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Low Radiation Dose and Low Cell Dose Increase the Risk of Graft Rejection in a Canine Hematopoietic Stem Cell Transplantation Model

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A B S T R A C T

The canine hematopoietic stem cell transplantation (HSCT) model has become accepted in recent decades as a good preclinical model for the development of new transplantation strategies. Information on factors associated with outcome after allogeneic HSCT are a prerequisite for designing new risk-adapted transplantation protocols. Here we report a retrospective analysis aimed at identifying risk factors for allograft rejection in the canine HSCT model. A total of 75 dog leukocyte antigen–identical sibling HSCTs were performed since 2003 on 10 different protocols. Conditioning consisted of total body irradiation at 1.0 Gy (n = 20), 2.0 Gy (n = 40), or 4.5 Gy (n = 15). Bone marrow was infused either intravenously (n = 54) or intraosseously (n = 21). Cyclosporin A alone or different combinations of cyclosporine A, mycophenolate mofetil, and everolimus were used for immunosuppression. A median cell dose of 3.5 (range, 1.0 to 11.8) total nucleated cells (TNCs)/kg was infused. Cox analyses were used to assess the influence of age, weight, radiation dose, donor/recipient sex, type of immunosuppression, and cell dose (TNCs, $CD34^+$ cells) on allograft rejection. Initial engraftment occurred in all dogs. Forty-two dogs (56%) experienced graft rejection at median of 11 weeks (range, 6 to 56 weeks) after HSCT. Univariate analyses revealed radiation dose, type of immunosuppression, TNC dose, recipient weight, and recipient age as factors influencing long-term engraftment. In multivariate analysis, low radiation dose ($P < .001$) and low TNC cell count ($P = .044$) were identified as significant independent risk factors for graft rejection. Peripheral blood mononuclear cell chimerism ≥30% ($P = .008$) and granulocyte chimerism ≥70% ($P = .023$) at 4 weeks after HSCT were independent predictors of stable engraftment. In summary, these data indicate that even in low-dose total body irradiation–based regimens, the irradiation dose is important for engraftment. The level of blood chimerism at 4 weeks post-HSCT was predictive of long-term engraftment in the canine HSCT model.

INTRODUCTION

The introduction of nonmyeloablative conditioning and reduced-intensity conditioning (RIC) to the hematopoietic stem cell transplantation (HSCT) procedure was a key step in the development of HSCT. RIC diminished the toxicity profile of conventional myeloablative regimens and consequently was associated with improved treatment-related mortality [1]. Thus, allogeneic HSCT became available to patients otherwise excluded from conventional HSCT because of comorbidities or an extensive treatment history. Today RIC and nonmyeloablative conditioning are well established as inherent parts of diverse transplantation protocols; however, RIC is associated with an increased risk of graft rejection and relapse [2,3].

Numerous factors have been identified as influencing HSCT outcomes. Besides conditioning intensity, type of immunosuppression; degree of HLA matching; cell content of the graft; stem cell source; age, weight, and sex of the donor and recipient; disease status; and previous treatment

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are some of the most frequently investigated of these factors [4,5]. Information on these factors is a prerequisite for designing new risk-adapted transplantation protocols; however, the impact of these factors on transplantation outcomes is controversial and remains to be elucidated in more detail [5-8].

Given that relapse and graft rejection are major causes of failure, particularly after RIC or nonmyeloablative conditioning HSCT, identifying suitable predictors that may help clinicians and researchers develop optimal interventions is important. Evaluation of donor–recipient chimerism in peripheral blood is done routinely to monitor engraftment status after HSCT. Recent studies indicate that the level of chimerism also may be a clinically useful parameter for prognosis [9,10].

RIC and nonmyeloablative conditioning HSCT was developed in the canine HSCT model [11,12]. This model has become accepted in recent decades as a suitable preclinical model for the investigation of new transplantation strategies, owing to the high transferability of canine data to humans. Data on potential risk factors and the prognostic impact of chimerism status for the canine model are scant, however.

Here we report on a retrospective analysis of the influence of potential risk factors on graft rejection after RIC or nonmyeloablative conditioning allogeneic HSCT in a canine model. The predictive value of peripheral blood chimerism was evaluated as well.

MATERIALS AND METHODS

Dogs and Study Design

Litters of purebred beagles were purchased from commercial kennels. Dogs were dewormed and immunized against rabies, parainfluenza, leptospirosis, distemper, hepatitis, and parvovirus.

All research protocols were formally approved by the Review Board of the State Institute for Agriculture, Food Safety, and Fishery, Mecklenburg–West Pomerania, Germany. Studies were performed according to the guidelines of the German Animal Welfare Act.

A total of 75 dog leukocyte antigen (DLA)-identical sibling HSCTs were performed since 2003 on 10 different protocols (Table 1) [13-18]. Histocompatibility of donor–recipient sibling pairs was determined by matching for highly polymorphic DLA class I and class II microsatellite markers [19,20]. For conditioning, dogs received total body irradiation (TBI) at doses of 1.0, 2.0, and 4.5 Gy at a dose rate of 0.25 or 0.1 Gy/minute from a high-energy linear accelerator (Primus 10 MV X-ray beam; Siemens, Buffalo Grove, IL). Donor bone marrow was collected from the femur, humerus, and iliac crest by aspiration under general anesthesia. Within 24 hours after TBI, bone marrow was infused either intravenously (n = 54) or intraosseously (n = 21). For intraosseous infusion, graft volume was reduced by buffy coat centrifugation with or without subsequent Ficoll density gradient centrifugation. Pretransplantation and post-transplantation immunosuppression consisted of cyclosporine A (CSA)/mycophenolate mofetil (MMF), CSA/everolimus (RAD), RAD/MMF, or CSA monotherapy at the following doses: CSA, 15 mg/kg; MMF, 10 and 20 mg/kg; RAD, 0.25 and 1.5 mg orally, twice daily. Dogs conditioned with 1 Gy TBI were also repetitively vaccinated with recipient blood cell lysates or given donor grafts supplemented with dendritic cells of either host or donor origin, to sensitize the donor cells in vivo to recipient hematopoietic antigens [13-18].

Chimerism Analysis

Donor–recipient hematopoietic chimerism was determined by analysis of variable number of tandem repeats using PCR and subsequent fluorescence capillary electrophoresis [21]. Chimerism levels were measured in the granulocytes and peripheral blood mononuclear cell (PBMC) compartment weekly up to day +70 after HSCT and in greater intervals thereafter. Granulocytes and PBMC fractions were separated by a standard Ficoll-Hypaque density gradient centrifugation (density 1.074 g/mL). Genomic DNA was isolated using the NucleoBond CB 100-Kit (Macherey-Nagel, Düren, Germany). Polymorphic tetranucleotide repeats were amplified by PCR using fluorescein-labeled primers (BioTez Berlin-Buch, Berlin, Germany) according to standard protocols and then analyzed.

The minimum follow-up was 26 weeks for protocols 1 to 8 and 16 weeks for protocols 9 and 10, but follow-up was extended whenever possible. Stable engraftment was defined as detection of >5% donor-derived granulocytes and PBMCs after the minimal observation period. Graft rejection was defined as detection of no donor-derived DNA in 2 subsequent chimerism analyses of the peripheral blood and 1 chimerism analysis of bone marrow.

Statistical Analysis

All data were stored and analyzed using SPSS version 22.0 (IBM, Armonk, NY). Results are expressed as frequency (%) or median (range) for the indicated number of dogs. Univariate and multivariate Cox regression analyses were used to assess the influence of age, weight, radiation dose, donor/recipient sex combination, type of immunosuppression, and cell dose (total nucleated cells [TNCs], $CD34^+$ cells) on allograft rejection. Predictor candidates with $P < .20$ on univariate analysis were entered into a subsequent multivariate regression model, and the adjusted hazard ratio (HR) with the respective P value and 95% confidence interval (CI) were calculated for each. In an additional approach, all predictors were used as covariates (for adjustment only) to evaluate potential post-transplantation predictors of graft rejection (granulocyte and PBMC chimerism at day +28). A P value < .05 was considered to indicate significance.

RESULTS

Overall Outcome

Dog and transplantation characteristics are summarized in Table 2. The closing date for analysis was June 30, 2015. Data regarding graft survival were available in all dogs. The median follow-up for the entire cohort was 13 weeks (range, 2 to 163 weeks).

Initial engraftment occurred in all animals. Forty-two dogs (56%) experienced graft rejection, at a median of 11 weeks (range, 6 to 56 weeks). Three of these animals showed late rejection beyond week 26. Graft rejection was always accompanied by prompt reconstitution of the recipient's hematopoiesis. Eleven dogs (15%) died, at a median of 9 weeks (range, 2 to 99 weeks) post-HSCT and were chimeric at the time of last sampling. Causes of death were suspected or proven infections (n = 6), graft-versus-host

Table 1
Overview of the Study Protocols

Protocol	No. of Dogs	Conditioning TBI, Gy	Application Route	Graft Processing	Immune Suppression	Adoptive Immunotherapy	Reference
1	9	2.0	i.v.	—	CSA/MMF	—	[13]
2	7	1.0	i.v.	—	CSA/MMF	Vaccination (cell $lysate_{Recipient}$)	[13]
3	6	1.0	i.v.	—	CSA/MMF	Graft enrichment ($MoDC_{Donor}$)	[13]
4	7	1.0	i.v.	—	CSA/MMF	Graft enrichment ($MoDC_{Recipient}$)	[14]
5	10	2.0	i.v.	—	CSA/RAD	—	[15]
6	7	2.0	i.o.	BC + DG	CSA/MMF	—	[16]
7	6	2.0	i.o.	BC	CSA/MMF	—	[16]
8	8	2.0	i.v.	—	RAD/MMF	—	[17]
9	7	4.5	i.v.	—	CSA	—	[18]
10	8	4.5	i.o.	BC	CSA	—	[18]

MoDC indicates monocyte-derived dendritic cell; i.o., intraosseous; BC, buffy coat; DG, density gradient.