**Increased Cancer Risk in Canine Radiation Chimeras**


One-hundred and eight dogs of various breeds were given 1200–1500 R (midline dose in air) total-body irradiation at 5–9.3 R/min delivered from 2 opposing 60Co sources, and 15 dogs were given cyclophosphamide, 100 mg/kg i.v., or dimethyl busulfan, 10 mg/kg i.v., in a single dose. They then received allogeneic (111 dogs) or autologous (12 dogs) marrow grafts. Radiation chimeras were observed for 6–121 mo (median 15) and chemotherapy chimeras for 6–97 mo (median 28). The incidence of malignant tumors in chimeras was compared to that in 215 untreated dogs observed for 2–187 mo (median 61). Seven malignancies were observed in 5 radiation chimeras. The tumors included hypernephroma, perianal gland carcinoma, mastocytoma (2), seminoma, mammary carcinoma, and Brenner tumor. Twenty-five tumors were seen in 25 control dogs. These included 8 thyroid carcinomas, 3 lymphosarcomas, 2 mammary carcinomas, 4 intestinal carcinomas, carcinoma, mastocytoma (2), seminoma, mammary carcinoma, and Brenner tumor. Twenty-five tumors were seen in 25 control dogs. These included 8 thyroid carcinomas, 3 lymphosarcomas, 2 mammary carcinomas, 4 intestinal carcinomas, and a number of other solid tumors. On the basis of a proportional hazards regression analysis, radiation chimeras have a risk of developing a malignancy that is estimated to be 6.90 times that of control dogs. A test of equality of these incidence rates gives a significance level of p < 0.001. No tumor has as yet been observed in the 15 canine chemotherapy chimeras. The increased risk of cancer among canine radiation chimeras suggests that high-dose total-body irradiation should be avoided in the conditioning for marrow transplantation of human patients with nonmalignant diseases.

MARROW transplantation has been used increasingly to treat patients with otherwise fatal malignant and nonmalignant disorders of the hematopoietic system. Ever since the observations by Schwartz and Beldotti7 and others10 of a high incidence of malignant lymphomas in F1 hybrid mice given parental hematopoietic grafts, there has been concern about the development of malignancies in marrow graft recipients. Furthermore, patients are conditioned for marrow transplantation by high doses of cytotoxic and immunosuppressive drugs and/or total-body irradiation (TBI), which are known for their carcinogenic potential.11–20 The present study analyzes the cancer risk in marrow graft recipients (chimeras) in a canine model that has been used to study many of the principles of marrow transplantation that have been applied to man. Specifically, the cancer incidence among 108 radiation chimeras and 15 chemotherapy chimeras observed from 6–121 mo is compared to that among 215 normal dogs observed for similar periods of time.

MATERIALS AND METHODS

**Dogs**

All 123 chimeras and 51 control dogs were maintained at the Fred Hutchinson Cancer Research Center (FHCRC), Seattle, Wash. They consisted of beagles, basenjis, Labrador retrievers, Dalmatians, red bone hounds, German shorthairs, Irish setters, and various crossbreeds, obtained from kennels in Washington, Oregon, Virginia, New York, Oklahoma, and Alberta. In addition, 164 untreated beagles at the Argonne National Laboratory (ANL), Argonne, Ill., served as controls. Among the chimeras, 96 were beagles and 27 were of other breeds; of the control dogs, 197 were beagles and 18 were of other breeds. Sixty-one of the chimeras were male and 62 female, compared to 119 males and 96 females among controls. Transplanted dogs were followed for at least 180 days after transplantation.

The kennel conditions, feeding, vaccination schedule, and dog maintenance were comparable at the FHCRC and ANL. Dogs at FHCRC were observed until their natural deaths or until sacrifice (because of lack of kennel space) by intravenous pentobarbital injection. Complete autopsies and histologic examinations of postmortem tissues were performed on all dogs that died.22–23

**Marrow Transplantation**

Conditioning for marrow transplantation consisted of TBI for 108 dogs and a chemotherapy regimen for 15 dogs. Irradiation was administered to the unanesthetized dogs housed in a polyurethane or aluminum cage placed midway between two opposing 2000 RPHM 60Co sources with the long axis perpendicular to the central beam. Eighty-four dogs with allogeneic marrow transplantation were conditioned with 1200 R (midline dose in air, corresponding to approximately 900 rad midline tissue dose) TBI at 9.3 R/min and 12 with 1500 R (1150 rad) at 5 R/min. Dogs receiving autologous grafts were given 1200 R at 9.3 R/min (6 dogs), 1500 R at 5 R/min (2 dogs) or 2000–2800 R (1500–2000 rad) at 9.3 R/min in 200-R fractions at intervals of 3 hr (4 dogs). A detailed description of the dosimetry has been given previously.24 Of the 15 chemotherapy-conditioned dogs, 2 were prepared by cyclophosphamide (CY), 100 mg/kg i.v. in a single dose,25 and 13 by dimethyl busulfan (DBM), 10 mg/kg i.v. in a single dose.26 Among the 96 allogeneic transplants in TBI-treated dogs, 69 were littermate grafts (58 DLA-identical, 10 DLA-nonidentical, 1
unknown), and 27 were grafts from unrelated dogs (6 DLA-matched, 14 DLA-mismatched, 7 unknown). All 15 chemotherapy-conditioned dogs were given transplants from DLA-identical littermates. The day of marrow infusion was designated day 0.

Sixty-six TBI and 13 chemotherapy chimeras were treated with the immunosuppressant methotrexate (MTX) for 102 days after grafting in an attempt to prevent graft-versus-host disease (GVHD). Details of postgrafting fluid, electrolyte, and antibiotic therapy, the assessment of hemopoietic engraftment by peripheral blood cell counts, marrow examinations, and blood genetic markers, and the clinical and histologic criteria for GVHD have been described.22,23

Statistical Methods

Chimeras and control dogs were compared in respect to age at first malignant tumor diagnosis using Kaplan-Meier plots28 and log-rank statistics.29–31 Dogs that died without cancer or were killed have censored “malignant tumor times” in these calculations. More refined methods were used to accommodate the fact that all Seattle dogs should properly be regarded as control dogs until the time of bone marrow transplantation. This led to the use of a “time-dependent” log-rank test in which group membership changed from control to chimera at the time of transplantation. The closely associated proportional hazards regression method,32,33 with time-dependent covariates, was used to estimate the relative cancer risk associated with transplantation and to examine the dependence of this risk on age and on age at transplantation. Similar statistical techniques have been used, for example, in the analysis of heart transplant survival data.33 A discussion of the meaning of such statistical tests and the relative risk estimates in the presence of competing risks (deaths unrelated to malignancy) has been published.35

RESULTS

Malignancies in Chimeras and Controls

During the observation period of 6–121 mo (median 15) after transplantation, 5 of the 108 radiation chimeras developed 7 malignant tumors (Table 1). Four died of this complication, while one (A859) is surviving. None of the 15 chemotherapy-conditioned chimeras, observed for 6–97 mo (median 28), has developed a malignancy as yet. Among the 215 control dogs, followed for 2–187 mo (median 6), 25 have developed malignancies (Table 2). Figure 1 illustrates the difference in cancer hazard between radiation chimeras and control dogs, presented in the form of Kaplan-Meier curves.

Causes of Death

Causes of death among dogs in the two colonies are summarized in Table 3. A large number of marrow recipients and controls at FHCRC were killed either because of lack of kennel space or use in other experiments. Deaths from infectious complications were equally distributed between marrow recipients and controls. Deaths from chronic GVHD and pancreatic insufficiency were restricted to chimeras. Pancreatic insufficiency appears to be radiation-related; it has been seen in dogs with autologous grafts but not in

Table 1. Malignancies in Radiation Chimeras

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Sex</th>
<th>Breed</th>
<th>Date of Birth</th>
<th>Date of Transplant</th>
<th>Radiation Dose (Sex)</th>
<th>Marrow Donor (Sex)*</th>
<th>MTX Post grafting</th>
<th>Tumor</th>
<th>Interval From Transplantation to Tumor Diagnosis in Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>755C</td>
<td>M</td>
<td>Beagle</td>
<td>3/61</td>
<td>3/62</td>
<td>1500 R Nonlittermate (F)</td>
<td>No</td>
<td>Hypernephroma metastatic to lung, liver, spleen, ileum, paraaortic nodes, spinal cord</td>
<td></td>
<td>2,920</td>
</tr>
<tr>
<td>977C</td>
<td>M</td>
<td>Beagle</td>
<td>2/61</td>
<td>2/64</td>
<td>1500 R Nonlittermate (F)</td>
<td>No</td>
<td>Perianal gland carcinoma metastatic to liver, spleen, inguinal and paraaortic nodes</td>
<td></td>
<td>2,467</td>
</tr>
<tr>
<td>655</td>
<td>M</td>
<td>Mongrel</td>
<td>8/68</td>
<td>2/69</td>
<td>1500 R Littermate (F)</td>
<td>No</td>
<td>1. Mastocytoma right hind leg</td>
<td></td>
<td>3,308</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2. Seminoma, metastatic to lymph nodes, liver, spleen, lungs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D7</td>
<td>F</td>
<td>Beagle</td>
<td>3/69</td>
<td>10/69</td>
<td>1200 R Littermate (M)</td>
<td>Yes</td>
<td>1. Mammary gland carcinoma metastatic to liver, spleen, bladder, skin, pericardium, and peritoneum</td>
<td></td>
<td>1,394</td>
</tr>
<tr>
<td>A859</td>
<td>M</td>
<td>Beagle</td>
<td>4/72</td>
<td>6/73</td>
<td>1200 R Littermate (M)</td>
<td>Yes</td>
<td>Recurrent mastocytoma of right hind leg</td>
<td></td>
<td>2,708</td>
</tr>
</tbody>
</table>

*The marrow donors for dogs 655, D7, and A859 were DLA-identical with the recipients. In dogs 755C and 977C, no histocompatibility typing was done.

†No immunosuppressive drugs other than Methotrexate were given.
Increased Cancer Risk Among Radiation Chimeras

Table 4 gives the number of TBI-treated and control dogs with cancer along with the "expected" number under the hypothesis of no association between cancer incidence and TBI. The difference between observed and expected cases of malignancies gives a (log-rank) significance level of $p = 0.008$, based on all dogs, or $p = 0.012$, based on beagles only. For the reason given in the methods section, this comparison is somewhat biased toward underestimating the significance of the increased risk. More specifically, marrow transplantation took place when dogs were anywhere from 3 mo to several years of age (median 9.4 mo). Very early cancers then, almost

Table 4. Cancer Incidence in Marrow Graft Recipients Conditioned by TBI and in Control Dogs

<table>
<thead>
<tr>
<th>Group (Number of Dogs)</th>
<th>Dogs With Cancer</th>
<th>Log-Rank Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Dogs TBI-treated (108)</td>
<td>5</td>
<td>2.07</td>
</tr>
<tr>
<td>Controls (215)</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Beagles TBI-treated (83)</td>
<td>4</td>
<td>1.53</td>
</tr>
<tr>
<td>Controls (198)</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>All 3-yr survivors TBI-treated (50)</td>
<td>5</td>
<td>1.05</td>
</tr>
<tr>
<td>Controls (156)</td>
<td>22</td>
<td>26.95</td>
</tr>
<tr>
<td>Beagles 3-yr survivors TBI-treated (37)</td>
<td>4</td>
<td>0.64</td>
</tr>
<tr>
<td>Controls (151)</td>
<td>22</td>
<td>26.36</td>
</tr>
</tbody>
</table>
necessarily, occurred in control dogs. A simple procedure to avoid this problem is to restrict attention to long-term survivors. For example, by 3 yr of age, all but two TBI-treated beagles had received their marrow grafts. Table 4 gives the observed and expected numbers of dogs with cancer and log-rank significance levels for test of equality of cancer incidence rates among 3-yr survivors. The significance levels are less than 0.001 for all dogs and for beagles alone.

A more refined method of taking account of waiting time to transplantation would permit the group membership to change from control to radiation chimera at the time of marrow grafting. This gave almost identical significance levels to those based on 0.001 for all dogs and for irradiation and transplantation, and thus, were not yet at risk for beagles alone.

The idea of group membership that changes at the time of transplantation can be incorporated in a proportional hazards regression analysis to estimate the magnitude of the increased cancer risk among radiation chimera. Suppose \( r(t) \) represents the (instantaneous) rate of cancer at age \( t \) among control dogs. Suppose that following transplantation, the cancer incidence rate at any age \( t \) becomes \( kr(t) \). The proportional hazards method permits the estimation of the relative risk parameter, \( k \), without any assumption concerning the function \( r(t) \). Application to data on the 83 TBI-treated beagles and 198 control beagles gave a relative risk estimate of 6.90, with an associated approximate 95\% confidence interval of 1.97–24.13. This means that TBI-treated dogs are estimated to have a cancer incidence rate of 6.90 times that of control dogs.

**Effect of Age on Cancer Risk**

In spite of the small number of cancers among the radiation chimera, an attempt was made to study the dependence of the relative risk on both age and age at transplant. There is an indication that the risk associated with irradiation and transplantation may be increasing as the animal ages and may be higher in dogs transplanted late, though neither trend is clear with these sample sizes. More explicitly, permitting the relative risk to vary as a power of age gave the following relative risk estimates:

\[
\begin{align*}
\text{Age (yr):} & \quad 4 \quad 5 \quad 6 \quad 7 \\
\text{Relative risk:} & \quad 3.55 \quad 5.59 \quad 8.11 \quad 11.10
\end{align*}
\]

The significance level of a test for trend is \( p = 0.16 \).

Similarly, if the relative risk is permitted to vary exponentially with age at transplant, one obtains:

\[
\begin{align*}
\text{Age (yr at transplant):} & \quad 0.5 \quad 1 \quad 1.5 \quad 2 \quad 3 \\
\text{Relative risk:} & \quad 4.28 \quad 5.19 \quad 6.28 \quad 7.61 \quad 11.17
\end{align*}
\]

The significance level of a test for no association is \( p = 0.09 \).

**Effect of Radiation Dose, Degree of Histocompatibility, or GVHD on Tumor Incidence**

As shown in Table 1, two chimeras with malignancies had been given 1200 R, and three had received 1500 R. There was no significant difference in cancer incidence between dogs given 1200 and 1500 R, respectively (\( p = 0.82 \)). Also, in allogeneic transplants, there was no correlation between tumor incidence and the degree of donor–recipient histocompatibility or presence/absence of GVHD.

**DISCUSSION**

This study shows that long-term canine radiation chimeras have an increased risk of developing malignant tumors when compared to nonirradiated dogs. No tumor has as yet been observed in a smaller number of chemotherapy-conditioned chimeras followed for similar periods of time. The tumors observed in TBI chimeras fail to show a predilection for the hematopoietic or lymphoreticular systems. Rather, they fall within the spectrum of malignancies seen in control dogs. There may be an increase in cancer risk with age at transplantation and with time after transplantation.

Because of the greatly varying observation times and the large number of animals in the experimental group that were killed upon termination of an experiment, a statistical approach had to be chosen that took into account these differences as well as the waiting time of experimental animals from birth until transplantation. This was important as three tumors in nonirradiated dogs occurred long before the first tumor in an irradiated dog was diagnosed, and at ages at which many dogs were still waiting for irradiation and transplantation, and thus, were not yet at risk for postirradiation complications. Time-dependent versions of the log-rank test and the proportional hazards regression methods accommodated these special features of the data.

A high incidence of lymphoreticular tumors of both host and donor type has been described in irradiated and nonirradiated F1 mice of various strains given grafts of parental spleen cells. It has been postulated that the donor cells were chronically stimulated by the histocompatibility antigens of the host and that
this chronic stimulation led to activation of murine leukemia virus, which, in turn, would infect host or donor cells, whichever would be susceptible to the virus.\textsuperscript{9,38} The development of malignant lymphomas following allogeneic or xenogeneic transplants has been described in other species as well.\textsuperscript{39} Also, the high incidence of malignant lymphomas in human organ graft recipients might be the result of chronic antigenic stimulation of host cells by histocompatibility antigens in the graft in the presence of incomplete immunosuppression.\textsuperscript{40,41}

Finally, there has been one report on a human patient with acute leukemia conditioned for marrow transplantation by TBI who developed an immunoblastic sarcoma shortly after transplantation.\textsuperscript{42} This patient's course was complicated by GVHD treated with high doses of corticosteroids and antithymocyte globulin. He died 84 days after grafting. No other similar case had been reported. Evidence has been presented, however, that viral infections in severely immunosuppressed patients may lead to reactive lymphocyte transformation resembling lymphoma.\textsuperscript{43} Also, several patients have been observed to develop single and multiple extramedullary tumors at variable times after marrow transplantation and before marrow relapse.\textsuperscript{44,45} These chloromas may suggest various differential diagnoses and should be considered before the diagnosis of a second malignancy is established.

The etiology of tumors in the canine radiation chimeras presented here is likely to be different from virus activation or chronic antigenic stimulation. In contrast to rodents with hemopoietic grafts and humans with organ transplants, canine radiation chimeras have not shown lymphoreticular neoplasms, although lymphoma is a common spontaneous tumor in dogs.\textsuperscript{46}

An increased risk for malignancies, primarily of the lympho-hemopoietic system, has also been reported in human patients with various congenital immunodeficiencies.\textsuperscript{47} Canine chimeras experience a severe combined immunodeficiency during the first 200–300 days after transplantation,\textsuperscript{48} a period during which no malignancy has as yet been observed. Thereafter, they regain normal immunologic reactivity, which makes it unlikely that the development of tumors diagnosed 1394–3308 days (46–110 mo) after transplantation is related to immunologic dysfunction.

It appears that TBI is the most likely cause for the increased cancer risk in radiation chimeras. The carcinogenicity and leukemogenicity of ionizing radiation have been shown beyond doubt in animals and man.\textsuperscript{16,19} Dose rate and total dose of exposure seem to be important, although a threshold could not be established.\textsuperscript{15} Thyroid, bronchogenic, and breast carcinomas have been found to be increased after exposure to a broad dose range of irradiation in different settings.\textsuperscript{16–18} More recently, the development of myeloproliferative syndromes has been shown in beagle dogs following TBI at very low dose rates.\textsuperscript{49} Whatever the mechanism of tumor induction may be, hemopoietic malignancies should not be likely in our radiation chimeras since their own hemopoietic system has been exposed to very high doses of radiation, destroying precursor cells rather than inducing damage that might lead to the development of abnormal clones.\textsuperscript{50}

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The donor marrow that gave rise to their current hemopoietic system, however, has never been exposed to radiation. Thus, the development of nonhemopoietic tumors was more likely and, in fact, could be confirmed by our findings. It is of interest that no tumor has as yet been observed among the smaller number of chemotherapy chimeras followed for prolonged periods of time.

Dogs in the present study have been observed for a great part of their natural lives. Comparably long observation times are not available for human marrow transplant recipients, and it is not clear whether the finding of an increased cancer incidence among canine radiation chimeras can be extrapolated to man. Twenty-four human CY chimeras transplanted in Seattle are long-term survivors between 4 and 8 yr after marrow transplantation and an additional 52 have been observed from 1 to 4 yr without development of a malignancy\textsuperscript{3} (and unpublished observation). Twenty human radiation chimeras (including six patients with monozygous twin grafts) are living between 4 and 9 yr after marrow transplantation, and an additional 59 allogeneic and 16 syngeneic radiation chimeras have been observed from 1 to 4 yr.\textsuperscript{1} Two of the human radiation chimeras developed nonhematologic malignancies approximately 1 yr after grafting (unpublished observations). Whether these malignancies are due to a generally increased risk of developing a secondary tumor in patients with hematologic malignancies or whether they are related to irradiation is unknown at present. Despite this uncertainty, the increased risk of cancer in canine radiation chimeras reported here suggests that TBI, at least at high doses, should be avoided, if possible, in the conditioning for marrow transplantation of human patients with nonmalignant diseases.

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