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Interleukin-3 plays dual roles in osteoclastogenesis by promoting the development of osteoclast progenitors but inhibiting the osteoclastogenic process ☆

Huixian Hong [a,b], Zhenqi Shi [b], Ping Qiao [b,d], Hui Li [c], Erin M. McCoy [b], Ping Mao [a], Hui Xu [c], Xu Feng [b], Shunqing Wang [a,*]

[a] Department of Hematology, Guangzhou First People's Hospital, Guangzhou Medical University, Guangzhou, Guangdong 510180, China
[b] Department of Pathology, University of Alabama at Birmingham, Birmingham, AL 35294, USA
[c] Department of Dermatology, University of Alabama at Birmingham, Birmingham, AL 35294, USA
[d] Department of Pharmacology, Norman Bethune Medical College, Jilin University, Changchun, Jilin 130021, China

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ABSTRACT

Interleukin (IL)-3, a multilineage hematopoietic growth factor, is implicated in the regulation of osteoclastogenesis. However, the role of IL-3 in osteoclastogenesis remains controversial; whereas early studies showed that IL-3 stimulates osteoclastogenesis, recent investigations demonstrated that IL-3 inhibits osteoclast formation. The objective of this work is to further address the role of IL-3 in osteoclastogenesis. We found that IL-3 treatment of bone marrow cells generated a population of cells capable of differentiating into osteoclasts in tissue culture dishes in response to the stimulation of the monocyte/macrophage-colony stimulating factor (M-CSF) and the receptor activator of nuclear factor kappa B ligand (RANKL). The IL-3-dependent hematopoietic cells were able to further proliferate and differentiate in response to M-CSF stimulation and the resulting cells were also capable of forming osteoclasts with M-CSF and RANKL treatment. Interestingly, IL-3 inhibits M-CSF-/RANKL-induced differentiation of the IL-3-dependent hematopoietic cells into osteoclasts. The flow cytometry analysis indicates that while IL-3 treatment of bone marrow cells slightly affected the percentage of osteoclast precursors in the surviving populations, it considerably increased the percentage of osteoclast precursors in the populations after subsequent M-CSF treatment. Moreover, osteoclasts derived from IL-3-dependent hematopoietic cells were fully functional. Thus, we conclude that IL-3 plays dual roles in osteoclastogenesis by promoting the development of osteoclast progenitors but inhibiting the osteoclastogenic process. These findings provide a better understanding of the role of IL-3 in osteoclastogenesis.

1. Introduction

Osteoclasts, the bone-resorbing cells, play an important role in skeletal development and adult bone remodeling [1,2]. Osteoclasts differentiate from hematopoietic cells of the monocyte/macrophage lineage involving several different stages [3]. Hematopoietic stem cells (HSC) give rise to common myeloid progenitors (CMP) with stimulation of various factors including stem cell factor (SCF), IL-3 and interleukin 6 (IL-6). IL-3 and/or the granulocyte/macrophage-colony stimulating factor (GM-CSF) further promote development of CMP into granulocyte/macrophage progenitors (GMP). CMP and GMP are collectively considered osteoclast progenitors. M-CSF then promotes GMP to differentiate into cells of the monocyte/macrophage lineage [4,5], which are osteoclast precursors. M-CSF and RANKL are two essential and sufficient factors driving osteoclast precursors to differentiate into osteoclasts [6,7].

Abbreviations: α-MEM, α-minimal essential medium; APC, allophycocyanin; BMC, bone marrow cells; BRC, bone remodeling compartment; Car2, carbonic anhydrase 2; CMP, common myeloid progenitors; Ctsk, cathepsin K; FBS, fetal bovine serum; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GMP, granulocyte/macrophage progenitors; GM-CSF, granulocyte/macrophage colony stimulating factor; IL-3, interleukin 3; IL-6, interleukin 6; HSC, hematopoietic stem cells; M-CSF, monocyte/macrophage-colony stimulating factor; MSC, mesenchymal stem cells; MMP9, matrix metalloproteinase 9; PBS, phosphate-buffered buffers; PE, phycoerythrin; RANKL, receptor activator of nuclear factor kappa B ligand; RT-PCR, reverse transcription-polymerase chain reaction; SCF, stem cell factor; SEM, scanning electron microscopy; TRAP, tartrate resistant acid phosphatase.

* Corresponding author. Address: Department of Hematology, Guangzhou First People's Hospital, 1 Panfu Road, Guangzhou Medical University, Guangzhou, Guangdong 510180, China. Fax: +86 20 81066581.

E-mail addresses: shqwang_cn@yahoo.com, drwangshq@medmail.com.cn (S. Wang).

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IL-3 is a multilineage hematopoietic growth factor that promotes the proliferation, differentiation and/or survival of early multilineage hematopoietic progenitors [8]. In particular, this cytokine plays a key role in stimulating the proliferation and survival of myeloid precursors. By the early 1980s, it had been well established that osteoclasts differentiate from hematopoietic cells of the monocyte/macrophage lineage [9]. Given the role of IL-3 in the proliferation and survival of myeloid precursors, a number of groups investigated the potential role of IL-3 in osteoclastogenesis *in vitro* in the late 1980s [10–14]. Collectively, these early investigations demonstrated that IL-3 stimulates osteoclastogenesis *in vitro* using either organ cultures or whole bone marrow cultures.

Intriguingly, numerous recent studies showed that IL-3 inhibits osteoclast formation in *in vitro* osteoclastogenesis assays in which osteoclast precursors were treated with the two essential osteoclast factors M-CSF and RANKL [15–19]. Importantly, these studies indicate that the inhibitory regulation in the osteoclastogenesis assays results from the direct effect of IL-3 on osteoclast precursors. Thus, the role of IL-3 in osteoclastogenesis remains controversial.

In this study, we seek to further address the role of IL-3 in osteoclastogenesis. Our results demonstrate that IL-3 stimulates the development of osteoclast progenitors from bone marrow cells, but it inhibits differentiation of osteoclast precursors into osteoclasts.

2. Materials and methods

2.1. Chemicals and biological reagents

Recombinant mouse IL-3 was obtained from R&D System, Inc. (Minneapolis, MN). Mouse M-CSF was prepared as culture supernatants from CMG14-12 cells, an M-CSF-producing cell line kindly provided by Dr. Sunao Takeshita [20]. Recombinant GST-RANKL was prepared in our laboratory as previously described [21]. Phycoerythrin (PE)-conjugated anti-mouse CD11b antibody and allophycocyanin (APC)-conjugated rat IgG2a k isotype control antibody were obtained from eBioscience (San Diego, CA). APC-conjugated anti-mouse CD115 (c-Fms) antibody was purchased from BioLegend (San Diego, CA). PE-conjugated rat IgG2a k isotype control antibody was from BD Pharmingen (San Jose, CA).

2.2. Preparation and culture of mouse bone marrow cells

C57BL/6 mice were obtained from Harlan Industries (Indianapolis, IN). The experiments involving mice were approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham. Bone marrow cells were obtained from long bones of young (4–6 week-old) mice and cultured in α-minimal essential medium (α-MEM) containing 10% heat-inactivated fetal bovine serum (FBS) in the presence of different factors as indicated in individual experiments.

2.3. In vitro osteoclastogenesis assay

Different numbers of cells as specified in individual assays were seeded in wells of 24-well tissue culture plates and cultured in α-MEM supplemented with 44 ng/ml M-CSF plus RANKL 100 ng/ml for 5 days. The osteoclastogenesis cultures were then stained for tartrate resistant acid phosphatase (TRAP) activity with the Leukocyte Acid Phosphatase kit (387-A) from Sigma–Aldrich (St. Louis, MO).

2.4. Flow cytometry

1×10^6 cells were washed with cold phosphate-buffered buffers (PBS) and resuspended in 200 μl blocking buffer (PBS/0.5% BSA/0.1% Azide) containing 2.4G2 antibody (5 μg/ml) for 30 min on ice. Cells were then washed with 500 μl PBS/azide before addition of 0.5 μl PE-conjugated anti-CD11b antibody and APC-conjugated anti-CD115 antibody or corresponding control IgG antibodies. The mixtures were incubated on ice for 30 min under dim light and then washed with 1 ml cold PBS/azide twice. Cells were fixed with 400 μl cold 1% formaldehyde and analyzed using Accuri C6 Flow Cytometor.

2.5. In vitro bone resorption assay

Cells are seeded on bovine bone slices in 24-well culture plates and cultured as indicated in individual experiments to promote osteoclastogenesis and bone resorption. Bone slices were harvested and cells were removed with 0.25 M ammonium hydroxide and mechanical agitation. Bone pits were analyzed by scanning electron microscopy (SEM) using a Philips 515 scanning microscope.

2.6. Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated from the cells using TRIzol reagent from Invitrogen (Carlsbad, CA) and 1 μg of total RNA was reversed-transcribed to cDNA with oligo(dT) using the ThermoScript™ RT-PCR system (Invitrogen). Semi-quantitative RT-PCR experiments were performed using the primers and conditions described previously [22].

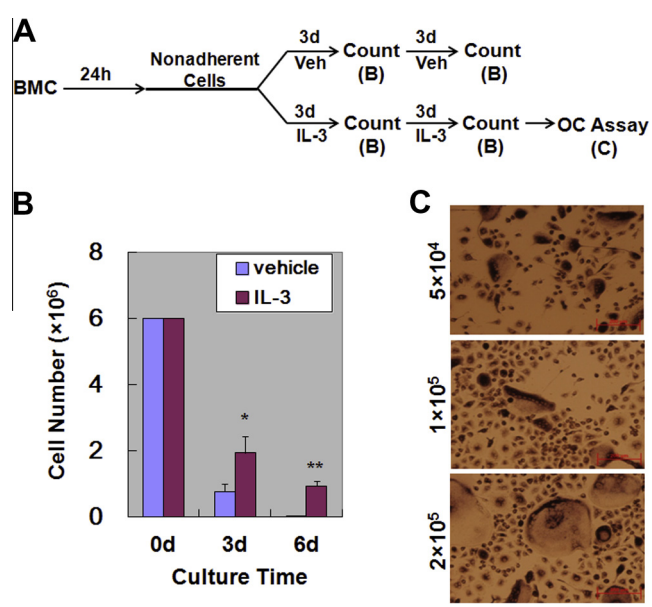

Fig. 1. IL-3 treatment of bone marrow cells generates a population of cells capable of forming osteoclasts. (A) A brief description of the experimental procedure. Bone marrow cells (BMC) were cultured for 24 h (h) in tissue culture dishes. Nonadherent cells were then moved to new tissue culture dishes and cultured with vehicle (Veh, i.e., PBS) or IL-3 (1 ng/ml) for up to 6 days (d). At day 3 and day 6, surviving cells were counted and quantified in panel B. Cells from the 6 days cultures were used to perform osteoclast (OC) assays, and the results are shown in panel C. (B) 6×10^6 Cells were added to one 60-mm tissue culture dish and the assay was performed in triplicate. Data are expressed as mean ± S.D. $^{*}p < 0.05$; $^{**}p < 0.01$. (C) Different numbers of surviving cells from the 6 days culture described in (A) were seeded in 24-well tissue culture plates and cultured with 44 ng/ml M-CSF and 100 ng/ml RANKL for 5 days. The cultures were then stained for TRAP activity. The assay was independently repeated 3 times and a representative area from each condition is shown.

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