

# Chapter 1

## The Scientific Foundation of Marrow Transplantation Based on Animal Studies

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*It should be noted that marrow grafting could not have reached clinical application without animal research, first in inbred rodents and then in outbred species, particularly the dog.*  
E. Donnall Thomas, The Nobel Prizes, 1990; Les Prix Nobel, Norstedts Tryckeri AB, Stockholm, 1991, p. 227

With modern demonstration of conservation of so many genes across species, it is apparent that human beings have much in common with all animals and other living things. It should be no surprise, therefore, that so many of the biological principles underlying transplantation immunology are transferable from one species to another. The successful application in humans of not only bone marrow transplantation (BMT), but also transplantation of other organs has been achieved as a result of extensive animal studies.

The experiments of Alexis Carrel at the beginning of this century established a paradigm of biology—that cells or organs transferred from one individual to another would always be recognized as foreign and therefore be rejected. That this paradigm might not always be true was suggested by Owen, who noted that freemartin cattle contained cells of different genetic origin. The paradigm was further undermined by the hypothesis of Burnett and by the animal experiments of Medawar and colleagues. They provided the scientific basis for an understanding of tolerance induced in utero or in newborns. However, their studies indicated that tolerance between genetically divergent animals could not be achieved once the immune system had developed sufficiently to distinguish self from non-self. The numerous studies of transplantation biology performed before 1960 are presented in the monograph by Woodruff (1). Studies of BMT and immunosuppressive drugs would eventually provide at least a partial solution to the immunological barrier against transplantation.

### **Marrow Transplant Studies in the Murine Model**

The possibility of BMT came first from studies in the mouse. At the end of World War II following the atomic bomb explosions, there was a great deal of

interest in how radiation damages living organisms. It became recognized that the marrow is the organ most sensitive to radiation and that death following low-lethal exposures was due to marrow failure. Jacobson and associates (2) made the observation that mice could withstand an otherwise lethal exposure to whole-body irradiation if the spleen were protected by a lead foil. Shortly thereafter, Lorenz and colleagues (3) found that similar radiation protection could be conferred by infusion of bone marrow. At first it was thought that the radioprotective effect was due to a humoral factor derived from the spleen or marrow, which stimulated marrow recovery.

In the mid-1950s, several reports offered a different explanation for the “radiation protection” effect. In 1955, Main and Prehn (4) showed that a mouse given lethal irradiation and a marrow infusion from a different strain would accept a subsequent skin graft from the donor, and in 1956, Trentin (5) showed that the skin graft acceptance was specific for the donor strain. Also in 1956, Ford and associates (6) showed that the cytogenetic characteristics of the marrow in such mice were those of the donor and not the recipient, and Nowell and colleagues (7) demonstrated the presence of rat granulocytes in mice protected with rat marrow. These experiments made it clear that protection against radiation was due to the transfer of living cells and that a form of tolerance had been induced.

In 1958, Schwartz and Dameshek (8) found that 6-mercaptopurine could induce a form of specific immune tolerance in rabbits when given at the time of antigen exposure. This observation was followed by the development of azathioprine by Elion and associates (9). Murray (10) showed that these agents could prolong the life of kidney grafts in dogs. These studies of immunosuppression by drugs led the way to solid organ grafts and to control of the graft-versus-host (GVH) reaction following a marrow graft.

The availability of inbred mice made possible a wide range of studies of immunology, immunogenetics, and radiation biology, too numerous to review completely herein. The book by van Bekkum and de Vries (11) summarizes many of the early

## 4 CHAPTER ONE

observations. Some of the more important studies demonstrated that:

1. Marrow given intravenously was just as effective in repopulating the marrow spaces as marrow given by any other route (12).
2. Marrow, an immunologically competent organ, could mount an immune attack against the host, resulting in graft-versus-host disease (GVHD) (11).
3. The severity of the immune reaction of donor cells against the host was controlled by genetic factors (13).
4. Methotrexate (MTX) could prevent or ameliorate the GVH reaction (14,15).
5. Cyclophosphamide (CY) alone could provide immunosuppression sufficient for allogeneic engraftment (16).

Finally, in mice, the importance of the thymus, T cells, B cells, and other lymphoid subsets in immunogenetics and transplantation biology began to be understood (17-19).

### Marrow Transplant Studies in Other Animal Models

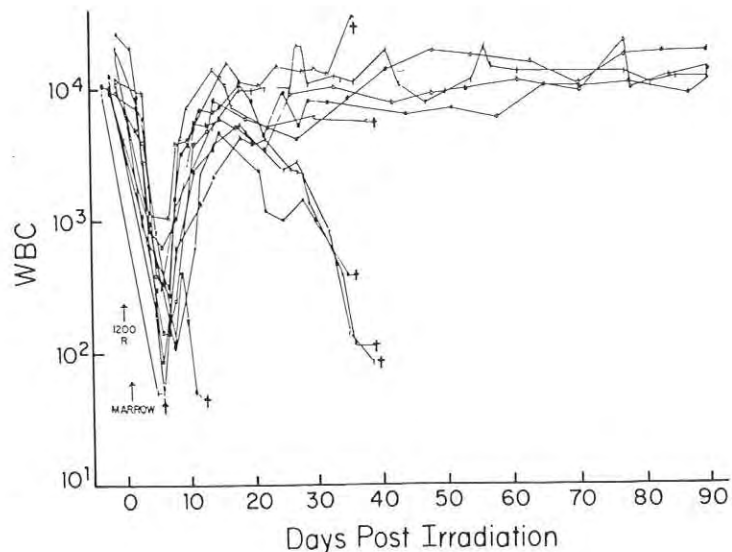
Other animal species have had important roles in transplantation biology. The inbred rat has made possible the extension of observations in the mouse, particularly the study of immunosuppressive drugs (20). Busulfan was first introduced into marrow transplant preparative regimens in the rat (21). The rabbit has been widely used in studies of skin grafting and in studies of humoral immunity (1,8). The pig has

been particularly informative in liver transplantation (22) and in studies of chimerism after marrow grafting (23). The monkey has provided important information on the pathology of GVHD (24). In preclinical studies applicable to human patients, the dog has been the most widely used and informative species.

### Marrow Transplant Studies in the Canine Model

For more than three decades the dog has served as a random-bred animal model for studies of principles and techniques of BMT applicable to humans. Early studies of radiation and marrow grafting in dogs, carried out before the modern knowledge of the histocompatibility complex, identified the problems of graft rejection and GVHD and resulted in some long-term canine chimeras that survived in good health for a number of years (Figure 1-1) (25,26).

The dog offers several advantages over other animal species for transplantation research. Dogs are generally available and relatively inexpensive. They can be kept disease-free in a suitable colony and are easy to work with. They are large enough to obtain serial blood and marrow samples and organ biopsies and to allow external gamma-camera scanning of the infusion of radiolabeled antibodies. Large canine families are regularly available for genetic studies of istocompatibility antigens, and they provide matched littermate pairs, stimulating HLA-identical human sibling pairs. The major canine histocompatibility complex, DLA, is now well defined. An added attraction of the dog is the availability of animals with spontaneous malignant and nonmalig-



**Figure 1-1.** Results of marrow transplants in dogs before tissue typing became available. Eleven dogs were given TBI, 12 R (measured in air), and post-grafting MTX. This figure shows the white blood cell count in these animals demonstrate failure of engraftment, graft rejection, or GVHD (with normal counts) all followed by death (+). Some animals became long-term survivors. (Reproduced by permission from Crouch BG, van Putten LM, van Bekkum DW, de Vries MJ. Treatment of total-body x-irradiated monkeys with autologous and homologous bone marrow. *J Natl Cancer Inst* 1961;27:53-65.)



**Figure 1-2.** Recipients of allogeneic marrow grafts 2 to 12 years after transplant. Dogs with graying hair color were conditioned by 9.2 Gy TBI, whereas the large hound was conditioned by cyclophosphamide.

nant hematological diseases resembling those encountered in humans.

### Conditioning Regimens

Conditioning programs serve to suppress the recipient's immune system for acceptance of the marrow graft and to eradicate the recipient's underlying disease that made treatment by BMT necessary. Total body irradiation (TBI) has been the most commonly used conditioning program for BMT. Dogs not given a marrow infusion usually die from complications of marrow failure when exposed to a TBI dose of 4 Gy, although dogs given intensive care may occasionally survive that exposure (27). Consistent survival following otherwise lethal doses of TBI can be obtained when dogs are given an infusion of autologous marrow, fresh or cryopreserved (28,29). Successful allogeneic marrow grafts from DLA-identical littermates are consistently obtained only after a TBI dose of more than 9 Gy, delivered at a dose rate of 7 cGy/min (30). Grafts from DLA-nonidentical unrelated or littermate donors require TBI doses in excess of 15 Gy (31). Dogs with successful grafts have, as a rule, been complete chimeras (i.e., cells from marrow, peripheral blood, lymph nodes, and bronchoalveolar fluid have all been of donor origin).

The fact that many leukemic recurrences were seen after human marrow grafting stimulated research into increasing the dose of TBI by the use of dose fractionation. Hematopoietic cells are less capable of undergoing DNA repair after a dose of fractionated TBI than other tissue cells. Thus, administering multiple fractions of, for example, 200 cGy of TBI with at least 3-hour interfraction intervals would lead to severe marrow toxicity, whereas other organs would be much less affected. Bodenberger and colleagues (32) administered doses of TBI ranging from 18 to 23 Gy, given in fractions of 4.5 to 9 Gy, at rates of 4 cGy/

min over periods of 3 to 7 days. Most dogs survived these very high doses of TBI with the help of a subsequent infusion of autologous marrow. Deeg and associates (33) explored fractions of 1.5 to 2 Gy, given at intervals of 3 to 6 hours, for total doses of 12 to 21 Gy, with dose rates ranging from 2 to 20 cGy/min. The acute radiation toxicity of single- and fractionated-dose TBI regimens was comparable at equal total doses. Lower dose rates permitted higher total doses and vice versa. Long-term complications were significantly less in dogs given fractionated TBI compared with those given a single exposure. Late radiation deaths were related to liver failure, malnutrition, and pancreatic atrophy. Although the effects of fractionated and single-dose TBI on the myeloid elements of the marrow are comparable, the immunosuppressive effects of fractionated TBI are inferior to those of single-dose TBI. Studies in dogs have shown a much higher rate of graft failure when fractionated TBI was used in preparation for the transplant (34).

Consistent with findings in mice, rats, and monkeys, the alkylating agent CY can be substituted for TBI to prepare dogs for allogeneic marrow transplants (35). CY-conditioned dogs often show persisting mixtures of host and donor hematopoietic cells. This finding may be undesirable in efforts to condition patients with hematopoietic malignancies for marrow transplants, but can be disregarded in patients with nonmalignant disorders of the marrow.

Following studies with busulfan (BU) in the rat, the BU derivative, dimethylbusulfan, was studied extensively in dogs (36,37). The major advantage of dimethylbusulfan over BU is that it can be injected intravenously. The LD<sub>99</sub> was 7.5 mg/kg in the absence of autologous marrow infusion. When autologous marrow was infused, dogs were able to survive a dose of 10 mg/kg. Marrow grafts were not successful when donors were DLA-nonidentical. However, when DLA-identical littermate marrow was used, approximately

## 6 CHAPTER ONE

half the animals showed long-term sustained engraftment. The other half rejected their grafts, presumably as a result of disparity for minor histocompatibility antigens outside of DLA. Graft failure could be prevented by the addition of immunosuppression with antithymocyte serum to the dimethylbusulfan preparative regimen.

## Resistance to DLA-nonidentical Marrow Grafts

Graft resistance reflects the action of host cells that are insensitive to the usual doses of TBI and do not require prior sensitization to donor histocompatibility antigens to destroy an allogeneic marrow graft. Although the phenomenon of hybrid resistance—rejection of marrow grafts from homozygous parents by the F1 hybrid recipients—had been well described in mice, studies in the dog model drew attention to allogeneic resistance (reviewed in 38). After 9.2 Gy of TBI, grafts of  $4 \times 10^8$  marrow cells/kg were successful when donors were genotypically DLA-identical littermates, but usually did not succeed in DLA-nonidentical recipients (31). The genetic determinants involved in resistance appear to be separate from but linked to the recognized antigens of the DLA complex. The recipient cells mediating resistance appear to be large granular lymphocytes that do not express classic T-cell markers. They are not susceptible to treatment of recipients with antithymocyte serum or cyclosporine (CSP). Also, injections of recipients with silica particles, L-asparaginase, *Corynebacterium parvum*, or CY to overcome resistance were either unsuccessful in enhancing engraftment or gave inconsistent results (31,38). Success was achieved by treating the recipients with a monoclonal antibody directed against a leukocyte adhesion molecule, CD44 (39). The mechanism of this enhancement is currently under investigation.

Hematopoietic engraftment could be achieved by increasing the radiation dose or by adding viable peripheral blood mononuclear cells to the marrow inoculum (31). Similar success was seen when donor thoracic duct cells were added, indicating that the graft-enhancing effect is mediated by donor lymphocytes, either through immunological destruction of the host cells involved in resistance or by providing an accessory function promoting both differentiation and self-renewal of hematopoietic stem cells (31). In vitro irradiated mononuclear cells were ineffective.

## Hematopoietic Precursor Cells From Sources Other Than the Marrow

Dogs, as well as mice, guinea pigs, rats, and baboons, have circulating pluripotent hematopoietic stem cells in their blood (40,41). These cells can be collected effectively with leukapheresis techniques and are capable of repopulating marrow in otherwise lethally irradiated animals. Long-term repopulation of canine marrow by cells of donor type has been shown (41,42).

## The Role of Histocompatibility Matching

Histocompatibility is an important factor governing graft acceptance, development of lethal GVHD, or eventual survival of the recipient after BMT. DLA is similar to the major histocompatibility complex in other species. Two serologically determined loci, DLA-A and DLA-B, and a third locus, DLA-D, defined by mixed leukocyte culture, have been recognized (43,44). The molecular structure of the canine histocompatibility complex also resembles that of other species (45,46). There is considerable polymorphism of antigens at each of the loci.

The dog was the first random-bred species in which the prospective value of in vitro histocompatibility typing for the outcome of marrow transplants was shown (47). Littermates genotypically identical for DLA survived significantly longer after BMT than their DLA-nonidentical counterparts. Despite DLA genotypic identity, GVHD was severe in some animals, indicating the need for immunosuppression after grafting (48). Presumably, GVHD in this setting is directed at "minor" transplantation antigens other than DLA. These studies emphasized the need for immunosuppression after transplantation, even in histocompatible situations. Encouraged by findings in dogs, human BMT began in the late 1960s to use siblings who were genotypically identical with their recipients for HLA. Subsequent studies in unrelated dogs of different breeds suggested that long-term survival can be achieved in some recipients of phenotypically DLA-identical unrelated marrow, although with a higher incidence of GVHD (49).

## The Influence of Transfusions on Subsequent Marrow Grafts

Transfusions given before a marrow graft can influence the outcome (reviewed in 31). Marrow rejection was seen in all dogs given three preceding whole blood transfusions from their littermate marrow donors before TBI. Even with only one preceding transfusion from the DLA-identical marrow donor, 75% of dogs rejected a subsequent marrow graft. These results can be explained by sensitization of recipients to polymorphic minor histocompatibility antigens outside of DLA, which are undetected by the usual in vitro histocompatibility typing techniques. Sensitizing cells seem to be dendritic cells contained in the transfused blood. The 100% incidence of rejection after three transfusions of whole blood from the marrow donor is consistent with at least two polymorphic histocompatibility systems outside of DLA being involved in sensitization. The notion that several minor polymorphic loci have a role in sensitization made it likely that graft rejection would be a problem after blood transfusions from unrelated donors. Under those circumstances, graft rejection would be seen if one or more of the blood transfusion donors had minor antigens in common with the marrow donor and if

these antigens were not present on the cells of the recipient. Indeed, our studies showed that 9 preceding blood transfusions from randomly selected, unrelated donors resulted in rejection of marrow from DLA-identical littermates in 40% of cases.

Subsequent studies in dogs pointed the way to avoiding or overcoming transfusion-induced sensitization. The use of buffy coat-poor blood products reduced the incidence of rejection, presumably because of the removal of antigen-presenting mononuclear cells from the transfusion. The combination of an alkylating agent and antithymocyte serum successfully overcame transfusion-induced sensitization. Most recent studies have shown that the sensitizing ability of blood transfusions can be abrogated by treatment of blood products with either ultraviolet light irradiation (50) or gamma irradiation (51). In the future, therefore, problems resulting from transfusion-induced sensitization should be significantly diminished in magnitude.

Graft-versus-host Disease

Theoretically, GVHD can be expected in all allogeneic marrow graft recipients because of the multiple differences of polymorphic histocompatibility antigens. T cells in the marrow inoculum recognize host histocompatibility antigens as foreign, become sensitized, proliferate, and attack the tissue cells of the recipient, thereby producing the clinical picture of GVHD in skin, gut, and liver.

Studies in dogs showed that GVHD can occur even among DLA-identical littermates (reviewed in 52). When transplants are carried out between DLA-nonidentical recipient pairs, GVHD occurs more rapidly and is fatal in all animals when no postgrafting immunosuppression is given (Figure 1-3). The inci-

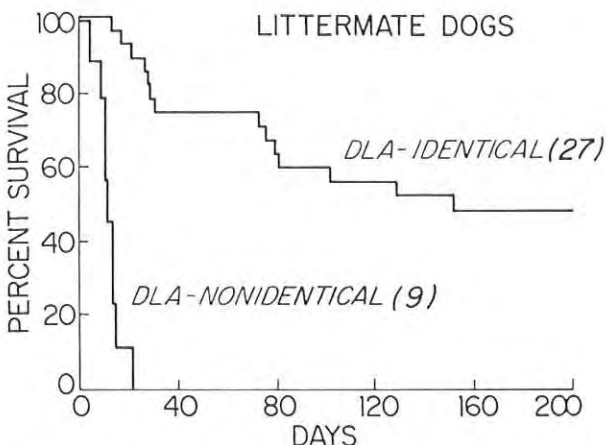


Figure 1-3. Survival of dogs given 9.2 Gy TBI and hematopoietic grafts from either DLA-identical or DLA-haploidentical littermates. No immunosuppression was given after transplant.

dence of GVHD after grafts between unrelated dogs phenotypically DLA-identical was much higher than that seen among DLA-identical littermates. This finding is likely to be related to the greater degree of disparity for minor histocompatibility antigens in the unrelated pairs.

On the basis of studies in mice (14,15), MTX was used in dogs and was shown to be effective in preventing GVHD in some recipients (25). The drug was more effective when given for 3 months after grafting than when given for shorter periods (Figure 1-4). Eventually, it could be discontinued. Azathioprine and 6-mercaptopurine were somewhat effective, but were inferior to MTX. Procarbazine, CY, cytosine arabinoside, 15-deoxyspergualine, and prednisone were all ineffective in preventing GVHD. Antithymocyte serum had only a marginal effect on GVHD when given prophylactically, but was of value in treating established GVHD. On the basis of the early canine studies, MTX was administered prophylactically in most human marrow transplant recipients during the 1970s, and antithymocyte globulin was used to treat acute GVHD, once established.

In the late 1970s, the immunosuppressive drug CSP became available and was found to be as effective as MTX in preventing GVHD in dogs. The equivalency of the two drugs was subsequently confirmed in randomized prospective human studies. A very effective prophylactic regimen in the dog was a combination of a short course of MTX and long-term CSP after BMT (Figure 1-5) (53,54). This combination was then used in human patients in 1981. Randomized prospective trials showed the combination to be more effective than either drug alone. This is now a frequently used regimen for GVHD prophylaxis.

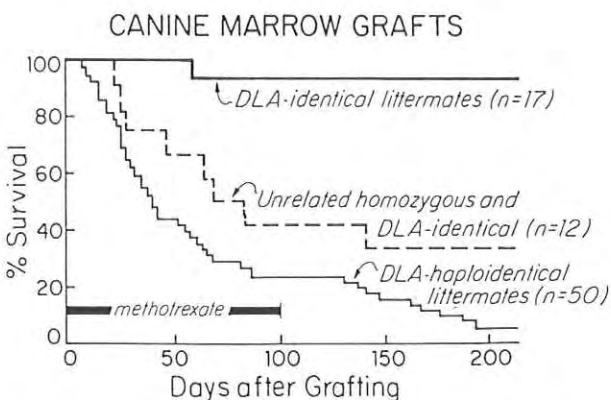
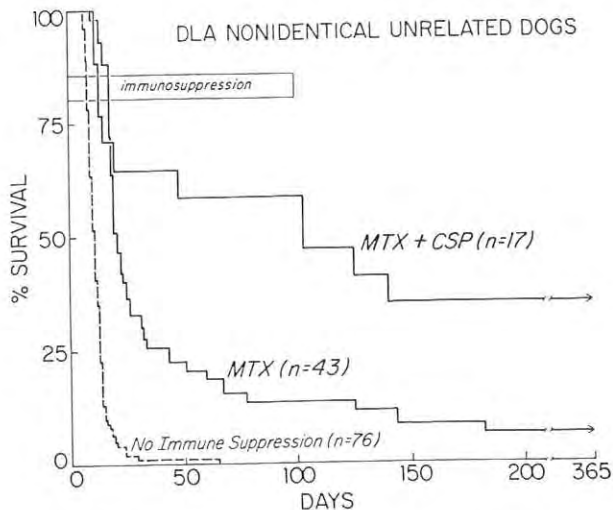


Figure 1-4. Survival of dogs given 9.2 Gy TBI and hematopoietic grafts from genotypically DLA-identical littermates, phenotypically DLA-identical unrelated donors, or DLA-haploidentical littermates. All dogs were given immunosuppression with intermittent methotrexate for 100 days after grafting.

## 8 CHAPTER ONE



**Figure 1-5.** Survival of dogs given 9.2 Gy TBI and hematopoietic grafts from DLA-identical unrelated donors. Dogs were given no immunosuppression, methotrexate for 100 days after grafting, or a short course of methotrexate (0.4 mg/kg IV) on days 1, 3, 6, and 11, along with cyclosporine from day 0 to day 100.

### Graft-host Tolerance

Lifelong graft-host tolerance has been documented repeatedly in canine recipients of marrow allografts, even though immunosuppressive therapy was discontinued after a few weeks or months. These dogs have served in studies on the nature of the operational tolerance involved in maintaining the stable chimeric state (55–58). In vitro tests showed that donor lymphocytes circulating in recipient dogs were specifically nonresponsive to lymphocytes of host origin while responding to those from unrelated animals. In turn, lymphocytes from dogs with GVHD showed strong reactivity to host lymphocytes in mixed leukocyte culture. Serum blocking factors were ruled out as a mechanism of maintaining unresponsiveness in stable chimeras, and results of further studies suggested that clonal abortion was operative whereby newly generated lymphocytes potentially reactive with host tissue are removed. Transplantation studies with a combination of marrow and peripheral blood cells showed that tolerance to host tissue could be transferred, a finding which is in agreement with the concept of suppressor cells. Consistent with findings in mice, grafts of solid organs from marrow donors could be transplanted into recipients without the need of immunosuppression.

### Reconstitution of Immunological Function

Extensive studies were carried out on the recovery of humoral and cellular immune responses after canine BMT (59). Regardless of the type of graft, dogs were found to be profoundly immunodeficient for the first 200 to 300 days after transplantation. Thereafter,

immunological reconstitution appeared to be nearly complete except for dogs with chronic GVHD. As a clinical correlate to these observations on immune function, long-term chimeras regained their health and were able to live in an unprotected environment without an increased incidence of infection.

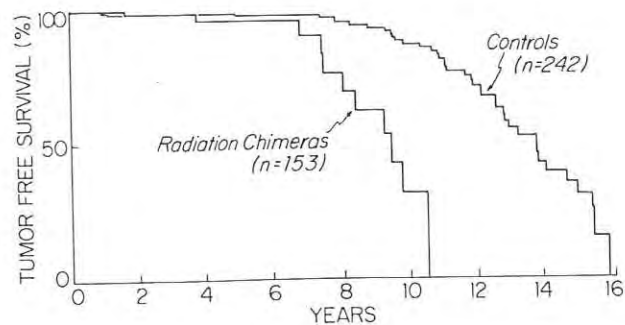
### Secondary Malignant Tumors and Other Long-range Irradiation Effects

Many canine chimeras have been observed for more than 10 years after BMT. Careful studies on gonadal function in canine chimeras have not been carried out. However, as a rule, chimeras prepared with TBI appeared to be sterile, but some dogs have become impregnated and others have sired normal litters. Chimeras prepared with CY had normal fertility. Radiation-induced cataracts developed in most dogs.

The increased risk of development of cancer after irradiation has been known since the days of the early radiologists, a knowledge that has been extended by the observations in the victims of the atomic bomb explosions in Hiroshima and Nagasaki. There has therefore been concern about the possibility of the development of malignant tumors after TBI in preparation for marrow grafting. A comparison of the cumulative cancer incidence among 153 long-term canine irradiation chimeras and 242 untreated dogs observed for 6 to 188 months (median, 81) showed a 5 times higher incidence of cancer in irradiation chimeras (60) (Figure 1-6). No tumor was seen in a smaller number of chemotherapy-conditioned animals followed for a comparable period. The canine findings suggested that TBI should be avoided whenever possible in conditioning of human marrow transplant recipients for nonmalignant disease.

### Marrow Grafting for Spontaneous Canine Diseases

Diseases such as hemolytic anemia associated with hereditary pyruvate kinase deficiency; cyclic neutro-



**Figure 1-6.** Kaplan-Meier product limit estimates of tumor-free survival of otherwise normal dogs (controls) and dogs given 9.2 Gy TBI followed by either autologous or allogeneic hematopoietic grafts (radiation chimeras).

penia; hemophilia, spontaneous malignancies, including lymphoma, leukemia, and nonhematopoietic solid tumors; as well as inborn errors of metabolism are valuable models for studying the use of therapeutic marrow transplantation.

It was possible to show that cyclic neutropenia is not the result of a defect of marrow regulation, but rather of a stem-cell defect that is correctable by BMT (61,62). Orthotopic transplantation of a normal liver into a hemophilic dog completely corrected the factor VIII deficiency (63). Marrow grafting studies ruled out the hematopoietic and lymphoid systems as sources of factor VIII production (64). Severe life-threatening hemolytic anemia due to pyruvate kinase deficiency has been corrected by marrow grafting (65,66); increased iron stores in the liver associated with the hemolytic process decreased with time after transplantation. Studies in canine lymphosarcomas have shown that one fourth of dogs at an otherwise incurable stage of disease could be cured by autologous marrow grafts following TBI (67). BMTs in dogs with ceroid lipofuscinosis and GM<sub>1</sub> gangliosidosis, although technically successful, showed that these genetic defects are not reversible using this approach (reviewed in 68). Fucosidosis and mucopolysaccharidosis have been shown to be correctable.

### Gene Transfer

The ability of pluripotent stem cells not only to self-renew but also to differentiate into the various committed hematopoietic precursors has made them potential targets for attempts at somatic gene therapy. It is now reasonable to contemplate the replacement of missing or defective genes in hematopoietic tissues to cure potentially lethal hereditary or acquired disorders. Currently, retroviral vectors represent the preferred method for gene transduction because they provide high efficiency of transduction and stable integration of the provirus into the host genome. Gene transduction into long-term repopulating marrow cells has been shown in the mouse. In 1991, Schuening and colleagues (69) reported successful long-term gene transfer into canine hematopoietic cells. Amphotropic helper-free retrovirus vectors containing the bacterial neomycin phosphotransferase gene (neo) and the human adenosine deaminase (ADA) gene were used to transduce canine marrow cells, which were kept in a long-term culture system. The marrow was then transplanted into the dog of origin after an otherwise lethal dose of TBI consisting of 9.2 Gy. The longest surviving dogs are now approaching 3 years after BMT. Their marrows show intermittently between 1 to 11% neomycin-resistant CFU-GM colonies. Concurrent polymerase chain reaction analysis demonstrates the presence of both the neo and human ADA genes in marrow cells, granulocytes, and blood and lymph node lymphocytes. These findings provide evidence for gene transduction into pluripotent hematopoietic stem cells. Peripheral blood samples of all dogs were free of helper virus, and no long-term side effects of

the gene transduction were observed. Similar findings have now been reported by Carter and associates (70).

### Radiolabeled Monoclonal Antibodies

Monoclonal antibodies directed against antigens on tumor cells offer an opportunity for directed anticancer therapy. Monoclonal antibodies injected in vivo can concentrate on tumor cells, but they generate only a limited antitumor effect because some tumor cells lack target antigens, whereas others, although coated by antibody, are not killed. Studies are ongoing with antibodies linked to toxins, such as the ricin-A chain, for more effective cancer cell kill. Another possible way to use monoclonal antibodies is to attach them to short-lived radioactive isotopes. Not only cells expressing the target antigens, but also neighboring cells, which may be antigen-negative, would be killed.

Studies in dogs have shown appropriate antibody isotope conjugates to localize preferentially in marrow and spleen, and to some extent also in lymph nodes; the amount of isotope in the marrow compared with other organs achieved a ratio of 28:1 or more (71,72). Such radiolabeled antibodies can produce fatal marrow aplasia, which can then be reversed by infusion of cryopreserved autologous marrow several days later when very little radioactivity is left. These studies are continuing, and various combinations of chemotherapy, TBI, and radiolabeled antibody are being explored for their efficacy in preparing dogs for grafts of T-cell-depleted marrow. It is hoped that refinements of this approach, particularly the use of high-energy beta-emitting isotopes with short linear energy transfer, will ultimately result in less toxic and more efficient conditioning programs, which would not only provide better eradication of malignant disease, but also lessen the problem of graft failure.

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## 10 CHAPTER ONE

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