

HISTOCOMPATIBILITY STUDIES IN A CLOSELY BRED COLONY OF DOGS

V. Mechanisms of Cellular Adaptation in Long-Term DL-A Identical Radiation Chimeras*

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Significant advances have been made in the definition of the serologically detectable (SD)¹ and lymphocyte surface-reactive (LD) products of the main histocompatibility complex in a variety of mammalian species, including the dog (1-18) and man (19-33). Such progress has not, however, produced dramatic improvements in the results of bone marrow transplantation. Reports from Thomas' group (34-36) have indicated that attempts to reconstitute supralethally irradiated dogs with DL-A identical bone marrow frequently result in lethal graft-vs.-host (GVH) disease in the recipients. Clinical application of bone marrow transplantation has been hampered similarly by GVH reactions of unpredictable severity and/or mortality in patients treated with marrow obtained from an HL-A SD and LD identical sibling donor (37-40).

Different results have been achieved, however, in the colony of selectively bred beagles maintained at The Mary Imogene Bassett Hospital in Cooperstown, N. Y. Transplantation of bone marrow from prospective genotypically DL-A identical Cooperstown donors into supralethally irradiated littermate and nonlittermate recipients has regularly resulted in the establishment of a long-term state of chimerism, with no evidence of GVH disease in the recipients (41-46). Such chimeras have also been rendered specifically tolerant to skin (43), kidney (41), heart (A. D. Boyd, personal communication), lung (47), liver (49), and pancreatic (49) allografts obtained from the bone marrow donor. Evidence has been presented, however, that donor-recipient pairs selected on the basis of

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¹Abbreviations used in this paper: GVH, graft-vs.-host, LD, lymphocyte surface reactive; SD, serologically detectable.

DL-A identity are genetically heterogeneous for non-DL-A histocompatibility determinants (4, 6, 16, 43, 50-53) and a number of other markers (43-45).

The availability of a population of stable long-term chimeras and of their respective bone marrow donors has provided an opportunity to investigate the mechanism(s) whereby bone marrow cells transplanted into a host bearing non-DL-A incompatibilities become engrafted and proliferate. The long-term adaptation of such allogeneic cells to their new incompatible milieu is also of relevance to the question of whether somatic mutations may have occurred in the transplanted cells (44). The studies presented in this report support the conclusion that the inhibition of responses of transplanted bone marrow cells and their progeny to the non-DL-A alloantigens present in the irradiated host is a consequence of a central state of cellular unresponsiveness, and is not due to humoral factors. Reversal of tolerance has been achieved by irradiation of the original marrow donor, followed by return to this donor of marrow obtained from its corresponding chimera. Upon recovery, the reconstituted dogs had the capacity to reject skin allografts obtained from their chimeral partner. Repeated exposure to skin obtained from the original chimera has resulted in accelerated skin graft rejection responses in the reconstituted dogs. It has also conferred on the immunologically competent cells of such dogs the capacity to induce lethal secondary disease in their respective chimeras. The evidence also indicates that bone marrow cells and their progeny can retain their germ-line characteristics for as long as 4.5 yr after transplantation into a (non-DL-A) histoincompatible environment.

Materials and Methods

Selection of Donors and Recipients. 14 of a series of 20 adult male and female bone marrow transplant donor-recipient pairs of beagles of the Cooperstown Colony (Tables I, II) weighing 17-27 lbs were used. Each pair of dogs had been selected for bone marrow transplantation on the basis of genotypic evidence of DL-A identity (20, 43), confirmed by lymphocytotoxicity tests performed with DL-A antisera capable of detecting 12 SD specificities, as described previously (43). Identity for LD products of the DL-A complex was tested in standard fashion by the mixed leukocyte culture technique (12). Erythrocyte group antigens A, C, and D were determined by the techniques of Swisher and Young (55). In referring to each pair of dogs presented in this study, the original donor of marrow will be termed "A" and the original recipient chimera, "B".

Method of Irradiation and Bone Marrow Transplantation. The method of supralethal total body irradiation from two opposing⁶⁰Co sources, yielding 1,200-1,400 R used in this study has been described in detail previously (43). Bone marrow transplantation was performed by the intravenous infusion into the recipient of a suspension of $3.0-3.5 \times 10^6$ nucleated bone marrow cells obtained by needle aspiration of the long bones and sternum of the donor (43). This was given within 12 to 18 h after irradiation. Leukocyte and platelet levels were determined in the recipients three times weekly for the first 21 days and at weekly intervals thereafter. The return of leukocyte and platelet levels to normal values after transplantation and absence of detectable GVH disease provided evidence of successful take and proliferation of the bone marrow transplant (43). Persistence of chimerism was periodically confirmed in informative pairs by testing for the continuing presence of donor erythrocyte antigens on the recipient's erythrocytes and of sex characteristics of donor cells in the recipient's peripheral leukocytes (43, 56).

Method of Skin Grafting and Criteria for the Diagnosis of Allograft Rejection. The method of skin grafting has been described earlier (43). Briefly, it consists of the transplantation of full-thickness skin specimens measuring 2×2 cm, using the intact panniculus carnosus of the recipient as the graft bed. The grafts were examined daily after the 7th postoperative day; standard

criteria (43) were used for the diagnosis of allograft rejection; the latter was confirmed by subsequent sloughing of the eschar. All grafts were performed in duplicate.

Basic Experimental Sequence

ATTEMPT TO PROLONG SKIN ALLOGRAFT SURVIVAL WITH CHIMERAL SERUM. Beginning within 6–8 m after the establishment of chimerism, 10-ml serum samples were drawn weekly from eight “B” dogs and from an untreated DL-A identical littermate of an “A” dog. The sera from each dog were pooled and stored at -70°C before use. Each of the eight corresponding “A” dogs was given skin grafts from its “B” partner, and received, 5, 10, or 20 ml of serum from its chimera intravenously on the day of skin transplantation and daily thereafter, until rejection of the grafts. One dog received 5 ml of normal serum, following a similar schedule, and was grafted in the same manner. The results were compared with those obtained with grafts placed on untreated dogs under the same conditions of DL-A identity (Table III).

IRRADIATION AND RECONSTITUTION OF THE ORIGINAL DONORS OF MARROW WITH BONE MARROW OBTAINED FROM THEIR CORRESPONDING CHIMERAS. Within 14–21 days after rejection of the “B” skin grafts, the nine sensitized “A” dogs and 5 additional “A” dogs were irradiated and reconstituted with a transplant of marrow obtained from their “B” partner (Table IV). After recovery, all 14 “A” dogs were given skin grafts from the corresponding “B” animal (Table V). The first nine “A” dogs were grafted within 36–295 days after reconstitution with “B” marrow (Table V). Each of these dogs had rejected “B” skin grafts before being subjected to irradiation and transplantation with “B” marrow. The other five “A” animals, which had not been sensitized earlier, received their first “B” skin grafts within 171–206 days after reconstitution with “B” marrow (Table V).

TEST OF THE ABILITY OF THE RECONSTITUTED ORIGINAL DONORS OF MARROW TO REJECT SUCCESSIVE SKIN GRAFTS FROM THEIR CHIMERAS. “B” skin grafts were given in consecutive fashion to the corresponding “A” partner, within 2–3 wk after rejection of each preceding graft, until each “A” dog received four sets of “B” grafts. One “A” dog (no. 21–74) did not reject its first-set “B” graft; this animal received a second-set graft at 122 days after the first graft, a third-set at 68 days after the second, and a fourth-set at 37 days after the third-set graft (Tables V and VI).

REIRRADIATION OF THE ORIGINAL CHIMERAS AND PRETREATMENT WITH MARROW AND BLOOD CELLS FROM THE ORIGINAL DONOR SENSITIZED BY FOUR SUCCESSIVE SETS OF CHIMERA SKIN GRAFTS. The final series of experiments were aimed at an assessment of the ability of bone marrow and/or blood leukocytes obtained from “A” dogs sensitized with four sets of “B” skin grafts to produce GVH disease in their corresponding “B” partner. For this purpose, each of the “B” dogs was irradiated and received bone marrow from its “A” partner; this was given within 2–8 days after that particular “A” dog had rejected its fourth-set “B” graft. Five “B” dogs were treated with $1.2\text{--}2.0 \times 10^9$ nucleated “A” bone marrow cells and 2–12 transfusions of 125-ml aliquots of whole “A” blood which had been irradiated by exposure to 1,000 R for 18–20 min, using a standard ^{60}Co source. Another three “B” dogs were given $2.1\text{--}3.0 \times 10^9$ “A” marrow cells and three to six transfusions of unirradiated “A” blood (Table VII). Five “B” dogs received $1.01\text{--}2.8 \times 10^9$ “A” bone marrow cells and 1.29 to 8.36×10^9 “A” leukocytes suspended in donor plasma, given intravenously at the time of marrow transplantation, and at 5- to 8-day intervals thereafter (Table VIII). These dogs were also treated with two to six transfusions of 125-ml aliquots of irradiated whole blood obtained from a normal DL-A identical donor. The doses of bone marrow cells and the degree of contamination of such cells with peripheral blood leukocytes were roughly comparable in the groups of dogs treated with irradiated whole blood, unirradiated blood, or blood leukocytes.

Results

Background Data. Table I presents the long-term follow-up of the series of bone marrow chimeras maintained at the Cooperstown Colony for the purposes of this study. It consists of 11 littermate and 9 nonlittermate pairs of supraethally irradiated and reconstituted animals (“B”) and their donors (“A”), with a survival of 882–1,466 days in the 20 consecutive recipients of marrow (“B”). Persistence of chimerism was confirmed by determination of the presence of

TABLE I
Results of Bone Marrow Transplantation in Prospectively Selected Genotypically DL-A Identical Beagles*

Donor	Recip- ient	DL-A genotype	Relation	Survival of bone marrow transplant	Swisher erythrocyte groups of:				Sex markers of:	
					Donor	Recipient after trans- plantation	Donor	Recipient after trans- plantation	Recipient after trans- plantation	Recipient after trans- plantation
				<i>days</i>						
21-59	21-60	bkhfm/bkhfm	Littermates	> 1,466	C	A	C	M	M	M
21-62	21-61	bkhfm/bkhfm	"	> 1,446	AC	C	AC	F	M	F
21-65	21-66	gln/be	"	> 1,426	C	AC	C	M	M	M
21-74	21-73	bkhfm/bkhfm	"	> 1,405	C	A	C	M	M	M
22-21	22-22	gln/gln	"	> 1,352	C	C	C	M	M	M
21-95	21-96	bkhfm/bkhfm	"	> 1,352	A	AC	A	M	F	M
22-32	22-29	gln/gln	"	> 1,261	C	C	C	F	M	F
22-03	22-04	bcdn/bkhfm	"	> 1,255	AC	A	AC	F	F	F
22-43	22-40	bkhfm/gln	"	> 1,185	AC	AC	AC	F	M	F
22-55	22-48	bkhfm/bkhfm	"	> 1,136	AC	AC	AC	F	M	F
22-95	22-94	bkhfm/gln	"	> 965	AC	AC	AC	M	M	M
21-87	21-56	bkhfm/bkhfm	Nonlittermates	> 1,301	AC	A	AC	F	M	F
21-90	21-97	bkhfm/bkhfm	"	> 1,300	AC	A	AC	F	F	F
22-06	22-24	gln/gln	"	> 1,239	C	C	C	M	M	M
22-51	22-57	bkhfm/bkhfm	"	> 1,101	AC	AC	AC	M	F	M
22-52	22-56	bkhfm/bkhfm	"	> 1,100	AC	A	AC	F	M	F
22-46	22-07	gln = gln	"	> 1,032	AC	AC	AC	F	M	F
22-13	22-73	gln/gln	"	> 963	AC	C	AC	M	M	M
23-09	21-94	bkhfm/bkhfm	"	> 893	AC	A	AC	M	M	M
23-03	23-08	bkhfm/bkhfm	"	> 882	C	AC	C	M	M	M

* 31 July 1973.

donor Swisher erythrocyte antigens and leukocyte sex characteristics in the blood of the recipient. Table II illustrates the survival of each chimera, with normal renal function, at 833–1,402 days after bilateral nephrectomy and transplantation of a kidney from the donor of marrow. Skin grafts from the same donor continued to survive at 928–1,020 days (one graft was rejected at 84 days). The “A” skin or kidney grafts to “B” dogs were not affected by any of the subsequent procedures.

TABLE II
*Responses to Donor-Specific Kidney and Skin Allografts in DL-A Identical Bone Marrow Chimeras**

Donor	Recipient	Relation	Survival time of:	
			Renal allograft from donor of marrow	Skin allograft from donor of marrow‡
			<i>days</i>	<i>days</i>
21-59	21-60	Littermates	>1,402	84
21-62	21-61	“	>1,401	>999
21-65	21-66	“	>1,378	>983
21-74	21-73	“	>1,303	—
22-21	22-22	“	>1,275	—
21-95	21-96	“	>1,262	>943
22-32	22-29	“	>1,199	>1,020
22-03	22-04	“	>1,198	—
22-43	22-40	“	>1,134	>937
22-55	22-48	“	>1,055	>932
22-95	22-94	“	>885	—
		Nonlittermates		
21-87	21-56	“	>1,226	>964
21-90	21-97	“	>1,182	>964
22-06	22-24	“	>1,170	>949
22-51	22-57	“	>1,042	>931
22-52	22-56	“	>1,021	>931
22-46	22-07	“	>1,012	>931
22-13	22-73	“	>918	>928
23-09	21-94	“	>833	—
23-03	23-08	“	>839	—

* 31 July 1973.

‡ DL-A-incompatible skin grafts were rejected by the same animals within 10.5–26 days (43).

Effect of Chimeral Serum Upon Skin Allograft Survival. As noted in Table III, “A” dogs treated with serum obtained from their long-term bone marrow chimera partners (“B”) rejected “B” skin grafts at 26, 39, 13, 75, 27, 19, 22, and 26 days, respectively. There was no obvious relationship between the duration of graft survival and the total volume of serum administered. One “A” dog given normal serum rejected its “B” graft at 24 days. The survival time accorded to skin allografts in normal, untreated DL-A identical Cooperstown dogs was 22–29 days (43).

TABLE III
Reactivity to Skin Allografts Obtained from Long-Term Bone Marrow Chimeras in the Corresponding Donors of Marrow Treated with Chimeral Serum

Bone marrow donor	Recipient	Relationship	Daily dose of chimera serum	Treatment of donor of marrow	Total volume of serum given	Survival of skin grafts from chimera placed on the donor of marrow (days)*
			<i>ml</i>	<i>days</i>	<i>ml</i>	<i>days</i>
21-62	21-61	LM	5	24	120	24‡
22-51	22-57	Non-LM	5	26	130	26
21-66	21-65	LM	10	38	380	39
22-06	22-24	Non-LM	10	13	130	13
21-95	21-96	LM	10	75	750	75
22-43	22-40	LM	10	27	270	27
22-32	22-29	LM	10	19	190	19
21-90	21-97	Non-LM	20	22	440	22
22-46	22-07	Non-LM	20	26	520	26

* Survival of skin allografts in untreated DL-A identical beagles, 22-29 days (mean survival time, 25.5 days) (43).

‡ Recipient given normal serum from a DL-A identical littermate donor.

Irradiation and Reconstitution of Normal and Preimmunized Original Donors of Marrow ("A") with Bone Marrow from the Corresponding Chimeras ("B"). Table IV compares the results of reconstitution of irradiated "A" dogs with bone marrow from their corresponding "B" chimera, with and without prior sensitization of "A" with "B" skin allografts. When "A" dogs sensitized with "B" skin were irradiated and given "B" marrow, uniformly successful engraftment occurred, and the "A" dogs are surviving with no evidence of secondary disease for 727, 755, 756, 713, 538, 759, 719, and 712 days, respectively. "A" dogs not grafted with "B" skin before marrow transplantation from "B" are surviving in similar fashion for 750, 715, 708, 663, and 633 days, respectively

Response of Reconstituted "A" Dogs to Successive "B" Skin Grafts. As summarized in Table V, first-set "B" skin allografts were rejected by unirradiated "A" dogs within 13-39 days; one graft survived for 75 days. First-set "B" skin grafts placed on the five unsensitized "A" dogs at 196, 171, 171, 159, and 206 days, respectively, after irradiation and reconstitution with "B" marrow cells (dogs 21-74, 22-55, 22-95, 22-52, and 23-03) were rejected at 88, 24, 62, and 34 days, respectively; one graft (dog 21-74) continues to survive at 654 days (Table V). Challenge of the first nine "A" dogs (dogs 21-62, 21-66, 21-95, 22-43, 22-32, 22-06, 22-51, 21-90, and 22-46) with second-set skin grafts from the corresponding "B" partner within 168, 133, 105, 274, 45, 36, 295, 34, and 59 days, respectively, after irradiation and reconstitution with "B" marrow, resulted in skin graft survivals of 16, 14, 23, 22, 21, 12, 20, 24, and 14 days, respectively (range, 12-24 days). Subsequent "B" skin grafts placed upon the corresponding "A" dog after rejection of the immediately preceding transplant were accorded progressively decreasing survival times, as is illustrated in Table VI. One exception was "A" dog 21-74, which remained unresponsive to all skin grafts from its "B" partner. The latter transplants are surviving for 654, 532, 465, and 428 days, respectively.

Reirradiation of "B" Dogs and Reconstitution with Cells from the "A" Partner

TABLE IV
Results of Irradiation and Reconstitution of Normal and of Preimmunized Original Donors of Marrow (Dogs A) with Bone Marrow Obtained from Their Corresponding Long-Term DL-A Identical Chimeras (Dogs B)

Dog A	Dog B	Donor-recipient relationship	Duration of chimerism in B before present experiment	Immunological status of dog A before present experiment	Results of survival of repopulation of dog A with bone marrow obtained from its chimera (B)*
			<i>days</i>		<i>days</i>
21-62	21-61	LM	>1,446	+‡	>727
21-65	21-66	LM	>1,426	+	>755
21-95	21-96	LM	>1,352	+	>756
22-43	22-40	LM	>1,185	+	>713
22-32	22-29	LM	>1,261	+	>538
22-06	22-24	Non-LM	>1,239	+	>538
22-51	22-57	Non-LM	>1,101	+	>759
21-90	21-97	Non-LM	>1,300	+	>719
22-46	22-07	Non-LM	>1,032	+	>712
21-74	21-73	LM	>1,405	-	>750
22-55	22-48	LM	>1,136	-	>715
22-95	22-94	LM	>965	-	>708
22-52	22-56	Non-LM	>1,400	-	>663
23-03	23-08	Non-LM	>882	-	>663

* All reconstituted A dogs are surviving uneventfully, with no evidence of secondary disease, as of 1 December 1974.

‡ +, immunized against dog B tissues by skin grafting (all grafts rejected; mean survival time, 34.8 days).

§ -, No pretreatment with B tissues.

Sensitized by "B" Skin Grafts. Within 2-8 days after rejection of the fourth-set skin graft, (the interval was 20 days after fourth-set grafting in dog 21-74, which failed to reject "B" skin grafts), the "B" dogs were irradiated and received marrow from their corresponding "A" partner, in which evidence of hypersensitization to "B" skin grafts had been obtained in all but one instance. As illustrated in Table VII, four of five "B" dogs reconstituted with "A" marrow and given transfusions of irradiated "A" blood survive uneventfully for 336, 484, and 482 days, respectively; one animal succumbed of secondary disease within 76 days after marrow transplantation. In contrast, three consecutive "B" dogs treated with "A" marrow, but given *unirradiated* blood transfusions from the sensitized "A" donor, had an initial engraftment of the transplanted marrow, but died of severe secondary disease within 28, 44, and 49 days.

A third group of five "B" dogs was irradiated and reconstituted with "A" marrow and received intravenous injections of peripheral blood leukocytes isolated from the same sensitized "A" donor of marrow. As outlined in Table VIII, two of the recipients (dogs 21-73 and 22-48) showed no evidence of engraftment and succumbed at 12 and 15 days, respectively; two other animals

TABLE V
Response of Irradiated and Reconstituted A Dogs to Successive Skin Allografts Obtained from their Corresponding B Partners

Dog A	Dog B	Survival of B skin grafts before and after irradiation and reconstitution with marrow from B		Survival of successive B skin grafts applied to A dogs at 2-3 wk intervals after rejection of the preceding graft from the same donor (listed as graft numbers postirradiation and reconstitution of A dogs)		
		Before	After	Graft no. 2	Graft no. 3	Graft no. 4
		<i>days</i>	<i>days</i>	<i>days</i>	<i>days</i>	<i>days</i>
21-62	21-61	24	16	14	13	—
21-66	21-65	39	14	10.5	12	—
21-95	21-96	75	23	25	12	—
22-43	22-40	27	22	19	16	—
22-32	22-29	19	21	13	13	—
22-06	22-24	13	12	11	11.5	—
22-51	22-57	26	20	18	17	—
21-90	21-97	22	24	13	10	—
22-46	22-07	26	14	13	14	—
21-74	21-73	—	>654*	>532	>465	>428
22-55	22-48	—	88	20	19	14
22-95	22-94	—	24	12.5	13	10
22-52	22-56	—	62	100	33	23
23-03	23-08	—	34	31	23	17

* As of 1 December 1974.

TABLE VI
Sensitization of A Dogs to Specific Non-DL-A Antigens Through the Transplantation of Successive Skin Allografts from the Same B Partners

Type of graft	No. of A dogs	No. of dogs rejecting skin allografts on postoperative day:										MST*	
		0-10	10-15	16-20	21-25	26-30	31-35	36-40	41-60	61-80	81-90		>90
1st-set grafts	14		1	1	3	3	1	1		2	1	1	<i>days</i> 36.8
2nd-set grafts	"		4	3	4		1					2	25.3
3rd-set grafts	"		7	3	2		1					1	17.2
4th-set grafts	"	2	7	3	1							1	14.0

* MST, mean survival time.

had an early graft take, followed by severe secondary disease and death at 16 and 28 days, respectively; one dog is surviving at 343 days. The "B" recipient (no. 21-73) of marrow and leukocytes from "A" dog 21-74, which had not rejected its "B" grafts, succumbed after failure of engraftment.

Discussion

Progress in organ transplantation continues to await the perfection of currently available techniques to produce allograft tolerance without interference with the

TABLE VII

Reconstitution of Irradiated B Dogs with Bone Marrow from their Original A Partner, After Sensitization with B Skin Allografts; Effect of Blood Transfusion from the Donor of Marrow

Dog A	Dog B	Time between rejection of last B graft by A and transplantation of A marrow into B	Dose of A marrow	Blood transfusions from donor of marrow:			Survival of B recipient*	
				Treatment of transfused blood	Total volume of transfusions	Timing of transfusions after marrow transplantation		
		<i>days</i>			<i>ml</i>	<i>days</i>	<i>days</i>	
21-62	21-61	2	2.0×10^9	Irradiated	250	12,23	2	>482
21-65	21-66	8	1.6×10^9	"	875	9,11,16,21,25,32,38	7	>484
22-06	22-24	8	1.2×10^9	"	375	10,14,16	3	>484
22-51	22-57	6	1.8×10^9	"	625	9,14,19,23,26	5	>336
21-95	21-96	8	1.5×10^9	"	1,500	12,15,19,23,28,35, 44,54,56,58,61,75	12	76
22-32	22-29	8	2.1×10^9	Unirradiated	500	11,13,18,25		44
22-46	22-07	6	2.5×10^9	"	750	10,13,15,20,24,31		49
21-90	21-97	8	3.0×10^9	"	375	4,10,21		28

* 1 December 1974.

remainder of the host's defense mechanisms. The feasibility of this approach was originally demonstrated by the induction of immunological tolerance by Billingham et al. (57). The distinctions between the latter type of classical immunological tolerance (i.e., central) and a variety of other modalities, including immunological enhancement (58-60) and the facilitation of allograft survival by pretreatment of adult recipients with histocompatibility antigens(s) (61-63), have become increasingly tenuous in recent years (64).

In further studies of this question in the canine species, Ferrebee and associates in Cooperstown, New York (65-68), produced chimerism in randomly selected dogs after supralethal total body irradiation and bone marrow transplantation, and demonstrated that the recipients which occasionally survived treatment tolerated allografts obtained from the donor of marrow (67). Methods were not available at that time, however, for the prospective selection of donors and recipients, so as to permit the creation of long-term bone marrow chimeras in predictable fashion. Recent advances in the genetic definition of the main histocompatibility complex in the dog (DL-A) have provided an opportunity to achieve this goal through the establishment of selectively bred lines of beagles of known DL-A genotype in the Cooperstown Colony (6, 7, 16, 44-46). Extension of the approach of Thomas and Ferrebee (67) to genotypically DL-A identical littermate and nonlittermate Cooperstown Colony dogs has resulted in the regular long-term survival of irradiated bone marrow chimeras, with evidence of persisting chimerism (56) and no detectable GVH disease (43). Evidence of the genetic heterogeneity of these pairs of donors ("A" dogs) and recipients ("B" dogs) has been secured by detection of markers other than the products of the DL-A complex, particularly for non-DL-A alloantigens (43, 46). Nevertheless, these chimeras have shown specific tolerance to allografts of other organs obtained from the donor of marrow (references 43 and 47-49; and A. D. Boyd, personal communication).

TABLE VIII
Reconstitution of Irradiated B Dogs with Bone Marrow from their Original A Partner after Sensitization with B Skin Allografts; Effect of A Donor Peripheral Blood Leukocytes

Dog A	Dog B	Time between rejection of last B graft by A and transplantation of A marrow into B	Dose of A marrow	Irradiated blood given to B dogs		A leukocytes given to B dogs after marrow transplantation		Survival of B recipient
				Volume	Timing after marrow	No. given	Timing after marrow	
21-74	21-73	days 20	1.2×10^9	ml 250	days 8,12	2	days 1,6	days 12*
23-03	23-08	3	2.8×10^9	—	—	—	1,6,13	16
22-55	22-48	4 6	1.01×10^9	250	9,13	2	1,6	15*
22-95	22-94	4	1.78×10^9	750	11,18,20,22,25,28	6	1	28
22-52	22-56	7	2.1×10^9	750	12,15,22,25,32,41	6	1,7,14,21, 26,33,40,48*	343‡

* Hyperacute loss of marrow.

‡ This dog had a serum glutamic oxaloacetic-acid transamine elevation on the 40th day after marrow transplantation; survival listed as of 1 December 1974.

In contrast to the survival rates attained in the Cooperstown Colony, DL-A identical bone marrow transplants performed in other canine populations (34-36), as well as HL-A identical marrow transplants in man (37-40) have been associated with a significantly high incidence of lethal GVH disease. A possible explanation for the success of the long-term chimeras in Cooperstown dogs and in a certain proportion of the dogs tested in other canine populations (34-36) is that such donor-recipient combinations may share sufficient non-DL-A alloantigens to avoid triggering GVH disease after transplantation of marrow (43, 44). Although such a sharing appears to be a consequence of selective breeding in the restricted genetic pool of the Cooperstown Colony (44), it has been a chance occurrence in the other reported series (34-36). In spite of the sharing of some non-DL-A alloantigens, untreated genotypically DL-A identical Cooperstown dogs do, however, differ by sufficient concentrations of such antigens to cause rejection of first-set allografts of all organs tested in this colony, including skin, kidney, heart, lung, and liver (4, 6, 16, 43, 50-53).

The precise nature of the mechanism(s) of allogeneic unresponsiveness operative in radiation chimeras has remained uncertain. The detection of blocking antibodies of the Hellström et al. type (69) in long-term canine bone marrow chimeras similar to those of the Cooperstown Colony has prompted the suggestion that this type of humoral factor(s) might be responsible for the establishment and maintenance of chimeral tolerance (70). The results of the present study do not support this conclusion. Treatment of "A" dogs with serum obtained from long-term "B" chimeras reconstituted by "A" marrow has failed to significantly prolong the survival of "B" skin grafts placed on the "A" partner. In addition, the continuous exposure of marrow, whole blood, or leukocyte suspensions of sensitized "A" dogs to chimeric serum had no effect upon the ability of such cells to produce severe secondary disease in irradiated "B" recipients. Tsoi et al. (71) have also independently and simultaneously (46) observed that serum from bone marrow chimeras has no effect upon the survival of chimeral skin allografts placed on the donor of marrow, even if occasional serum samples had a blocking effect *in vitro*, and Brent et al. (72) have reported similar negative *in vivo* results in mice rendered tolerant during the neonatal state.

Epstein et al. (56) have provided evidence pointing to the repopulation of radiation chimeras by donor bone marrow cells and their progeny. There are no other definitive data, however, on the possible regeneration of some of the long-term chimeric dogs' own clones of immunologically competent cells. It is also not clear whether somatic mutations can occur in the transplanted cells or their progeny, as a consequence of their continuous exposure to an incompatible (non-DL-A alloantigens) milieu (54). In the course of this study, untreated "A" dogs and "A" dogs which had rejected skin allografts from their corresponding "B" chimera were irradiated and given bone marrow from "B". All grafts were successful, with no evidence of GVH disease. There was no difference in the outcome of marrow transplantation in "A" dogs sensitized to first-set "B" skin grafts, and in those which had not received "B" grafts. Storb et al. (73) have shown that allogeneic sensitization of a prospective recipient by blood transfu-

sion before irradiation acts as a barrier to the success of bone marrow transplantation from the same donor. The results of this study therefore indicate that the contribution of the irradiated "B" host's cells to repopulation of its bone marrow and derived cells was nil or insufficient to be detectable by this test system. The engraftment and proliferation of marrow from "B" in "A" also militate against the possibility that the originally transplanted "A" stem cells and their progeny developed somatic mutations against "self" during their prolonged sojourn in the "B" host. Rather, the results are compatible with the conclusion that such cells preserve their original germ-line properties (54), without changes in information storage in the foreign *in vivo* milieu.

After reconstitution with marrow cells obtained from their "B" chimera, all but 1 of 14 "A" dogs had the capacity to reject skin allografts from "B". Previous contact with "B" skin caused differences in the type of response accorded to "B" grafts. Challenge of "A" dogs with a second-set "B" skin graft after irradiation and reconstitution with "B" marrow resulted in an accelerated rejection response, which occurred when the grafts were transplanted within 34-59 days after reconstitution of the "A" dogs, as well as when a longer waiting period of 143-295 days was allotted, in order to ensure recovery of immunological function by the host (74). In contrast, four of five "A" dogs given first-set "B" grafts at 159-206 days after irradiation and reconstitution with "B" marrow rejected the skin grafts at 24, 34, 62, and 88 days, respectively; one animal remains unresponsive to "B" grafts.

The ability of "A" dogs reconstituted with "B" marrow to reject skin grafts from "B" suggests that the "A" marrow stem cells originally transplanted into the "B" dogs (and their progeny) have retained the capacity to recognize and to react against the non-DL-A histoincompatibilities present in "B" host tissues. The ability to muster an allograft response (such as a GVH reaction) was inhibited, however, while "A" cells remained in the "B" environment. Upon the return of such cells to their original "A" milieu, the "A" dogs gradually regained the ability to reject "B" tissues. Taken together, the evidence suggests that the allogeneic unresponsiveness resulting from exposure of "A" cells to the "B" milieu may be mediated by a mechanism akin to classical immunological tolerance, with inhibition of the clonal proliferation required to produce antigen-reactive lymphocytes (55, 75, 76), and without clonal elimination due to terminal differentiation.

It is of interest that second-set "B" skin grafts placed on reconstituted "A" dogs which had rejected a first-set of "B" graft before irradiation were accorded a decrease in survival time. The similar results obtained early and late after irradiation suggest that this response may be a consequence of the persistence in the irradiated host of radioresistant immunologically competent primed cells. Such a possibility is consonant with the observation that preirradiation blood transfusions can interfere with the success of bone marrow transplantation (73, 77), and highlights the potential importance of host-vs.-graft reactions as determinants of the success of bone marrow transplantation.

Once the reconstituted "A" dogs rejected a "B" skin graft, subsequent grafts from the same donor were accorded progressively decreasing survival times, a

possible indication of increasing rates of lymphocyte proliferation in response to repeated antigenic stimulation. The interval between each successive graft was deliberately kept at 2-3 wks, in order to induce accelerated rejection, rather than white graft responses (78), in preparation for a cellular transfer of allograft sensitivity from the hypersensitized "A" dogs to their "B" partner. Previous reports indicate that leukocytes obtained from individuals sensitized by repeated skin grafts from the same donor have the capacity to transfer specific allograft sensitivity to third-party recipients (78). In the present study, the intravenous injection of (anti-"B") "A" marrow and irradiated whole blood has successfully reconstituted four out of five supralethally irradiated "B" recipients; secondary disease, with death at 76 days, occurred in one dog. When unirradiated "A" blood was used in conjunction with "A" marrow, however, three out of three "B" dogs died of severe secondary disease within 28-49 days. Only one of five "B" dogs given "A" marrow and blood leukocytes survived; two animals had a total failure of engraftment, and succumbed at 12 and 15 days, and the remaining two dogs died of severe secondary disease at 16 and 28 days, respectively.

These results are consistent with the conclusion that bone marrow and unirradiated blood, or intact blood leukocytes, from a specifically sensitized donor can transfer allograft sensitivity to supralethally irradiated canine recipients, with the production of lethal secondary disease in the target animals. The ability of "A" cells to transfer allograft sensitivity under the conditions of this experiment supports the *de novo* nature of the transferred sensitivity. Indeed, "A" cells had been shown earlier to lack the capacity to produce detectable secondary disease in the same "B" recipient, and only acquired this capacity after the "A" dogs had been hypersensitized with "B" skin grafts. This observation may be of relevance to the suggestion that cellular transfers of hypersensitivity might be an expression of the elevation of latent host sensitivity, rather than conferring a *de novo* type of reactivity (79, 80).

Cosgrove et al. (81) have reported that bone marrow obtained from parental donors sensitized with F_1 hybrid thymus, liver, or spleen cells can induce severe early GVH reactions in lethally irradiated F_1 hybrid mice. The results of this study suggest that marrow stem cells may not be as effective as blood leukocytes in transfers of allograft sensitivity in the canine species. Rather, such transfers appear to be mediated primarily by radiosensitive blood leukocytes, a conclusion that is in harmony with the role of lymphocytes in mechanisms of cellular hypersensitivity (75, 76). The results also support the conclusion that the GVH reaction is an expression of cell-mediated immunity amenable to further study and/or modification by transfer techniques (82, 83).

Summary

20 Cooperstown beagles of known DL-A genotypes ("B" dogs) were exposed to supralethal total body irradiation and received a bone marrow allograft from a DL-A identical donor ("A" dog); the resulting chimeras have survived uneventfully for 882, 1466 days, with no evidence of secondary disease, and have been tolerant to kidney and skin allografts obtained from the donor of marrow.

Treatment of "A" dogs with serum obtained from their long-term "B" chimeras had no significant effect upon the ability of the recipients to reject "B"

skin allografts. Such grafts were rejected within 13–39 days; one graft survived for 75 days. Subsequent irradiation and reconstitution of the grafted dogs and of five additional “A” animals with the marrow obtained from the corresponding “B” chimeras resulted in the successful engraftment and long-term survival of the transplanted marrow for 538–759 days. There was no difference between the results observed in “A” pretreated with a “B” skin graft and in “A” dogs not given “B” skin grafts.

Rechallenge of skin-grafted “A” dogs with “B” skin transplants after irradiation and reconstitution with “B” marrow resulted in accelerated graft rejections at 12–24 days; in contrast, “B” skin grafts placed on five irradiated “A” dogs given “B” marrow without a previous skin graft survived for 24–88 days; one dog failed to reject such “B” grafts, which continue to survive at 654 days. Subsequent “B” skin grafts placed upon the corresponding “A” partners with 2–3 wk after rejection of the immediately preceding graft were accorded progressively diminished survival times.

When each “B” dog was reirradiated and reconstituted with bone marrow from its “A” partner, which had been sensitized by four consecutive “B” skin grafts, four of five “B” dogs given “A” marrow and irradiated “A” blood survived uneventfully. In contrast, three “B” dogs given “A” marrow and unirradiated “A” blood died of secondary disease within 28–49 days, and, four of five “B” dogs given “A” marrow and “A” leukocytes died. There was failure of engraftment in two instances and acute secondary disease at 16 and 28 days, respectively, in two other dogs.

The prospective selection of genotypically DL-A identical pairs of dogs of the Cooperstown Colony has produced the regularly predictable survival of supralethally irradiated recipients of bone marrow allografts. The resulting chimeras have survived with no evidence of secondary disease for as long as 4.5 y after transplantation. The mechanisms of graft-host accommodation operative in this experimental system may be the consequence of a central inhibition of clonal proliferation of lymphocytes capable of responding to non-DL-A alloantigens present in “B” and absent in “A” tissues. Humoral factors do not appear to be implicated in the mediation of this state of immunological tolerance.

The ability of cells obtained from “A” dogs sensitized by “B” skin grafts to produce secondary disease in the irradiated “B” hosts appears to be localized primarily to radiosensitive peripheral blood leukocytes. The results highlight the potential usefulness of cell transfer techniques as a model for further studies of the type of cell-mediated allograft sensitivity expressed by the graft-vs.-host reaction.

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