Dexamethasone Regulates IL-1β and TNF-α-Induced Interleukin-8 Production in Human Bone Marrow Stromal and Osteoblast-Like Cells

L. R. Chaudhary, L. V. Avioli
Division of Bone and Mineral Diseases, The Jewish Hospital of St. Louis, Washington University Medical Center, St Louis, Missouri 63110, USA
Received: 12 October 1993 / Accepted: 22 December 1993

Abstract. We have investigated both constitutive- and cytokine-induced secretion of interleukin-8 (IL-8) and its regulation by dexamethasone and 17β-estradiol in normal human bone marrow stromal (HBMS), osteoblast-like cells (hOB), and osteosarcoma MG-63 cells. Although HBMS cells secrete low levels of IL-8 constitutively, treatment with IL-1β and tumor necrosis factor-α (TNF-α) induced IL-8 secretion. Their effects were synergistic but IL-8 production was not affected by 17β-estradiol. Human osteosarcoma MG-63 cells also secreted low levels of IL-8 constitutively; the production was induced by IL-1β and TNF-α and was also not affected by 17β-estradiol. The magnitude of the response to cytokine stimulation of IL-8 in MG-63 cells was much lower than that of HBMS and hOB cells, indicating differences in response in normal human and osteoblastic osteosarcoma cells. Dexamethasone (10⁻⁷ M) significantly inhibited IL-1β plus TNF-α stimulated IL-8 production in HBMS, MG-63, and hOB cells. The accumulated results demonstrate that IL-8 is secreted by HBMS, MG-63, and hOB cells, suggesting that IL-8 may play a role in the regulation of bone cell function. These data also emphasize the importance of glucocorticoids in controlling cytokine secretion in HBMS, hOB, and MG-63 cells.

Key words: Glucocorticoids — Interleukin-8 — Bone marrow stromal — Osteoprogenitor — 17β-Estradiol.

Interleukin-8 (IL-8) is synthesized as a 99-amino acid precursor, secreted after cleavage of a signal sequence of 20 residues and processed by repeated N-terminal cleavage yielding several biologically active variants (reviews, 1, 2). The major form consists of 72 amino acid residues with a molecular weight of 8383 daltons, pI 8.3 and four cysteines that form two disulfide bridges. It is also referred to as neutrophil activating peptide-1 (NAP-1) and monocyte-derived neutrophil chemotactic factor (MDNCF). IL-8 elicits pleiotropic biological effects including neutrophil activation, chemotaxis, cell shape change, exocytosis of secretory vesicles and azurophil granules, expression of surface adhesion molecules, production of superoxide and hydrogen peroxide reactive oxygen metabolites [1, 2], rapid and transient increase in cytosolic calcium in monocytes and neutrophils [3], as well as the release of cell matrix resorbing enzymes gelatinase and elastase [2, 4].

Bone marrow stromal cells have long been considered as the source of osteoprogenitor cells in animal models [5, 6]. The renewal of the osteoblast population at the bone surface is considered to occur via differentiation of osteoprogenitor cells along the osteoblast lineage [7, 8]. Estrogen and glucocorticoid receptors have been identified in normal human osteoblasts [9, 10] and osteosarcoma cell lines [11], and estrogen replacement therapy decreases the rate of bone loss and bone fractures in postmenopausal women [12-14]. Long-term treatment of human bone-derived cells with physiological concentrations of glucocorticoids induced alkaline phosphatase activity and parathyroid hormone (PTH)-stimulated cAMP production [15]. Results from our laboratory have also shown that glucocorticoids induce differentiation of human bone marrow stromal cells into cells which display osteoblastic phenotype, i.e., increased alkaline phosphatase activity, cAMP production in response to PTH, osteocalcin production in response to 1,25(OH)₂D₃ [16]. These observations along with others [17-21] support a role of glucocorticoids in the differentiation of bone marrow osteoprogenitor cells into osteoblast lineage cells.

Cytokines, hematopoietic cells, and "chemotactic" substances have also been incriminated in the activation and recruitment of osteoclast precursor cells from the bone marrow [22-32], as well as in conditioning the proliferation of osteoblasts [33, 34]. In addition to IL-1β [35], TNF-α [36], GM-CSF [37], and IL-6 [32, 37], normal human osteoblast-like (hOB) cells have been shown to produce IL-8 [37]. In fact, the magnitude of IL-8 stimulation by cytokines such as IL-1β and TNF-α was much greater than the amount of IL-6 and GM-CSF [37], suggesting a role of IL-8 in bone cell function. Furthermore, 17β-estradiol, a major regulator of bone metabolism, might affect cytokines production in osteoprogenitor and preosteoblast-like cells rather than mature osteoblasts.

Therefore, we studied the constitutive and cytokines stimulated production of IL-8 by normal human bone marrow stromal (osteoprogenitor) and the less differentiated preosteoblastic human osteosarcoma MG-63 cells, and also assessed the effects of dexamethasone and 17β-estradiol.

Materials and Methods

17β-Estradiol and dexamethasone were obtained from Sigma Chem-
ical Company (St. Louis, MO); concentrated stock solutions of 17β-
estriadiol and dexamethasone were made in absolute ethanol and
appropriate amounts were added directly to the medium and thor-
oughly mixed. Recombinant human IL-1β and recombinant human
TNF-α (4.75 × 10^−8 M) were generously provided by Dr. Diana Boraschi
(Sclavo Pharmaceutical Co., Siena, Italy) and Gene-
tech, Inc. (South San Francisco, CA), respectively. Appropriate
amounts of IL-1β and TNF-α were added directly to the media.
Cytokine enzyme-linked immunosorbent assay (ELISA) kit for IL-8
was purchased from R & D Systems (Minneapolis, MN). Dulbecco’s
phosphate-buffered saline (PBS), crude bacterial collagenase,
trypsin-EDTA, Histopaque-1077, fetal bovine serum (FBS) and Dul-
becco’s modified Eagle medium (DMEM): Ham’s F-12 medium (1:1)
were obtained from Sigma. The protein assay kit was purchased
from Bio Rad (Richmond, CA).

**Human Bone Marrow Stromal Cell Culture**

Human ribs obtained from surgery patients were transported to the
laboratory in tissue culture flasks containing DMEM/Ham’s F-12
medium and were processed immediately or after storage overnight
in the refrigerator. The ribs were cleaned of cartilage and muscle and
cracked open with a bone cutter. Bone marrow was harvested by
gently flushing the marrow compartments with DMEM/F-12 me-
dium containing heparin (10 U/ml) and DNase (1 μg/ml). Marrow
cells were pelleted by centrifugation at 500 × g for 10 minutes at
room temperature. The cells were resuspended in 20 ml of α-MEM
without ribonuclease acid (αMEM) containing 10% FBS and trans-
ferred to 50 ml plastic centrifuge tube. Fifteen milliliters of His-
toqucpe-1077 (Sigma) was added to the bottom of the marrow cell
suspension. The tube was centrifuged at 500 × g at room tempera-
ture for 30 minutes. Cell layer at the interface was harvested and
washed three times with the medium. The marrow cells were seeded
in culture flasks T-175 at a density of 4 × 10^5 cells/cm^2 and allowed
to attach to the flask without disturbance for 7 days. The cells were
then fed with half of the medium and thereafter refed with the fresh
medium every 3 days. After cells reached confluence, bone marrow
stromal cells were passaged and seeded in 24-well culture plates at
a density of 40,000 cells/well.

**Human Bone Cell Culture**

Human ribs used for bone marrow collection as described above
were used to scrape out trabecular bone with a size 4 bone currette.
Human osteoblast-like (hOB) cells were cultured from trabecular bone
chips by the method of Robey and Termine [38] with mod-
fications. Briefly, bone chips were washed several times with
DMEM:Ham’s F-12 medium and digested with collagenase (Boeh-
ringer Mannheim, 250 U/ml) and DNase type I (Sigma, 1 μg/ml) in
DMEM:F-12 medium for 2 hours at 37°C. After digestion, chips
were washed with DMEM:F-12 (calcium-free) containing 10% FBS
and plated in calcium-free DMEM:F-12 medium containing 10% FBS
and penicillin-streptomycin (100 U/ml and 100 μg/ml, respec-
tively) in T-175 culture flasks. Cells that grew out from the bone
chips were passaged and plated in 24-well culture plates (60,000
cells/well) and maintained in a humidified atmosphere of 95% air and
5% CO₂ at 37°C. All assays were performed on the first passage
cells. The hOB cells were shown to possess osteoblast phenotype
including high cellular concentrations of alkaline phosphatase, in-
creased secretion of osteocalcin, a matrix protein made only by
osteoblasts and odontoblasts [39], in response to 1,25(OH)₂D₃ and
increased cAMP production in response to PTH [40]; they main-
tained the full phenotype of the mature osteoblast.

**Preparation of HBMS Cell-Conditioned Medium**

HBMS cells were trypsinized and subcultured into 24-well culture
plates at 40,000 cells/well in αMEM medium (1 ml/ml) without phe-

nol red supplemented with 10% FBS for 48 hours; then cells were
washed with PBS and fresh αMEM medium supplemented with 1% charcoa

Preparation of hOB Cell-Conditioned Medium

When cells reached confluency in primary cultures, hOB cells were
trypsinized and subcultured into 24-well culture plates at 60,000
cells/well in DMEM:Ham’s F-12 medium supplemented with 10%
FBS (1 ml/well). The cells were allowed to attach and to recover from
tryptsin for 48 hours in this medium; then they were washed with PBS and placed in fresh medium containing charcoal-stripped
1% FBS (v/v). After 48 hours, the cells were washed with PBS and treated with IL-1β, TNF-α, and 17β-estradiol for 48 hours. Each
treatment was performed in triplicate wells for each cell strain. After treatment, the conditioned media were collected, centrifuged, and used for assaying IL-8.

**Preparation of MG-63 Cell-Conditioned Medium**

Human osteosarcoma MG-63 cells (40,000 cells/well) were grown in
24-well plates in αMEM medium containing 10% FBS and were
and standards for hOB cells.

Protein in the cell layer was measured by the method of Bradford
[41] using BSA as standard.

**Assay of Cytokines**

The IL-8 was assayed in the conditioned medium using the highly
specific quantitative "sandwich" enzyme-linked immunoassay

technique, as described in the R & D Systems IL-8 ELISA kit. The
minimum detectable amount by this assay is 4.7 pg/ml. The data are
expressed as total amount (ng/ml) of cytokine produced by HBMS,
MG-63, and hOB cells. Data were also corrected for cell number by
measuring protein (data not shown).

**Statistical Analysis**

Statistical analysis of data was performed with unpaired Student’s t

**Results**

Human bone marrow stromal cells secreted low basal levels of
IL-8 (mean ± SE, 91 ± 56 pg/ml). When compared with
untreated controls, treatment of HBMS cells with IL-1β (1
ng/ml) and TNF-α (10 ng/ml) stimulated IL-8 production by
297- and 416-fold respectively, whereas 17β-estradiol (10⁻⁸
M) had no effect on both IL-1β- and TNF-α-induced IL-8
production. IL-1β and TNF-α exhibited a synergistic 2340-
fold increment on IL-8 secretion. As shown in Figure 1,
dexamethasone significantly inhibited IL-1β and TNF-α
stimulated IL-8 secretion. As the cells were subjected to the
cytokines and steroids simultaneously, it is possible that
they may have required steroid pretreatment for priming the
response to cytokines. Consequently, HBMS cells were ini-
tially pretreated with 17β-estradiol and dexamethasone
for 24 hours; IL-1β (1 ng/ml) and TNF-α (10 ng/ml) were sub-
sequently added to cultures and the cells were treated for
another 24 hours. As shown in Figure 2, pretreatment with
17β-estradiol had no effect on the ability of the cytokines to