IL-1α Stimulates the Formation of Osteoclast-like Cells via RANK-RANKL Signaling System by Osteoblasts and Monocytes

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Abstract:We examined the effect of the inflammatory mediator interleukin-1α (IL-1α) on the expression of macrophage colony-stimulating factor (M-CSF), osteoprotegerin (OPG), and prostaglandin E_2 (PGE_2) in rat osteoblasts, and the indirect effect of IL-1α on the formation of osteoclast-like cells. The expression of M-CSF and OPG were estimated by determining protein levels using Western blot analysis. PGE_2 expression was determined using ELISA. The formation of osteoclast-like cells was estimated using tartrate-resistant acid phosphatase (TRAP) staining of osteoclast precursors in culture with conditioned medium from IL-1α-treated osteoblasts and the soluble receptor activator of NF-κB ligand (RANKL). M-CSF and PGE_2 expression in osteoblasts increased markedly in cells cultured with IL-1α, whereas OPG expression decreased. The conditioned medium containing M-CSF and PGE_2 produced by IL-1α-treated osteoblasts and soluble RANKL increased the TRAP staining of osteoclast precursors. These results suggest that IL-1α stimulated the formation of osteoclast-like cells via an increase in M-CSF and PGE_2 production, and a decrease in OPG production by osteoblasts.

Keywords: IL-1α; macrophage colony-stimulating factor; osteoprotegerin; PGE_2

Introduction

IL-1 is a multifunctional cytokine that regulates various cell and tissue functions. Activated monocytes/macrophages produce a potent bone-resorbing factor initially called “osteoclast-activating factor”, which was later identified as IL-1. Several lines of evidence suggest that IL-1 plays a role in the pathogenesis of bone lesions associated with multiple myeloma, rheumatoid arthritis, and osteoporosis[1].

Osteoclasts are multinucleated cells that are responsible for bone resorption. Osteoblasts are involved in osteoclast differentiation and function via cell-to-cell contact[2]. Osteoclast precursors express receptor activator of NF-κB (RANK), a member of the TNF receptor family, recognize RANK ligand (RANKL) in cell-to-cell interactions with osteoblasts, and differentiate into mononuclear perfusion osteoclasts (POC) in the presence of M-CSF. RANKL is also involved in POC survival and fusion and mature osteoclast activation. The expression of RANKL in osteoblasts is enhanced by 1α,25-dihydroxyvitamin D_3, parathormone (PTH), PGE_2, and IL-11. OPG is a soluble receptor for RANKL that acts as a decoy receptor in the RANK-RANKL signaling system[2].

We hypothesized that IL-1α not only suppresses osteogenesis, but also promotes bone resorption. Previously, we demonstrated that IL-1α inhibits the formation of bone nodules through decreases in alkaline phosphatase activity and type I collagen production in rat osteoblasts[3]. Therefore, we conducted this study to determine the effect of IL-1α on the formation of osteoclast-like cells via the RANK-RANKL signaling system using rat osteoblasts.

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Materials and Methods

Cell culture

The rat clonal cell line ROS 17/2.8[4] was used as the osteoblastic cell line in this study. The cells were maintained in α-minimal essential medium (α-MEM) containing 10% heat-inactivated fetal bovine serum (FBS), and 1% penicillin-streptomycin solution.

Western blotting and ELISA

Immunotransfer analysis was performed on a semidry transfer unit with a continuous buffer system at a constant amperage of 0.8 mA/cm^2. The transfer membrane was incubated with anti-M-CSF, anti-OPG, or anti-α-tubulin, and then incubated with appropriate second antibodies conjugated with biotin. The membrane was incubated with horseradish peroxidase-conjugated streptavidin. Immunoreactive proteins were visualized by producing chemiluminescence using a commercial kit, and exposing the blots to X-ray film.

The amount of PGE_2 in the culture medium was determined using a commercially available ELISA kit.

TRAP staining of osteoclast precursors

ROS 17/2.8 cells were cultured with or without 100 U/ml IL-1α for up to 14 days. The cell culture medium was changed to serum-free α-MEM without IL-1α on day 14 of culture, and then the cells were cultured for 24 h. Each sample of culture medium collected was concentrated and used in this experiment as conditioned medium.

RAW 264.7 cells[5] were obtained from a commercial source, and used in this experiment as osteoclast precursors. To determine the formation of osteoclast-like cells, RAW 264.7 cells were cultured in diluted conditioned medium supplemented with 100 ng/ml soluble RANKL for up to 7 days. Cells cultured under the two different conditions were fixed and stained using a TRACP & ALP double-stain kit according to the manufacturer’s protocol on day 7 of culture. TRAP-positive multinucleated cells with over three nuclei were considered osteoclast-like cells.

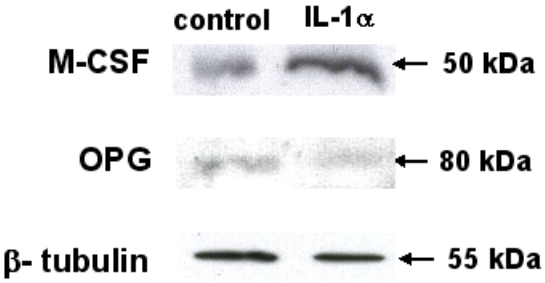

Fig. 1.Effect of IL-1α on M-CSF and OPG protein expression. The expression of M-CSF and OPG was determined using Western blotting at days 14 and 5 of culture, respectively.

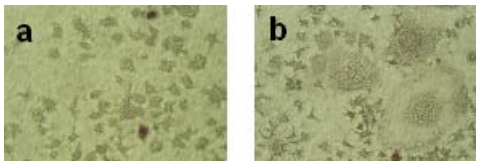

Fig. 2. Effect of IL-1α on PGE_2 production. PGE_2 production was determined using ELISA. The data shown are the mean ± SD for three separate experiments; **p < 0.01, IL-1α treatment versus control.

Results and Discussion

The most definitive studies demonstrating a role for M-CSF in osteoclast recruitment are those in the osteopetrotic (op/op) mouse. Mice homozygous for this mutation have a severe deficiency of osteoclasts and mononuclear phagocytes and are totally devoid of serum and tissue M-CSF activity. This deficiency results from a single base pair insertion in the coding region of the M-CSF gene, resulting in the production of defective M-CSF. Treatment of mutant mice with M-CSF corrects the defect in bone remodeling[6]. In addition, osteoblasts derived from op/op mice do not support osteoclast development *in vitro*, but exogenous M-CSF induces osteoclast formation in op/op hematopoietic cells[7]. In our study, IL-1α strongly induced M-CSF production by osteoblasts (Fig.1). Furthermore, the conditioned medium containing much M-CSF derived from IL-1α-treated osteoblasts increased the numbers and staining of TRAP-positive multinucleated cells (Fig. 3). These results suggest that M-CSF derived from IL-1α-treated osteoblasts increased the formation of osteoclast-like cells.

RANKL binds to the RANK receptor to induce bone resorption[8]. OPG, a member of the TNF receptor family expressed by osteoblasts, strongly inhibits bone resorption by binding to RANKL with high affinity, thereby preventing RANKL from engaging the RANK receptor. PGE_2 increased RANKL expression and decreased OPG expression[2]. In our study, OPG expression decreased gradually with culture time (Fig. 1), whereas PGE_2 expression increased gradually with culture time (Fig. 2), with the addition of IL-1α. Furthermore, the addition of IL-1α suppressed OPG expression in the initial stage of culture, whereas no RANKL expression was detected, irrespective of the addition of IL-1α (data not shown). These results suggest that IL-1α inhibits OPG production via increased PGE_2 production, and that IL-1α increases RANKL expression via increased PGE_2 production. Furthermore, these results suggest that the decreasing of OPG production from IL-1α-treated osteoblasts increases the opportunity of cell-to-cell interactions between osteoblasts and osteoclast precursors via the RANK-RANKL signaling system, and greatly contributes to the formation of osteoclasts.

In conclusion, our results indicate that IL-1α stimulates the formation of osteoclast-like cells via increased M-CSF and PGE_2 production and decreased OPG production by osteoblasts.

References

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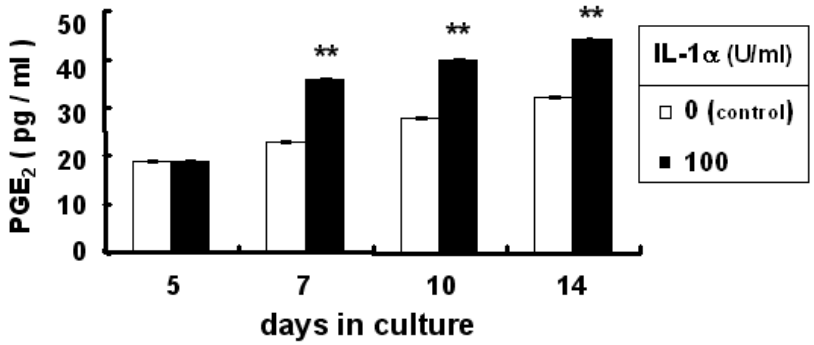

Fig. 3.Effect of the conditioned medium of IL-1α-treated and -untreated ROS 17/2.8 cells on the formation of osteoclast-like cells. (a) RAW 264.7 cells cultured in soluble RANKL and conditioned medium from IL-1α-untreated ROS 17/2.8 cells; (b) RAW 264.7 cells cultured in soluble RANKL and conditioned medium from IL-1α-treated ROS 17/2.8 cells.