BIOLOGY

The Triterpenoid CDDO-Me Delays Murine Acute Graft-versus-Host Disease with the Preservation of Graft-versus-Tumor Effects after Allogeneic Bone Marrow Transplantation

Minghui Li,[1] Kai Sun,[2] Doug Redelman,[3] Lisbeth A. Welniak,[2] William J. Murphy[2]

The occurrence of acute graft-versus-host disease (aGVHD) and tumor relapse represent the two major obstacles impeding the efficacy of allogeneic bone marrow transplantation (BMT) in cancer. We have previously shown that the synthetic triterpenoid 2-cyano-3, 12-dioxooleana-1, 9-dien-28-oic acid (CDDO) can inhibit murine early aGVHD, but antitumor effects were not assessed. In the current study, we found that a new derivative of CDDO, CDDO-Me, had an increased ability to inhibit allogeneic T cell responses and induce cell death of alloreactive T cells in vitro. Administration of CDDO-Me to mice following allogeneic BMT resulted in significant and increased protection from lethal aGVHD compared to CDDO. This correlated with reduced TNF-α production, reduced donor T cell proliferation, and decreased adhesion molecule ($\alpha_4\beta_7$ integrin) expression on the donor T cells. CDDO-Me was also superior to CDDO in inhibiting leukemia growth in vitro. When CDDO-Me was administered following an allogeneic BMT to leukemia-bearing mice, significant increases in survival were observed. These findings suggest that CDDO-Me is superior to CDDO in delaying aGVHD, while preserving or possibly even augmenting GVT effects.

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INTRODUCTION

Allogeneic bone marrow transplantation (BMT) is currently used for the treatment of a variety of hematologic malignancies. It has also shown promise in solid cancers such as renal cell carcinoma [1,2] and in autoimmunity [3,4], but significant obstacles regarding acute graft-versus-host disease (aGVHD) still limit the broader use of this procedure. GVHD is an immune-mediated disease in which donor T cells are primed mainly by recipient antigen-presenting cells and then recognize and attack the genetically disparate recipient. Proinflammatory cytokines produced by donor T cells and other immune cells, have been shown to be critical for GVHD generation and play an important role in orchestrating the entire process [5,6]. In general, efforts to reduce the incidence of GVHD have also diminished beneficial graft-versus-tumor (GVT) responses with increased tumor relapse, suggesting that these two processes are intimately linked [7].

Triterpenoids, biosynthesized in some plants by the cyclization of squalene, have been used for medicinal purposes in many Asian countries because of their anti-inflammatory and anticarcinogenic properties [8,9]. 2-Cyano-3, 12-dioxooleana-1, 9-dien-28-oic acid (CDDO) is a novel synthetic triterpenoid that is much more potent than its parent compound. It has potent differentiating, proapoptotic, and antiproliferative capabilities on tumors, and anti-inflammatory properties as well [10,11]. CDDO is an agonist ligand for the peroxisome proliferator-activated receptor γ (PPARγ); however, its antitumor function can be PPARγ-dependent or independent [12,13]. Recently, we have shown that CDDO can suppress allogeneic T cell response and inhibit murine aGVHD, but the effects of CDDO on GVT were not explored [14].

CDDO-Me, a synthetic C-28 methyl ester of CDDO, has been shown to have even more potent antitumor properties than CDDO [13,15-18], and

From the [1]Department of Microbiology and Immunology, University of Nevada School of Medicine, Reno, Nevada; [2]Department of Dermatology, University of California at Davis, Sacramento, California; and [3]Department of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, Nevada.

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Correspondence and reprint requests: William J. Murphy, PhD, Department of Dermatology, University of California, Davis, Res 3 Room 3300, 4645 2nd Avenue, Sacramento, CA 95817 (e-mail: wmjmurphy@ucdavis.edu).

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currently is in phase I/II clinical trials as a novel therapeutic agent for malignancies [18]. In the current study, we compared the abilities of CDDO and CDDO-Me to suppress murine aGVHD. We found that administration of CDDO-Me was superior to CDDO and significantly protected mice from aGVHD in a fully major histocompatibility complex (MHC)-mismatched murine BMT model. This was associated with reduced donor T cell proliferation, decreased adhesion molecule ($\alpha4\beta7$ integrin) expression on the donor T cells, and reduced production of the proinflammatory cytokine tumor necrosis factor (TNF)-α. Importantly, GVT effects were preserved when GVHD was delayed. These results suggest that CDDO-Me may be of significant benefit in suppressing aGVHD, while preserving or possibly even augmenting GVT effects if it is applied together with allogeneic BMT in cancer.

MATERIALS AND METHODS

Animals

Female C57BL/6 (B6) and BALB/C mice were obtained from the Animal Production Area of the National Cancer Institute (Frederick, MD). The mice were kept in specific pathogen-free conditions. All animal protocols were approved by the Ethics Committee for Animal Experimentation at the University of Nevada, Reno. The mice were between 8 and 16 weeks of age at the start of the experiments.

Reagents and Media

CDDO-Me and CDDO were manufactured through the NIH RAID Program and provided by Reata Pharmaceuticals, Inc., Irving, TX. CDDO-Me and CDDO were prepared in a vehicle solution of 10% DMSO (Sigma, St. Louis, MO), 10% Cremophor EL (Sigma), and 80% 0.9% sodium chloride (Baxter, Deerfield, IL). All mitogen assays and mixed lymphocyte reactions (MLRs) were cultured in complete media containing RPMI 1640 (Cambrex, Walkersville, MD), supplemented with 10% fetal bovine serum (Gemini, Woodland, CA), 10 mM HEPES, 2 mM L-glutamine (Cambrex), 50 IU/mL penicillin/50 μg/mL streptomycin (Mediatech, Herndon, VA), 1 mM nonessential amino acids, 1 mM sodium pyruvate, and 50 μM 2-ME (Invitrogen, Grand Island, NY).

Mitogen Assay

Naïve unfractionated BALB/C splenocytes (SCs) were plated in quadruplicate in 96-well plates at a concentration of 2.5×10^5 cells per well, and were treated with 2.5 μg/mL concanavalin A (ConA) (Sigma) in the presence of various doses of CDDO-Me. Cells were incubated for 24 hours at 37°C and 5% CO_2, and were then pulsed with 1 μCi/well [^{3}H] thymidine (MP Biomedicals, Solon, OH) for an additional 16 to 18 hours prior to harvesting, and read on a 1450 Microbeta Trilux scintillation counter (Wallac, Turku, Finland).

MLR

In the primary MLR, unfractionated BALB/C SCs (2.5×10^5/well) and 30 Gy irradiated B6 SCs (2.5×10^5/well) were plated in 96-well round-bottomed plates in quadruplicate in complete media and incubated at 37°C with 5% CO_2 for 3 days, then pulsed with [^{3}H]-thymidine (1 μCi /well) for 16 to 18 hours prior to harvesting and counting.

In the secondary MLR assay, B6 SCs were T cell depleted by incubating with anti-thy1.2 (30H12) and rabbit complement, and then irradiated and incubated with unfractionated BALB/C SCs in the presence of CDDO-Me or vehicle control. After 3 days, cells were harvested and washed twice, and then T cells were negatively purified with a MACS system (Miltenyi Biotec, Auburn, CA). Next, the same number of purified trypan blue-exclusive T cells (0.8×10^5/well) from different treatment groups were cultured with irradiated B6 SCs (2.5×10^5/well) for 3 days in the absence of CDDO-Me and then pulsed with [^{3}H] thymidine for 16 hours prior to harvesting and counting.

Carboxyfluorescein Succinimidyl Ester (CFSE) Labeling and Cell Death Assessment

Unfractionated BALB/C SCs were labeled with 2.5 μM CFSE (Molecular Probes, Eugene, OR) as per the manufacturer's instructions. T cell-depleted SCs from B6 mice were prepared by incubating with anti-Thy 1.2 monoclonal antibody (mAb) (clone 30H-12), followed by incubation with the rabbit complement. CFSE-labeled BALB/C SCs (2.5×10^5 cells per well) were cultured along with 2.5×10^5 irradiated (30 Gy) T cell-depleted B6 SCs in the presence of various concentrations of CDDO-Me. On day 4 in the culture, cells were harvested and labeled with PE-Cy5-conjugated CD4 or CD8 antibodies and PE Annexin V (BD Pharmingen, San Diego, CA). During flow cytometry analysis, gates were drawn on CD4 or CD8-positive cells, then on $CFSE^{high}/CFSE^{low}$ cells, and the percentages of Annexin V-positive cells were determined.

Flow Cytometry

Cells (1×10^6) were labeled with fluorochrome-conjugated antimouse antibodies, and nonspecific binding was corrected with isotype-matched controls. FITC-anti-$H2D^d$ (clone 34-2-12), PE-anti-LPAM-1 (clone DATK32), PE-anti-CD3 (clone 17A2), PE-Cy5-anti-CD8a (clone 53-6.7), PE-Annexin V, and 7-AAD, were purchased from BD Biosciences. PE-Cy5-anti-CD4 (GK1.5) was purchased from eBioscience. All results were obtained on a BD FACScan