl	—	15	2
		19-45	19-46	gl	b, c, d, h, k, fm	b, c, d, h, k fm	—	13	
10		19-03	19-94	b, c, h, k, fm	e, gl	e, gl	—	12	1
		19-04	19-03	e, gl	b, c, h, k, fm	b, c, h, k, fm	—	11	
11	Non-LM	19-30	19-44	b, e, k, gl	b, c, k, gl	c	A, C	18	5
		19-44	19-30	b, c, k, gl	b, e, k, gl	e	—	13	
12	"	18-37	18-27	b, k, fm, gl	e, h, k, fm	e	A, C	13	2
		18-27	18-37	e, h, k, fm	h, k, fm, gl	gl	—	11	
13	"	19-32	19-36	b, e, h, k, fm	gl	gl	—	13	3
		19-36	19-32	gl	b, e, h, k, fm	b, e, h, k, fm	C	10	
14	"	18-38	18-25	b, k, gl	b, h, k, fm	h, fm	A, C	13	3
		18-25	18-38	b, h, k, fm	b, k, gl	gl	—	10	
15	"	19-12	18-53	b, h, k, fm	gl	gl	C	12	1
		18-53	19-12	gl	b, h, k, fm	b, h, k, fm	—	11	
16	"	16-72	16-45	b, e, j, gl	b, h, j, k, fm, gl	b, k, fm	A	12	1
		16-45	16-72	b, h, j, k, fm, gl	b, e, j, gl	e	—	11	
17	"	19-27	19-05	b, e, h, k, fm	b, e, k, gl	gl	—	11	0
		19-05	19-27	b, e, k, gl	b, e, h, k, fm	h, fm	—	11	

* LM, littermates.

‡ Non-LM, nonlittermates.

Sex differences did not appear to play a prominent role in the observed results. Three renal allografts transplanted from male donors to female recipients in the absence of detectable leukocyte group incompatibilities were rejected at 20, 34, and 34 days, respectively (dogs 18-28 and 18-21, 19-17 and 19-25 and 19-33 and 19-35 in Table II). Three female-to-male transplants

TABLE V

Influence of Donor-Recipient Incompatibilities for One Detectable Leukocyte Group Antigen or Pair of Associated Antigens upon Kidney Allograft Survival

Recipient number	Donor number	Genetic status	Recipient leukocyte group phenotype	Donor leukocyte group phenotype	Detectable leukocyte group incompatibility	Renal allograft survival time
						days
19-00	18-99	LM*	b, h, k, fm, gl	b, c, h, k, fm	c	20
19-30	19-44	Non-LM‡	b, e, k, gl	b, c, k, gl	c	18
19-38	19-23	Non-LM	b, h, k, fm, gl	b, e, h, k, fm, gl	e	16
19-11	19-06	"	b, h, k, fm	b, e, h, k, fm	e	15
19-44	19-30	"	b, c, k, gl	b, e, k, gl	e	13
18-37	18-27	"	h, k, fm, gl	e, h, k, fm	e	13
16-45	16-72	"	b, h, j, k, fm, gl	b, e, j, gl	e	11
18-31	18-30	LM	b, e, h, k, fm	b, e, h, k, gl	gl	18
19-46	19-45	"	b, c, d, h, k, fm	gl	gl	15
19-13	19-10	"	b, h, k, fm	b, h, k, fm, gl	gl	14
19-32	19-36	Non-LM	b, e, h, k, fm	gl	gl	13
19-12	18-53	"	b, h, k, fm	gl	gl	12
19-27	19-05	"	b, e, h, k, fm	b, e, k, gl	gl	11
18-27	18-37	"	e, h, k, fm	b, k, fm, gl	gl	11
18-25	18-38	"	b, h, k, fm	b, k, gl	gl	10

* LM, littermates.

‡ Non-LM, nonlittermates.

done under similar conditions survived for 35, 45, and 28 days, respectively (dogs 19-25 and 19-17, 18-46 and 18-55 and 18-57 and 18-56, in Table II).

DISCUSSION

Interest in host responses to bone marrow transplants stimulated the establishment, some years ago, of a closely bred colony of beagles (29). Occasional lone-term survivals of some marrow transplants indicated that varying degrees of histocompatibility might be present between some of the members of this colony. Attempts to predict the duration of graft survival on the basis of

donor-recipient coefficients of relationship or by matching erythrocyte antigens were not successful (23). More recently, however, Epstein, Storb, Ragde, and Thomas (26) demonstrated that cross-immunization of canine littermates with buffy coat cells stimulated the formation of leukocyte group-specific antisera, and that the use of such antisera for the selection of donors and recipients resulted in prolonged bone marrow graft survivals in littermates (26) and in unrelated dogs (30). In an extension of this method, Mollen, St. John, Cannon, and Ferrebee (24) developed a battery of antisera capable of detecting 10 leukocyte antigen specificities in their closely bred colony of beagles, and reported that skin allografts performed across detectable leukocyte group incompatibilities were rejected more rapidly (MST = 13.7 days) than transplants obtained from compatible donors (MST = 25.1 days). Ferrebee, Cannon, Mollen, and St. John (23) have indicated recently that such typing sera may also be of value in the selection of donor-recipient combinations for the transplantation of other organs.

The results of the present study indicate that the leukocyte group antigens currently detectable by Mollen, St. John, Cannon, and Ferrebee's (24) battery of typing antisera play an important role in conditioning the survival time of renal allografts in the closely bred colony of beagles under study. As has been noted previously for bone marrow (29) and skin transplants (24), donor–recipient coefficients of correlation or erythrocyte group compatibility do not appear to play a decisive role as determinants of renal allograft survival. Performance of kidney transplants across detectable leukocyte group incompatibilities caused as sharp a decrease in allograft survival time in siblings (littermates) as in nonlittermates. Conversely, renal transplants performed in the absence of such incompatibilities were accorded comparable prolongations in survival times in the littermate and nonlittermate groups. The results of transplantation in the closely bred colony of beagles in the face of known incompatibilities were better, however, than those observed in randomly selected mongrel dogs. These observations, taken together with evidence that the leukocyte antigens detected by Ferrebee, Cannon, Mollen, and St. John (23) behave as Mendelian autosomal dominants, suggest that the same general rules of histocompatibility encountered in the murine H-2 system (31) and in the human HL-A system (32) may be operative in the canine species. It is anticipated that further genetic and serologic studies of the canine leukocyte antigens will result in the identification of additional antigenic specificities, and in a further definition of what may constitute the main system of histocompatibility in dogs, for which the term DL-A is proposed.

It is of interest that, under present experimental conditions, incompatibilities for the Swisher erythrocyte antigens A, C, and/or D did not appear to have an adverse effect upon renal allograft survival. This observation is in harmony with similar results of Altman (33) in mongrel dogs, and with the studies of

Rubenstein, Morgado, Blumenstock, and Ferrebee (34) with regard to skin allograft survival in the same colony of beagles.

The transplants exchanged within six pairs of littermate beagles with no detectable donor-recipient leukocyte group incompatibilities may be deserving of further comment. The pronounced differences in survival noted between the individual members of some of these pairs would appear to suggest that a significant number of antigenic specificities capable of conditioning allograft survival may as yet not have been detected. Almost identical and prolonged allograft survivals of 35 and 34 days, respectively, only occurred in one pair of animals (No. 19-17 and 19-25). These results are in keeping with the observation that siblings may be either very good, or potentially unfavorable allograft donors, depending upon their inherited parental haplotypes (13). Differences in survival time of transplants exchanged within pairs of leukocyte group-incompatible transplants were not as marked as those noted in the first group. It is possible that, in the latter situation, the already identified incompatibilities may have obscured the effects of as yet undetected donor–recipient differences. It is of interest, however, that the most pronounced difference in survival time occurred in a donor–recipient pair (No. 19-30 and 19-44) incompatible for antigen c in one direction and antigen e in the other direction. The kidney bearing antigen c was rejected in 18 days, while the kidney bearing antigen e survived for only 13 days. In this regard, Mollen, St. John, Cannon, and Ferrebee (24) have reported that donor–recipient incompatibilities for antigens b, c, j, and k did not cause a decrease in the survival time of skin allografts in this beagle colony—i.e., they might be "weak" transplantation antigens. Renal transplants incompatible for antigen c were accorded longer survival times (20 and 18 days) than transplants incompatible for antigen e (MST = 13.6 days) or gl (MST = 13.0 days). It would therefore appear that antigen c may also not be as potent in renal transplantation as e or gl.

As was noted in Table V, the survival of gl-incompatible renal transplants ranged from 18 to 10 days. This result would appear to further highlight the need for a definition of additional as yet undetected antigenic specificities present in the colony of beagles under study. It also supports the concept that any interpretation of the individual "potency" of a leukocyte antigen may be as much a function of its immunogenicity as it is dependent upon the antigenic makeup of the recipient. Indeed, gl might have acted as a "strong" antigen in recipient 18-25, resulting in a short renal allograft survival of 10 days, and as a "weak" antigen in recipient 18-31, whose transplant survived for 18 days. In the first instance, there were probably no antigens similar to or cross-reacting with gl in the nonlittermate kidney transplant recipient, while the presence of such antigens in the second animal, which received a renal allograft from a littermate, might have attenuated the effects of the gl incompatibility in that case. A similar situation has recently also been documented in human subjects

with regard to antigens HL-A 2 and Da 15 of the HL-A system (35). Ferrebee, Cannon, Mollen, and St. John (23) are currently attempting to classify their closely bred colony of beagles into several substrains on the basis of patterns of inheritance of the leukocyte group antigens. This approach may provide further insight into the immunogenetics of what may be the principal system of canine histocompatibility, and will be of value in the definition of as yet unknown leukocyte antigenic specificities in this population. In the meantime, however, the availability of carefully characterized canine donors and recipients has provided a valuable new approach to further studies of organ transplant responses in unmodified recipients.

Further progress in the development of this population of beagles may be particularly useful in the assessment of the comparative immunogenicity of the major transplantable organs, and for experimental studies of the facilitation of organ transplant survival in the mammalian host.

SUMMARY

The establishment of a closely bred colony of beagles with known leukocyte group phenotypes has permitted an assessment of the role of leukocyte group antigens in conditioning the survival of renal allografts in the unmodified host. 22 kidney transplants obtained from leukocyte group-compatible donors were accorded a mean survival time of 25.5 days, as compared with 13.1 days for 27 transplants obtained from incompatible donors. Donor–recipient coefficients of correlation and Swisher erythrocyte group incompatibilities did not appear to affect the observed results. The mean survival time of 21 renal allografts performed in randomly selected mongrel dogs was 9.5 days.

Availability of a carefully characterized and phenotyped canine population may be useful in further studies of the comparative immunogenicity of the major transplantable organs, and of methods designed to facilitate prolonged organ transplant survival in the mammalian host.

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BIBLIOGRAPHY

1. Lawrence, H. S. 1959. Homograft sensitivity — An expression of the immunological origins and consequences of individuality. *Physiol. Rev.* **39:**811.
2. Ceppellini, R., E. S. Cortoni, G. Leigheb, P. L. Mattivz, V. C. Maggiano, and M. Visetti. 1965. An experimental approach to genetic analysis of histocompatibility in man. *Histocompatibility Testing.* **11:**13.
3. Amos, D. B., B. G. Hattler, P. Hutchin, R. McCloskey, and C. M. Zimewsky. 1966. Skin donor selection by leukocyte typing. *Lancet.* **1:**300.
4. Rapaport, F. T., H. S. Lawrence, J. M. Converse, and J. M. Mulholland. 1960. The specificity of skin homograft rejection in man. *Ann. N. Y. Acad. Sci.* **87:** 217.

5. Rapaport, F. T., H. S. Lawrence, L. Thomas, J. M. Converse, W. S. Tillett, and J. H. Mulholland. 1962. Cross-reactions to skin homografts in man. *J. Clin. Investig.* **41**:2166.
6. Wilson, R. E., L. Henry, and J. P. Merrill. 1963. A model system for determining histocompatibility in man. *J. Clin. Invest.* **42**:1497.
7. Brent, L., and P. B. Medawar. 1963. Tissue transplantation: A new approach to the typing problem. *Brit. Med. J.* **2**:269.
8. Gray, J. G., and P. S. Russell. 1965. The lymphocyte transfer test in man. *Nat. Acad. Sci. Nat. Res. Counc. Publ.* **1229**:105.
9. Ramsier, H., and J. W. Streilein. 1965. Homograft sensitivity reactions in irradiated hamsters. *Lancet.* **1**:622.
10. Hirschhorn, K. 1965. Method of studying lymphocyte interaction and other immunologic and cytogenetic studies of human lymphocytes. *Nat. Acad. Sci. Nat. Res. Counc.* **1229**:177.
11. Hirschhorn, K., F. Bach, F. T. Rapaport, J. M. Converse, and H. S. Lawrence. 1964. The relationship of in vitro lymphocyte compatibility to homograft sensitivity in man. *Ann. N. Y. Acad. Sci.* **120**:303.
12. 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.
13. Colombani, J., and J. Dausset. 1969. L'Histocompatibilité Humaine. *Pathol. Biol.* **17**:281.
14. Dausset, J., 1958. Iso-leuco-anticorps. *Acta Haematol.* **20**:156.
15. Berah, M., J. Hors, and J. Dausset. 1969. A study of HL-A antigens in human organs. *Transplantation.* In press.
16. Bach, F., and D. B. Amos. 1967. Hu-1, Major histocompatibility locus in man. *Science* (*Washington*). **156**:1506.
17. Dausset, J., R. L. Walford, J. Colombani, L. Legrand, N. Feingold, A. Barge, and F. T. Rapaport. 1969. The HL-A system sub-loci and their importance in transplantation. *Transpl. Proc.* **1**:331.
18. Dausset, J., F. T. Rapaport, P. Ivanyi, and J. Colombani. 1967. Tissue alloantigens and transplantation. *In* Histocompatibility Testing. 63.
19. Van Rood, J. J., A. Van Leeuwen, A. M. J. Schippers, W. H. Vooys, E. Frederiks, H. Balner, and J. G. Eernisse. 1965. Leukocyte groups, the normal lymphocyte transfer test and homograft sensitivity. *In* Histocompatibility Testing. **11**:37.
20. Dausset, J., and F. T. Rapaport. 1969. Histocompatibility studies in haploidentical genetic combinations. *Transpl. Proc.*, **1**:649.
21. Van Rood, J. J., and J. D. Eernisse. 1968. The detection of transplantation antigens in leukocytes. *Seminars Hematol.* **2**:187.
22. Sakai, A., M. Simonsen, and E. Jensen. 1969. Kidney transplantation in siblings. Is immunosuppressive treatment invariably needed? *Transplantation.* **7**:444.
23. Ferrebee, J. W., F. D. Cannon, N. Mollen, and D. St. John. 1969. Inheritance of lymphocyte types and their relationship to histocompatibility in a closely-bred colony of beagles. *Transplantation.* In press.
24. Mollen, N., D.St. John, F. D. Cannon, and J. W. Ferrebee. 1968. Lymphocyte typing in allografted beagles. *Transplantation.***6**:939.

25. Swisher, S. N., and L. E. Young. 1961. The blood grouping systems of dogs. *Physiol. Rev.* **41**:495.
26. Epstein, R. B., R. Storb, H. Ragde, and E. D. Thomas. 1968. Cytotoxic typing antisera for marrow grafting in littermate dogs. *Transplantation.* **6**:45.
27. Rapaport, F. T., A. S. Markowitz, R. T. McCluskey, T. Hanaoka. 1969. Induction of glomerulonephritis in renal autografts. *Surgery.* **66**:34.
28. Al-Askari, S. 1968. Urologic aspects of renal transplantation. *In* Human Transplantation. F. T. Rapaport and J. Dausset, editors., Grune & Stratton, Inc., New York. 100.
29. Thomas, E. D., and J. W. Ferrebee. 1962. Transplantation of marrow and whole organs: experiences and comments. *Can. Med. Ass. J.* **86**:435.
30. 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.
31. 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.
32. Dausset, J., J. Colombani, L. Legrand, and N. Feingold. 1969. Les sub-loci du système HL-A—le système principal d'histocompatibilité de l'homme. *La Presse Méd.* **77**:849.
33. Altman, B. 1965. Hemmaglutinin in skin and kidney homotransplantation in dogs. *Transplantation.* **3**:326.
34. Rubinstein, P., F. Morgado, D. A. Blumenstock, and J. W. Ferrebee. 1968. Isohemagglutinins and histocompatibility in the dog. *Transplantation.* **6**:961.
35. Dausset, J., J. Colombani, M. Colombani, L. Legrand, and N. Feingold. 1968. Un nouvel antigène du système HL-A (Hu-1): l'antigène 15, allèle possible des antigènes 1, 11, 12. *Nouv. Rev. Fr. Hématol.* **8**:398.