HISTOCOMPATIBILITY STUDIES IN A CLOSELY BRED COLONY OF DOGS

IV. Tolerance to Bone Marrow, Kidney, and Skin Allografts in DL-A-Identical Radiation Chimeras*

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The main obstacle to successful organ transplantation today is the recipient's recognition of the implanted cells as foreign, and his reaction to this intrusion through cellular and/or humoral mechanisms normally triggered by infectious microorganisms (1). The tempo and intensity of this immunological response are conditioned significantly by the degree of genetic or antigenic disparity existing between donor and recipient (2). This consideration has stimulated an intensive search for techniques capable of selecting optimally compatible donor-recipient pairs for organ transplantation. Such techniques include in vivo tests performed with skin allografts (third man test) or with lymphocytes (normal lymphocyte transfer test) obtained from prospective donors and recipients, and in vitro cellular (mixed lymphocyte cultures) and serological (leukocyte group antigens) reactions (3). The latter effort, largely stimulated by the classical studies of Gorer and Snell and their associates (4–7), has provided cogent evidence for the existence in different mammalian species of a major system of histocompatibility whose components are amenable to serological analysis and definition. Such histocompatibility systems include the murine H-2 system (6), HL-A in man (8), Ag-B (9) or RtH-1 (10) in rats, GPL-A in guinea pigs (11), PL-A in pigs (12), ChL-A in chimpanzees (13), and RhL-A in rhesus monkeys (14).

Another important approach to the transplantation problem has been concerned with the search for specific immunological techniques capable of inducing allograft tolerance without interference with the remainder of the host's immunological defenses (1). Historically, this endeavor began with the studies of Medawar and his associates in 1951 (15), and led to Billingham, Brent, and Medawar's (16) definition of immunological tolerance as a weakening or suppression of allograft responsiveness through exposure of the host to specific antigenic stimulation before the development of immunological competence. Since then, this concept has been broadened considerably, and the dividing lines between classical immunological tolerance and

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serologically mediated allograft enhancement (17, 18) have become increasingly tenuous (15). It has also been reported that treatment of adult animals with transplantation antigens after the development of immunological competence may decrease allograft reactivity in the recipients (19–22). As noted in the classical monograph of van Bekkum and de Vries (23), the combined use of total body irradiation and bone marrow transplantation in rodents has provided an alternative and uniquely important approach to this problem.

In their attempt to extend the latter experimental approach to the canine species, Ferrebee and Thomas and their associates established a selectively bred colony of beagles at the Mary Imogene Bassett Hospital (24), and observed that only an occasional dog exposed to supralethal total body irradiation and given a bone marrow transplant from a randomly selected donor survived this treatment without untoward effects (25). Such survivors were also unresponsive to skin allografts obtained from their donor of marrow (25). The one long-term renal allograft of Mannick et al. (26) and the one long-term lung allograft of Blumenstock et al. (27) performed under similar experimental conditions suggested that these radiation chimeras might also be unresponsive to other tissues obtained from the donor of marrow. The principal obstacle to more extensive study of this experimental model was the unavailability of methods of donor selection capable of providing a regularly reproducible take and a long-term survival of a bone marrow transplant in the dog. Most bone marrow transplants either failed to take or caused fatal secondary graft-*versus*-host (GVH)[1] disease in the recipients (28). Some means of prospective selection of compatible donor-recipient pairs thus appeared to be essential to permit progress of this endeavor.

In further studies of this question, Kasakura, Thomas, and Ferrebee (29), Puza et al. (30), Rubinstein and Ferrebee (31), Cleton, van Es, Ponsen, and van Rood (32), Cohen and Kozari (33), and Epstein, Storb, Ragde, and Thomas (34) observed that immunization of dogs, and particularly of littermates (34), with buffy coat cells resulted in the appearance of leukocyte group-specific isoantibodies in the recipients. In an extension of the elegant studies of Epstein et al. (34), Rubinstein and associates (35), and Mollen et al. (36) produced a battery of typing sera capable of detecting 10 different leukocyte antigen specificities (or sets of specificities) in the Cooperstown colony of beagles. Transplantation studies performed on the basis of donor-recipient compatibility for these antigens in the Cooperstown Colony have shown that allografts of skin (36), kidney (37), heart (38), lung (39), and liver (40) are accorded prolonged survivals in compatible animals. Conversely, allografts transplanted across major leukocyte group incompatibilities are rejected rapidly. This result, taken together with evidence that such leukocyte antigens behave as Mendelian autosomal dominants in the Cooperstown Colony (41), suggested that these leukocyte antigens were components of a single major system of histocompatibility, for which the term DL-A was proposed (37). Bos et al. (42), Westbroek et al. (43), Templeton and Thomas (44), and Vriesendorp et al. (45) have recently provided additional evidence to support the existence of this major histocompatibility locus in the canine species. Recent studies of the segregation of the components of the DL-A system in 679 offspring of 141 consecutive matings in the Cooperstown Colony have identified 23

[1] *Abbreviations used in this paper:* DIH, distemper-infectious hepatitis vaccine; GVH, graft-*versus* host; MST, mean survival time.

different DL-A haplotypes (i.e., specificities, or sets of specificities, determined by the DL-A region of one chromosome) in this colony (46), and have demonstrated that all currently known DL-A antigen(s) are regularly transmitted en bloc from parents to offspring, with no evidence of independent segregation. These studies have also resulted in the definition of the DL-A genotypes of 1302 offspring of 517 matings in the Cooperstown Colony (46). Preliminary studies (47, 48) have raised the possibility that the application of such genotypic criteria of donor-recipient DL-A identity might considerably improve the results of bone marrow transplantation in the canine species (49).

This report describes the long-term survival of 17 consecutive irradiated recipients of bone marrow allografts obtained from prospectively selected genotypically DL-A-identical donors, 11 littermate and 6 nonlittermate. The recipients are thus far surviving without evidence of GVH disease for periods of 211–649 days, and are unresponsive to kidney and skin allografts obtained from their donor of marrow. They have, however, rejected DL-A-incompatible skin allografts obtained from other donors at the same rate as untreated dogs. The results suggest that total body irradiation and transplantation of bone marrow from a genotypically DL-A-identical donor induces in the recipient a state of unresponsiveness to other tissue allografts from the same donor. The immunological specificity of this tolerant state is demonstrated by the ability of the recipients to reject promptly allografts obtained from other donors.

Materials and Methods

Selection of Donors and Recipients.—Adult male and female beagles of the Cooperstown Colony weighing 18–25 lb. were used throughout this study. Donor-recipient pairs for bone marrow transplantation were selected on the basis of genotypic evidence of DL-A identity. The DL-A genotypes of all of the animals used were obtained as a result of the earlier definition of the DL-A haplotypes and of their segregation in each succeeding generation of the various different lines of selectively bred animals maintained in the colony (46). Deduction of each genotype was confirmed by serological testing with a standard battery of lymphocytotoxic DL-A antisera. The method of preparation of these antisera and the typing technique used have been described previously (34–37). 12 different DL-A specificities (or sets of specificities) were detected by this method, including *b*, *c*, *d*, *e*, *f*, *g*, *h*, *k*, *l*, *m*, *n*, and *o*. Erythrocyte group antigens A, C, and D were detected with the typing sera and by the technique of Swisher and Young (50). Coefficients of relationship of each donor and recipient and of their respective parents were determined in each experiment.

Method of Total Body Irradiation and Bone Marrow Transplantation.—The method of supralethal total body irradiation has been described in detail previously (51). Each recipient was exposed to 1200–1400 R of continuous irradiation from two opposing ^{60}Co sources, at a source-to-target distance of 2–2.5 m, with an exposure rate of 3–4 R/min. 12–18 hr later, a bone marrow transplant obtained from a prospectively selected genotypically DL-A-identical donor was performed by a modification of the standard technique (52), consisting of the intravenous infusion of a suspension of 3–3.5 $\times$ 10^9 nucleated bone marrow cells obtained by needle aspiration of the long bones and sternum of the donor (52). Leukocyte and platelet counts were performed three times weekly for the first 21 days after irradiation and at weekly intervals thereafter. Return of leukocyte and platelet levels to normal values after treatment and the absence of GVH disease constituted evidence of successful take and proliferation of

the bone marrow transplant (53). Persistence of chimerism was confirmed at regular intervals by the continuing presence of donor erythrocyte group antigens in the recipient, and by the appearance of the sex characteristics of donor cells in the recipient's peripheral leukocytes (54).

Method of Study of Serum Antibody Responses.—Each animal received an intramuscular injection of 1 ml of distemper and infectious hepatitis (DIH) vaccine (prepared by Dr. James A. Baker, Veterinary Virus Research Institute, Cornell University, Ithaca, N. Y.) at the age of 3 months (55). Serum antibody levels were determined immediately before irradiation. The animals received additional booster immunizations of 1 ml of vaccine before kidney transplantation and at various intervals thereafter. Anti-distemper and anti-infectious hepatitis virus antibody determinations were performed in Dr. Baker's laboratory by standard viral neutralization tests (56, 57).

Methods of Grafting and Criteria for the Assessment of Allograft Survival.—Within 43–120 days after bone marrow transplantation, each recipient underwent bilateral nephrectomy under general halothane anesthesia, followed immediately by transplantation of a kidney obtained from the bone marrow donor. The technique of transplantation has been described previously (37). Complete urinalysis and blood urea nitrogen determinations were performed daily during the 1st month after operation, and at biweekly intervals thereafter. Criteria for the continued survival of each kidney transplant included normal renal function tests and evidence of the ability of the transplanted kidney to maintain the host's life without weight loss, anorexia, and/or vomiting.

The method of skin grafting has been described in an earlier report (35). Briefly, the recipients were anesthetized and the hair of the lateral aspects of the chest was removed. Under sterile precautions, skin surfaces measuring 2 × 2 cm were excised, leaving the intact panniculus carnosus as the graft bed. Skin specimens of the same size were excised from the donors; the subcutaneous fat was removed from each graft by sharp dissection, and the graft was approximated to the recipient bed with interrupted 5-0 nylon sutures. A maximum of four grafts was applied in duplicate to each recipient. Vaseline gauze and compressive gauze dressings were placed on the transplants, and then covered with an adhesive bandage. Dressings were removed on the 7th postoperative day, and the grafts were then examined daily. A graft was considered to have undergone rejection when 75% of the transplanted tissue had become hard, opaque, or necrotic. The diagnosis of rejection was confirmed in each instance by sloughing of the eschar.

For the purposes of this study, each recipient was tested with skin allografts within 230–450 days after bone marrow transplantation. The skin grafts were obtained from the bone marrow donor and from a maximum of three other donors selected on the basis of varying degrees of DL-A compatibility with the recipients.

RESULTS

The DL-A genotypes and coefficients of relationship of the donor-recipient pairs presented in this report are outlined in Table I. 11 animals received bone marrow transplants from DL-A-identical littermates. The sires and dams of each pair of animals in this group were completely unrelated in three instances; the coefficients of relationship of the other eight sets of parents varied from $\frac{7}{16}$ to $\frac{1}{128}$. Six dogs received bone marrow transplants from DL-A-identical nonlittermate donors. The donor-recipient coefficients of relationship ranged from $\frac{1}{8}$ to $\frac{87}{512}$; the coefficients of relationship of the parents of each individual donor and recipient in this group ranged from $\frac{23}{64}$ to zero.

The hematological effects of supralethal total body irradiation and bone

marrow transplantation are summarized in Table II. There was a uniform decrease in leukocyte and platelet levels during the first 10–12 days after irradiation, with leukocyte counts in the range of 99–311/mm^3, and platelet counts in the range of 4600–63,000/mm^3. These values returned to normal levels within 20–110 days after bone marrow transplantation, with leukocyte counts ranging from 6300 to 12,000/mm^3, and platelet counts from 107,000 to 257,000/mm^3. The serological responses of each recipient to immunization with

TABLE I

DL-A Genotypes and Coefficients of Relationship of Bone Marrow Donors and Recipients and Their Parents

Donor	Recipient	DL-A genotype of each donor and recipient pair	Donor-recipient relation	Donor-recipient coefficient of relationship	Coefficient of relationship of parents of	
					Donor	Recipient
21-53	21-54	bkhfm/gln	Littermates	1/2	23/64	23/64
21-59	21-60	bkhfm/bkhfm	"	1/2	0	0
21-62	21-61	bkhfm/bkhfm	"	1/2	0	0
21-65	21-66	gln/be	"	1/2	7/16	7/16
21-74	21-73	bkhfm/bkhfm	"	1/2	3/32	3/32
22-21	22-22	gln/gln	"	1/2	0	0
21-95	21-96	bkhfm/bkhfm	"	1/2	7/32	7/32
22-32	22-29	gln/gln	"	1/2	3/16	3/16
22-03	22-04	bkcdn/bkhfm	"	1/2	7/64	7/64
22-43	22-40	bkhfm/gln	"	1/2	37/128	37/128
22-55	22-48	bkhfm/bkhfm	"	1/2	1/128	1/128
21-87	21-56	bkhfm/bkhfm	Nonlittermates	85/256	13/64	23/64
21-90	21-97	bkhfm/bkhfm	"	87/512	13/64	7/32
22-06	22-24	gln/gln	"	1/8	25/256	0
22-51	22-57	bkhfm/bkhfm	"	77/512	1/128	13/64
22-52	22-56	bkhfm/bkhfm	"	77/512	1/128	13/64
22-46	22-07	gln/gln	"	163/512	3/32	25/256

DIH vaccine are also summarized in Table II. There was a relative decrease in antibody titers to distemper and infectious hepatitis viruses during the early postirradiation and transplantation period. As noted in Table II, however, such titers generally returned to normal levels within 3–6 months after bone marrow transplantation. At that time, the titers were roughly comparable to antibody levels in the recipients before irradiation.

The results of bone marrow transplantation are outlined in Table III. Bone marrow allografts obtained from prospectively selected DL-A-identical donors are currently surviving for 211–649 days without evidence of GVH disease in 17 consecutive recipients. These include 11 recipients of bone marrow obtained from littermates (315, 364, 424, 440, 531, 531, 584, 605, 625, 645, and 649 days, respectively) and 6 recipients of transplants from nonlittermate donors (211, 279, 280, 418, 479, and 480 days, respectively). Evidence of persisting chimerism

has been obtained at regular intervals in eight informative donor-recipient pairs on the basis of changes in the recipients' erythrocyte group antigens after bone marrow transplantation (recipients 21-60, 21-61, 21-66, 21-73, 21-96, 22-04, 21-97, 22-56). Similar evidence has been obtained in eight pairs by the observation that the recipients' peripheral blood leukocytes had acquired the

TABLE II

Effects of Supralethal Total Body Irradiation and Bone Marrow Transplantation in Selectively Bred DL-A-Identical Donor-Recipient Pairs of Beagles

Donor	Recipient	Relation	Leukocyte/platelet levels* of recipients at		Serum antibody titers in recipients			
			7–12 days after irradiation	22–110 days after bone marrow transplantation	Before irradiation		3–6 months after bone marrow transplantation	
					Distemper	Infectious hepatitis	Distemper	Infectious hepatitis
21-53	21-54	Littermate	99/4,666	10,800/241,000	>1/100	ND	1/180	1/1,250
21-59	21-60	" "	266/17,500	12,000/257,000	>1/100	ND	1/125	1/16,000
21-62	21-61	" "	240/24,250	10,300/220,000	>1/100	ND	1/800	1/25,000
21-65	21-66	" "	177/9,600	8,800/213,000	>1/100	ND	1/2000	1/800
21-74	21-73	" "	177/17,500	12,900/160,000	>1/100	ND	1/1250	1/12,500
21-95	21-96	" "	33/48,000	6,300/195,000	>1/100	ND	1/800	1/8,000
22-03	22-04	" "	288/63,000	11,600/225,000	>1/100	ND	1/310	1/15,800
22-32	22-29	" "	133/20,500	9,800/127,000	1/3100	1/690	1/3100	1/310
22-21	22-22	" "	155/25,000	10,500/107,000	1/2000	1/3,100	1/5000	1/3,100
22-43	22-40	" "	211/19,000	10,300/137,000	1/2000	1/6,300	1/5000	1/8,000
22-55	22-48	" "	200/30,000	8,000/200,000	1/2000	1/16,000	1/80	1/12,500
21-87	21-56	Nonlittermate pairs	300/32,000	14,600/171,000	1/500	1/3,100	1/2000	1/3,100
21-90	21-97	" "	311/36,500	12,700/190,000	1/310	1/310	1/400	1/25,000
22-06	22-24	" "	133/26,000	11,200/189,000	1/1800	1/3,100	1/2000	1/12,500
22-52	22-56	" "	277/49,000	6,500/169,000	1/125	1/25,000	1/310	1/12,500
22-51	22-57	" "	166/37,500	11,400/220,000	1/400	1/25,000	1/100	1/25,000
22-46	22-07	" "	144/15,400	10,000/156,000	1/310	1/4,000	1/800	1/2,500

* Per mm^3 of blood.

sex characteristics of their corresponding bone marrow donors (recipients 21-61, 21-96, 22-29, 22-40, 22-48, 22-56, 22-57, 22-07).

As noted in Table IV, renal allografts obtained from the bone marrow donors are currently maintaining normal function in 11 littermate recipients (234, 313, 377, 378, 441, 444, 482, 557, 580, 581, and 586 days, respectively) and in 6 nonlittermate recipients (191, 200, 221, 349, 361, and 405 days, respectively). In contrast, renal allografts in 13 untreated littermate recipients under comparable conditions of DL-A compatibility were rejected in 13–38 days (mean survival

time [MST] = 28.3 days). Renal allografts in untreated recipients of DL-A-incompatible transplants survived 11–20 days in eight littermates (MST = 14.8 days) and 10–18 days in 19 nonlittermates (MST = 12.4 days).

The responses to skin allografts observed in the bone marrow chimeras are outlined in Table V. 13 of 14 grafts obtained from the donors of marrow are surviving in a manner indistinguishable from the behavior of skin autografts.

TABLE III

Results of Bone Marrow Transplantation in Prospectively Selected Genotypically DL-A-Identical Pairs of Beagles

Donor	Recipient	Relation	Survival of bone marrow transplant*	Swisher erythrocyte groups of			Sex markers of		
				Donor	Recipient	Recipient after transplantation	Donor	Recipient	Recipient after transplantation
			days						
21-53	21-54	Littermate pairs	>649	AC	AC	AC	M	M	M
21-59	21-60	" "	>645	C	A	C	M	M	M
21-62	21-61	" "	>625	AC	C	AC	F	M	F
21-65	21-66	" "	>605	C	AC	C	M	M	M
21-74	21-73	" "	>584	C	A	C	M	M	M
21-95	21-96	" "	>531	A	AC	A	M	F	M
22-03	22-04	" "	>531	AC	A	AC	F	F	F
22-32	22-29	" "	>440	C	C	C	F	M	F
22-21	22-22	" "	>424	C	C	C	M	M	M
22-43	22-40	" "	>364	AC	AC	AC	F	M	F
22-55	22-48	" "	>315	AC	AC	AC	F	M	F
21-87	21-56	Nonlittermate pairs	>480	AC	AC	AC	F	F	F
21-90	21-97	" "	>479	AC	A	AC	F	F	F
22-06	22-24	" "	>418	C	C	C	M	M	M
22-52	22-56	" "	>280	AC	A	AC	F	M	F
22-51	22-57	" "	>279	AC	AC	AC	M	F	M
22-46	22-07	" "	>211	AC	AC	AC	F	M	F

* All recipients are surviving uneventfully, with no evidence of secondary disease, as of 15 May 1972.

In seven of eight recipients of littermate bone marrow, the current skin allograft survival is 199, 178, 162, 122, 116, 116, and 110 days, respectively. In six nonlittermate recipients, the current survival is 107, 110, 110, 128, 143, and 143 days, respectively. One allograft (recipient 21–60) developed slow progressive rejection changes which were complete by the 84th day after transplantation.

Six recipients of littermate marrow were tested with skin grafts from a DL-A-identical sibling of the bone marrow donor. These grafts were rejected within 20–36 days (MST = 25.8 days). Five recipients of nonlittermate marrow were tested in similar fashion with skin grafts from a DL-A-identical sibling of

the marrow donor. These transplants survived for 27–76 days (MST = 56.2 days).

Nine recipients of bone marrow obtained from littermate donors and six recipients of nonlittermate marrow were tested with one or more skin allografts obtained from DL-A-incompatible donors. The nine dogs rejected 15 DL-A-incompatible skin grafts in 12–26 days (MST = 16.3 days); the second group

TABLE IV

Responses to Donor-Specific Kidney Allografts in DL-A-Identical Bone Marrow Chimeras

Experimental group	Relation between donor and recipient	No. of dogs	Kidney allograft survival times (No. of transplants per days listed)									Mean survival time
			10–15	16–30	31–45	46–100	101–190	191–250	251–400	401–500	501–600	
												days
Bone marrow* chimeras	LM*	11	—	—	—	—	—	1 (> 234)	3 (> 313) (> 377) (> 378)	3 (> 441) (> 444) (> 482)	4 (> 557) (> 580) (> 581) (> 586)	*
	Non-LM	6	—	—	—	—	—	3 (> 191) (> 200) (> 221)	2 (> 349) (> 361)	1 (> 405)	—	*
Untreated DL-A compatible	LM	13	1	5	7	—	—	—	—	—	—	28.6
	Non-LM	9	—	6	3	—	—	—	—	—	—	28.3
Untreated DL-A incompatible	LM	8	6	2	—	—	—	—	—	—	—	14.8
	Non-LM	19	17	2	—	—	—	—	—	—	—	12.4

* All renal allografts are surviving with normal function as of the last date of follow-up, 15 May 1972.
LM = littermates.

of six dogs rejected 11 DL-A-incompatible skin grafts in 10.5–18 days (MST = 14.5 days).

As noted in Table VI, skin allografts performed in untreated genotypically DL-A-identical pairs of littermates survived for 20–29 days (MST = 25.5 days). This survival was not significantly different from that of DL-A-identical skin allografts in littermate bone marrow chimeras, 22–36 days (MST = 25.8 days) (Table V). It is of interest, however, that reciprocal exchange of skin allografts between DL-A-identical pairs of normal littermates did not regularly result in the same survival times in both animals. In a number of instances, the differences in survival of two such grafts were as great as 5 days.

DISCUSSION

The results of this study indicate that the use of combined genetic and serological criteria of DL-A compatibility in the prospective selection of DL-A-

TABLE V

Reactivity to Skin Allografts in DL-A-Identical Bone Marrow Chimeras Bearing Donor-Specific Kidney Allografts

Bone marrow and kidney donor	Recipient	DL-A genotype	Relationship	Survival time of skin allografts (days) obtained from					
						Donors with known DL-A incompatibilities			
				Bone marrow donor	DL-A-identical sibling of donor	Donor 1	Incompatible antigen(s)	Donor 2	Incompatible antigen(s)
21-53	21-54	bkhfm/gln	Littermates	—	—	—	—	—	—
21-59	21-60	bkhfm/bkhfm	"	84	20	12.5	gln	12.5	cdn
21-62	21-61	bkhfm/bkhfm	"	>178*	36	19	gln	19	cdn
21-65	21-66	gln/be	"	>162	—	—	—	—	—
21-74	21-73	bkhfm/bkhfm	"	>116	—	26	gln	—	—
21-95	21-96	gln/gln	"	>122	32.5	16.5	gln	14	cdn
22-03	22-04	bkhfm/bkhfm	"	—	22.5	18	gl	—	—
22-32	22-29	gln/gln	"	>199	—	12	bkhfm	13	cdn
22-21	22-22	bkcdn/bkhfm	"	—	—	12	bkcdn	21	bkhfm
22-43	22-40	bkhfm/gln	"	>116	22	13	o	—	—
22-55	22-48	bkhfm/bkhfm	"	>110	22	20	gln	16.5	cdn
21-87	21-56	bkhfm/bkhfm	Nonlittermates	>143	76	16	gln	14	cdn
21-90	21-97	bkhfm/bkhfm	"	>143	27	13.5	gln	16	cdn
22-06	22-24	gln/gln	"	>128	—	12	bkhfm	11	bkcdn
22-52	22-56	bkhfm/bkhfm	"	>110	34	18	gln	16	cdn
22-51	22-57	bkhfm/bkhfm	"	>110	72	18	gln	15	cdn
22-46	22-07	gln/gln	"	>107	72	10.5	bkhfm	—	—

* > = allografts continue to survive uneventfully as of the last date of follow-up (15 May 1972).

TABLE VI

Reactivity to Skin Allografts in Untreated DL-A-Identical Littermate Pairs of Beagles

Donor	Recipient	DL-A genotype	Skin allograft survival
			days
14-57	14-58	gln/bkhfm	26
14-58	14-57		26
16-68	16-69	bkcdn/gln	28
16-69	16-68		26
15-82	15-80	bkhfm/bkhfm	25
15-80	15-82		20
16-91	16-90	gln/gln	29
16-90	16-91		25
16-89	16-88	gln/gln	27
16-88	16-89		22
16-76	16-78	gln/gln	26
16-78	16-76		24
16-79	16-77	be/gln	26.5
16-77	16-79		26

identical donor-recipient pairs may regularly produce long-term survivals of bone marrow chimeras. A significant array of evidence supports the contention that this effect is specific in nature and is not a reflection of random inbreeding within the Cooperstown Colony. The coefficients of relationship of the bone marrow donors and recipients and of their preceding generation, and the establishment of long-term chimerism with equal ease in littermate and in nonlittermate recipients would appear to militate against such a possibility. In addition, it may be pertinent to note the following: (*a*) The Cooperstown Colony does not constitute a randomly inbred population; rather it includes a number of selectively bred lines (46), some of which bear the same DL-A genotypes, while others may be phenotypically DL-A compatible but genotypically DL-A different (46); (*b*) studies in progress at this time indicate that DL-A-incompatible bone marrow transplants regularly fail to survive, even when performed in littermates; (*c*) genotypically DL-A-identical allografts of other tissues transplanted into untreated Cooperstown Colony recipients are accorded relative prolongations in survival time, but invariably undergo rejection, in littermates as well as in nonlittermates (46); (*d*) DL-A-incompatible allografts are rejected in an even more rapid manner by both littermates and nonlittermates (46). The relevance of the DL-A marker system to the results observed is highlighted, in addition, by the heterogeneity of the animals studied for other markers present on other chromosomes, such as the Swisher erythrocyte groups, for example.

The long-term survival of 17 consecutive DL-A-identical bone marrow allografts presented in this study differs significantly from earlier studies by Epstein et al. (49), who reported a survival rate of less than 50% at 120 days and less than 30% at 240 days in 17 recipients of littermate marrow allografts selected on the basis of serological criteria of DL-A compatibility (49). These results may reflect the different canine population and serological reagents utilized by Epstein et al. (49) and their use of phenotypic rather than genotypic criteria of compatibility. It must be noted, however, that the data of Epstein et al. (49), as well as the incidence of GVH reactions observed in human recipients of HL-A-identical bone marrow transplants (58, 59), may have been a consequence of donor-recipient incompatibilities for H-antigens which are not as yet detectable by currently available techniques. In this regard, the regularly reproducible bone marrow allograft survivals obtained in this study on the basis of combined genetic and serological criteria of DL-A compatibility in selectively bred lines of beagles may provide an experimental model for the further investigation of such H-antigens and of their role in triggering GVH disease. Rather than localizing such determinants on chromosomes other than those bearing the DL-A region, however, the results of this study raise the alternative possibility that these determinants are genetically linked, i.e., they occur on one major autosomal complex, for which the DL-A genotype serves as an effective marker in the Cooperstown colony. Recent reports (60–64)

indicate that the fate of tissue and of bone marrow allografts in man and in the mouse may depend upon the products of at least two and possibly more closely linked genetic systems. Such systems include the serologically detectable HL-A and H2 systems, as well as products detectable by the mixed leukocyte culture test (MLC), and a possible cellular immunity locus (61).

The observation that bone marrow transplantation in DL-A-compatible, MLC-negative littermate dogs (65) and in HL-A-identical, MLC-negative recipients of human sibling marrow (66) are associated with significant failure rates, raises the possibility that, in addition to the two postulated DL-A loci, and to the MLC locus, the proposed main canine histocompatibility complex may include one or more additional closely linked but genetically separable determinants, one of which might be termed a "bone marrow transplantation" locus. Cudkowicz's (67) recent findings of hybrid resistance genes for bone marrow transplantation within the analogous histocompatibility complex in mice would appear to support this possibility. The success rate of bone marrow transplants observed in the Cooperstown colony may, in this regard, have been related to the criteria employed for the definition of DL-A genotypic identity between each littermate or nonlittermate donor-recipient pair selected for transplantation. Such identity was based upon evidence of the transmission of the same DL-A genetic material from the same ancestral source (46) for at least three or four successive generations. The resulting pairs of dogs differed for other genetic markers, located on other chromosomes. It is possible, however, that the criteria of selection of donors and recipients for this study identified, within the Cooperstown colony, those animals sharing components of the major histocompatibility complex other than DL-A and/or MLC determinants.

The unresponsiveness of the radiation chimeras to kidney and skin allografts obtained from the bone marrow donors is consonant with the hypothesis of Main and Prehn (68) that bone marrow transplantation may induce a state of tolerance to other tissues obtained from the same donor. The immunological specificity of the induced unresponsiveness is supported by the ability of the chimeras to reject skin allografts from both DL-A-compatible and DL-A-incompatible donors. The capacity of the chimeras to muster other forms of immune responses is attested by their production of normal serum antibody levels against DIH vaccine within 3–6 months after bone marrow transplantation. This observation is in agreement with the recent studies of Ochs et al. (69), who have provided extensive evidence of normal cellular and humoral immunological function in long-term radiation chimeras.

13 of 14 chimera recipients of skin allografts obtained from the bone marrow donors have thus far tolerated such transplants as if they were autografts. One recipient (recipient 21-60) has rejected such an allograft in slow fashion, with complete destruction of the transplant by the 84th postoperative day. This chronic response resembles the long-term behavior of skin allografts recently described in chimeric cattle twins by Stone et al. (70). The fate of the remaining

marrow donor-specific skin allografts is consistent, however, with the thesis that the chimeras are generally rendered tolerant to skin as well as to kidney allografts from the bone marrow donors. This conclusion evidently requires confirmation by a longer period of follow-up of these skin grafts. In this regard, it may be pertinent to note that opinions concerning the ability of bone marrow transplants to induce tolerance to skin are somewhat divided. Some authors suggest that bone marrow transplantation only induces an incomplete or split form of tolerance to other tissues (71–73), and Lance (74) has recently described skin-specific differentiation alloantigens in mice. On the other hand, Main and Prehn (68) and Thomas and Ferrebee (25) have described long-term survivals of skin allografts in radiation chimeras, and Wood et al. (75) have recently observed tolerance to skin allografts in mice treated with antilymphocyte serum and bone marrow cells.

The rejection of skin allografts obtained from other DL-A-identical donors further supports the individual-specific nature of the state of allograft unresponsiveness induced in the recipients. It also highlights the immunological consequences of host reactivity to non-DL-A or "weak" histocompatibility antigens (76–78) present in the skin graft donors but absent in the recipients. This result, taken together with the differences in survival of skin allografts exchanged reciprocally between untreated pairs of DL-A-identical littermates (Table VI), supports the conclusion of Graff et al. (79) that, in the absence of strong donor-recipient incompatibilities, otherwise weak immunogens may exert a cumulative effect upon allograft survival. The results also suggest that the bone marrow donors and recipients reported in this study, while genotypically DL-A identical, may differ by a varying, albeit probably limited number of other H determinants. The long-term survival of bone marrow transplants in the absence of GVH disease in such recipients suggests that relative degrees of accommodation to such non-DL-A histocompatibility barriers may have been developed by the immunologically competent cells transplanted into irradiated recipients. This possibility may provide an explanation for the partial unresponsiveness accorded by radiation chimeras to skin allografts obtained from DL-A-identical littermate siblings of the marrow donors, when such allografts were applied to recipients of bone marrow cells obtained from nonlittermate DL-A-identical donors. As noted in Table V, these grafts survived for 27–72 days (MST = 56.2 days), while similar grafts performed in recipients of littermate bone marrow transplants were rejected within 20–36 days (MST = 25.8 days). Such a difference in survival raises the possibility that the individual-specific nature of the unresponsiveness to skin allografts produced in radiation chimeras may have been broadened in nonlittermate marrow recipients to a partial tolerance to skin allografts obtained from DL-A-identical silbings of the bone marrow donors. Such an effect may have been due to the possibility that the bone marrow cells transplanted into nonlittermate recipients were compelled to adapt to a wider spectrum of non-DL-A H-determinants than

was the case in littermate marrow transplants. This possibility is supported by the relationships reported between the strength of histocompatibility barriers and the induction of immunological tolerance in mice (80), and is consistent with the recent observations of Hussey et al. (81) of the occasional success of bone marrow and renal allografts in mongrel dog radiation chimeras.

The precise nature of the mechanisms implicated in the type of allograft unresponsiveness described in this study is not clear at present. It is of interest, however, that blocking antibodies of the Hellström type (82) have recently been described in long-term canine bone marrow chimeras (83), and that such antibodies have also been reported in long-term recipients of human renal allografts (84). These observations may be of direct relevance to further studies of mechanisms of allogeneic unresponsiveness in radiation chimeras.

SUMMARY

17 Cooperstown beagles of known DL-A genotypes were exposed to supralethal total body irradiation and received a bone marrow allograft from a DL-A-identical donor; 11 littermate and 6 nonlittermate donor-recipient pairs were studied. The recipients are surviving uneventfully for 315, 364, 424, 440, 531, 531, 584, 605, 625, 635, and 649 days in the littermate group and for 211, 279, 280, 368, 479, and 480 days in the nonlittermate group.

The radiation chimeras underwent bilateral nephrectomy and received a kidney allograft obtained from their respective marrow donor within 43–120 days after bone marrow transplantation. The renal allografts are surviving for 191, 200, 221, 234, 313, 349, 361, 377, 378, 405, 441, 444, 482, 557, 580, 581, and 586 days, respectively.

12 of 13 skin allografts obtained from the marrow donor are at present surviving in the recipients for 107, 110, 110, 110, 116, 122, 128, 143, 143, 162, 178, and 199 days, respectively; one graft was rejected at 84 days. In contrast, the radiation chimeras rejected 25 skin allografts obtained from DL-A-incompatible donors within 10.5–21 days (MST = 15.2 days). Skin transplants obtained from DL-A-identical siblings of the bone marrow donors were rejected within 20–36 days (MST = 25.8 days) in recipients of bone marrow cells obtained from littermate donors. Recipients of nonlittermate bone marrow transplants accorded such allografts a prolonged survival time of 27–76 days (MST = 56.2 days).

Prospective selection of genotypically DL-A-identical donor-recipient pairs results in the regularly reproducible long-term survival of bone marrow allografts. The radiation chimeras produced in this manner have developed a donor-specific state of unresponsiveness to kidney and skin allografts. The results are consistent with the existence in the canine species of at least three closely linked genetic systems relevant to transplantation, including DL-A, MLC, and a possible bone marrow transplantation locus.

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