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The Triterpenoid CDDO-Me Promotes Hematopoietic Progenitor Expansion and Myelopoiesis in Mice

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The synthetic triterpenoid CDDO-Me has been shown to directly inhibit the growth of myeloid leukemias and lends itself to a wide array of therapeutic indications, including inflammatory conditions, because of its inhibition of NF-κB. We have previously demonstrated protection from acute graft-versus-host disease after CDDO-Me administration in an allogeneic bone marrow transplantation model. In the current study, we observed that CDDO-Me promoted myelopoiesis in both naive and transplanted mice. This effect was dose dependent, as high doses of CDDO-Me inhibited myeloid growth in vitro. All lineages (granulocyte macrophage colony-forming unit, BFU-E) were promoted by CDDO-Me. We then compared the effects with granulocyte colony-stimulating factor, a known inducer of myeloid expansion and mobilization from the bone marrow. Whereas both drugs induced terminal myeloid expansion in the spleen, peripheral blood, and bone marrow, granulocyte colony-stimulating factor only induced granulocyte macrophage colony-forming unit precursors in the spleen, while CDDO-Me increased these precursors in the spleen and bone marrow. After sublethal total-body irradiation, mice pretreated with CDDO-Me further displayed an accelerated recovery of myeloid progenitors and total nucleated cells in the spleen. A similar expansion of myeloid and myeloid progenitors was noted with CDDO-Me treatment after syngeneic bone marrow transplantation. Combined, these data suggest that CDDO-Me may be of use posttransplantation to accelerate myeloid recovery in addition to the prevention of graft-versus-host disease.

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INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is frequently used in the treatment of various hematological malignancies, immune deficiencies, and anemias. The recovery period after HSCT is characterized by prolonged neutropenia, thus increasing susceptibility to opportunistic infections and related mortalities [1,2]. Granulocyte colony-stimulating factor (G-CSF, filgrastim) is used clinically to accelerate donor myeloid recovery and to minimize the duration of neutropenia after HSCT. Administration of G-CSF after HSCT has been shown to decrease the time required for absolute neutrophil counts to reach above 500/μL, a commonly examined clinical threshold, and is regularly used in the clinic [3].

CDDO-Me (methyl-2-cyano-3, 12-dioxooleana-1, 9-diene-28-oate; bardoxolone methyl) is a synthetic triterpenoid that has been previously shown to induce apoptosis and inhibit growth in a number of hematologic and nonhematologic malignancies [4-7], as well as to improve renal function in patients with chronic kidney disease [8]. CDDO-Me induces the Keap1-Nrf2 pathway, thereby upregulating many antioxidant and cytoprotective genes [8,9]. CDDO-Me has been shown to protect against oxidative stress caused by inducible nitric oxide synthase. An elevation of NAD(P)H-quinone oxidoreductase and heme oxygenase, both of which protect cells from redox cycling and oxidative stress, are believed to mediate the antioxidant properties of CDDO-Me [10]. However, growth and survival inhibition have also been shown by CDDO-Me, most likely mediated by the blockade of NF-κB through the inhibition of IκBα kinase-β (IKK-β) [11]. Based on the antioxidant role of CDDO-Me, a recent study found that CDDO-Me can inhibit the suppressive activity of myeloid-derived suppressor cells, which utilize reactive oxygen species as a mechanism of suppression

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and thus improve immune function and antitumor responses [12]. Studies in our laboratory have additionally demonstrated that CDDO-Me inhibits the severity and development of acute murine graft-versus-host disease (GVHD) after allogeneic HSCT in mice and was shown to induce cell death in proliferating lymphocytes in a mixed lymphocyte reaction [13]. To our surprise in these studies, mice that received CDDO-Me were not only protected from GVHD but also displayed an expansion of myeloid progenitor grabulocyte macrophage-colony forming unit (GM-CFU) cells within the spleen and bone marrow.

In the current study, we examined the effects of CDDO-Me on hematopoiesis and compared administration of CDDO-Me with G-CSF in terms of their respective abilities to induce myelopoiesis and expand myeloid cells in resting and syngeneic HSCT mice. We found that both CDDO-Me and G-CSF expanded $CD11b^+$ $Gr\text{-}1^+$ myeloid cells in the bone marrow and spleen. However, CDDO-Me administration also increased the amount of GM-CFU and BFU-e colonies in the spleen, bone marrow, and blood, while G-CSF administration only increased GM-CFU and BFU-e colonies in the spleen and blood. Additionally, the number of GM-CFU and hematopoietic progenitor cells (HPCs) in the spleen and bone marrow showed a significant increase in mice that received CDDO-Me. In vitro studies showed that CDDO-Me was sufficient to stimulate colony growth of naive bone marrow cells in suboptimal colony-stimulating cytokine conditions. Given the broad effects of CDDO-Me, the myeloid expansion we observed may offer advantages in HSCT outcome.

MATERIALS AND METHODS

Animals

Female C57BL/6 (B6) mice were obtained from the Animal Production Area of the National Cancer Institute (Frederick, MD). Mice were kept in specific pathogen-free conditions at the University of Nevada, Reno (Reno, NV) or at the University of California Davis Medical Center (Sacramento, CA). All mice were between 8 and 12 weeks of age at the start of the experiments. Animal protocols were approved by the Institutional Animal Care and Use Committees at the respective institutions in which studies were performed.

Cells and Reagents

CDDO-Me was manufactured through the NIH RAID Program and provided by Reata Pharmaceuticals, Inc. (Irving, TX). CDDO-Me was prepared in a vehicle solution of 10% dimethyl sulfoxide (DMSO) (Sigma, St. Louis, MO), 10% Cremophor EL (Sigma), and 80% Dulbecco's phosphate-buffered saline (DPBS). A vehicle control consisting of 10% DMSO, 10% Cremophor EL, and 80% DPBS was used as a control in all experiments. G-CSF (Neupogen®, Amgen, Thousand Oaks, CA) was diluted to the appropriate concentration in DPBS. Except where indicated, mice received a treatment schedule of CDDO-Me (120 μg/dose, twice daily i.p.) or vehicle control for 7 days or G-CSF (2.5 μg/dose, twice daily s.q.) for 5 days. In naive mice, a treatment schedule of 120 μg per injection for 7 days was chosen because it resulted in the greatest increase in colony formation and was well tolerated.

Flow Cytometry

Single-cell suspensions (1×10^6) were prepared from the spleen and bone marrow as described previously [14] and labeled with fluorochrome-conjugated anti-mouse antibodies, and nonspecific binding was corrected with isotype-matched controls. FITC anti-CD11b (M1/70), PE-Cy5 anti-Gr-1 (RB6-8C5), PE-Sca-1 (D7), FITC c-kit (2B8), and 7-AAD were purchased from BD Biosciences (San Jose, CA). Lineage-positive cells were excluded by simultaneous staining with PE-Cy5-labeled anti-CD3ε, (145-2C11), anti-Gr-1(RB6-8C5), anti- CD11b (M1/70), anti-CD45R/B220 (RA3-6B2), and anti-(TER-119), all purchased from BioLegend (San Diego, CA). Flow cytometric acquisition was performed on a BD LSR Fortessa (Becton Dickinson, San Jose, CA) or Stratedigm S1400 (San Jose, CA). Data sets were analyzed using FlowJo software (Tree Star, Ashland, OR).

Colony Assays

GM-CFU, BFU-e, and CFU-HPP colony assays were performed as previously described [14,15]. For GM-CFU and BFU-e assays, 5×10^4 bone marrow cells or 5×10^5 splenocytes were plated in 35-mm Petri dishes with methylcellulose-containing medium. Colony formation was stimulated with 10 ng/mL recombinant murine granulocyte macrophage-colony stimulating factor (GM-CSF), 10 ng/mL recombinant murine interleukin (IL)-3, and 5 U/mL erythropoietin. Plates were incubated 7 days at 37°C at 5% CO_2. Colonies were defined as aggregates of 50 or more cells that contained only red cells (BFU-e) or only white cells (GM-CFU). In assays with suboptimal cytokines, colony growth was stimulated with 300 pg/mL GM-CSF (without IL-3 or erythropoietin), and CDDO-Me or vehicle control was added to dishes at the indicated concentrations with the amount of vehicle in each dish remaining constant. In CFU-HPP assays, bone marrow cells or spleen cells were plated in 60-mm Petri dishes at 5×10^4 or 5×10^5 cells per plate, respectively. Colony formation was stimulated