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HOMOLOGOUS BONE MARROW TRANSPLANTATION IN DOGS RECEIVING XRADIATION PLUS URETHANE OR 6-MERCAPTOPURINE

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ADMINISTRATIVE INFORMATION

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ABSTRACT

Experience has now been accumulated with homologous marrow transplantation in 13 mongrel dogs (Group 1) treated with 6-mercaptopurine (6-MP) or urethane prior to X irradiation (900 r, delivered at a dose rate of 15 r/minute). The marrow dose was 7-11 x 10^9 cells, given on the day following irradiation; urethane (175 or 350 mg/kg) and 6-MP (12.5 or 25 mg/kg) were administered at 3 or 4 daily intervals during the week prior to irradiation. Mean survival time (MST) in Group 1 was 23 days, with a maximum of 63 days. MST in a group of dogs (Group 2) given homologous marrow after 900 r, but not treated with the chemicals, was 10 days. Group 1 animals characteristically showed good recovery of peripheral blood granulocyte count by 8-10 days, together with objective evidence of marrow "take"; recovery of mononuclear cell count was not observed, except in the single case which survived for 63 days. None of the Group 2 animals showed any rise in the peripheral blood count after initial depression, and all died with marrow aplasia. Secondary disease in Group 1 dogs was characterized by anorexia weight loss, infection, and lymphoid tissue aplasia in all the animals; skin atrophy, liver lesions, jaundice and anemia were seen in some of the animals. The marrow showed active hematopoiesis and moderate to good cellularity in most of the Group 1 animals, although megakaryocyte activity was

deficient in some. Pneumonia and pulmonary edema were found in many of the dogs, at autopsy. It is evident that the use of these anti-metabolites permits the successful transplantation of homologous marrow in dogs at a dose of X radiation (900 r) which, by itself, is insufficient. These compounds (urethane and 6-mercaptopurine) are, therefore, additive to X radiation with respect to suppressing the homograft reaction in dogs, as well as in mice.

SUMMARY

The Problem:

The major source of our knowledge on bone marrow transplantation in irradiated animals has been from studies carried out on highly inbred strains of laboratory rodents. The attempt to bridge the gap between such studies and their eventual application to man requires investigations on larger mammalian species, such as dogs or monkeys, in which pure bred strains do not exist, and in which each animal is, in effect, an individual case or "patient". The problem of transplantation of marrow from one dog (not irradiated) into an irradiated dog differs in another important way from that in rodents: in the case of mice, exposure to a dose of X radiation in the lethal range is sufficient to suppress the immune response sufficiently to allow the "take" of a homologous bone marrow graft; whereas, in the case of the dogs, the monkey, and probably man, the immune system is relatively more radioresistant than the hematopoietic system, so that exposure to a lethal dose of radiation does not readily permit successful bone marrow transplantation. The present report deals with this problem by making use of certain antimetabolite chemicals, which when given in combination with X radiation make possible successful "takes" of bone marrow grafts from one dog to another.

The Findings:

Mongrel dogs were treated with 6-mercaptopurine (6-MP) or urethane prior to X irradiation (900 r, delivered at a dose rate of 15 r/minute). The marrow dose was 7-11 x 10^9 cells, given on the day following irradiation; urethane (175 or 350 mg/kg) and 6-MP (12.5 or 25 mg/kg) were administered at 3 or 4 daily intervals during the week prior to irradiation. Mean survival time in this group of 13 dogs was 23 days, with a maximum of 63 days. MST in a group of dogs given homologous marrow after 900 r, but not treated with the chemicals, was 10 days. The treated animals characteristically showed good recovery of peripheral blood granulocyte count by 8-10 days, together with objective evidence of marrow "take"; recovery of monomuclear cell count was not observed, except in the single case which survived for 63 days. None of the control animals showed any rise in the peripheral blood count after initial depression, and all died with marrow aplasia. Secondary disease in treated dogs was characterized by anorexia weight loss, infection, and lymphoid tissue aplasia in all the animals; skin atrophy, liver lesions, jaundice and anemia were seen in some of the animals. The marrow showed active hematopoiesis and moderate to good cellularity in most of the treated animals, although megakaryocyte activity was deficient in some. Pneumonia and pulmonary edema were found in many of the dogs at autopsy. It is evident that the use of these antimetabolites permits the successful transplantation of homologous marrow in dogs at a dose of X radiation

(900 r) which, by itself, is insufficient. These compounds (urethane and 6-mercaptopurine) are, therefore, additive to X radiation with respect to suppressing the homograft reaction in dogs, as well as in mice.

INTRODUCTION

The demonstration of the feasibility of homologous (i.e., genetically foreign) bone marrow transplantation as a life-saving procedure in lethally X-irradiated rodents (1,2,3) has stimulated attempts to extend such observations to larger mammals such as dogs (4,5) and monkeys (6), and also to man (7). In the case of dogs, in contrast to mice, it appears that exposure to doses several-fold larger than the LD_{50} does not suppress or abrogate the cellular systems involved in homograft rejection to the point necessary for graft survival. This is indicated by the difficulty in obtaining successful homologous marrow transplants in this species (4,5,8). Thomas et al (5) have attempted to deal with this problem by splenectomizing the dogs 2 weeks before irradiation, giving high X-radiation doses (1200 r given in 3 daily exposures of 400 r each), and treating the irradiated recipients with ACTH. In more recent studies, Thomas et al (9) achieved some successful marrow homografts in dogs after exposure of the graft recipients to Co^{60} gamma radiation doses of 1200 - 1600 r at a dose rate of 4.2 - 4.4 r/min.

Our present experiments on homologous bone marrow transplantation in X-irradiated dogs were designed to test whether transplantation would be more readily attained when X radiation was combined with antimetabolites known to depress the immune response. The two antimetabolites investigated were 6-mercaptopurine (6-MP), and urethane.

Schwartz and Damashek (10) had first reported that 6-MP suppresses humoral antibody production in the rabbit; and that this compound could induce immunological tolerance to purified protein antigens. Meeker et al (11) observed a definite prolongation of skin homograft survival in rabbit receiving 12 mg/gm of 6-MP for 14 days. Studies on mice in this Laboratory (12) have suggested that urethane in combination with X radiation potentiates the inhibitory effect of X radiation on the homograft reaction in mice, although urethane did not, by itself, prolong the survival of skin homografts.

MATERIALS AND METHODS

The experimental animals reported on here were adult mongrel dogs, of both sexes, weighing 10 - 15 kg. The dogs had all undergone a period of quarantine and observation, during which time the standard procedures for immunization against canine hepatitis, distemper, and rabies were carried out. Approximately 1 week before irradiation the dogs were placed in individual laboratory cages, at which time control hematological measurements were carried out.

Irradiation: The radiation source was a Westinghouse 250 KVP constant potential therapy unit, operated at 250 KVP, 15 ma, with filtration of 0.5 mm. Cu and 1 mm al, and HVL of 1.8 mm Cu. Each dog was anesthetized (Nembutal), and was mechanically rotated about its long axis during radiation exposure. The dose rate, measured in air at the potential midline, was approximately 15 r per minute, and the

target-to-midline distance was 115 cm.

Bone Marrow: The procedures for collection of the bone marrow from the donors and for infusion into the irradiated recipients were essentially the same as those described by Alpen and Baum (7) in their experiments showing protection of lethally X-irradiated dogs with autologous marrow. Total nucleated cell count was determined on the pooled marrow suspension, and correction was made, on the basis of white cell counts on a peripheral blood sample, for the number of peripheral blood leukocytes in the suspension. The marrow cell suspension ($5-10 \times 10^9$ cells) was administered intravenously by rapid infusion into the external jugular vein, usually on the day following irradiation.

Treatment of the Recipients Animals: Several different experimental treatment schedules were used; the dogs received multiple injections of either 6-MP plus urethane, 6-MP alone, or urethane alone, given at various intervals during the week prior to X irradiation. The drug dose (12.5 or 25 mg/kg 6-MP; 175 or 350 mg/kg urethane) and time relationships were varied somewhat from experiment to experiment, in the attempt to provide for optimal effectiveness and minimal toxicity. Fresh solutions of 6-MP were prepared by dissolving 500 mg of the compound in 5 ml of 0.1N NaOH, and then making appropriate dilutions with isotonic saline. Urethane was dissolved in isotonic saline. Both compounds were administered intravenously.

Food intake was a problem in the postirradiation period. In many cases it was found that the animals would eat canned chicken or fish rather than the usual horse meat and dog chow diet. Rations of these foods were given ad lib, whenever needed. Antibiotics (Tetracycline and Penicillin/Streptomycin) were given routinely during the postirradiation period, either daily or on alternate days.

Hematology: Blood samples were taken 2 or 3 times weekly following marrow transfusion, and determinations were made of total nucleated cell count, granulocytes, mononuclear cells, and hematocrit. In the cases where female marrow donors and male recipients were employed, the percentage of neutrophils showing the female sex chromatin "drumstick" was also determined, as a marker for the presence of donor cells (13). The limitations in the use of this procedure as a marker for homologous marrow cell transplantation in lethally X-irradiated monkeys have been discussed by Crouch et al (6). Survival time, and the hematological picture were the major criteria employed for evaluating the effect of the marrow cell infusions. Complete autopsies and histopathological studies were carried out on most of the animals.

RESULTS

The X-ray dose employed in most of these experiments was 900 r, a dose approximately 3 times the usual LD_{50}. It will be noted (Table I) that even at this supralethal radiation level, transfusion of fresh homologous bone marrow cells (5-10 x 10^9 nucleated cells per recipient)

TABLE I

MARROW TRANSPLANTATION IN LETHALLY X-IRRADIATED DOGS:
EFFECT OF HOMOLOGOUS BONE MARROW IN
DOGS GIVEN PRIOR TREATMENT WITH URETHANE OR 6-MERCAPTOPURINE

GROUP	X-RAYS DOSE	NO. of DOGS	TREATMENT	SURVIVAL NO at DAY 21	30	60	MEAN SURVIVAL TIME (days)
I	900 r	4	none	0	—	—	11
II	900 r	4	homologous marrow	0	—	—	10
III	900 r	13	6-MP or urethane plus homologous marrow	8	2	1	23
IV	900 r	3	autologous	3	3	3	>1 year*

*One of these dogs whelped a litter of 5 healthy pups 9 months after irradiation.

had no apparent effect on survival time, as compared with control irradiated dogs which received no marrow injection. There was, further, no demonstrable marrow "take" under these conditions as shown either by an increase in leukocyte counts (Figure 1), or by the presence of polymorphonuclear leukocytes bearing female "drumstick" chromatin, in those cases where marrow from female donors was transfused in irradiated male recipients. Thus, these results in the dog are in sharp contrast to those in mice, in which excellent homologous marrow transplantation is seen under comparable conditions of radiation-induced leukopenia. At death, all hematopoietic elements in the marrow were effaced, and only a delicate reticular stroma supporting a few plasma cells and red cells remained.

Transplantation of Homologous Marrow Following Treatment With Chemicals And X radiation: A total of 13 dogs treated with the antimetabolite chemicals and 900 r of X radiation prior to homologous marrow infusion are listed in Table II. It is evident from the data that the injection of homologous marrow cells under these conditions prolonged the survival of these dogs beyond the 8 - 11 day survival usually seen at this dose. The longest survival time observed in this series was 63 days. Objective evidence for the successful "take" of the donor marrow cells is seen in the cases where female "marker" polymorphonuclear leukocytes persisted over periods of 2 weeks or more (in one dog for 63 days) in the male recipients. In addition, definite

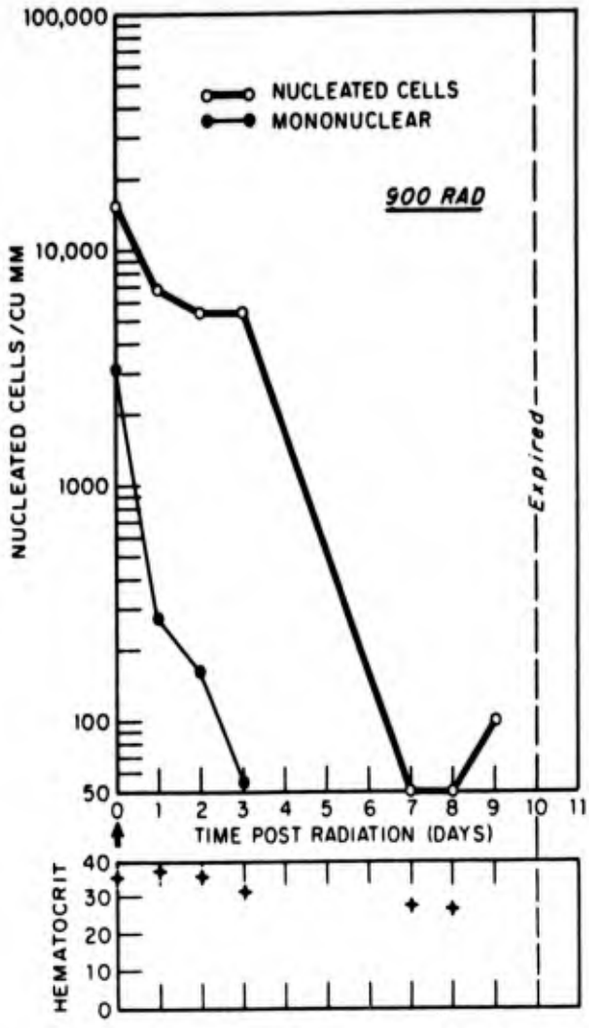

Fig. 1 Nucleated cell mononuclear cell and hematocrit response in peripheral blood of a dog after 900 r of X rays.

TABLE II

SURVIVAL DATA ON DOGS TRANSFUSED WITH HOMOLOGOUS MARROW AFTER RECEIVING CHEMICALS PLUS LETHAL X RADIATION (900 r)

DOG NO.	SEX	CHEMICALS (days injected preirradiation)	NO. MARROW CELLS ($x\ 10^9$)	DONOR SEX	SURVIVAL TIME (days)	COMMENTS
21	M	6-MP (7,6,5)	20*	—	23	——
45	F	6-MP + urethane (7,6,5,3)	11.3*	—	23	icterus, conjunctivitis
48	M	6-MP 3 urethane (6,5,4,3)	11.3**	M	13	+ markers
56	M	6-MP 3 urethane (6,5,4)	7.6**	M	16	+ markers; edema, congestion and hemorrhage of lungs
57	M	6-MP 3 urethane (7,6,5,4)	11.3	F	63	3 markers; secondary disease
60	M	urethane (6,5,4)	7.8**	M	21	no markers found; icterus; hematocrit 18 at death; pneumonia
62	M	6-MP (6,5,2,1)	11	F	31	+ markers at 14 days, none at 21 days; icterus; intestinal mucosal necrosis

*5 different marrow donors employed.

**2 different marrow donors employed.

TABLE II (con't)

SURVIVAL DATA ON DOGS TRANSFUSED WITH HOMOLOGOUS MARROW AFTER RECEIVING CHEMICALS PLUS LETHAL X RADIATION (900 r)

DOG NO.	SEX	CHEMICALS	NO. MARROW CELLS (x 10^9)	DONOR SEX	SURVIVAL TIME (days)	COMMENTS
63	M	6-MP (7,6,5,2)	10.3	F	29	+ markers; hematocrit 26 at death; pneumonia; hemorrhagic lungs
80	M	urethane (3,2,1)	10	F	17	+ markers; precipitous drop in granulocytes (100 cells/cu mm) at day 13.
81	M	urethane (3,2,1)	9.	F	18	+ markers; lung edema; hypocellular marrow at death. Corticosteroids given on day 13,14,15.
84	M	urethane (11,7,6,4)	15.	F	22	markers not det'd; pneumonia, marrow hypoplasia
93	M	urethane (3,2,1)	12.	F	15	+ markers; bloody urine
99	M	urethane (3,2,1)	8.5	F	22	+ markers; Beagle donor and recipient; antibiotics not given from days 12-20.

increase in the peripheral granulocyte count from the usual low values was already in evidence by 8 days after irradiation in essentially every dog listed. This is particularly meaningful in the 6 cases which received a single injection of marrow one day postirradiation. Dog No. 21 received marrow taken from 5 different donors, and was injected ($3\text{-}4 \times 10^9$ cells per injection) on day 0, 2, 4, 6, 9, after irradiation; similarly, dog No. 45 received marrow ($1\text{-}5.3 \times 10^9$ cells per injection) on day 0, 1, 3, 4 and 7. In these two instances, therefore, peripheral blood counts during the first postirradiation week are not necessarily reliable indicators of hematopoietic recovery.

The time-course of the hematological picture in individual animals is given in Figures 2, 3, 4, and 5. In all cases shown, there was a precipitous rise in granulocyte count occurring around 8 days postirradiation, and attaining values in the normal range by 15 days. In some cases (Figure 3) a subsequent decline in granulocyte count occurred, leading to death of the animal. In other instances, on the contrary, the granulocyte count remained high over a period of 3 to 4 weeks, with the animal succumbing, nevertheless. In several animals marked leukocytosis occurred, with peripheral white counts approaching 100,000/cu mm.

In all dogs but one (No. 57) successful transplantation of homologous bone marrow was not accompanied by a significant or sustained recovery in the mononuclear cell count in peripheral blood (see Figure 2).

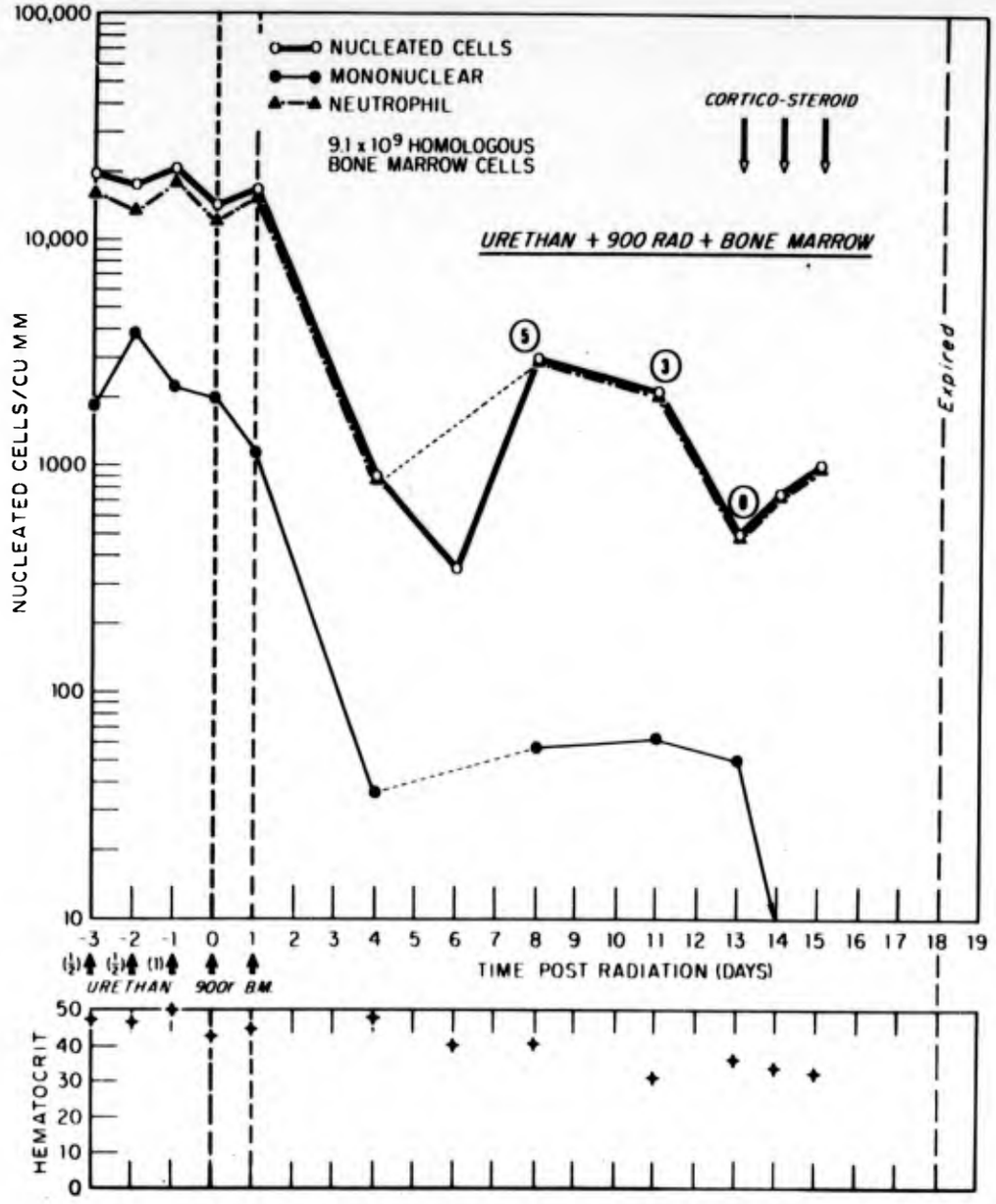

Fig. 2. Peripheral blood leukocyte counts and hematocrit in a male dog receiving a single transfusion of homologous bone marrow after treatment with urethane and 900 r of X rays. Circled numbers denote % of neutrophils bearing female "drumstick" chromatin.

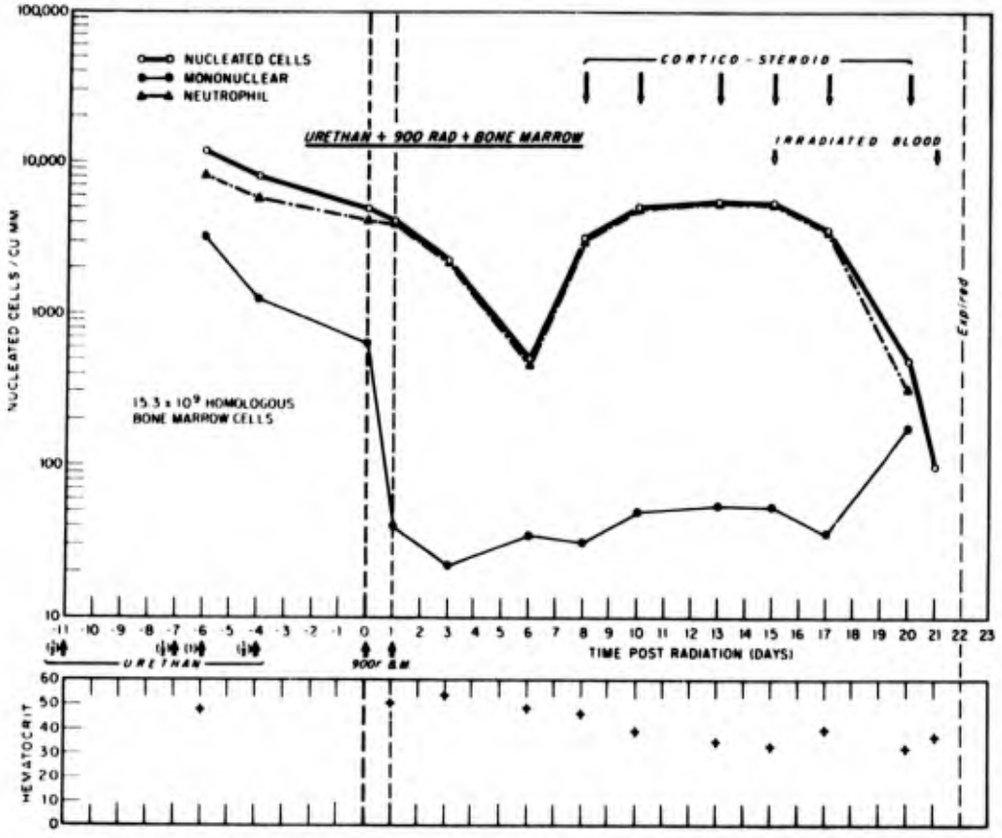

Fig. 3. Attempt at prevention of secondary disease by treatment of irradiated dog bearing homologous marrow graft, by means of corticosteroids and irradiated blood.

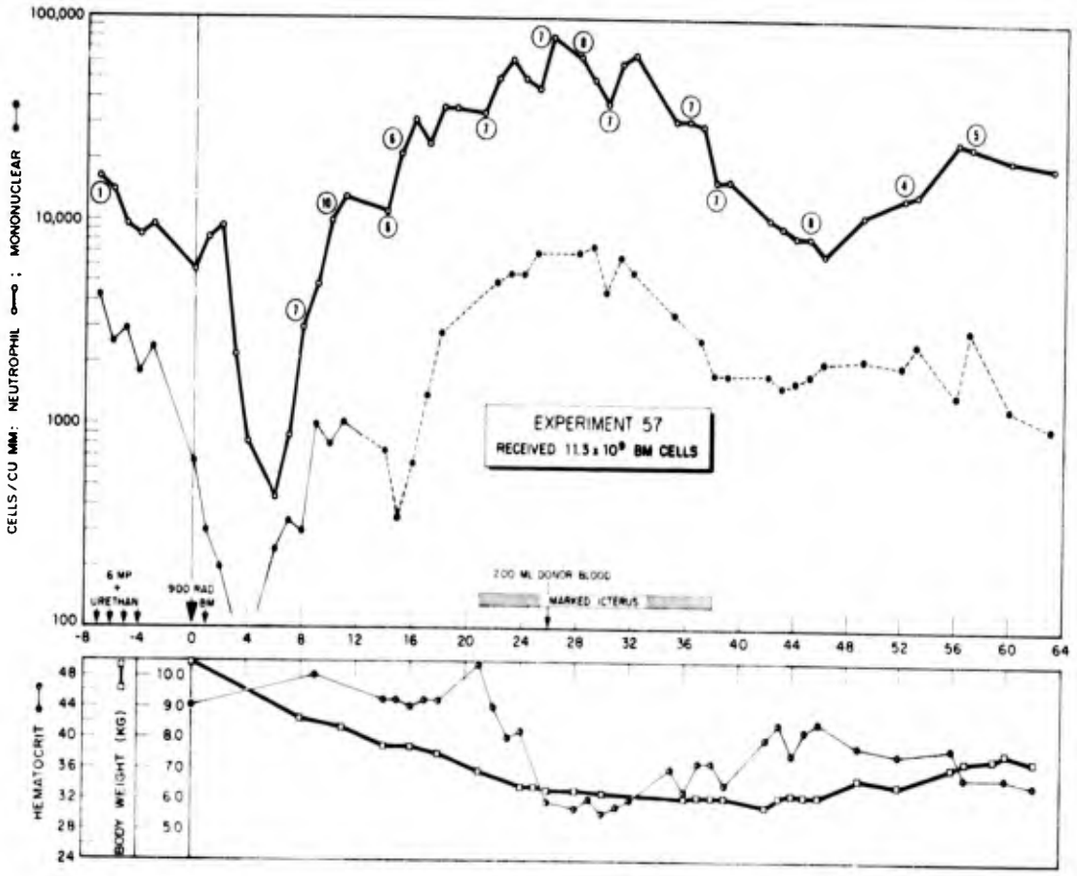

Fig. 4. Hematological and body weight response in a male dog surviving for 63 days after homologous bone marrow transplantation following X-irradiation (900 r) plus urethane and 6-MP.

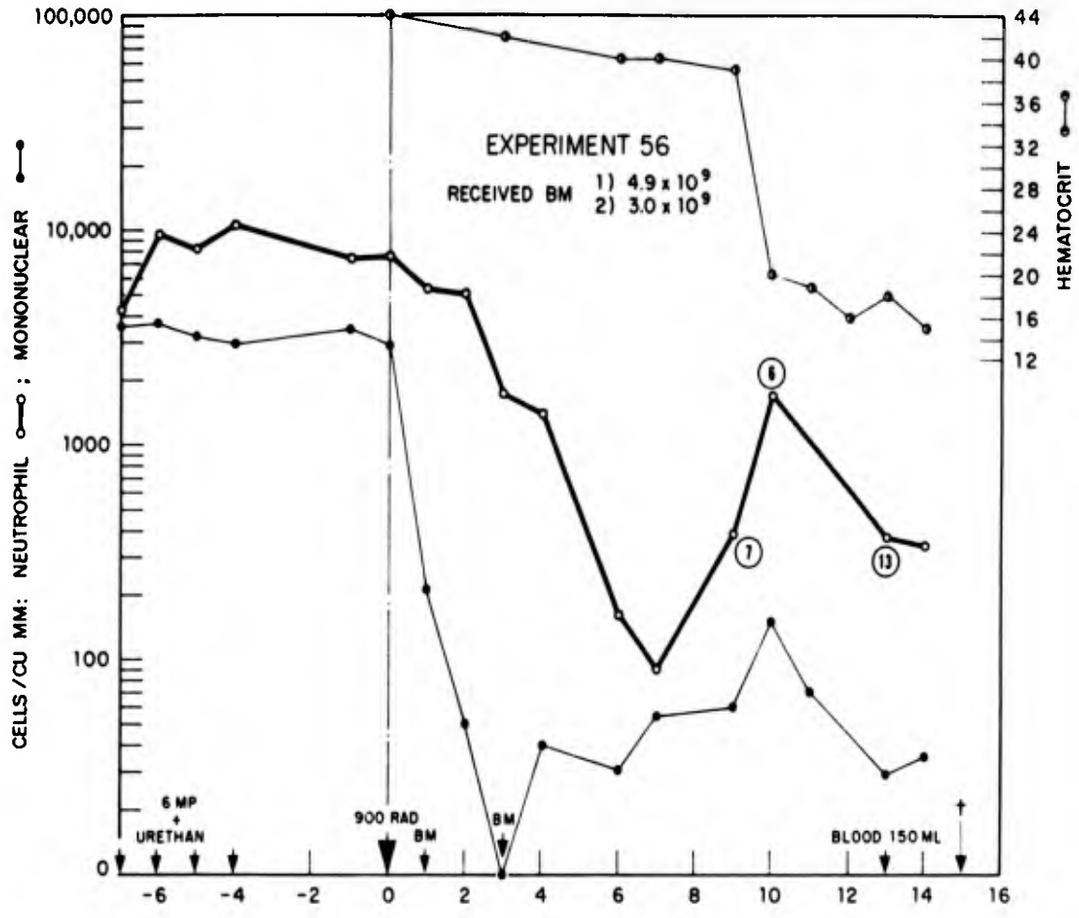

Fig. 5 Transplantation of bone marrow from a female dog to a male dog following X irradiation (900 r) plus urethane and 6-MP injections of marrow on days 1 and 3.

The fact that rapid recovery of mononuclear cell count is observed following autologous marrow transfusion after 900 r of X radiation (Figure 6) suggests that the lack of recovery in the former case is related to the antigenic differences between marrow donor and host.

Secondary Disease in Irradiated Dogs Bearing Homologous Marrow Transplants: Clinical signs and symptoms reminiscent of secondary disease in mice were observed in several of the dogs which died later than 3 weeks postirradiation. This is perhaps best described and illustrated in the case of dog No. 57. This male mongrel dog received injections of urethane and 6-MP prior to irradiation as follows: on days -7 and -6, 175 mg/kg and 12.5 mg/kg, respectively; on days -5 and -4, 350 mg/kg and 25 mg/kg, respectively. X radiation (900 r) was given at a dose rate of 15.4 r per minute, and 11.3 x 10^9 bone marrow cells from a healthy female mongrel dog were injected on the next day. The time-course of the hematological response is presented in Figure 4 . Following the initial drop in granulocyte level to 400/cu mm at 6 days postirradiation, there was excellent recovery in granulocytes by 8 days (3000 cells per cu mm), attaining preirradiation levels by day 11. That this hematological recovery was the consequence of a "take" of the donor marrow was indicated by the presence of female "drumstick" chromatin in 7% of the granulocytes in the blood sampled at 9 days. By comparison, 8% of the granulocytes in the blood of the marrow donor exhibited female chromatin.

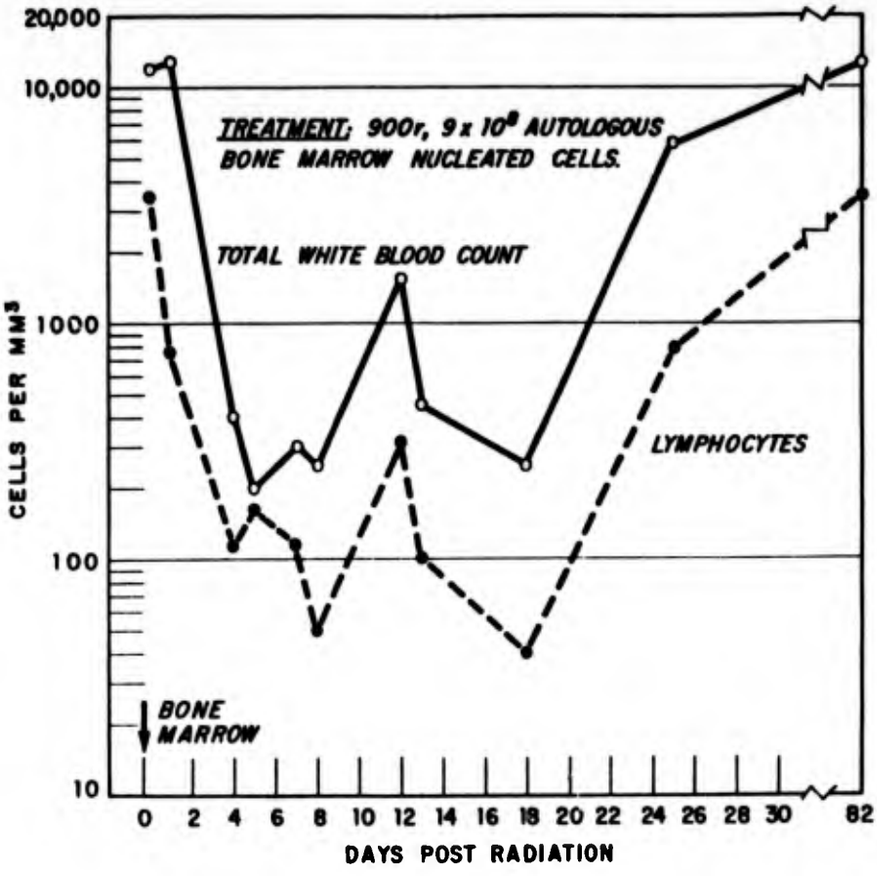

Fig. 6 Peripheral blood leukocyte and lumphocyte response in an X-irradiated dog (900 r) after transfusion of 9×10^8 autologous bone marrow cells.

The mononuclear cell level in this dog reached a minimum (less than 100 cells per cu mm) at 3 days postirradiation, and then rose steadily to attain a level of 1000 cells by 9 days, a level which was maintained until the middle of the second week; the mononuclear cell count then increased again, reaching a maximum of 9000 cell per cu mm at 25 days. Hematocrit values were relatively steady during this period (about 45); and the blood plasma was water-clear until day 21, when it became distinctly icteric. The urine was bloody, the stools quite watery, the eyes were filled with purulent fluid at this time, and the dog refused to eat. By day 25, the hematocrit level was 32, and the total nucleated cell count in the peripheral blood has reached approximately 50,000 cells per cu mm. Severe weight loss was evident (6.5 kg vs 10.8 kg initially). These evident signs of acute infection were treated by intramuscular injections of Penicillin and Tetracycline and by Bacitracin inunction in the eyes. A transfusion of 200 ml fresh blood (from which the buffy coat leukocytes had been removed (cf14)) derived from the marrow donor was given on day 25. The infection was apparently brought under control, as indicated by the dog's clinical status, and by the gradual return of the granulocytes to normal levels by the 5th week postirradiation. Female "drumstick" chromatin was observed in 7% of the peripheral blood granulocytes at this time; and in 5% of the granulocytes at 57 days postirradiation. At 62 days the dog exhibited muscular weakness, was unable to walk, and refused to take food. Female "marker" chromatin

was present in 3% of the granulocytes at this time, the granulocyte count was in the normal range (20,000 cells per cu mm), while the mononuclear cell level was only 1000 per cu mm. This animal expired 63 days after irradiation.

Evidence that this dog had succumbed to a secondary disease syndrome, quite analogous to that seen in rodents, comes from the histopathologic findings:

The marrow was moderately cellular, with blast cells of all cell lines, including plasma cells, seen. Areas of nuclear pyknosis and karyolysis were observed. The lymph nodes consisted of reticuloendothelial networks with no appreciable follicle activity, and with no definite organization of germinal centers. Fragmented cells and pyknotic nuclei were common within the follicles. The medullary sinusoids contained large mononuclear cells, some with iron pigment. The spleen parenchyma was extremely condensed. No active follicle structures could be recognized; some tiny erythroid nests, but no myeloid activity could be seen. The red pulp sinusoidal cells all contained large quantities of engulfed iron pigment. The liver showed diffuse intensive, periportal fibrosis around some central veins. The liver cell cords were in disarray, and consisted of broken clumps and islands of liver cells separated by irregularly anastomosing sinusoids. A few small islets of active necrosis were seen. Kupffer cells were filled with iron pigment and were quite prominent. The sinusoids and the proliferating

triadal connective tissue were infiltrated with plasma cells and mononuclear cells. There was massive atrophy of ear skin, with loss of appendages. In some places the germinal layer was only one cell thick. The vascular bed was intact, and no proliferative endarteritis was seen. The tubules, glomeruli and vessels of the kidney appeared normal. There was a generous infiltrate of plasma cell type cells in the interstitial tissue of the medulla. The vascular bed of the lung was congested and the alveoli were filled with precipitated proteinaceous material. Nests of plasma cells and mononuclear cells were scattered through the interstitial tissue and in the subpleural lymphatics. Foci of pneumonia were not seen. Apart from absence of lymphatic patches no lesions in the small intestine were identified. Marked edema of the lamina propria and submucosa of the colon was noted. A delicate sprinkling of plasma cells in the pia-arachnoid, and moderate cerebral edema were seen.

DISCUSSION

The foregoing results evoke two major points for discussion: 1) the question of the "take" of homologous donor marrow cells in the irradiated dog; 2) the secondary disease syndrome in irradiated dogs bearing marrow homografts.

It is evident under the conditions of these experiments, and with the radiation dose rate employed (15 rad/min), that an X-ray dose as high as 900 rad (which is 3 times the LD_{50} in dogs), did not depress the homograft response of the hosts sufficiently to allow successful transplantation of homologous marrow grafts. This conforms with the experience of Thomas et al (9) who state that grafts of homologous marrow in irradiated dogs are rarely accepted below doses of 1200 r. These workers did observe good "takes" of such grafts in dogs after exposure to gamma radiation doses in the range of 1200-1600 r, given at a dose rate of 4.2 - 4.4 r/min. Therefore, successful marrow homotransplantation in the recipient dogs which received 6-MP or urethane plus 900 rad of X radiation, in the present study show the <u>additive</u> effect of these chemicals and X radiation in suppressing the homograft reaction.

<u>Secondary Disease</u>: In an analogous study on lethally X-irradiated monkeys (650 r or higher), Crouch et al (6) noted an increased average survival time in the monkeys treated with homologous marrow, but none survived beyond 31 days. A secondary disease syndrome consisting of

anorexia, diarrhea, wasting, often occurring together with jaundice or dermatitis, was observed in these animals; this syndrome did not occur in irradiated monkeys treated with autologous marrow. Thus, the secondary disease syndrome in monkeys and dogs is not a consequence of the high dose of radiation per se but rather, results from the introduction of genetically foreign viable bone marrow cells into such irradiated animals. The histopathologic description of secondary disease in the present study is quite similar to that presented by De Vries et al (15) for monkeys. In both situations the animals succumb with the donor hematopoietic elements still active at the time of death. By contrast, the extensive and severe lymphoid tissue aplasia observed in the dogs and in the monkeys are precisely analogous with what occurs in secondary disease in mice. This is probably best attributed in our present state of knowledge, to a graft-versus-host reaction, initiated by immunologically competent cells in the inoculated marrow (cf 16).

The consequences of such lymphoid aplasia, with respect to increased susceptibility of radiation chimeras to various infectious processes and agents have been discussed by several authors. However, it is still an open question as to whether infection is the prime (or only) cause of death in the secondary disease syndrome. Is it possible, for example, to invoke pathological effects of the graft-versus-host reaction on tissues (other than lymphoid) which might

contribute to the demise of the animal? De Vries et al (15), observed lesions in the liver, intestinal tract epithelium, and skin in their monkeys bearing homologous marrow grafts; skin and liver lesions were observed in the present study. The pronounced fall in hematocrit accompanied by a marked icterus, as seen in dog No. 57 during the 4th week postirradiation and marrow transplantation, may also be a reflection of a graft-versus-host reaction. It is of interest that this effect was observed concomitantly with high levels of mononuclear cells in the peripheral blood.

Previous studies from this Laboratory (17) have suggested that the pathological manifestations of secondary disease in mice may be explicable in terms of antimitotic effects of the graft-versus-host reaction in tissues of the host. Such effects have been observed in skin wound healing (18), kidney mitosis following unilateral nephrectomy (17), intestinal epithelium (19), and bone marrow (20). The investigation of such reactions in the secondary disease syndrome in large mammals is of evident interest. From a more practical standpoint, however, the major problem facing us is that of prevention or amelioration of the secondary disease syndrome, following homologous marrow transplantation in large mammals. Recent studies on mice (21) have suggested some new approaches to this problem, specifically the use of suitable preirradiation of the marrow donor, and the administration of isoantiserum (anti donor) at appropriate times after

marrow transplantation. It is our intent to apply such procedures in our studies on homologous marrow transplantation in the dog.

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HOMOLOGOUS BONE MARROW TRANSPLANTATION IN DOGS RECEIVING X RADIATION PLUS URETHANE OR 6-MERCAPTOPURINE by L.J. Cole and E.L. Alpen
19 July 1962 36 p. tables illus. 21 refs.
UNCLASSIFIED

Experience has now been accumulated with homologous marrow transplantation in 13 mongrel dogs (Group 1) treated with 6-mercaptopurine (6-MP) or urethane prior to X-irradiation (900 r, delivered at a dose rate of 15 r/minute). The marrow
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1. Bone marrow - Transplantation.
2. X radiation - Biological effects.
3. Urethane - Biochemical effects.
4. 6-mercaptopurine - Biochemical effects.

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II. Alpen, E.L.
III. MR005.08-5200.

UNCLASSIFIED

dose was 7-11 x 10^9 cells, given on the day following irradiation; urethane (175 or 350 mg/kg) and 6-MP (12.5 or 25 mg/kg) were administered at 3 or 4 daily intervals during the week prior to irradiation. Mean survival time (MST) in Group 1 was 23 days, with a maximum of 63 days. MST in a group of dogs (Group 2) given homologous marrow after 900 r, but not treated with the chemicals, was 10 days. Group 1 animals characteristically showed good recovery of peripheral blood granulocyte count by 8-10 days, together with objective evidence of marrow "take"; recovery of mononuclear cell count was not observed, except in the single case which survived for 63 days. None of the Group 2 animals showed any rise in the peripheral blood count after initial depression, and all died with marrow aplasia. Secondary disease in Group 1 dogs was characterized by anorexia weight loss, infection, and lymphoid tissue aplasia in all the animals; skin atrophy, liver lesions, jaundice and anemia were seen in some of the animals. The marrow showed active hematopoiesis and moderate to good cellularity in most of the Group 1 animals, although megakaryocyte activity was deficient in some. Pneumonia and pulmonary edema were found in many of the dogs, at autopsy. It is evident that the use of these antimetabolites permits the successful transplantation of homologous marrow in dogs at a dose of X radiation (900 r) which, by itself, is insufficient. Therefore, urethane and 6-MP as additive to X-radiation, suppress the homograft reaction in dogs, as well as in mice. UNCLASSIFIED

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