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A Preclinical Model of Double- versus Single-Unit Unrelated Cord Blood Transplantation

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Cord blood transplantation (CBT) with units containing total nucleated cell (TNC) dose $>2.5 \times 10^7$/kg is associated with improved engraftment and decreased transplant-related mortality. For many adults no single cord blood units are available that meet the cell dose requirements. We developed a dog model of CBT to evaluate approaches to overcome the problem of low cell dose cord blood units. This study primarily compared double- versus single-unit CBT. Unrelated dogs were bred and cord blood units were harvested. We identified unrelated recipients that were dog leukocyte antigen (DLA)-88 (class I) and DLA-DRB1 (class II) allele-matched with cryopreserved units. Each unit contained $\leq 1.7 \times 10^7$ TNC/kg. Recipients were given 9.2 Gy total-body irradiation (TBI) and DLA-matched unrelated cord blood with postgrafting cyclosporine and mycophenolate mofetil. After double-unit CBT, 5 dogs engrafted and 4 survived long term with 1 dominant engrafting unit and prompt immune reconstitution. In contrast, 0 of 5 dogs given single-unit CBT survived beyond 105 days ($P = .03$, log-rank test); neutrophil and platelet recovery was delayed (both $P = .005$) and recipients developed fatal infections. This new large animal model showed that outcomes were improved after double-unit compared to single-unit CBT. After double-unit CBT, the nonengrafted unit facilitates engraftment of the dominant unit.

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INTRODUCTION

Cord blood transplantation (CBT) has emerged as an effective treatment for patients with malignant and nonmalignant hematologic diseases. Clinical experience has shown that the infused cell dose and degree of human leukocyte antigen (HLA) matching of cord blood units are associated with engraftment and outcome. Several studies have shown that total nucleated cell (TNC) count and $CD34^+$ cell doses above minimum thresholds are associated with improved engraftment and decreased transplant-related mortality (TRM) [1-4]. As a result, several transplant center guidelines recommended that the minimum threshold cell dose for a single cord blood unit is at least $\geq 2.5\text{-}3.0 \times 10^7$ TNC/kg for a 5/6 or 6/6 HLA-matched unit (with low resolution typing at HLA-A and -B and high resolution at −DRB1) [5-7]. With greater HLA disparity, the threshold cell dose is even greater: the minimal cell dose for a single 4/6 HLA-matched unit is at least $\geq 5.0 \times 10^7$ TNC/kg [6]. For many adults and older children no single units are available that meet these cell dose requirements. To overcome this limitation, investigators at the University of Minnesota introduced double-unit cord transplantation in which 2 cord units are infused simultaneously to increase the total infused cell dose [8,9]. Nonrandomized clinical studies indicate that compared to low cell dose single-unit CBT, double-unit CBT appears to have increased engraftment and decreased transplant related mortality [8,10,11]. Many transplant centers now routinely use double-unit CBT for adults, and there is currently a randomized clinical trial in progress comparing single- versus double-unit CBT in children. After double-unit CBT,

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in the vast majority of cases, a single cord blood unit emerges as the sole source of long-term hematopoiesis. Although TNC, $CD34^+$, $CD3^+$ cell count, sex-mismatch, ABO blood group, HLA mismatch, and order of infusion have been evaluated, to date, no factors have been identified that consistently predict which unit will emerge as the dominant engrafting unit [12]. Recent data, however, suggest the unit with higher $CD34^+$ cell viability at the time of thawing predicts the subsequent dominant engrafting unit, particularly when the nonengrafting unit has <75% $CD34^+$ viability [13].

Because of limitations with the clinical trials of CBT, questions still remain as to the benefit of double-unit CBT and the biologic determinants of engraftment following double-unit CBT. To address these questions we aimed to develop a large animal model of CBT. Because of the long track record of successful translation of experimental findings in the outbred dog model to the clinic [14,15], we investigated if the dog model of CBT could begin to address questions of cell dose and major histocompatibility complex (MHC) barriers to engraftment and survival after high dose total-body irradiation (TBI).

MATERIAL AND METHODS

Animals and Surgical Procedures

All procedures and research protocols were approved by the Institutional Animal Care and Use Committee of the Fred Hutchinson Cancer Research Center (FHCRC). Research and animal housing was conducted according to the principles outlined in the *Guide for Laboratory Animal Facilities and Care* prepared by the National Academy of Sciences and National Research Council. Dogs were raised at the FHCRC or obtained from commercial kennels licensed by the U.S. Department of Agriculture. Adult female, outbred beagle and mixed-breed minimongrel and hound dogs from which cord blood units were harvested weighed 8.0 to 38.0 (median: 10.4) kg and were 18-122 (median: 23.5) months old. CBT recipient beagle and mixed breed dogs weighed 7.0-32.0 (median: 9.8) kg and were 9.5-22.0 (median: 14.4) months old. All dogs were examined at least twice daily. Recipient dogs were euthanized after transplantation when established clinical criteria were met for poor condition with infectious complications.

Details of the canine cord blood unit collection and characterization are described in the Supplemental Materials/Methods section (available online). Briefly, at days 53 to 58 of gestation (estimated at 3-6 days before full term), gravid dogs were placed under general anesthesia and underwent a Cesarean section procedure. There were 2 to 12 fetuses per gravid female; each fetus had a placenta that was separate and distinct from the other littermates. Throughout the fetal/cord blood collection procedure, each fetus was closely monitored for complete anesthesia. Because of the small size of the canine umbilical cord, blood collection from the isolated, clamped umbilical cord resulted in insufficient cell dose yield needed for subsequent transplantation experiments. The CD34 cell and colony forming unit (CFU) content of fetal jugular vein and umbilical cord blood were equivalent (Figure S.1 and Tables S.1 and S.2). To minimize the number of animals used as cord blood donors, and to increase the cell dose yield from each fetus, we combined fetal and umbilical cord blood as the source of blood cells for all subsequent experiments. For this manuscript, CBT in dogs is defined as the transplantation of the combined fetal and umbilical cord blood.

Immediately after collection of each venous fetal/cord blood unit, aliquots of cord blood were collected for DLA typing and hematologic characterization. Immunophenotyping of blood was completed (Tables S.3 and S.4) [16]. After the cord blood unit cell dose was determined, the cells were cryopreserved.

DLA Typing

DLA-identical sibling units for CBT were chosen on the basis of complete family studies showing identity for highly polymorphic MHC associated class I and II microsatellite markers and by direct sequencing of DLA-DRB1 [17,18]. Initial experiments with DLA-identical siblings used recipient dogs from a prior litter of the same breeding pair. DLA-matched unrelated units for CBT were chosen on the basis of direct sequencing for both DLA DRB1 (class II) and DLA-88 (class I) allelic identity [18,19]. Breeding to generate the unrelated cord blood units was performed after the DLA type of the female and male was determined and dogs heterozygous for the most common DRB1 alleles, 00101, 00102, 00201, 00601, 00801, and 01501 were selected. The breeding pairs were unrelated by at least 6 generations. The CBT recipients were selected after DLA allele sequencing of adult dogs. Subsequently, DLA-matched unrelated recipient dogs were identified from randomly selected dogs that were unrelated to the cord blood donors by at least 6 generations in a separate, genetically diverse outbred colony.

Transplantation

Total-body irradiation (TBI) 9.2 Gray (Gy) was delivered as a single fraction at 7 cGy/min from a 4 MEV (for the initial experiments with DLA-identical siblings units) and, more recently, with a 6-MEV linear accelerator (CLINAC 4/80 and CLINAC 600 C/D, respectively, Varian Associates, Palo Alto, CA). This is considered to be a myeloablative TBI dose [20,21]. CBT consisted of intravenous infusions of unwashed,