Transplantation of X-Linked Severe Combined Immunodeficient Dogs with CD34+ Bone Marrow Cells

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ABSTRACT
X-linked severe combined immunodeficiency (X-SCID) is the most common form of human SCID and is caused by mutations in the common γ chain (γc), a shared component of the interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15, and IL-21 receptors. BMT for human X-SCID results in engraftment of donor T-cells and reconstitution of normal T-cell function but engraftment of few, if any, donor B-cells and poor reconstitution of humoral immune function. Canine X-SCID is also caused by mutations in the γc and has an immunological phenotype identical to that of human X-SCID. We have previously reported that transplantation of nonconditioned X-SCID dogs with unfractionated histocompatible bone marrow results in engraftment of both donor B- and T-cells and reconstitution of normal T-cell and humoral immune function. In this study, we assessed the ability of purified canine CD34+ bone marrow cells to reconstitute lymphoid populations after histocompatible BMT in 6 nonablated X-SCID dogs. All dogs showed engraftment of donor T-cells, with T-cell regeneration occurring through a thymic-dependent pathway, and had reconstituted normal T-cell function. In contrast to our previous studies, only 3 dogs had engraftment of donor B-cells and reconstituted normal antigen-specific B-cell function post-BMT. The variable donor B-cell engraftment and reconstitution of normal humoral immune function observed in this study are similar to the outcomes observed in the majority of human X-SCID patients following BMT. This study demonstrates that canine CD34+ cells contain progenitors capable of immune reconstitution and is the first study to document the ability of CD34+ bone marrow cells to reconstitute normal B- and T-cell function in a nonablated large-animal model of BMT. This study also demonstrates that the quality of immune reconstitution following CD34+ BMT may be dosage dependent. Thus canine X-SCID provides a large-animal preclinical model that can be used not only to determine the optimal conditions for both donor B- and T-cell engraftment following CD34+ BMT, but also to develop and evaluate strategies for gene therapy protocols that target CD34+ cells.

KEY WORDS
Canine • X-linked severe combined immunodeficiency • CD34 • Bone marrow transplantation • Immune reconstitution

INTRODUCTION
X-linked severe combined immunodeficiency (X-SCID) is caused by mutations in the gene encoding the common γ chain (γc), a shared signal transducing subunit of the interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15, and IL-21 receptors [1-10]. Defects in the γc account for approximately 50% of cases of SCID and most cases of T–B+ SCID [11]. In humans, the disease is characterized by low to absent peripheral T-cells and natural killer (NK) cells and normal or elevated levels of peripheral B-cells and by a marked inability to mount effective cell-mediated and humoral immune responses. If not successfully treated by bone marrow transplantation (BMT), X-SCID is fatal within the first 2 years of life [12]. This severe phenotype of X-SCID can be attributed to the pleiotropic effects of γc-containing receptors on the development and maintenance of immune...
CD34 BMT for Canine X-SCID

ing that CD34 is also a marker for dog bone marrow progenitor cells [39]. To further evaluate the role of CD34+ cells in lymphohematopoietic development, we have used the CD34+ population of normal canine bone marrow for transplants in X-SCID dogs. The results indicate that CD34+ canine marrow cells contain T- and B-lymphoid progenitors capable of immune reconstitution after transplantation into X-SCID dogs, with outcomes similar to those observed after human clinical transplantation. These experiments establish the X-SCID dog as a valuable model for evaluating and enhancing both BMT and gene therapy protocols for X-SCID.

MATERIALS AND METHODS

X-SCID Genotyping and Dog Leukocyte Antigen Typing

X-SCID dogs were derived from a breeding colony established from a carrier female that harbored a 4-base pair (bp) deletion in the first exon of the γc gene [27]. Diagnosis of X-SCID pups employed a polymerase chain reaction (PCR)–based mutation detection assay that was performed at less than 1 week of age [27]. Inheritance of dog leukocyte antigen (DLA) alleles within families was determined by a PCR technique based on the inheritance of a microsatellite locus within the class II region of the DLA and was used to select matched healthy sibling donors [40]. Primer sequences LUC2202 reverse 5′-TCTGTGGGAAGAG GTAAGTTCA-3′ and LUC2202 forward 5′-CGTTCT GTGCTACATTTCTGGTAT-3′ were used with 30 cycles of PCR programs 94°C-1′, 62°C-1′, and 72°C-1′. Amplicons were visualized on an 8% nondenaturating polyacrylamide gel. The presence of matching bands was used to identify a DLA-matched sibling donor for each affected X-SCID dog.

Transplantation in X-SCID Dogs

All experiments were performed in accordance with the protocols approved by the University of Pennsylvania Institutional Animal Care and Use Committee (IACUC #1360600). Healthy DLA-matched donors were killed and their bones obtained. The bones were cracked, scraped, and flushed with RPMI 1640 medium. The marrow was teased apart and passed through a sterile 70-µm cell strainer (Becton Dickinson, Franklin Lakes, NJ). The resulting cell suspension was centrifuged on a Ficoll-Hypaque 1.077 gradient. The presence of matching bands was used to identify a DLA-matched sibling donor for each affected X-SCID dog.

B & M T
Monitoring of X-SCID Dogs after Transplantation

The X-SCID dogs that underwent transplantation were monitored for evidence of engraftment at 4-week intervals. Assays that were performed included proliferation assays, complete blood cell count, flow cytometric analysis of lymphocyte subsets, and determination of serum IgG levels. For proliferation assays and flow cytometry, EDTA anticoagulated blood was obtained and diluted 1:1 in Hanks’ balanced salt solution (HBSS) without Ca++ or Mg++, layered over a 1.066 Ficoll-Hypaque gradient, and centrifuged at 400g for 20 minutes [42]. Peripheral blood mononuclear cells (PBMCs) were subsequently washed twice in HBSS, counted, and resuspended in RPMI 1640 supplemented with 10% fetal calf serum.

Proliferation Assays

PBMCs were plated in triplicate at 10⁴ cells per well in a 96-well microtiter plate (Costar, Corning, NY) and incubated for 2 days at 37°C in a humidified 5% CO₂ incubator in triplicate with concentrations of phytohemagglutinin (PHA-P) (Sigma) varying from 1 to 4 in triplicate with concentrations of phytohemagglutinin thymidine, 0.5 μg/mL. Tritiated thymidine, 0.5 μCi (ICN, Costa Mesa, CA), was added to cultures after 2 days, and cells were harvested on glass microfiber filters (Whatman, Maidstone, England) using an automated cell harvester after 18 hours of additional incubation. Filter disks were removed, suspended in liquid scintillation cocktail (Ecolume, ICN), and counted in a LS 6500 scintillation counter (Beckman Coulter, Fullerton, CA).

Flow Cytometry

Staining was performed on aliquots of 1 x 10⁶ Ficoll separated peripheral blood lymphocytes. PBMCs were washed in PBS containing 1% BSA, centrifuged, resuspended in PBS-BSA, and incubated for 20 minutes at 4°C with fluorescein isothiocyanate (FITC)-labeled antibodies to canine surface Ig (Rockland, Gilbertsville, PA) or unlabeled monoclonal antibodies to canine CD3 (CA17.2A12), CD4 (LSM-12.125), CD8 (LSM-4.78), CD21 (CA2.1D6), CD45RA (CA4.1D3), and CD3-like (LSM-8.338). For indirect labeling, PBMCs were washed twice with PBS-BSA and incubated with labeled secondary antibodies, FITC antimuscle-specific IgM, or PE-labeled antimouse IgG-specific antibodies (Jackson Immunoresearch). Analysis of 10,000 events was performed using a FACScalibur flow cytometer (Becton Dickinson). For cell sorting, PBMCs were stained with FITC-labeled anticanine CD3 and PE-labeled anticanine CD21. Cells were sorted by fluorescence into B-cell and T-cell fractions using FACSvantage and FACStar flow cytometers (Becton Dickinson).

Quantification of Serum IgG

Serum IgG was determined by radial immunodiffusion using canine IgG-specific plates (Bethyl, Montgomery, TX).

Assessment of Antigen-Specific Antibody Response

After donor cells were detected in dogs that underwent transplantation, the dogs were immunized intramuscularly with 0.5 mL tetanus toxoid (Lederle, Pearl River, NY). Animals were reimmunized with tetanus toxoid 2 weeks after the initial immunization. Serum samples were obtained from the immunized animals at weekly intervals for 4 weeks after the initial immunization. IgG-specific tetanus toxoid-specific antibody was determined using an enzyme-linked immunosorbent assay (ELISA). Ninety-six-well ELISA plates (Costar) were coated overnight at 4°C with 200 μL of carbonate buffer (pH 9.6) containing 0.5 Lf units/mL of tetanus toxoid (Wyeth-Ayerst Laboratories, Marietta, PA). The plates were washed 3 times with PBS-0.05% Tween 20 (PBS-T). Serial 2-fold serum dilutions starting at 1:250 were made in buffer, and 200 μL was added to the antigen-coated wells. All samples were tested in duplicate. The plates were incubated at room temperature for 2 hours and washed 3 times with PBS-T. Subsequently, the plates were incubated for 2 hours at room temperature with peroxidase-labeled antidog IgG (γ heavy chain specific, Bethyl) diluted to 1:5000 in PBS-T. Plates were washed 3 times with PBS-T and then incubated for 20 minutes with 200 μL of o-phenylenediamine + 0.014% H₂O₂ substrate solution (Sigma). The reaction was stopped by the addition of 50 μL of IN H₂SO₄ to each well. Absorbance at 490 nm was measured by a microplate ELISA reader (Molecular Devices, Menlo Park, CA). Endpoint titers were determined by calculating the reaction of each test sample to the negative preimmunization sample. A ratio of >2.0 with an optical density reading >0.2 was considered positive.

Chimerism Detection Assay

DNA was extracted from sorted cell populations (Gentra Systems, Minneapolis, MN), and PCR was performed using primers that flank the 4 bp of the γc gene that are missing in affected X-SCID dogs. The 5’ primer was labeled with 32P. Products from the PCR reaction were separated on a standard 6% denaturing polyacrylamide sequencing gel by electrophoresis at 2000 V for 7 hours. Gel data were captured using a GS525 storage phosphorimaging system (BioRad, Hercules, CA) and analyzed using Multianalyst software. Background counts were subtracted, and then the data were normalized to the actual ratio of 32P in the larger (169-bp normal) and smaller (165-bp mutant) PCR products seen from the control carrier female. This procedure corrects for any differences in PCR efficiency between the 163- and 169-bp products. After the data were adjusted based on the control carrier female (which was consistently between 47% and 53%), the percentage of donor cells was calculated as the percentage of counts in the normal band compared to the total counts in both bands. All data were within the capacity of the phosphoimager to detect 32P in a linear range. Consequently, the percentage of donor cells reported is more accurate than the percentage calculated by visual examination of the computer-generated gel image.

RESULTS

Bone Marrow Transplantation

Six dogs that were between 1 and 3 weeks of age received CD34⁺ cell doses that ranged from 1.2 to 40.0 x 10⁶ cells/kg (Table). The purity of the CD34⁺ cells was greater than 96% except in 1 recipient (R862).
Normalization of Lymphocyte Subsets

During the first 4 weeks of life, X-SCID dogs may have absolute lymphocyte counts and B-cell numbers that are normal, whereas T-cells have invariably been absent at the time of BMT [25,26,29]. At 1 month post-BMT, R860, the animal that received the highest dose of CD34+ cells, had normal absolute lymphocyte numbers (Figure 1). The remaining animals had abnormally low absolute lymphocyte numbers at 1 month post-BMT. By 2 months post-BMT, lymphocyte counts had normalized in all of the animals except R775, the animal that received the lowest dose of CD34+ cells. This dog continued to have marginal to sub-normal lymphocyte counts throughout the study period of 6 months. Percentages of T-cells (Figure 2A) and absolute numbers of T-cells (Figure 2B) were normalized in all the animals except R775 at 2 months post-BMT. In R775, the percentage of T-cells was not normalized until 3 months after transplantation, and the absolute number of T-cells did not reach normal levels within the study period. Percentages of B-cells (Figure 2C) remained above normal in 4 of the animals at 1 month after transplantation but had normalized in all the animals but R862 by 2 months post-BMT. Absolute numbers of B-cells were at or above normal in all the animals throughout the study period (Figure 2D).

Reconstitution of T-Cell Function

Proliferation to PHA was used to assess the development of mitogen-responsive T-cells (Figure 3). At 1 month post-BMT, only 1 animal, R860, had normal lymphocyte proliferation. By 2 months post-BMT, all of the animals except R775, the dog that received the lowest dose of CD34+ cells, had normal responses to PHA. This dog did not attain normal levels of proliferation to PHA until 3 to 4 months after transplantation. After normalization, proliferative responses were stable throughout the remainder of the study.

The percentage of T-cells that were positive for the RA isoform of CD45 was assessed to distinguish newly generated naive T-lymphocytes from peripherally expanded T-cells [43]. This assessment was important because over time X-SCID dogs that do not receive transplants can develop nonfunctional T-cells that express an activated CD45RO phenotype [44]. By 2 months post-BMT, all animals except R775 showed normal percentages of CD45RA+ T-cells (Figure 4). Additionally, sorted PBMC fractions from the dogs that underwent transplantation showed that virtually all of the T-cells present in these animals after 2 months of age were donor derived. The development of donor-derived T-cells was similar to that observed with unfractionated bone marrow [29,30].

Reconstitution of B-Cell Function

The reconstitution of B-cell function in animals that underwent transplantation was assessed by measuring serum IgG levels, evaluating the IgG antibody response to tetanus toxoid, and evaluating the percentage of engrafted donor B-cells. IgG levels in these dogs showed a generally increasing trend after transplantation in all of the animals except R775 and R889 (Figure 5). The 4 dogs with increasing IgG levels had reached normal levels by 6 months post-BMT. High initial readings for IgG can be explained by the presence of maternally derived IgG.

In our previous transplantation studies using unfractionated bone marrow, virtually all of the T-cells post-BMT were of donor origin, whereas 30% to 50% of the total peripheral B-cells were of donor origin. The engraftment of donor B-cells in the recipients of CD34+ cells was assessed using a similar PCR-based assay (Figure 6). Two of the recipients had at least 15% donor B-cells. Two of the recipients had low levels (2%-4%) of donor B-cells, and 2 animals had <2% donor B-cells.

Animals were also tested for their ability to produce a specific IgG antibody response to a neoantigen, tetanus toxoid (Figure 7). The 2 animals that showed the highest level

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Details of Infused Bone Marrow Cells

<table>
<thead>
<tr>
<th>Animal</th>
<th>Donor</th>
<th>Total Cells, $\times 10^6$</th>
<th>Total Cell Dose, $\times 10^6$ cells/kg</th>
<th>CD34+, %†</th>
<th>CD34+ Cell Dose, $\times 10^6$ cells/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>R743-M</td>
<td>M</td>
<td>13.2</td>
<td>11.1</td>
<td>98.5</td>
<td>10.93</td>
</tr>
<tr>
<td>R775-M</td>
<td>M</td>
<td>1.0</td>
<td>1.2</td>
<td>97.9</td>
<td>1.17</td>
</tr>
<tr>
<td>R860-M</td>
<td>F</td>
<td>50.8</td>
<td>43.2</td>
<td>97.3</td>
<td>40.03</td>
</tr>
<tr>
<td>R862-M</td>
<td>M</td>
<td>16.2</td>
<td>19.5</td>
<td>91.1</td>
<td>17.76</td>
</tr>
<tr>
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<td>56.8</td>
<td>37.5</td>
<td>95.6</td>
<td>35.85</td>
</tr>
<tr>
<td>R889-M</td>
<td>F</td>
<td>27.5</td>
<td>30.3</td>
<td>96.7</td>
<td>29.3</td>
</tr>
</tbody>
</table>

*Total nucleated cells.
†Lymphoid gate.
of donor B-cell engraftment, R860 and R743, also had near-normal levels of antitetanus IgG. R862, R775, and R889 had undetectable or low levels of detected donor B-cells and also had correspondingly low tetanus toxoid IgG antibody responses. One animal (R868) had low levels of donor B-cell engraftment and a good tetanus toxoid response.

**DISCUSSION**

Previous studies have documented the ability of autologous and allogeneic DLA-identical canine CD34⁺ cells to rescue lethally irradiated normal dogs with kinetics of neutrophil and platelet recovery similar to those observed using unfractionated bone marrow [38,39]. This study extends those observations by demonstrating that highly purified DLA-identical canine CD34⁺ bone marrow cells are capable of engrafting donor B- and T-cells in nonablated X-SCID dogs. Although studies have shown that a population of human CD34⁻ cells also contains hematopoietic stem cell activity, it is unlikely that CD34⁻ cells contributed to the immunologic reconstitution seen in the dogs that underwent transplantation [45,46]. In one study, transplantation of 10⁷ autologous canine CD34⁺ cells/kg failed to rescue lethally irradiated normal adult dogs, with all 3 dogs dying by 20 days posttransplantation [38].

The canine X-SCID transplantations described in this study are most similar to those performed in human X-SCID patients who undergo transplantation within the first few months after birth. Two recent studies evaluating a large cohort of SCID patients who underwent BMT showed that young patient age at transplantation influences survival outcome [16,47].

**Figure 2.** Percentage and absolute numbers of B- and T-cells in X-SCID dogs after BMT. The percentages of peripheral T-cells (A) and B-cells (C) were determined through flow cytometry. Absolute numbers of T-cells (B) and B-cells (D) were calculated using absolute lymphocyte counts. Normal values for B-cell and T-cell percentages for dogs older than 1 month are 5% to 20% and 60% to 90%, respectively. Normal values for B-cell and T-cell absolute numbers for dogs older than 1 month are 177 to 708 cells/mm³ and 2124 to 3186 cells/mm³, respectively.

**Figure 3.** Proliferative response of peripheral blood lymphocytes to PHA in X-SCID dogs after BMT. Peripheral blood lymphocytes were obtained from dogs at monthly intervals after transplantation and stimulated with PHA. Normal proliferation values for animals older than 1 month are greater than 30,000 CPM.
The kinetics of T-cell engraftment observed with our CD34+ transplants were comparable to both those seen in human X-SCID patients receiving transplants of T-cell–depleted HLA-identical or haploidentical bone marrow cells and those seen in our previous studies of canine X-SCID using unfractionated bone marrow containing less than 1.5% mature T-cells. The proportions and absolute numbers of T-cells reached normal levels between 2 and 3 months posttransplantation in all but 1 dog, R775, which received the lowest dose of CD34+ bone marrow cells. R775 remained T-lymphopenic throughout the study. It is unlikely that any contaminating mature T-cells in the CD34+ cell preparations contributed to the T-cell engraftment in the dogs that underwent transplantation, because the majority of the T-cells were naïve CD45RA+ lymphocytes consistent with their being recent thymic emigrants [43,48]. It is likely that the majority of the CD3+ CD45RA+ cells observed post-BMT were CD4+ T-cells and were not from extrathymic expansion of CD8+ T-cells [49], because only 9.1% ± 3.1% of the T-cells in the animals were CD8 at 2 months post-BMT (data not shown). The in vitro proliferative response to T-cell mitogenic stimulation followed similar kinetics, with normal responses observed between 2 and 3 months posttransplantation with the exception of R775, in which the development of a normal proliferative response was delayed.

The consistent development of donor-derived T-cells in the nonmyeloablated X-SCID dogs is in contrast to the results seen with B-cell engraftment. In the present study, 2 of 6 dogs developed significant numbers of donor-derived B-cells (>15% of total B-cells), whereas 2 of the dogs had <2% donor B-cells. Both dogs with high levels of B-cell engraftment produced normal levels of antigen-specific IgG antibodies after immunization with tetanus toxoid. In contrast, the animals with low levels of B-cell engraftment generally had deficient humoral function. These results differ from those of our previous studies using unfractionated bone marrow, in which all X-SCID dogs that developed T-cell reconstitution also developed 20% to 50% donor-derived B-cells and normal levels of antigen-specific IgG antibody [29,30].

Although this study was not designed to test the effect of CD34 cell dose, it appears that cell dose does influence the quality of T-cell reconstitution. The dog that received the lowest dose of cells, 1 x 10^6 CD34+ cells/kg, exhibited a delayed T-cell reconstitution with the absolute number of T-cells never reaching normal values and an inability to produce IgG-specific antibody. In contrast, all dogs that received >10 x 10^6 CD34+ cells/kg exhibited normal T-cell reconstitution based on the parameters examined. Although it is clear that the dose of CD34+ cells appears to influence T-cell reconstitution, the effect of cell dose on reconstitution of normal humoral immune function is less clear. In general, the dogs that received the higher doses of CD34+ bone marrow cells appeared to be better able to produce IgG-specific antibody following immunization. Previous studies have also reported an association between CD34+ cell dose and rate of engraftment, with dogs receiving less than 10^6 CD34+ cells/kg exhibiting delayed neutrophil recovery and a markedly delayed platelet recovery [39].

The development of donor-derived T-cells but variable engraftment of donor B-cells in the present study is similar to the outcomes observed in published reports of HLA-identical and T-cell–depleted haploidentical BMTs in human X-SCID patients. All human patients with successful transplantation show engraftment of donor T-cells and attain normal T-cell function, but few patients have engraftment of donor B-cells, with the majority having continual humoral immune deficiencies that require many of these patients to be maintained on intravenous immune globulin (IVIG) therapy [16,17]. The presence of low levels of IgG antibody in canine and X-SCID patients who have not had engraftment of donor B-cells after BMT may be due to a γc-independent IL-4 signaling pathway [50,51]. However, recent reports have suggested that normal effective humoral immunity in human X-SCID patients who undergo BMT can only be achieved by the engraftment of donor B-cells, even at low levels [17,18]. Together, these findings indicate

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**Figure 4.** Proportion of CD45RA+ T-cells in X-SCID dogs at indicated time points after BMT. Peripheral blood lymphocytes were obtained from X-SCID dogs at monthly intervals and analyzed through flow cytometry to determine the percentage of CD3+ T-cells that expressed the RA isoform of CD45. Normal percentages for animals 1 through 6 months of age is greater than 85% CD45RA+ CD3+ T-cells.

**Figure 5.** Serum IgG concentrations in X-SCID dogs at indicated time points following BMT. Normal serum IgG concentration for 6-month-old healthy dogs is 1028 mg/dL ± 312 mg/dL.
that the best way to attain functional humoral immunity following BMT is to ensure the engraftment of significant numbers of normal donor B-cells.

The failure of many transplantation-treated X-SCID patients to show engraftment of significant numbers of donor B-cells suggests that much of the observed T-cell reconstitution is derived from either a small number of $\gamma c$ normal stem cells or committed lymphoid progenitors [52]. This derivation is possible because there is a selective advantage for a normal $\gamma c$ in lymphoid progenitors committed to the T-cell lineage. This developmental selective advantage does not apply to cells of the B- or myeloid lineage in humans or dogs. Therefore, the number of circulating $\gamma c$-positive B-cells and myeloid cells is an indicator of the extent of engraftment of true donor or gene-corrected progenitor cells. High levels of such engraftment should be a major goal for any BMT or gene therapy approach to treatment of X-SCID, not only to ensure normal B-cell function and obviate the need for IVIG treatment, but also to ensure durable T-cell function, especially considering that the production of thymically derived T-cells appears to occur throughout adult life [53,54].

One method of achieving this result could be the use of pretransplantation conditioning. Nonmyeloablative conditioning of recipients using busulfan (8 mg/kg) and cyclophosphamide (200 mg/kg) has recently been reported not to influence donor B-cell engraftment following BMT of human X-SCID patients; however, true myeloablative therapy could potentially improve the likelihood of donor B-cell engraftment [17]. Although true cytoreductive therapy prior to transplantation may improve the likelihood of donor B-cell engraftment, elucidation of the conditions allowing full immunologic reconstitution and engraftment of both donor B- and T-cells in X-SCID dogs receiving transplants without pretransplantation conditioning needs to be explored because cytoablation increases the risk of life-threatening infections during the posttransplantation period and requires lengthy hospitalization, thus increasing the cost of BMT. In addition, long-term sequelae, including growth retardation, sterility, learning disabilities, and possible malignancies, have to be considered following the use of pretransplantation cytoreductive therapy [55,56]. Pretransplantation conditioning may also have an adverse effect on the bone marrow, and possibly thymic, microenvironments, which would influence the outcome of the BMT [57-59]. In the absence of such a conditioning regimen, the engraftment of transplanted cells can essentially be viewed as a competitive repopulation in which the number of normal cells that are

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![Figure 6](image1.png)

**Figure 6.** PCR analysis of peripheral blood B-cells in X-SCID dogs after BMT. Peripheral blood B-cells were flow sorted from X-SCID dogs that underwent transplantation. Shown are the products of a PCR reaction that amplifies a region spanning the 4-bp deletion that occurs in our colony. The upper band represents the 169-bp normal PCR fragment, and the lower band represents the 165-bp mutant fragment. The percentage of total counts that were acquired in the normal band is expressed above each lane. Carrier indicates heterozygous female.

![Figure 7](image2.png)

**Figure 7.** IgG-specific antibody responses to tetanus toxoid in X-SCID dogs after BMT. Serum samples were obtained prior to immunization and at weekly intervals following primary and secondary immunization ($\uparrow$). An ELISA specific for tetanus toxoid was performed using 2-fold dilutions of serum starting at 1:250. Results of the dogs that underwent transplantation are compared with pooled sera from immunized healthy dogs.
infused is correlated with the amount of donor cell chimerism seen in the periphery [60]. Therefore, maximizing the number of normal cells that are infused, whether from healthy donors or transduced autologous cells, is of great importance.

Donor B-cell engraftment was observed in our previous studies of transplantation of unfractionated bone marrow in X-SCID dogs [29,30] as well as in some of the recipients in the present study in which CD34+ donor cells were used. In human X-SCID patients who receive transplants of either unfractionated marrow or T-cell–depleted marrow without myeloablative conditioning, donor B-cell engraftment is rarely observed [16,17]. The higher frequency of donor B-cell engraftment in the X-SCID dogs may reflect either the larger number of CD34+ progenitor cells transplanted in the canine experiments or the increased B-cell progenitor content of the canine CD34+ cells from young puppies. We have observed that approximately 14% of the nucleated marrow cells from 2- to 3-week-old puppies are CD34+ cells (Suter et al., unpublished data). As a result, the recipients of unfractionated marrow received estimated doses of CD34+ cells as high as 20 × 10^6 cells/kg. Similar high cell doses were given to most of the recipients of purified CD34+ cells in the present study. These doses of CD34+ cells are significantly higher than those used in human histocompatible or haploidentical BMT and may contribute to the higher frequency of B-cell engraftment observed in the dogs. Additionally, the CD34+ cells from very young dogs may have a higher frequency of B-lymphoid progenitors. Data from transplantation of human CD34+ cells into immunodeficient mice or patients have suggested greater B-lymphoid potential from umbilical cord blood cells [61-64]. It is possible that the CD34+ cells from the very young puppies used as donors in the present experiments are similar to umbilical cord blood cells in that they have a high potential for B-cell engraftment and generation. Thus, the canine experiments suggest several strategies to increase B-cell engraftment after nonmyeloablative transplantation, notably the use of high cell doses and very young donors.

Historically, the dog has been a valuable model for BMT, with many of the advances achieved in the dog being directly transferable to human clinical BMT protocols [65,66]. In canine X-SCID, unlike mouse γc-deficiency, the role of the γc in lymphoid development and function is similar to humans, allowing canine X-SCID to serve as a unique large-animal preclinical model to determine the optimal conditions for both donor B- and T-cell engraftment following allogeneic CD34+ BMT. Determination of the optimal conditions for engraftment of CD34+ cells will also be directly applicable to gene therapy studies because CD34+ cells are currently the target for transduction protocols.

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