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Denileukin Diftitox as Prophylaxis against Graft-versus-Host Disease in the Canine Hematopoietic Cell Transplantation Model

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ABSTRACT

Denileukin diftitox (Ontak) was evaluated in combination with methotrexate (MTX) for preventing acute graft-versus-host disease (GVHD) in dogs given 9.2 Gy of total body irradiation and DLA-nonidentical hematopoietic cell grafts. Six dogs were given denileukin diftitox 9 μg/kg/day intravenously (IV) on days 2, 4, 5, 7, 8, and 10, in combination with MTX 0.4 mg/kg/day IV on days 1, 3, 6, and 11 after transplantation. Median survival of the dogs given MTX in combination with denileukin diftitox was 16 days (range, 13-18 days), similar to that of 35 historical controls given MTX alone (median survival, 20 days). Five of the 6 denileukin diftitox–treated dogs had clinical and pathological evidence of 3-system GVHD; 1 dog died of canine herpes virus infection without evidence of GVHD. In conclusion, denileukin diftitox did not prevent, mitigate, or delay acute GVHD in this stringent and predictive (with respect to outcomes in human patients) hematopoietic cell transplantation model.

KEY WORDS

Hematopoietic cell transplantation • graft-versus-host disease • denileukin diftitox • canine model

INTRODUCTION

A main purpose of immunosuppressive therapy after allogeneic hematopoietic cell transplantation (HCT) is the prevention of graft-versus-host disease (GVHD), a major cause of mortality and morbidity after transplantation. The immunosuppressive regimens most widely in clinical use combine calcineurin inhibitors with antimetabolites. Using a combination regimen of cyclosporine (CSP) and a short course of methotrexate (MTX) developed in the dog leukocyte antigen (DLA)-nonidentical canine transplant model [1,2], for example, incidence and severity of GVHD could be lowered substantially [3]. However, even with postgrafting CSP and MTX, acute GVHD occurs in 40%-80% of human recipients of HLA-matched grafts, illustrating the need for more effective GVHD prophylaxis [4,5].

Denileukin diftitox (Ontak) is a recombinant fusion protein composed of amino acid sequences for diphtheria toxin fragments A and B followed by the sequences for human interleukin (IL)-2 [6]. Denileukin diftitox preferentially binds to the high-affinity IL-2R expressed on activated T cells [7] and, after internalization of the receptor–ligand complex, kills T cells by inhibiting protein synthesis [8]. Denileukin diftitox has been shown to have antitumor effects in patients with cutaneous T-cell lymphoma and non-Hodgkin's lymphoma whose malignant cells express IL-2R [9,10]. More recently, denileukin diftitox has been used to treat steroid-refractory acute GVHD and showed promising activity, with complete response rates of 50%-59% [11,12]. We therefore hypothesized that denileukin diftitox would be effective in the *prevention* of acute GVHD. After demonstrating cross-reactivity of denileukin diftitox with canine T-cells in vitro, we tested our hypothesis in the DLA-nonidentical canine transplantation model, which has been highly predictive of outcome in humans [3].

METHODS

Canine Mixed Lymphocyte Cultures

A total of 10^5 responder peripheral blood mononuclear cells (PBMCs) were cultured with 10^5 irradiated (24 Gy), allogeneic, DLA-mismatched stimulator PBMCs at 10^6/mL for 6 days according to established methods [13]. Defined concentrations (10^{-8}-$10^{-11.5}$ molar) of denileukin diftitox (Ontak; Ligand Pharmaceuticals, San Diego, CA) or sera from denileukin diftitox–treated dogs were added to canine mixed lymphocyte cultures (MLCs) at the beginning of the culture. Cells were labeled with 1 μCi/well of ^{3}H-thymidine for the final 16 hours of culture, harvested onto glass fiber filters, and counted for isotope incorporation.

Hematopoietic Cell Transplantation

The Institutional Animal Care and Use Committee of the Fred Hutchinson Cancer Research Center approved this study. Hematopoietic cell grafts were carried out using beagle or miniature mongrel–beagle crossbreeds, and standard care was provided as described previously [2,14,15]. Recipients (autologous, n = 3; allogeneic, n = 6) were age 8.0-73.8 months (median, 12.9 months) and weighed 8.5-12.9 kg (median, 10.7 kg). The donors (n = 6) were age 6.7-15.4 months (median, 13.1 months), of different pedigrees for at least 5 generations, and chosen on the basis of nonidentity for highly polymorphic major histocompatibility complex class I and II microsatellite marker polymorphisms and DLA-DRB1 alleles [16,17].

On day 0, donor marrow was harvested and infused intravenously (IV) into autologous (n = 3) or allogeneic (n = 6) recipients within 4 hours after a single 9.2-Gy dose (7 cGy/min) of total body irradiation (TBI) (Varian Clinac 4; Varian Medical Systems, Palo Alto, CA).

Allogeneic transplant recipients were given MTX 0.4 mg/kg IV on days 1, 3, 6, and 11 and denileukin diftitox 9 μg/kg/day IV on days 2, 4, 5, 7, 8, and 10. Autologous marrow transplantation with postgrafting MTX and denileukin diftitox (9 μg/kg/day × 6 days, n = 2; 18 μg/kg/day × 6 days, n = 1) was done to determine denileukin diftitox toxicity after myeloablative conditioning in the absence of allogeneic effects. The median number of nucleated marrow cells infused was 5.4×10^8 cells/kg (range, 3.8-6.9 $\times 10^8$ cells/kg). In addition, PBMCs were harvested from allogeneic donors by leukapheresis (Cobe BCT, Lakewood, CO) and infused IV on day 1 (median number, 4.2×10^8 cells/kg; range, 1.8-11.7 $\times 10^8$ cells/kg).

Clinical signs of cute GVHD included diarrhea, skin erythema, and elevated liver enzyme values. Any dog in poor clinical condition was euthanized, and a complete necropsy was performed to allow histopathologic distinction between GVHD and regimen-related toxicities. Engraftment was assessed based on sustained hematopoietic recovery after the postirradiation nadir, histological features of the marrow, and documentation of donor microsatellite marker polymorphisms in blood and marrow by polymerase chain reaction [18].

Results from current recipients were compared with those of 3 groups of previously published and concurrent DLA-nonidentical, unrelated HCT recipients, all of which received 9.2 Gy TBI and HCT, as did the dogs in the current study [2,18-21]. The dogs in control group 1 received no postgrafting immunosuppression, the dogs in group 2 were given MTX alone, and the dogs in group 3 received a combination of MTX and CSP.

RESULTS

Denileukin Diftitox Inhibits T-Cell Proliferative Responsiveness in Canine Allogeneic MLCs

Figure 1 shows the dose-dependent inhibition of canine allogeneic MLCs by defined concentrations of denileukin diftitox added at the initiation of culture. The results demonstrate a complete abrogation of alloantigen-induced T-cell proliferation in the presence of 10^{-10} molar concentrations of denileukin diftitox.

Sera Obtained from Dogs after IV Injection of Denileukin Diftitox Exhibit T-Cell–Inhibitory Activity

We then investigated whether dogs given single IV injections of denileukin diftitox achieved serum concentrations sufficient to inhibit proliferation of alloactivated T cells. We found that sera obtained from dogs 20 minutes after a single 9-μg/kg IV injection of denileukin diftitox completely inhibited the

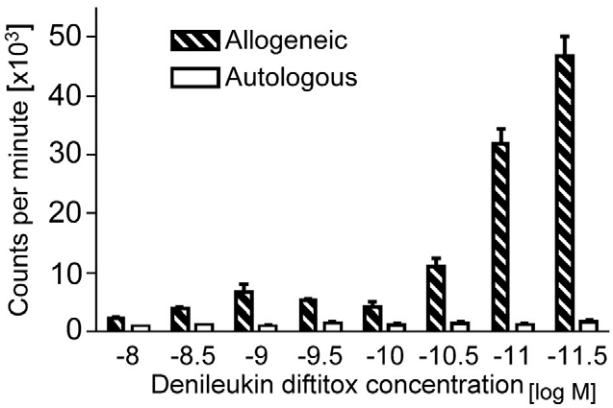

Figure 1. Inhibition of proliferative responsiveness in canine allogeneic MLCs by denileukin diftitox. Defined concentrations of denileukin diftitox were added to allogeneic MLCs at the beginning of culture, as described in Methods. Shown are means (± standard errors) derived from 3 replicates per experimental condition. The experiment shown is representative of 2 experiments.