Effects of physiological concentrations of steroid hormones and interleukin-11 on basal and stimulated production of interleukin-8 by human osteoblast-like cells with different functional profiles

A. Dovio[1], M.L. Sartori[1], R.G. Masera[1], L. Peretti[2], L. Perotti[3], A. Angeli[1]

[1]Internal Medicine Unit, Department of Clinical and Biological Sciences, University of Turin, Orbassano (TO), Italy; and the [2]Pathology Unit and [3]Internal Medicine Unit, A.S.O. S. Croce e Carle, Cuneo, Italy.

Abstract

Objective

IL-8 is a CXC chemokine involved in the pathogenesis of articular damage in rheumatoid arthritis. Local hyperproduction of IL-8 has been suggested to play a role in subchondral bone loss, since it suppresses osteoblast activity and promotes osteoclasts recruitment. Osteoblasts are a source of IL-8; its secretion is regulated by a number of hormones and cytokines. The aim of the present study was to evaluate the single and combined effects of physiological concentrations of cortisol, 17β-estradiol and IL-11 upon basal and IL-1β-inducible production of IL-8 in two human osteoblast-like cell lines, Saos-2 and MG-63.

Methods

Cells were incubated with cortisol (0.01 to 1 μM), 17β-estradiol (10 to 1000 pg/ml), IL-11 (1 to 100 ng/ml), in presence or absence of IL-1β (10 ng/ml), for 20 h. Combinations of 17β-estradiol and cortisol, and of IL-11 and cortisol, were also tested. After incubation, IL-8 levels in supernatants were measured by ELISA.

Results

Cortisol dose-dependently inhibited spontaneous IL-8 secretion in both cell lines, although statistical significance was attained in the MG-63 cells only ($P < 0.01$); no effect of 17β-estradiol was apparent. With regard to IL-1β–inducible production, cortisol dose-dependently inhibited IL-8 release in both cell lines ($P < 0.01$); 17β-estradiol resulted in only a non-significant decrease in Saos-2, but not in MG-63 cells. 17β-estradiol did not alter the effects of cortisol in experiments involving co-incubation. IL-11 did not have any effect on spontaneous IL-8 release, but exerted a significant inhibitory effect on IL-1β-inducible release in MG-63 cells ($P < 0.05$); no additional effect was observed upon the degree of cortisol-dependent inhibition.

Conclusion

Cortisol is a potent physiological inhibitor of IL-8 production by osteoblast-like cells. The results of the present study support the use of exogenous supplemental glucocorticoids to prevent the deleterious effects of excess IL-8. The estrogenic milieu and local concentrations of IL-11 have little if any effect on the IL-8-dependent mechanisms of disease.

Key words

Cortisol, estradiol, IL-11, IL-8, osteoblast, rheumatoid arthritis.

Andrea Dovio, MD; Maria Luisa Sartori, BSc; Rosa Gabriella Masera, MD; Laura Peretti, MD; Livio Perotti, MD; Alberto Angeli, MD, PhD.

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Please address correspondence and reprint requests to: Alberto Angeli, MD, PhD, Internal Medicine Unit, Department of Clinical and Biological Sciences, University of Turin–Regione Gonzole 10, 10043 Orbassano (TO) – Italy.
E-mail: alberto.angeli@unito.it

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Introduction

Interleukin-8 (IL-8) – formerly referred to as neutrophil attractant/activating protein 1 (NAP-1), neutrophil activating factor (NAF), or granulocyte chemotactic protein (GCP) – is a chemoattractive cytokine (chemokine) belonging to the CXC family that is characterized by a single non-cysteine amino acid situated between two N-terminal cysteines, and chemotactic properties primarily for neutrophils. Its systematic name in the established nomenclature for chemokines is CXCL8 (1).

IL-8 is produced and released by many cellular types, including cells such as osteoblasts derived from multi-potent mesenchymal progenitors (2-5). It is a pro-inflammatory substance that contributes to the recruitment and trafficking of neutrophils during the course of an immunologic or inflammatory response. Moreover, it is credited with angiogenic properties, and is able to recruit osteoclasts (1, 6-7).

IL-8 can induce synovial inflammation. Administration of recombinant IL-8 into the knee joints of rabbits resulted in neutrophilic infiltration followed by a later infiltration of mononuclear cells and proliferation of synovial lining cells (8). In the synovial fluid and tissue of patients suffering from rheumatoid arthritis (RA), IL-8 levels are remarkably higher than the levels seen in other rheumatic diseases, including osteoarthritis (9-10). Interactions in RA between inflammation in the synovial space, breakdown of cartilage and resorption of the subchondral bone are presently the subject of intense investigation. IL-8 is expressed by both mesenchymal (i.e., osteoblasts, osteocytes, stromal and endothelial cells) and mononuclear cells in trabecular bone biopsies obtained from RA patients (3). A local hyperproduction of IL-8 as a response to inflammatory stimuli has been suggested to play an important role in suppressing osteoblast activity and promoting osteoclast recruitment (11). Chemoattraction of neutrophils is also likely to contribute to the degradation of bone matrix since neutrophils possess a range of potent proteinases and hydrolases, as well as the ability to generate a series of reactive oxygen intermediates (12).

IL-8 expression by osteoblasts is regulated by a number of factors, including cytokines and hormones. Interleukin-1β (IL-1β), which admittedly is a key mediator of the rheumatoid inflammation in the peripheral joints, markedly increases such expression (3-5, 13). With regard to hormonal signals, glucocorticoids have been consistently shown to be effective inhibitors of basal and stimulated IL-8 production by osteoblasts, while studies on estrogen action have yielded negative results (13-15).

Interleukin-11 (IL-11) is an anti-inflammatory cytokine that mediates its effects through gp130-dependent pathways, leading to inhibition of the transcriptional activator nuclear factor (NF)-κB (16-18). NF-κB is known to be active in the promoter region of the human IL-8 gene and it reportedly contributes to the up-regulatory effect of inflammatory cytokines (19). IL-11 is currently being investigated as a potential therapeutic tool in inflammatory autoimmune diseases, including RA (20).

The aim of the present study was to evaluate the single and combined effects of physiological concentrations of cortisol, 17β-estradiol and IL-11 upon basal and IL-1β-inducible production of IL-8 in two human osteoblast-like cell lines, Saos-2 and MG-63. These cell lines represent a valuable model since they produce IL-8 (2, 21), express glucocorticoid and estrogen receptors (22-23), and are glucocorticoid and estrogen responsive (22, 24-25). They also express the IL-11 receptor and are IL-11 responsive (2, 26-29). Importantly, they show divergent functional profiles in terms of alkaline phosphatase, cytokines, and receptors, which possibly reflect different degrees of osteoblastic differentiation (Table I).

Materials and methods

Materials

Human recombinant IL-1β and IL-11 (Sigma-Aldrich, Milan, Italy) were reconstituted using sterile buffered saline (PBS) containing 1% bovine serum albumin to a concentration of 1 μg/ml and stored at -20°C. Cortisol and 17β-

estradiol (Sigma-Aldrich) were dissolved in absolute ethanol and stored at -20°C. Before the experiments steroids were diluted to the final concentrations in complete medium; the maximum concentration of ethanol in the culture media was 0.01% (vol/vol). This concentration was previously found to have no significant effect on the osteoblastic phenotypes or functions being studied.

Cells and culture conditions

The human osteosarcoma cell lines Saos-2 and MG-63 have been maintained and extensively studied in our laboratory. Saos-2 cells were kindly provided by Prof. M.L. Brandi (University of Florence, Italy); MG-63 cells were purchased from Interlab Cell Line Collection (National Institute for Cancer Research, Genoa, Italy). Saos-2 and MG-63 cell lines were cultured in Coon's F12 modified medium (Oxoid, Milan, Italy) and DMEM (Euroclone, UK), respectively, enriched with 10% (vol/vol) heat inactivated fetal bovine serum (FBS), 2 m*M* glutamine, 100 IU/ml penicillin and 100 µg/ml streptomycin (Sigma-Aldrich), in a humidified atmosphere of 95% air / 5% CO_2 at 37°C. The pattern of expression of osteoblastic markers, cytokines and hormone receptors assessed in our laboratory is shown in Table I.

Before the experiments cells were seeded into 6-well plates (Sterilin, Celbio, Milan, Italy). Since phenol red, commonly used as a pH indicator in cell culture media, is known to possess estrogen activity, cells were incubated with complete phenol red free (PhRF) media enriched with 10% charcoal-treated FBS. When they were near confluence, the media were replaced with fresh media containing different concentrations of cortisol (0.01 – 1 µ*M*), 17β-estradiol (10 – 1000 pg/ml), or IL-11 (1 – 100 ng/ml), alone or in combination with IL-1β (10 ng/ml), and the cells were cultured for 20 h.

IL-8 enzyme-linked immunosorbent assay (ELISA)

After treatment, cell cultures media were harvested for assay, spun for 10 min at 2500 g at 4°C and the supernatants were frozen at –20°C. Supernatants were diluted 10- to 1000-fold when necessary before analysis. IL-8 was measured in duplicate by a sensitive ELISA using a commercial kit (R&D Systems, Abingdon, UK). Level of sensitivity was < 10 pg/ml; the intra-assay and inter-assay CV values were < 5%. Data were expressed as the amount of IL-8 released from viable cells cultured under different experimental conditions (pg/10^6 cells).

Statistical analysis

The results are expressed as the mean ± SE. Statistical analysis of the data was carried out using Statistica 6.0 (Statsoft Inc., Tulsa, OK, USA). The effects of IL-1β, cortisol, 17β-estradiol and IL-11 were analyzed by multiple measures ANOVA, followed by the Newman-Keuls multiple comparison test when appropriate. $P < 0.05$ was considered to represent statistical significance.

Table I. Cytokines and glucocorticoid receptor expression profiles of the two human osteoblast-like cell lines, Saos-2 and MG-63.

		Saos-2	MG-63
IL-6	(pg/10^6 cells)	1.2 ± 0.3 (n=4)	7725 ± 2005 (n=7)
sIL-6R	(pg/10^6 cells)	126 ± 1 (n=2)	303 ± 45 (n=3)
IL-11	(pg/10^6 cells)	621 ± 40 (n=3)	15.1 ± 0.8 (n=3)
IL-1β	(pg/10^6 cells)	undetectable (n=2)	undetectable (n=2)
TNF-α	(pg/10^6 cells)	undetectable (n=2)	undetectable (n=2)
IL-8	(pg/10^6 cells)	60 ± 11 (n=7)	32286 ± 6410 (n=10)
OPG	(pg/10^6 cells)	683 ± 73 (n=8)	708120 ± 118474 (n=8)
sRANKL	(pg/10^6 cells)	not detectable (n=3)	not detectable (n=3)
APh	(nmol/10^6cells/min)	1060 ± 1675 (n=4)	not detectable (n=3)
GR number	(sites/cell)	31783 ± 1847 (n=12)	110380 ± 4499 (n=11)
affinity	(nM)	3.5 ± 0.4 (n=12)	2.8 ± 0.3 (n=11)

IL: interleukin; sIL-6R: soluble IL-6 receptor; OPG: osteoprotegerin; APh: alkaline phosphatase; GR: glucocorticoid receptor.

Results

In both cell lines, IL-1β was a potent stimulator of IL-8 release in the extracellular medium ($P < 0.01$ by multiple measures ANOVA), the concentration of 1 ng/ml being close to the maximal effect which was attained at the concentration of 10 ng/ml (Fig. 1). This concentration therefore was chosen for subsequent experiments. In terms of the percent increase above baseline levels, Saos-2 cells responded to IL-1β significantly more than MG-63 cells (P

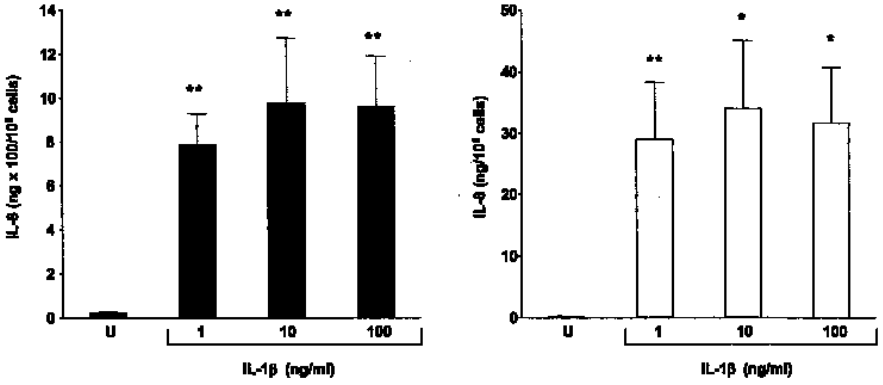

Fig. 1. Dose-response curve of IL-1β on IL-8 secretion by MG-63 and Saos-2 cells. The cells were exposed to IL-1β (1-100 ng/ml) for 20 h; IL-8 concentrations were measured by ELISA. ■ MG-63; ❒ Saos-2 cells; U: untreated. Data are expressed as means ± SE of 3 independent experiments. *P< 0.05, **P< 0.01 by multiple measures ANOVAfollowed by the Newman-Keuls post-hoc comparison test.

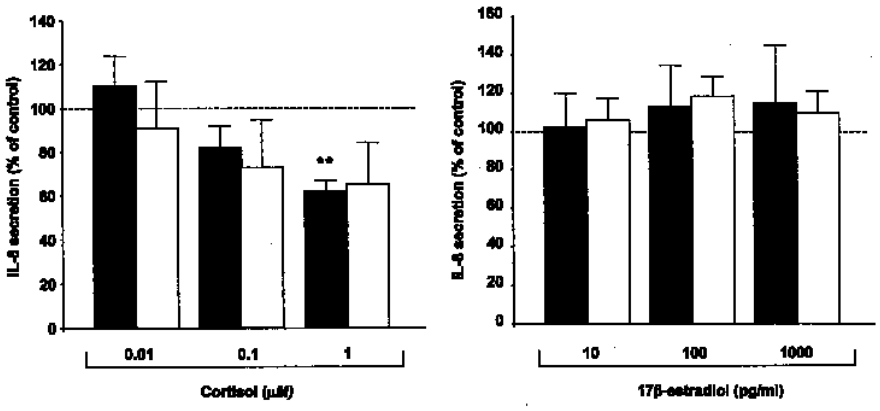

Fig. 2. Effects of cortisol and 17β-estradiol on constitutive IL-8 release by MG-63 and Saos-2 cells. The cells were exposed to cortisol (0.01 – 1 μ*M*) or 17β-estradiol (10 – 1000 pg/ml). ■ MG-63; ❒ Saos-2 cells. Data are expressed as means ± SE of four independent experiments. **P< 0.01 by multiple measures ANOVAfollowed by the Newman-Keuls post-hoc comparison test.

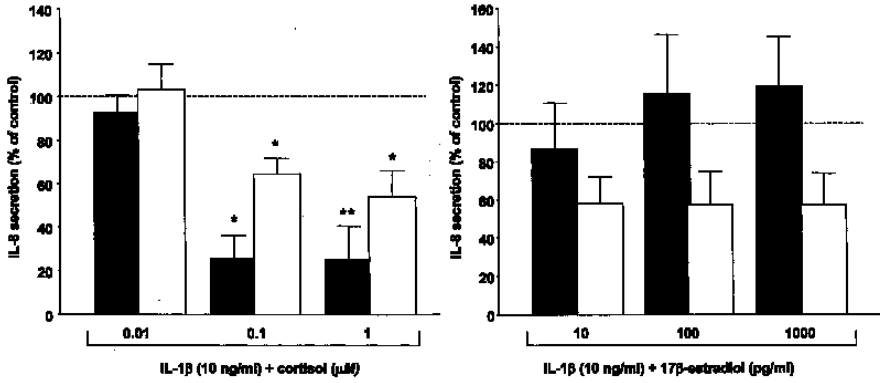

Fig. 3. Effects of cortisol and 17β-estradiol on IL-1β-inducible IL-8 release by MG-63 and Saos-2 cells. The cells were exposed to IL-1β (10 ng/ml), alone or in combination with cortisol (0.01 – 1 μ*M*) or 17β-estradiol (10 – 1000 pg/ml). ■ MG-63; ❒ Saos-2 cells. Data are expressed as means ± SE of four independent experiments. *P < 0.05, **P< 0.01 by multiple measures ANOVA followed by the Newman-Keuls post-hoc comparison test.

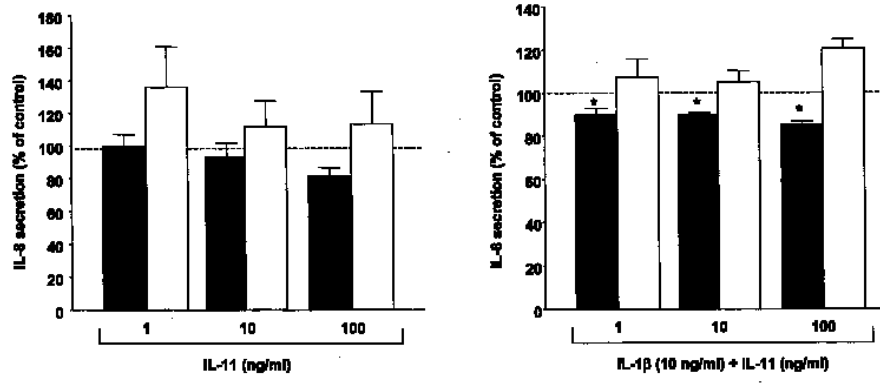

Fig. 4. Effects of IL-11 on spontaneous and IL-1β-inducible IL-8 release by MG-63 and Saos-2 cells. The cells were exposed to IL-11 (1 – 100 ng/ml) in the presence or in the absence of IL-1β (10 ng/ml). ■ MG-63; ❒ Saos-2 cells. Data are expressed as means ± SE of three independent experiments. *P < 0.05 by multiple measures ANOVAfollowed by the Newman-Keuls post-hoc comparison test.

< 0.02 by two-factor ANOVA). Exposure to cortisol yielded a dose-dependent decrease in spontaneous IL-8 production which was highly significant at the concentration of 1 μ*M*, notably in MG-63 cells (P< 0.01 by multiple measures ANOVA). On the contrary, no effect of 17β-estradiol was apparent in either cell line (Fig. 2).

Co-exposure of cells to glucocorticoid and estrogen did not change the degree of inhibition observed with glucocorticoid alone (data not shown). With regard to IL-1β-inducible production, cortisol dose-dependently inhibited (P < 0.01 by multiple measures ANOVA) and again was significantly more effective in MG-63 than in Saos-2 cells (P < 0.03 by two-factor ANOVA). 17β-Estradiol induced a non-significant decrease in Saos-2 cells, but not in MG-63 cells (Fig. 3). 17β-Estradiol did not change the effects of cortisol in experiments with co-incubation (data not shown). IL-11 did not show for either cell line appreciable effects on spontaneous IL-8 release, but exerted a significant inhibitory effect on IL-1β-inducible release in MG-63 cells (P < 0.05 by multiple measures ANOVA), while it was totally ineffective under the same experimental conditions in Saos-2 cells (Fig. 4). In MG-63 cells, however, no additional effect was observed upon the degree of cortisol-dependent inhibition when experiments involving co-exposure to glucocorticoids and IL-11 were performed (data not shown).

Discussion

Osteoblasts are specialized fibroblasts that express only a few specific genes and retain the ability to produce an array of cytokines, as do most cellular types derived from common mesenchymal progenitors. Subchondral osteoblasts are reportedly involved in the inflammatory processes that activate local cartilage and bone destruction in RA (3,4,30). In the present investigation we focused on two established human osteoblast-like cell lines that constitutively display different functional phenotypes and conceivably have different hierarchies of transcriptional activators of cytokine genes. Both cell lines reportedly express glucocorticoid, estrogen and IL-11 receptors and are responsive to their specific ligands (22-29). Moreover, both cell lines release IL-8, which is markedly induced by IL-1β, consistent with previous observations in different human osteoblastic models (2-5).

We first examined whether exposure to

physiological concentrations of cortisol and/or 17β-estradiol (the most representative endogenous glucocorticoid and estrogen hormones, respectively) was effective in challenging basal and/or IL-1β-inducible IL-8 production. IL-8 has an important pathogenetic role in the development and progression of many inflammatory diseases, notably RA (1). Pertinently, either a deficiency of glucocorticoid action or menopausal status have been associated with a higher risk of clinically apparent RA (31, 32). We found that cortisol did inhibit IL-8 release in the culture medium under all experimental conditions. The inhibitory effect, as inferred by percent changes in levels versus unexposed cells, was more apparent in MG-63 cells, that constitutively express more IL-8 and more glucocorticoid receptors as well. Keeping in mind the limitation that in this experiment we utilized osteosarcoma-derived osteoblast-like cells, our results are consistent with those of previous studies, and add support to the view that glucocorticoids play a pivotal role in modulating IL-8 production in cells of mesenchymal origin (13, 14, 19).

The clinical relevance of glucocorticoid inhibition of pro-inflammatory substances in patients with RA even at physiological concentrations has been underscored most recently in a study on the suppressive effects of cortisol on IL-8 release from primary early cultures of synoviocytes (33). Interestingly enough, we have previously found that IL-8 released by mononuclear cells of the synovial fluid obtained from untreated RA patients inversely correlated with synovial fluid cortisol concentrations (34). Taken together, all these data speak in favour of the thesis that in RAlocal hyperproduction of IL-8 gains pathogenetic importance also because it is unrestrained by endogenous glucocorticoids. It is pertinent to say that the use of corticoid derivatives as an early therapy for RA, possibly in combination with other "induction-therapy", is gaining value (35). In RA patients given glucocorticoids, the inhibitory effect upon IL-8 production offers an additional explanation for the apparent paradox of a beneficial effect on subchondral bone in the face of generalized glucocorticoid-induced osteoporosis.

Contrary to cortisol, we did not find any apparent effect of physiological concentrations of 17β-estradiol on IL-8 production by our cell lines. These negative results confirm previous observations (14,15). The mechanisms accounting for the clinical appearance and progression of RA at the time of menopause do not seem to involve IL-8 production, at least from subchondral cells of the osteoblastic lineage.

IL-11 is a gp130-mediated cytokine which is credited with anti-inflammatory properties. It has been shown to diminish the release of pro-inflammatory cytokines in different cellular models; the suggested mechanism was inhibition of NF-κB activity through the up-regulation of Iκ-Bα (16-18). Consequently, IL-11 has been investigated as a potential therapeutic agent in a number of inflammatory diseases, including RA (20). Surprisingly, we found a significant (albeit modest) inhibitory effect of IL-11 on IL-1β-inducible IL-8 release only in MG-63 but not in Saos-2 cells. IL-8 gene promoter possesses binding sites for NF-κB, and NF-κB has documented importance for the regulation of constitutive and IL-1β-stimulated expression (36). Inhibition of IL-8 production therefore is likely to occur only in the presence of an NF-κB activating signal such as IL-1β, and of remarkably increased production of the cytokine.

Moreover, we did not observe any additional effect of IL-11 upon cortisol-dependent inhibition. This observation has a number of possible explanations. First, it is consistent with a redundancy of mechanisms that may eventually lead to restrained IL-8 release. Indeed, both glucocorticoids and IL-11 are known to inhibit NF-κB activity. Glucocorticoids act both through a direct physical interaction of the glucocorticoid receptor with NF-κB, and via the up-regulation of Iκ-Bα (19). This latter effect has also been reported to be exerted by IL-11 (17). Second, it is possible that glucocorticoids and IL-11 mutually inhibit their action. We have recently shown that IL-11 dose-dependently decreases glucocorticoid receptor in MG-63 cells (37). Furthermore, Liu *et al.* have found that glucocorticoids inhibit IL-11 receptor expression, with no effect on gp130 (38). It should be noted that these authors reported on the prolonged exposure of rat osteoblasts to dexamethasone; moreover, in contrast with these findings, gp130 up-regulation by glucocorticoids has been described by others (39). In any case, it appears obvious that the potentiality of IL-11 as a negative modulator of IL-8 is much less, and even minimal, in comparison to that of cortisol at physiological concentrations, and that there is no synergism between the two substances for this particular action. Indeed, administration of IL-11 was found to be ineffective in patients with RA on glucocorticoid therapy (20).

In conclusion, we believe that cortisol is a potent physiological modulator of IL-8 production by cells of the osteoblastic lineage and that results of the present study support the use of exogenous supplemental glucocorticoids to prevent the deleterious effects of excess IL-8, hence of chemoattracted neutrophils in inflammatory diseases involving bone, notably active RA. In such conditions, the estrogenic milieu and local concentrations of IL-11 have little if any effect on IL-8-dependent mechanisms of disease.

References

1. WUYTS A, PROOST P, VAN DAMME J: Interleukin-8 and other CXC chemokines. *In* THOMPSON A (Ed.): *The Cytokine Handbook*, London, Academic Press 1998: 271-311.
2. BILBE G, ROBERTS E, BIRCH M, EVANS DB: PCR phenotyping of cytokines, growth factors and their receptors and bone matrix proteins in human osteoblast-like cell lines. *Bone* 1996; 19: 437-45.
3. LISIGNOLI G, TONEGUZZI S, GRASSI F *et al.*: Different chemokines are expressed in human arthritic bone biopsies: IFN-γ and IL-6 differently modulate IL-8, MCP-1 and RANTES production by arthritic osteoblasts. *Cytokine* 2002; 20: 231-8.
4. LISIGNOLI G, TONEGUZZI S, POZZI C *et al.*: Pro-inflammatory cytokines and chemokine production and expression by human osteoblasts isolated from patients with rheumatoid arthritis and osteoarthritis. *J Rheumatol* 1999; 26: 791-9.
5. SUNYER T, LEWIS J, COLLIN-OSDOBY P, OSDOBY P: Estrogen's bone protective ef-

fects may involve differential IL-1 receptor regulation in human osteoclast-like cells. *J Clin Invest* 1999; 103: 1409-18.

6. COLLIN-OSDOBY P, KIRSCH D, ANDERSON F, JOOST O, DEAN A, OSDOBY P: The chemokine IL-8 as an autocrine inhibitor of osteoclast bone resorptive activity via IL-8 receptors expressed by avian osteoclasts and human osteoclast-like cells. *J Bone Miner Res* 1996; 11: S357 (abstr.).
7. FULLER K, OWENS J, CHAMBERS T: Macrophage inflammatory protein-1 and IL-8 stimulate the motility but suppress the resorption of isolated rat osteoclasts. *J Immunol* 1995; 154: 6065–72.
8. ENDO H, AKAHOSHI T, TAKAGISHI K, KASHIWAZAKI S, MATSUSHIMA K: Elevation of interleukin-8 (IL-8) levels in joint fluids of patients with rheumatoid arthritis and the induction by IL-8 of leukocyte infiltration and synovitis in rabbit joints. *Lymphokine Cytokine Res* 1991; 10: 245-52.
9. KANEKO S, SATOH T, CHIBA J, JU C, INOUE K, KAGAWAJ: Interleukin-6 and interleukin-8 levels in serum and synovial fluid of patients with osteoarthritis. *Cytokines Cell Mol Ther* 2000; 6: 71-9.
10. FURUZAWA-CARBALLEDA J, ALCOCER-VARELA J: Interleukin-8, interleukin-10, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 expression levels are higher in synovial tissue from patients with rheumatoid arthritis than in osteoarthritis. *Scand J Immunol* 1999; 50: 215-22.
11. ROTHE L, COLLIN-OSDOBY P, CHEN Y *et al.*: Human osteoclasts and osteoclast-like cells synthesize and release high basal and inflammatory stimulated levels of the potent chemokine interleukin-8. *Endocrinology* 1998; 139: 4353-63.
12. EDWARDS SW, HALLETT MB: Seeing the wood for the trees: the forgotten role of neutrophils in rheumatoid arthritis. *Immunol Today* 1997; 18: 320-4.
13. CHAUDHARY LR, AVIOLI LV: Regulation of interleukin-8 gene expression by interleukin-1beta, osteotropic hormones, and protein kinase inhibitors in normal human bone marrow stromal cells. *J Biol Chem* 1996; 271: 16591-6.
14. CHAUDHARY LR, AVIOLI LV: Dexamethasone regulates IL-1 beta and TNF-alpha-induced interleukin-8 production in human bone marrow stromal and osteoblast-like cells. *Calcif Tissue Int* 1994; 55: 16-20.
15. CHAUDHARY LR, SPELSBERG TC, RIGGS BL: Production of various cytokines by normal human osteoblast-like cells in response to interleukin-1 beta and tumor necrosis factor-alpha: lack of regulation by 17 beta-estradiol. *Endocrinology* 1992; 130: 2528-34.
16. TREPICCHIO WL, DORNER AJ: Interleukin-11: a gp130 cytokine. *Ann NY Acad Sci* 1998; 856: 12-21.
17. TREPICCHIO WL, WANG L, BOZZA M, DORNER AJ: IL-11 regulates macrophage effector function through the inhibition of nuclear factor-kappaB. *J Immunol* 1997; 159: 5661-70.
18. TREPICCHIO WL, BOZZAM, PEDNEAULT G, DORNER AJ: Recombinant human IL-11 attenuates the inflammatory response through down-regulation of proinflammatory cytokine release and nitric oxide production. *J Immunol* 1996; 157: 3627-34.
19. MCKAY LI, CIDLOWSKI JA: Molecular control of immune/inflammatory responses: interactions between nuclear factor-kB and steroid receptor-signaling pathways. *Endocr Rev* 1999; 20: 435-59.
20. MORELAND L, GUGLIOTTI R, KING K *et al.*: Results of a phase-I/II randomized, masked, placebo-controlled trial of recombinant human interleukin-11 (rhIL-11) in the treatment of subjects with active rheumatoid arthritis. *Arthritis Res* 2001; 3: 247-52.
21. SIDDIQI A, BURRIN JM, WOOD DF, MONSON JP: Tri-iodothyronine regulates the production of interleukin-6 and interleukin-8 in human bone marrow stromal and osteoblast-like cells. *J Endocrinol* 1998; 157: 453-61.
22. DOVIO A, SARTORI ML, MASERARG, RACCA S, ANGELI A: Inhibitory effect of physiological concentrations of cortisol but not estradiol on interleukin (IL)-6 production by human osteoblast-like cell lines with different constitutive IL-6 expression. *Cytokine* 2001; 15: 47-52.
23. BLAND R: Steroid hormone receptor expression and action in bone. *Clin Science* 2000; 98: 217-40.
24. RAO LG, SUTHERLAND MK, REDDYGS, SIUCALDERA ML, USKOKOVIC MR, MURRAY TM: Effects of 1alpha,25-dihydroxy-16ene, 23yne-vitamin D3 on osteoblastic function in human osteosarcoma SaOS-2 cells: differentiation-stage dependence and modulation by 17-beta estradiol. *Bone* 1996; 19: 621-7.
25. LAJEUNESSE D: Effect of 17 beta-estradiol on the human osteosarcoma cell line MG-63. *Bone Miner* 1994; 24: 1-16.
26. LU ZY, ZHANG XG, GUZJ *et al.*: A highly sensitive quantitative bioassay for human interleukin-11. *J Immunol Methods* 1994; 173: 19-26.
27. ELIAS JA, TANG W, HOROWITZ MC: Cytokine and hormonal stimulation of human osteosarcoma interleukin-11 production. *Endocrinology* 1995; 136: 489-98.
28. BELLIDO T, STAHL N, FARRUGGELLA TJ, BORBA V, YANCOPOULOS GD, MANOLAGAS SC: Detection of receptors for interleukin-6, interleukin-11, leukemia inhibitory factor, oncostatin M, and ciliary neurotrophic factor in bone marrow stromal/osteoblastic cells. *J Clin Invest* 1996; 97: 431-7.
29. CHEREL M, SOREL M, LEBEAU B *et al.*: Molecular cloning of two isoforms of a receptor for the human hematopoietic cytokine interleukin-11. *Blood* 1995; 86: 2534-40.
30. KANEKO M, TOMITA T, NAKASE T *et al.*: Expression of proteinases and inflammatory cytokines in subchondral bone regions in the destructive joint of rheumatoid arthritis. *Rheumatology (Oxford)* 2001; 40: 247-55.
31. WLUKA AE, CICUTTINI FM, SPECTOR TD: Menopause, oestrogens and arthritis. *Maturitas* 2000; 35: 183-99.
32. CROFFORD LJ: The hypothalamic-pituitary-adrenal axis in the pathogenesis of rheumatic diseases. *Endocrinol Metab Clin North Am* 2002; 31: 1-13.
33. STRAUB RH, GUNZLER C, MILLER LE, CUTOLO M, SCHOLMERICH J, SCHILL S: Anti-inflammatory cooperativity of corticosteroids and norepinephrine in rheumatoid arthritis synovial tissue *in vivo* and *in vitro*. *FASEB* J 2002; 16: 993-1000.
34. PEROTTI LA, PERETTI L, LOVINO C, MASERARG, SARTORI ML, ANGELI A: Concentration of cortisol in the synovial fluid of patients with untreated rheumatoid arthritis. Relation to *in vitro* IL-8 production by synovial mononuclear cells. *Ann N Y Acad Sci* 1999; 876: 255-8.
35. MORELAND LW, O'DELLJR: Glucocorticoids and rheumatoid arthritis: back to the future? *Arthritis Rheum* 2002; 46: 2553-63.
36. GEORGANAS C, LIU H, PERLMAN H, HOFFMANN A, THIMMAPAYA B, POPE RM: Regulation of IL-6 and IL-8 expression in rheumatoid arthritis synovial fibroblasts: the dominant role for NF-kappa B but not C/EBP beta or c-Jun. *J Immunol* 2000; 165: 7199-206.
37. DOVIO A, SARTORI ML, MASERARG *et al.*: Autocrine down-regulation of glucocorticoid receptors by interleukin-11 in human osteoblast-like cell lines. *J Endocrinol* 2003; 177: 109-17.
38. LIU F, AUBIN JE, MALAVAL L: Expression of leukemia inhibitory factor (LIF)/interleukin-6 family cytokines and receptors during *in vitro* osteogenesis: differential regulation by dexamethasone and LIF. *Bone* 2002; 31: 212-9.
39. LIN SC, O'BRIEN CA, MANOLAGAS SC: Sex steroids decrease and glucocorticoids increase the levels of gp130 protein in bone marrow-derived stromal cells: correlation with altered IL-6 induced DNA-binding activity of STAT1 and STAT3 complexes. *J Bone Miner Res* 1996; 11: S161 (abstr.).