JCI The Journal of Clinical Investigation

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J Clin Invest. 1971;50(6):1272-1275. https://doi.org/10.1172/JCI106605.

Research Article

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In summary, fatal graft-*versus*-host disease was observed in a number of canine recipients despite matching with their sibling donor by serological histocompatibility testing and by mixed leukocyte culture in a manner similar to that employed to define human HL-A matched sibling pairs. The graft-*versus*-host disease in these matched siblings developed more slowly than that observed in mismatched dogs, but the ultimate death of approximately half of the matched recipients emphasizes the need for posttransplantation immunosuppression even in this “compatible” situation.

Marrow Grafts between Canine Siblings Matched by Serotyping and Mixed Leukocyte Culture

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ABSTRACT Marrow grafts were carried out between 16 canine sibling donor–recipient pairs. The pairs were matched by serological histocompatibility testing and were nonreactive in a one-way mixed leukocyte culture. Recipients were prepared for transplantation by 1500–1580 R of total body irradiation. Donor marrow was infused within 4 hr of irradiation. Recipients were not given immunosuppressive drug therapy after grafting. All 16 recipients showed evidence of prompt and sustained allogeneic marrow engraftment. Six died between 30 and 128 days after grafting with graft-*versus*-host disease, and three died between days 72 and 230 with pneumonia but no evidence of graft-*versus*-host disease with the exception of lymphoid atrophy. Seven recipients survived without graft-*versus*-host disease and are in excellent health between 200 and 684 after grafting.

In summary, fatal graft-*versus*-host disease was observed in a number of canine recipients despite matching with their sibling donor by serological histocompatibility testing and by mixed leukocyte culture in a manner similar to that employed to define human HL-A matched sibling pairs. The graft-*versus*-host disease in these matched siblings developed more slowly than that observed in mismatched dogs, but the ultimate death of approximately half of the matched recipients emphasizes the need for posttransplantation immunosuppression even in this "compatible" situation.

INTRODUCTION

The dog has been used as a model for studies of histocompatibility and marrow grafting in a randomly bred species. In initial studies, serological histocompatibility typing with a small panel of lymphocytotoxic antisera was used to select canine donor–recipient pairs for grafting. Clearly, the degree of matching with these antisera appeared to be an important determinant of marrow graft rejection, development of a lethal graft-*versus*-host (GVH)[1] disease or eventual survival of the recipient after infusion of allogeneic marrow (1–3).

Studies of serological histocompatibility typing in man have resulted in the recognition of a number of human histocompatibility or HL-A antigens (4). Despite polymorphism of the HL-A system, one can find HL-A–identical individuals among siblings because there are only four possible combinations of the parental haplotypes. More recently, family studies were conducted in which a one-way mixed leukocyte culture test (MLC) and serological typing were correlated (5). It was found that HL-A disparity caused stimulation in MLC while HL-A identity resulted in nonreactivity.

In some cases HL-A identity cannot be established by serotyping alone as insufficient family members are available for haplotype analysis. In these cases HL-A identity is verified by nonreactivity in MLC (5).

In the dog also, a good correlation has been found between serotyping and the results in MLC (6). Thus, canine sibling pairs matched for serologically detectable histocompatibility antigens and nonreactive in MLC make an excellent randomly bred animal model for marrow grafting experiments that may be applicable to HL-A–matched human siblings. This report presents the results of marrow grafting between 16 such canine sibling pairs.

METHODS

Pairs of canine siblings, 6–12 months of age and weighing 5–30 kg, were observed for 2 months for disease. They were typed for canine red cell antigen A (7), dewormed, and immunized against distemper and hepatitis. Each pair was selected on the basis of matching with eight canine lymphocytotoxic antisera. Four of the sera (anti-A, anti-B, anti-C, anti-D) have been developed in this laboratory and four (anti-d, anti-e, anti-f, anti-g) by Dr. J. W. Ferrebee, Cooperstown, N. Y. Details of the development of the sera

Received for publication 28 December 1970.

[1] *Abbreviations used in this paper:* GVH, graft-*versus*-host; MLC, mixed leukocyte culture test.

testing technique and data on the usefulness of the sera in selecting donor–recipient pairs for organ grafting have been described (1–3, 8, 9). Lymphocytes of all sibling pairs were tested in a one-way MLC (6). The published MLC method was altered in three respects: (*a*) the cells were cultured in Waymouth medium,[2] (*b*) a final concentration of 10^6 stimulating leukocytes per milliliter was used, and (*c*) each culture was exposed to ^{3}H-thymidine for 17 hr after 7 days of incubation at 37°C. Of 58 canine sibling pairs recently matched by serotyping in this laboratory, 43 were found to be nonreactive in MLC. Only pairs that were matched by serotyping and nonreactive in MLC were used in the present study. One member of each pair served as the marrow donor and the other as the marrow recipient.

Recipients were prepared for grafting by 1500–1580 R (midline air exposure) of whole body irradiation from two opposing ^{60}Co sources at a rate of 5.3 R/min. This corresponded to a midbody exposure of approximately 1200–1300 rads (10).

Donors were killed with pentobarbital sodium and marrow was then removed from all major bones and prepared for intravenous infusion (11). Donor marrow was infused within 4 hr of irradiation of the recipient.

Recipients were given parenteral fluids, electrolytes, and antibiotic therapy when clinically indicated. No immunosuppressive drugs were given after grafting. Peripheral blood cell counts were obtained at least three times weekly. Prompt rises in white blood cell and platelet counts after the postirradiation decline and histologic evidence of marrow repopulation were used as criteria for marrow engraftment. Other criteria were the development of a lethal GVH disease, the demonstration of a donor sex chromosome pattern in cells from marrow and peripheral blood, or a change to donor red cell type. Details of the cytogenetic technique have been described (12). Only cells with 78 chromosomes were evaluated. Complete autopsies were carried out on all recipients that died.

RESULTS

Table I summarizes data on the 16 recipients. Table II presents the MLC results. The activity in counts per minute represents means of duplicate cultures. Reproducibility was such that the average deviation of a duplicate from its mean was 13.8%. Each value represents the response of lymphocytes from a given dog to stimulation by irradiated leukocytes from that dog (autologous control), his sibling, and an unrelated control. The unrelated controls were included to be sure that the responding cells were functionally intact, and in most cases consisted of a mixture of irradiated cells from three unrelated dogs. In four cases this control consisted of irradiated human cells.

All 16 dogs showed prompt marrow engraftment as indicated by rising white blood cell (Fig. 1) and platelet counts after the postirradiation decline. None of the 16 rejected the graft. 9 of the 16 died (Table I). 6 died 30, 75, 80, 86, 101, and 128 days after grafting with GVH disease manifested clinically as anorexia, diarrhea, wasting, debility, jaundice, or erythematous skin eruptions. Histological findings included a cellular marrow, necrosis, or atrophy of the ileal and colonic mucosa with leukocyte infiltration, focal liver cell necrosis, dyskeratotic skin lesions, and lymphoid atrophy. These findings were consistent with those described in rodents and

TABLE I

Survival of Marrow Graft Recipients that were Matched with their Sibling Donors both by Serological Histocompatibility Typing and by MLC

Recipient No.	Breed	Lymphocyte type	No. of marrow cells infused $\times 10^9$	Survival
				days
9644	Hound	ACdefg	31.9	30
9505	Hound	Beg	33.8	72
886	Beagle	ABDefg	10.0	75
A166	Beagle	ACedfg	12.5	79
890	Beagle	BDefg	10.2	80
868	Beagle	fg	20.0	86
A73	Beagle	efg	5.1	101
766	Beagle	fg	10.1	128
A103	Mongrel	ABCDedfg	15.8	>200
8269	Hound	ABDedfg	38.5	>214
9549	Hound	ACefg	22.1	>245
773	Beagle	fg	12.9	230
A91	Mongrel	ABCDdfg	17.0	>303
655	Mongrel	ACefg	18.0	>680
719	German shepherd	ACef	30.4	>682
728	Beagle	AC	7.1	>684

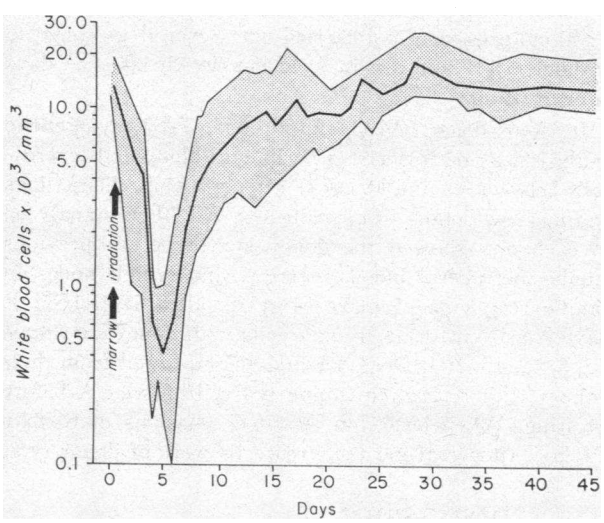

FIGURE 1 White blood cell changes (average) in 16 canine recipients given 1500–1580 R total body irradiation followed by marrow from histocompatible siblings. The shaded area represents the range.

[2] Grand Island Biological Company, Grand Island, N. Y.

TABLE II

^{3}H-Thymidine Uptake (cpm) per Mixed Leukocyte Culture

Dog. No.*	Autologous control	Sibling mixture	Unrelated control	Dog. No.*	Autologous control	Sibling mixture	Unrelated control
9642 D	632	407	6,340	A100 D	1,148	481	4,759
9644 R	511	487	6,640	A103 R	300	316	3,831
9503 D	381	345	4,165	8260 D	424	456	1,608
9505 R	440	382	3,753	8269 R	1,516	783	7,437
870 D	799	639	5,929	9547 D	473	301	9,270
886 R	464	682	4,842	9549 R	694	666	5,272
888 D	528	803	1,593	A89 D	728	852	3,156
890 R	623	420	3,043	A91 R	1,970	2,104	9,344
865 D	1,553	2,630	6,502	772 D	190	285	2,891
868 R	1,664	1,279	4,709	773 R	746	483	8,360
A75 D	446	654	1,335	658 D	313	299	1,796
A73 R	2,354	2,368	17,195	655 R	503	195	2,046
765 D	408	715	9,801	718 D	350	398	4,176
766 R	627	1,064	20,252	719 R	269	393	11,998
A165 D	1,521	1,441	28,650	731 D	365	334	2,274
A166 R	271	619	20,377	728 R	236	254	2,778

* D = donor; R = recipient.

monkeys (13, 14). Three dogs dying with pneumonia on days 72, 79, and 230 did not show evidence of GVH disease at autopsy with the exception of lymphoid atrophy. One of the three (A166) had shown transient GVH disease between days 25 and 45 manifested as erythematous skin lesions and diarrhea. The etiology of the pneumonia remained obscure. Bacteriological examination of lung and blood was negative in all three dogs. Viral cultures were not carried out. Seven dogs survived without GVH and are in excellent health 200–684 days after grafting.

In four dogs (A103, A166, 655, 728) cytogenetic analysis was performed on peripheral blood and marrow cells between 23 and 603 days after grafting. These dogs had marrow donors of opposite sex. All 40 cells analyzed in each dog showed the donor karyotype. In previous studies peripheral blood, marrow, and lymph nodes in canine recipients of marrow grafts after 1200–1580 R have consistently shown only cells with donor karyotype (2, 3, 12, 15, 16). Dogs 868 and 9549 differed from their donors with respect to canine red cell antigen A before grafting. When tested on repeated occasions more than 70 days after grafting their red cells were of donor type.

DISCUSSION

A number of canine recipients in the present study died with GVH disease despite matching of the sibling pairs by serological histocompatibility testing and MLC.

The high incidence of lethal GVH disease must be explained on the basis of "minor" histocompatibility differences undetected by currently employed in vitro typing techniques. The GVH disease in the matched siblings differed from that seen in dogs given grafts from mismatched donors in that it occurred between the 2nd and 4th month after grafting, and it's onset and course appeared to be more chronic. Mismatched dogs uniformly succumbed to violent GVH disease within 21 days of grafting (17).

Three matched siblings showed a combination of lymphoid atrophy and pneumonia in the absence of other findings of GVH disease at the time of autopsy. Lymphoid atrophy has been observed in other animal species after allogeneic marrow grafting (18, 19) and has been related to destruction of donor lymphoid cells ("allergic death") caused by an excess of host antigens (20, 21). Lymphoid atrophy, therefore, may be a consequence of GVH disease and it may explain the impairment of immunological defense against pathogens observed in animals with allogeneic marrow grafts (11, 19).

Recently, the effectiveness of the immunosuppressive drug methotrexate for control of canine GVH disease was demonstrated using unrelated donor–recipient pairs with known histoincompatibility. The acute form of GVH disease has been controlled and long-term survival for over 600 days has been achieved in some recipients when

intensive methotrexate therapy was begun immediately after marrow grafting and continued for 102 days (15). Cyclophosphamide proved to be ineffective in preventing rapidly fatal GVH disease in this incompatible combination (16). The present study underscores the need for immunosuppressive therapy after marrow grafting even in "compatible" siblings. Since methotrexate was effective in ameliorating GVH disease in incompatible recipients, we presume that it would be effective for these compatible siblings. Canine sibling pairs, matched by serotyping and by MLC in a manner similar to that used to define human HL-A–matched siblings, appear to offer an excellent model in a randomly bred species for evaluation of the optimal dose and schedule of methotrexate and other promising agents.

ACKNOWLEDGMENTS

We would like to thank Mr. Peter Wootton, Department of Radiology, for carrying out the radiation dosimetry studies. We are grateful to Dr. K. G. Lerner, Department of Pathology, for help in reviewing the histological sections. We would like to acknowledge the excellent technical assistance of Mrs. Virginia Erickson, Miss M. Murphy, Mr. T. C. Graham, Mr. Eric Mickelson, Mr. Cecil Allen, and Mr. B. Gong.

This research was supported by Grant AI 09419 and Contract 43-67-1435, National Institute of Allergy and Infectious Diseases, and Grant CA 10895, National Cancer Institute, National Institutes of Health, United States Public Health Service.

Dr. Thomas was supported by Research Career Program Award 1-K6-AI-2425 from the National Institute of Allergy and Infectious Diseases.

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