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Changes in Proinflammatory Cytokine Activity after Menopause

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There is now a large body of evidence suggesting that the decline in ovarian function with menopause is associated with spontaneous increases in proinflammatory cytokines. The cytokines that have obtained the most attention are IL-1, IL-6, and TNF-α. The exact mechanisms by which estrogen interferes with cytokine activity are still incompletely known but may potentially include interactions of the ER with other transcription factors, modulation of nitric oxide activity, antioxidative effects, plasma membrane actions, and changes in immune cell function. Experimental and clinical studies strongly support a link between the increased state of proinflammatory cytokine activity and postmenopausal bone loss. Preliminary evidence suggests that these changes also might be relevant to vascular homeostasis and the development of atherosclerosis. Better knowledge of the mechanisms and the time course of these interactions may open new avenues for the prevention and treatment of some of the most prevalent and important disorders in postmenopausal women. (*Endocrine Reviews* 23: 90–119, 2002)

Abbreviations: AF, Activation function; AP, activator protein; AT1, angiotensin-1; BMD, bone mineral density; CBP, cAMP response element binding protein-binding protein; COX, cyclooxygenase; DHEA, dehydroepiandrosterone; DHEAS, DHEA's sulfate conjugate; eNOS, endothelial NO synthase; ERE, estrogen response element; EVOS, European Vertebral Osteoporosis Study; GM-CSF, granulocyte-macrophage-colony stimulating factor; gp, glycoprotein; IL-1ra, IL-1 receptor antagonist; IκB, inhibitory NF-κB; M-CSF, macrophage colony-stimulating factor; iNOS, cytokine-inducible NO synthase; NF-κB, nuclear factor-κB; NO, nitric oxide; OPG, osteoprotegerin; OPG-L, OPG ligand; SERM, selective ER modulator; sIL-6R, soluble IL-6 receptor.

I. Introduction

NATURAL MENOPAUSE IS associated with a rapid decline in circulating estrogen (1). There is now a large body of evidence suggesting that, apart from the loss of reproductive function, the decline in sex hormones has many implications for nonreproductive tissues (2, 3). For example, it is well known that estrogen contributes to the maintenance of bone mass. Despite recent doubts arising from short-term placebo-controlled studies in women with established coronary artery disease, there is still substantial reason to believe that estrogen may prevent coronary heart disease in the long run. Because the average life expectancy for women in Western countries exceeds 80 yr and women spend more than a third of their lifetime in postmenopause, the possible implications of estrogen deficiency on the rates of cardiovascular disease and osteoporosis are of enormous public health importance.

There is no unifying mechanism that would be able to explain all consequences of menopause on the metabolism of

organs as diverse as bone, blood vessels, or adipose tissue (4–6). However, menopause-triggered changes in the activity of proinflammatory cytokines are beginning to emerge as a common theme that may have a significant impact on the function of all of these tissues. The following review focuses on what is currently known about the mechanisms of the interactions between estrogen and proinflammatory cytokines and attempts to outline possible implications for bone and the cardiovascular system.

II. Evidence for Increases in IL-1, TNF-α, and IL-6 after Natural or Surgical Menopause

Spontaneous increases in the expression and secretion of the proinflammatory cytokines IL-1, IL-6, and TNF-α with estrogen deficiency were first noted several years ago in *ex vivo* cultures of circulating monocytes (Refs. 7 and 8 and Fig. 1), bone marrow macrophages (9–12), and osteoblasts (13). Increases in these cytokines with estrogen deficiency are, however, subtle compared with the increases observed as a host reaction to infection or major tissue injury (14). Efforts to directly demonstrate cytokine increases with estrogen deficiency in tissue samples *in vivo* (15, 16) or in circulation (17–20) have been less successful. Nevertheless, using extremely sensitive techniques, such as IL-6 promoter luciferase constructs, increases in IL-6 promoter activity have been observed in the spleens of ovariectomized transgenic mice harboring these constructs (21). Moreover, several authors have noted increases (22) or at least a trend for increases (23, 24) in circulating IL-6 and TNF-α (25) after natural or surgical menopause.

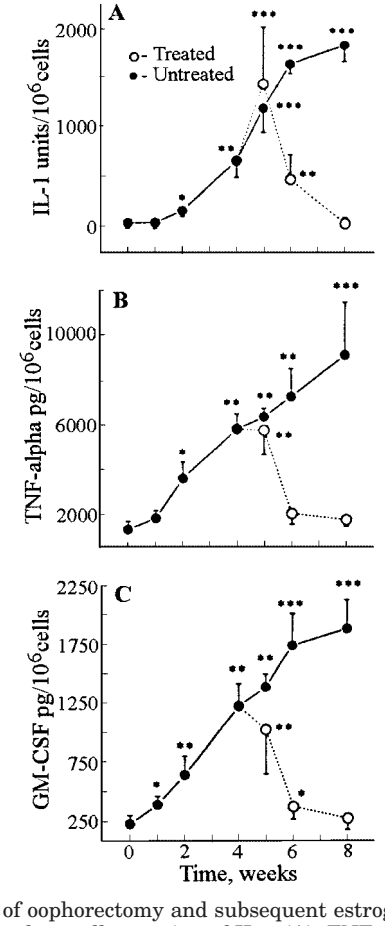

FIG. 1. Effect of oophorectomy and subsequent estrogen therapy on human mononuclear cell secretion of IL-1 (A), TNF-α (B), and phytohemagglutinin-induced secretion of GM-CSF (C). *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.0005$ compared with baseline. [Reproduced with permission from R. Pacifici *et al.*: *Proc Natl Acad Sci USA* 88:5134–5138, 1991 (8).]

Estrogen deficiency has also been shown to enhance the responsiveness of cells toward some of these cytokines by up-regulating cytokine receptor numbers and cofactors of cytokine action, thus amplifying the effects of the cytokine increases. For example, in *ex vivo* bone marrow cultures of ovariectomized mice, the expression of both the ligand-binding subunit of the IL-6 receptor [glycoprotein (gp) 80] and the signal-transducing subunit of the IL-6 receptor (gp130) were found to be increased (26). In humans, elevated soluble IL-6 receptor (sIL-6R) concentrations in circulation have been observed after surgical and natural menopause (23, 27). sIL-6R is derived from the extracellular domain of the 80-kDa receptor and is capable of presenting IL-6 to the signal-transducer gp130, thus enhancing cell responsiveness to IL-6. It is still unclear whether these changes in cell responsiveness are a direct consequence of estrogen deficiency or whether they are secondary to the increases in IL-6 concentration, because IL-6 is known to stimulate gp130 gene transcription (28). There is preliminary evidence that estrogen deficiency may also enhance the responsiveness to IL-6 through modulating IL-6 signaling pathways (29). For example, in multiple myeloma cells, 17-β-E2 completely abolished IL-6-inducible cell proliferation by inducing mRNA expression of the protein inhibitor of activated signal transducer and activator of transcription 3 (30).

III. Effect of Estrogen Treatment on IL-1, IL-6, and TNF

Because withdrawal of estrogen is associated with increases in cytokine activity, administration of estrogen might be expected to cause corresponding decreases in cytokine activity. Indeed, E2 treatment inhibited IL-1β and TNF-α expression and/or release in osteoblast-like cells (31, 32), monocytes/macrophages (8, 33–37), and whole-blood cultures (38). Likewise, E2 inhibited the release of IL-6 from a variety of cell species, including macrophages (37, 39), bone marrow cells (40), whole-blood cultures (38), bone marrow stromal cells, synoviocytes (41), osteoblasts (Refs. 42–45 and Fig. 2), and endothelial cells (46–48). E2 attenuated increases in circulating IL-6 levels in mice *in vivo* triggered by hemorrhage (49, 50). Estrogen-treated ovariectomized rhesus monkeys showed an attenuation of the peak ACTH level and the IL-6 response to an iv infusion of recombinant human IL-1 β (51). Similar inhibitory effects of estrogen on cytokine responses and the hypothalamic-pituitary-adrenal axis toward inflammatory stimuli were observed in postmenopausal women (52). Moreover, serum IL-6 levels appear to be lower in postmenopausal women on hormonal replacement therapy (22, 53, 54) and in estrogen-treated ovariectomized mice (19) compared with untreated controls. Other endog-

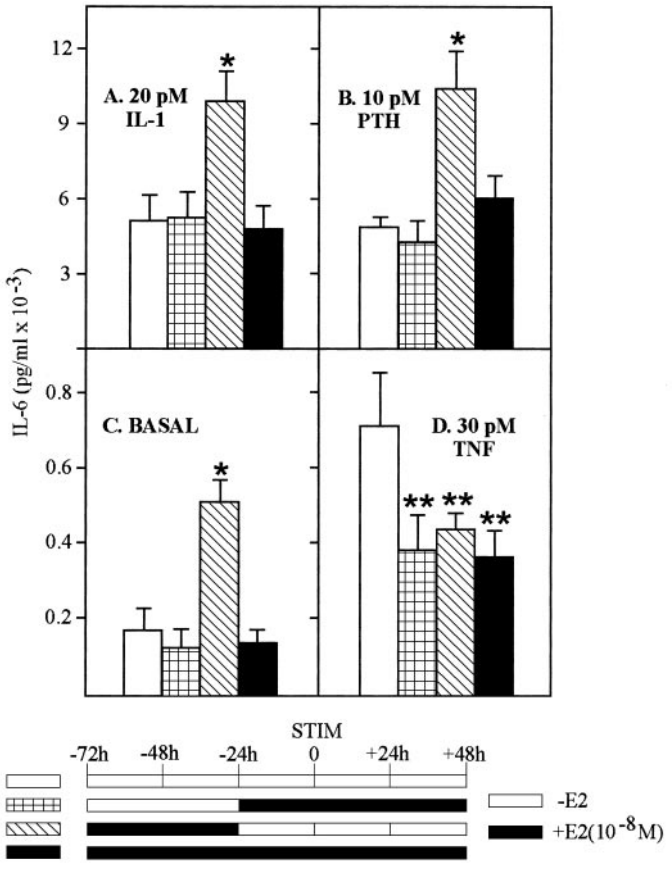

FIG. 2. Effect of withdrawal of 17β-E2 on basal and stimulated IL-6 production by primary cultures of calvaria cells. Primary cultures of calvaria cells were treated with 17β-E2, as indicated by the *black bars* at the *bottom* of the figure. At −24 h, medium was changed in all the cultures. At zero time, cells were stimulated with 20 pM recombinant human IL-1β (A), 10 nM bovine PTH(1–34) (B), left untreated (C), or treated with 30 pM recombinant murine TNF-α (D). After 48 h, supernatants were collected. *Bars* represent the mean amount of IL-6 (± SD) in supernatants from duplicate cultures. *, Significantly different from other treatment groups ($P < 0.01$). **, Significantly different from cells never treated with 17β-E2 ($P < 0.01$). [Reproduced with permission from G. Passeri *et al.*: *Endocrinology* 133:822–828, 1993 (13). © The Endocrine Society.]

enous estrogens such as estrone, which is the predominant estrogen in the menopausal years (55), and environmental phytoestrogens (56) appear to share these repressive effects of E2 on IL-6 gene expression.

Complementary changes with estrogen treatment have been observed for several other components that determine cytokine activity, such as cytokine receptors and specific binding proteins. The latter are crucial for keeping proinflammatory cytokine activities in check by interfering with cytokine receptor binding (57). Estrogen treatment increased the expression of the decoy type II IL-1 receptor in bone marrow cells and osteoclasts, while decreasing the steady-state levels of the type I IL-1 signaling receptor (58). Downregulation of the expression of the IL-6 receptor and signaling proteins by E2 was reported for bone marrow stromal cells (26).

Nevertheless, the relation between estrogen and these cytokines is far from being that simple. In fact, the existing literature is replete with seemingly contradictory data, because an equally large number of studies failed to demonstrate any effects of estrogen on the expression or protein concentrations of these cytokines. A lack of estrogen action has been reported for macrophages (59, 60, 61), bone marrow cells (45, 62, 63), bone marrow stromal cells/osteoblasts (31, 64–66), vascular smooth muscle cells (67), or on circulating levels of these cytokines (27, 63, 68–69). Even more puzzling, some researchers have even reported biphasic (70) or frankly stimulatory effects of estrogen on the release of IL-1, TNF-α, or IL-6 from monocytes/macrophages (19, 71–75) and fibroblast-like synoviocytes (76). Similar conflicting data have been reported for the effects of estrogen on the concentration of circulating TNF-α (39), IL-1α, and IL-6 (77).

These discrepancies are not readily explained by differences in species, cell type, time after menopause, estrogen concentrations, or culture conditions. They raise the hypothesis that there may be several distinct pathways by which estrogen may affect cytokine gene expression, eliciting either net increases or decreases in cytokine production, depending on the activation of these pathways in the individual cellular context. Alternatively, there may be some intrinsic or extrinsic coregulators of estrogen action that are able to switch the response from suppression to stimulation, again largely depending on the individual cellular context. Part of the discrepancies, particularly those involving *in vivo* studies, may also be due to pharmacological differences between hormonal replacement and endogenous estrogen secretion. There is no study as yet that would have reported spontaneous decreases in cytokine activity with natural menopause, suggesting that stimulatory effects of estrogen on cytokine production in nonreproductive tissues may perhaps only occur with nonphysiological conditions. For example, in a recent study, monocyte IL-6 secretion was inhibited by estrogen in healthy rats, but inhibition tended to be reversed under conditions of activated immune function (19). Better comprehension of the complex effects of estrogen on cytokine production will largely depend on the success in unraveling the underlying mechanisms of these effects at the molecular level.

IV. Potential Mechanisms of the Interaction between Estrogen and Proinflammatory Cytokines at the Molecular Level (Fig. 3)

At present, it appears as though most, if not all, effects of estrogen on cytokine activity in nonreproductive tissues occur by activating the ER. There are two well-characterized intracellular ERs, -α and -β (78). The tripartite structure of the ERs is defined by a central sequence-specific DNA binding domain, an N-terminal constitutive activation function (AF)-1, and a C-terminal E/F domain (AF-2). In the best understood mode of action, or classical pathway, ERs function as ligand-dependent transcription factors. Ligand binding is thought to cause a conformational shift in helix 12 in the C-terminal AF-2 domain. This is followed by the dissociation of tissue-specific corepressors and association of coactivators that favor the binding to specific consensus regulatory sequences, referred to as estrogen response elements

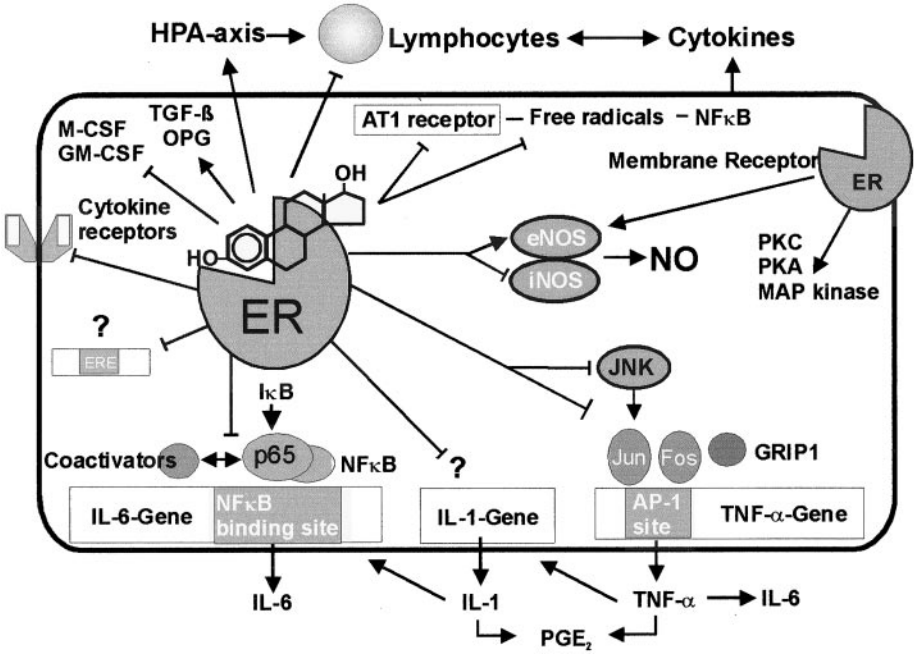

FIG. 3. Schematic presentation of the potential actions of estrogen on proinflammatory cytokine activity. The activated ER directly inhibits IL-6 and TNF-α gene expression via NF-κB- and AP-1-dependent mechanisms. Estrogen also modulates the activity of NO via eNOS and iNOS, exerts direct and indirect antioxidative actions, and interferes with the activity of the hypothalamic-pituitary-adrenal (HPA)-axis and lymphocyte function. Membrane ERs may also be involved in cytokine regulation. Cytokines that have been shown to be directly and/or indirectly modulated by estrogen include IL-1, IL-6, TNF-α, M-CSF, GM-CSF, OPG, and TGF-β. $\downarrow$, Stimulatory effects; $\perp$, inhibitory effects.

(EREs), located within the regulatory regions of target genes (79). The effects of estrogen on reproductive tissues are mediated this way. In contrast, the promoters of IL-1, IL-6, and TNF-α lack a classical ERE. Activation of the ER, therefore, is unlikely to result in a direct interaction with these promoters (43). Studies with the new class of selective ER modulators (SERMs) have provided further evidence for a different mode of interaction of the ER with target genes in nonreproductive tissues that is not mediated by a direct transcriptional effect of the ER (80–82). Consistent with such ERE-independent actions of the ER, several interactions of the ER with other transcription factors have been discovered in the past few years, some of which may also be involved in the inhibitory effects of estrogen on proinflammatory cytokine activity.

A. Interactions between estrogen and the IL-6 gene

Nuclear factor-κB (NF-κB) has a key role as a coordinating element in the response of the body to situations of stress, infection, or inflammation. NF-κB preexists in the cytoplasm of most cells in an inactive form bound to the inhibitory protein IκB. On receipt of an appropriate signal, NF-κB is released from IκB and translocates to the nucleus where it can up-regulate the expression of IL-1, IL-6, TNF-α, and other cytokines essential in an inflammatory response by binding to specific NF-κB binding sites in the promoter regions of these genes (83). Some of these cytokines, *e.g.*, IL-1 and TNF-α, activate NF-κB themselves, thus initiating an autoregulatory feedback loop.

The inhibitory action of the activated ER on binding of NF-κB to the IL-6-gene is one of the best-examined examples for a cross-talk between ligand-activated ERs and proinflammatory transcription factors. Expression of IL-6 at inflammatory sites is largely controlled by NF-κB (84). Antagonism of NF-κB activity has been observed with many nuclear receptors, including the receptors for glucocorticoids (85, 86), androgens (87), progesterone (88), the RXR (89), and the peroxisome proliferator-activated receptor γ (90). There is also ample evidence that the p65 subunit of NF-κB represses ER-α-mediated transactivation (86). However, in some cells, this repression appears to be reciprocal, and E2 can in return block the ability of NF-κB to bind to target sequences located within the regulatory regions of the IL-6 gene (44). These repressive effects of 17β-E2 on NF-κB activation have now been confirmed for a large number of different cell species, including astroglia cells (91), HeLa cells (92, 93), murine macrophages (94), human osteoblasts (44), human hepatoma HepG2 cells (95), and rat cardiac myocytes (96).

The cell type-specific manner in which this occurs is consistent with a cofactor-modulated process. Deletion analysis and the use of ER-α/β chimeras suggest that in osteoblasts, the A/B domain of the ER containing AF-1 is essential for preventing NF-κB-induced gene transcription (97). The exact mechanism by which the ER blocks NF-κB-induced IL-6 expression is, however, not agreed upon (98, 99). The association between ER and NF-κB may introduce conformational changes in both proteins that lead to the inability to bind DNA (98). Alternatively, the association may result in the formation of inactive complexes on the DNA by preventing the interaction with essential cofactors or the basal transcriptional machinery. The NF-κB protein RelA (p65) can recruit histone acetyltransferase activity to the promoter through interactions with the p300 and cAMP response element binding protein-binding protein (CBP) coactivator proteins (100). A competitive mechanism for limiting amounts of CBP and other coactivators has been proposed for the interaction between NF-κB and the GR to explain glucocorticoid-mediated inhibition of NF-κB (101). A similar competition may be involved in the estrogen-mediated inhibition of NF-κB-induced IL-6 transcription, because the ER also interacts with the p300 coactivator. In support of this hypothesis, Harnish

et al. (95) showed that in HepG2 cells, ligand-bound ER-α decreased histone acetyltransferase activity required for NF-κB transcriptional activity. CBP reversed NF-κB inhibition, supporting the hypothesis that reduced NF-κB function may occur by limiting the availability of this coactivator. Moreover, overexpression of p300 in human coronary smooth muscle cells significantly reduced the inhibitory effect of ER on RelA-dependent transcription in these cells (102). Nevertheless, some questions remain, because the latter effects were ligand dependent and may be explained by a direct inhibitory effect of p300 on ligand sensitivity (103).

In some cell species, estrogen may also act by modifying the expression of factors binding to the DNA or by altering nuclear translocation of NF-κB. Thus, in a study by Sun *et al.* (104), estrogen was observed to block the degradation of IκBα, suggesting that the inhibitory effect of estrogen on NF-κB function may also be, in part, due to the sustained presence of the inhibitory subunit of NF-κB.

It has been suggested that the effects of the ER on IL-6 transcription may extend beyond NF-κB, because the transcription factor CCAAT/enhancer-binding protein (C/EBP)β (nuclear factor-IL-6) has also been shown to interact with the human ER *in vitro* (44). Many immune response genes and acute-phase response genes contain both C/EBPβ and NF-κB sites, and cooperative interactions between nuclear factor-IL-6 and NF-κB may play an important role in the expression of these genes (105). Indeed, studies in HeLa cells transiently transfected with a pIL-6/chloramphenicol acetyltransferase plasmid and an ER expression vector, suggested that, in addition to an intact NF-κB binding site, estrogen repression of IL-6 responses requires the binding of C/EBP-family factors to the C/EBP site of the IL-6 promoter (93). However, the need for C/EBP for the inhibitory effects of estrogen on IL-6 in HeLa cells was not confirmed by Galien and Garcia (98) in a second study.

Estrogen-induced down-regulation of IL-6 expression has been independently observed in cells that only express ER-α or ER-β (96, 106), suggesting that both receptor types are capable of repressing NF-κB signaling to the IL-6 gene upon activation. Whether this may apply to all forms of the ER-β is not clear yet, because Bhat *et al.* (107) have identified a form of a human ER-β containing an N-terminal extension that attenuated cytokine-mediated NF-κB activation in contrast to a receptor form lacking this N-terminal extension. In human osteoblastic U2-OS cells that were transiently or stably transfected with ER-α or ER-β, ER-α was the major ER through which transcription of NF-κB-regulated genes was inhibited, suggesting that the extent of estrogen-induced IL-6 suppression may depend on the cell-specific pattern of ER expression (97).

The described interaction between the ER and NF-κB may also be relevant to the expression of other NF-κB-regulated proinflammatory cytokine genes (108). However, because many of these interactions appear to be cell- and gene-specific, extrapolations from the above findings to other cytokine genes are difficult to draw without detailed examination. This is illustrated by the observation that NF-κB complexes cooperate with ER-α to recruit cofactors into the complex and thereby synergistically activate the serotonin-1A receptor promoter (109).

B. Interactions between estrogen and the TNF-α gene

The activator protein (AP)-1 site in the TNF-responsive element of the −125 to −82 region of the TNF-α-promoter is thought to be critical for the ability of IL-1 and TNF-α to induce TNF-α gene expression. AP-1 consists of homodimers of Jun family proteins or heterodimers of the Jun family with Fos family proteins (110). Depending on the cellular context and the transcribed gene, estrogen has been shown to either activate (111–117) or suppress (118, 119) AP-1-mediated gene transcription. In monocytic cells, estrogen blocks AP-1-mediated transcription of the TNF-α gene. Interestingly, estrogen also repressed the collagenase promoter when cotransfected into the cells, despite the fact that estrogen is known to activate collagenase transcription in other cell types via an AP-1 element in the collagenase promoter. This clearly indicates that estrogen-mediated stimulation or repression of gene transcription at AP-1 sites occurs in a cell-specific fashion (120). Of note, soybean isoflavones are also capable of suppressing TNF-α transcription in monocytes by binding to the ER (121).

The direct interaction between the ER and the AP-1 site in the TNF-α promoter requires binding of the ER to other promoter-bound proteins, such as the GR-interacting protein 1 (121, 122), and the AF-2 surface in the ligand-binding domain of the ER (120, 121). This may explain why raloxifene, which prevents the formation of an active AF-2 surface, antagonized the AP-1-mediated repression of the TNF-α promoter (120, 122).

Estrogen may also suppress AP-1-dependent TNF-α-transcription by inhibiting Jun expression and suppressing the Jun N-terminal kinase pathway. Binding of estrogen to ER-β in the murine RAW 264.7 monocytic cells reduced the activity of the Jun NH_2-terminal kinase, diminished phosphorylation of c-Jun and JunD at their NH_2 termini, and resulted in a consecutive decrease in the ability of these nuclear proteins to autostimulate the expression of c-Jun and JunD genes, thus leading to lower production of c-Jun and JunD (123, 124).

Decreases in TNF-α transcription in monocytes were observed with transfection of both the estrogen α- and β-receptor, albeit the latter appears to be more efficient in suppressing TNF-α transcription (120, 123). There is increasing awareness of such a selective regulation of ER-α and -β mode and amplitude of gene transcription by different receptor ligands, as both receptors appear to have strong affinity preferences for particular coactivators (125). The manner by which the two different ERs are regulated is just about to be explored, but differential effects of ER-α and ER-β on both NF-κB-induced IL-6 production (97) and AP-1-dependent TNF-α gene transcription may offer a glimpse at one of several possible mechanisms explaining the cell-specific differences in the effects of estrogen on cytokine production.

Until now, no direct molecular mechanism has been proposed for a direct suppressive effect of estrogen on IL-1 transcription. NF-κB is a potent stimulator of IL-1 gene transcription (126), raising the possibility that repression of IL-1 transcription by estrogen may work through a similar mechanism as that implicated in the repression of IL-6 gene transcription. However, it is also possible that the increases in

IL-1 activity with estrogen deficiency may be merely secondary to an increase in TNF-α, as the two cytokines are known to stimulate the expression of each other (127, 128) and the secretion of the two cytokines is usually closely correlated. Consistent with this hypothesis, TNF-α neutralization blocked IL-1 production, whereas IL-1 neutralization did not block TNF-α (129). Likewise, in cell cultures prepared from neonatal calvariae, TNF-α-stimulated IL-6 production was inhibited by 17β-E2, whereas IL-1-induced IL-6 production was unaffected by 17β-E2 (42).

C. Estrogen, nitric oxide (NO), and cytokines

NO may be another potential target through which estrogen may regulate cytokine activity. NO has been the focus of much attention in the last few years. As a diffusible universal messenger, it plays a central role in the pathogenesis of inflammatory diseases (130). NO is an extremely pleiotropic molecule, and there are many contradictory reports in the literature concerning its role as an anti- or proinflammatory agent. These inconsistencies may be due to the multiple cellular actions of this molecule, the level and site of NO production, and the redox milieu into which it is released (131). Estrogen causes rapid stimulation of NO production in endothelial cells (132, 133), osteoblasts (134), and monocytes (135). This is achieved through up-regulation of the activity of the constitutively expressed endothelial NO synthase (eNOS/NOS III) and does not appear to be associated with changes in eNOS mRNA or protein (136). Correspondingly, ER-α-knockout mice have a significantly reduced basal release of NO in aorta (137). Moreover, plasma nitrate and nitrite levels, which are related to NO production, are decreased in a state of estrogen deficiency (16, 138, 139), and serum NO levels increase with estrogen replacement (140, 141).

Determining the precise mechanism through which estrogen regulates eNOS activity is an active area of investigation (142). There is increasing evidence that at least part of the acute stimulation of eNOS enzymatic activity may be mediated by coupling of specific plasma membrane ERs to Gα proteins (134, 143, 144). In human endothelial cells, the ER-α also appears to bind to the p85α regulatory subunit of PI3K, leading to the activation of protein kinase B/Akt (145, 146).

Interestingly, and emphasizing the complexity of estrogen actions on NO production, estrogen inhibits another form of NOS that is induced by IL-1 and TNF-α and is therefore called cytokine-inducible NO synthase [iNOS/NOS II (147–150)]. We still do not exactly understand the physiological implications of the differential effects of estrogen on the two NO synthase isoforms. However, the inhibitory effects of estrogen on iNOS activity might well contribute to its suppressive effects on proinflammatory cytokine activity (149).

The effects of NO on proinflammatory cytokines and cytokine-dependent tissue activities are mixed and may critically depend on cofactors in the cellular environment. Both stimulatory (151–157) and inhibitory effects (158–166) on the expression and/or secretion or proinflammatory cytokines have been described. These dual effects are nicely illustrated by the differential effects of NO in bone tissue. iNOS-deficient mice exhibit profound defects of IL-1-induced osteoclastic bone resorption through abnormalities in the translocation of the p65 component of NF-κB and in NF-κB-DNA binding, suggesting that the iNOS pathway is essential for IL-1-induced bone resorption, which is again thought to play a major role in menopause-associated bone loss (167). In contrast, NO donors have been rather shown to reverse ovariectomy-induced bone loss in rats (168, 169), and nitrate use appears to protect postmenopausal women against bone loss as effectively as estrogen use (140, 170).

At present, there are no data that would prove any relevance of these changes in NO activity to cytokine production with menopause. Nevertheless, given the central role of NO as a cellular mediator, the differential effects of estrogen on eNOS and iNOS activity on the one hand, and the variable interactions between NO and cytokine activity on the other hand, may be part of the "Janus-faced" effects of estrogen on cytokine production that have been encountered in some of the described experimental models.

D. Direct antioxidant effects of estrogen

Free radicals are potent stimuli of NF-κB-mediated proinflammatory cytokine expression. As estrogen has direct antioxidant effects *in vitro* and *in vivo*, increases in free radical production with the decline in estrogen concentrations may be another source of increase in cytokine production. In vascular tissue, increases in the oxidative modifications of molecules, such as low-density lipoproteins, after menopause may stimulate cytokine production by propagating the formation of cytokine-producing foam cells that are characteristic of early atherosclerotic lesions (171).

The mechanisms of the antioxidant effect of estrogen are incompletely understood and may vary in different tissues. Estrogen might play an inhibitory role either in production and/or scavenging of reactive oxygen species, or in protecting the level of endogenous antioxidants. An ER-dependent inhibition of superoxide anion production in microglial cells has been linked to the activation of MAPK (172). Direct antioxidative effects appear to be dependent on the A-ring hydroxyl group of the estrogen molecule, which acts as a highly effective electron donor and free radical scavenger. 3-Methoxyestrone, which lacks the phenolic hydroxyl group, does not interfere with free radical generation (102, 173). However, E2 may also affect the cellular antioxidant enzyme system that is necessary to maintain an optimal redox balance. Detoxification of superoxide anion and hydrogen peroxide, catalyzed by intracellular superoxided dismutase, catalase, and glutathione peroxidase enzyme activities, represents a major line of defense. Significantly lower glutathione peroxidase activity has been seen in blood samples from late menopausal women compared with that seen in premenopausal women (174–176). Some of the antioxidative properties of estrogen on the vessel wall also appear to be mediated through down-regulation of angiotensin-1 (AT1) receptor gene expression. Up-regulation of AT1 receptor expression with estrogen deficiency is thought to be a predominant source of free radical production in the vasculature. Indeed, increases in the generation of superoxide radicals in the vessel wall with ovariectomy in the rat can be normalized by AT1 receptor antagonists (177–179).

The impact of these antioxidative effects of estrogen on cytokine production has received little attention so far. Many of the antioxidative effects of estrogen have been observed at supraphysiological levels, but there are some data showing that these effects may also be relevant at physiological estrogen levels (180). For example, 17β-E2 inhibited *in vitro* vascular smooth muscle cell proliferation via a nongenomic antioxidant mechanism (181). 17β-E2, but also its stereoisomer 17α-E2, dose dependently and receptor independently inhibited reactive oxygen species generation in cytomegalovirus-infected smooth muscle cells and the consecutive increases in NFκB-activity. These effects occurred at physiological estrogen concentrations (102).

E. Rapid nongenomic actions

In a variety of cell types, E2 exerts rapid nongenomic actions that appear to be mediated by plasma membrane ERs (182). For example, E2 rapidly activates MAPK in endothelial cells (183) and in fibroblasts that have been transfected with cDNA clones encoding either ER-α or -β (184). Estrogen also rapidly activates early growth response gene-1 expression in cardiomyocytes via ERK 1 and 2 (185). These rapid effects of estrogen trigger several interactive signal transduction pathways that involve the PKC and PKA pathways as well as MAPK and resemble the effects of the more traditional membrane growth factor receptors. These pathways have the potential to enhance (186–193) or inhibit (194) the expression of IL-1, IL-6, and/or TNF-α, depending on the cellular context. The relevance of these estrogen membrane receptors for estrogen actions is just about to be explored, but it is possible that the stimulatory effects of estrogen on the production of proinflammatory cytokines that have been observed in some *in vitro* settings may be in part attributable to rapid estrogen actions and to membrane-receptor-mediated effects.

F. Effects of estrogen on lymphocytes

Of considerable interest both with respect to postmenopausal changes in bone metabolism and vascular function is the action of estrogen on lymphocyte function (195, 196). Alterations in T cell subsets have already been described several years ago in women with postmenopausal osteoporosis (197, 198). A recent study by Cenci *et al.* (199) suggested that the increase in TNF-α production in the bone marrow of ovariectomized mice may, at least in part, originate from activated T cells. Remarkably, this appeared to be due to the increase in the number of TNF-α-producing T cells, not to an increase in TNF-α gene expression. The expansion of the T lymphocyte pool in bone marrow after loss of ovarian function may thus be an important indirect mechanism for increases in proinflammatory cytokine concentration. In line with this, the inhibitory effect of estrogen on TNF-α production in an animal model of experimental autoimmune encephalomyelitis appears to be predominantly due to a reduction in the number of proinflammatory cytokine producing T cells in the central nervous system (200).

Estrogen deficiency is also associated with an increase in pre-B lymphopoiesis in bone marrow (201–205). *Vice versa*, B-lineage differentiation in highly purified early pro-B cells was abrogated when the cells were treated with E2 (195). In a mouse model of systemic lupus erythematosus, estrogen treatment altered the maturation of splenic B cell subsets with a diminished transitional population and an increase in marginal zone B cells (206). As cell-to-cell interactions between lymphocytes and mesenchymal cells greatly affect the production of IL-6 by mesenchymal cells (207, 208), the postmenopausal increases in lymphopoiesis may not only affect TNF-α secretion, but IL-6 synthesis as well.

At present, it is difficult to judge which may be first: the rise in proinflammatory cytokines or the enlargement of the activated lymphocyte pool. Arguing for the latter, both T and B cells express ERs, and estrogen deficiency may remove some direct restrictions imposed on lymphocyte proliferation and function. Alternatively, the increases in lymphocyte number and function may predominantly be the result of the increased availability of lymphocyte stimulators after menopause. RANKL, a member of the TNF ligand family whose interactions with estrogen will be described in *Section V* in more detail, is crucial for lymphocyte development. As the activity of RANKL is up-regulated by estrogen deficiency, this may in turn promote T and B cell development and activation (209). Kanematsu *et al.* (210) reported that in ovariectomized mice, increases in pre-B cells were abolished by the administration of the cyclooxygenase (COX) inhibitor indomethacin. This suggests that RANKL-induced increases in pre-B cells may be in part mediated by increased PGE_2 levels. Administration of a neutralizing antibody against IL-6 also has been shown to prevent ovariectomy-induced increases in the number of several hematopoietic progenitors in the bone marrow of mice (211). Mechanisms that involve the interaction between matrix proteins, such as osteopontin and lymphocyte function, may potentially play a role as well (212).

G. Central interactions between the gonadal axis and inflammatory processes

Apart from direct cellular interactions between estrogen and cytokines at the molecular level, several menopause-related changes in cell and tissue function have the potential to indirectly affect proinflammatory cytokine activity, although the relevance of these interactions is largely unknown at the present time. For example, estrogen is known to alter the hypothalamic-pituitary-adrenal axis through modulating the release of both hypothalamic and pituitary peptides, such as PRL (213) and GH, which in turn may modify immune responses and cytokine secretion (214). There are many other reciprocal interactions between the hypothalamic-pituitary-adrenal axis and immune-mediated inflammatory processes on the one hand and the gonadal axis on the other hand, which may potentially contribute to changes in inflammatory parameters with menopause. Estrogen directly enhances CRH gene expression and stimulates the central noradrenergic system, thereby positively affecting the two principal components of the stress system. (215). This may be associated with a suppression of the immune-inflammatory reaction and a shift from the T helper cell 1 to the T helper cell 2 profile in lymphocytes (216). It may

also, in part, explain the preponderance of some autoimmune disorders in women as compared with men.

H. Changes in visceral fat with menopause

Women tend to accumulate visceral fat with menopause. This appears to involve an estrogen-dependent mechanism of reduced energy expenditure (217–219) and can be prevented by hormonal replacement therapy (220, 221). Although still speculative at the present time, adipose tissue might turn out to be a significant source of increased proinflammatory cytokines in postmenopausal women. Indeed, there is increasing evidence that adipose tissue is a major determinant of circulating IL-6 (222). As adipocytes from visceral adipose tissue release 2–3 times more IL-6 than those from the sc depot (223), even small increases in visceral fat with menopause might have a large impact on circulating IL-6 levels. In accordance with this hypothesis, Straub *et al.* (54) observed a positive association between body mass index and serum IL-6 only in postmenopausal women, and this relationship was lost among women with hormone replacement. It appears that to some extent, IL-6 may in return increase estrogen synthesis in adipose tissue by stimulating the aromatase activity and, thus, the conversion of C19 androgenic steroids to estrogen (224). As adipose tissue is the major site of estrogen biosynthesis in postmenopausal women, this may serve as a negative feedback mechanism to limit IL-6 increases.

V. Effects of Estrogen on RANKL and Osteoprotegerin (OPG)

RANKL is a recently discovered member of the TNF superfamily. It is a membrane-associated factor that is expressed in a variety of cell species, including osteoblast/stromal cells, endothelial cells, and lymphocytes. As a result of its independent discovery by several investigators, it is also known as TNF-related activation-induced cytokine, OPG ligand (OPG-L), and osteoclast differentiation factor (225, 226). RANKL exerts its effects through binding to RANK, a TNF receptor family member. RANK is also known as osteoclast differentiation and activation receptor. The biological activity of RANKL is neutralized by binding to OPG, another member of the TNF receptor superfamily. OPG is also known as osteoclastogenesis inhibitory factor, follicular dendritic cell-derived receptor-1, or TNF receptor-like molecule 1. OPG does not contain a transmembrane domain and acts as a soluble receptor.

RANKL and OPG have emerged as essential mediators of proinflammatory cytokines on osteoclastogenesis and lymphocyte maturation. RANKL- and RANK-deficient mice have a complete block in osteoclast development and show significant alterations in both immune cells and immune cell organs with a lack of lymph node development (227, 228). Moreover, several studies have implicated OPG in the regulation of vascular homeostasis (229–230).

Estrogen dose dependently increased levels of OPG mRNA and protein in human osteoblastic cells (231) and stromal cells (232). Cotreatment with the antiestrogen ICI 182,780 abrogated these effects completely, demonstrating that they are ER mediated. In the mouse stromal cell line ST-2, enhanced induction of OPG expression was only observed in ER-α-overexpressing cells, but not in ER-β-transfected cells, indicating that, at least in some cell species, the stimulatory effects of estrogen on OPG are dependent on ER-α (233). Estrogen deficiency results in decreased OPG expression in bone, whereas estrogen replacement therapy prevents it (234). In accordance with these findings, postmenopausal women who use hormone replacement therapy were reported to have higher circulating OPG levels than those without hormone replacement (235). Apart from its direct effect on OPG synthesis, estrogen may indirectly block RANKL activity by limiting the availability of the major RANKL stimulators IL-1, IL-6, and TNF-α (236). However, IL-1-α and TNF-α are known to stimulate OPG secretion as well (237–240). RANK expression itself appears to be unaffected by estrogen deficiency, but estrogen deficiency may enhance RANK signaling in monocytes by down-regulating RANK-induced Jun N-terminal kinase 1 activity (241).

VI. Effects of Estrogen on Other Cellular Mediators Relevant to Inflammatory Processes

A. Effects of estrogen on TGF-β

TGF-β plays an important role in tissue recycling and tissue repair in response to injury. It exerts a large number of suppressive effects on inflammatory processes, ranging from the suppression of the growth and differentiation of hematopoietic precursor cells to inhibition of proliferation and apoptosis of immunocompetent cells. TGF-β thereby often antagonizes the effects of proinflammatory cytokines. The essential role of TGF-β in suppressing inflammatory processes is impressively demonstrated by the appearance of massive infiltrations of inflammatory cells in several organs of mice deficient in TGF-β1 activity (242–244).

Findings that estrogen positively affects TGF-β function have been very consistent for various tissues. Estrogen stimulates TGF-β expression and/or production by osteoclasts (245, 246), osteoblasts (247, 248), vascular smooth muscle cells (249), and fibroblasts (250). Transdermal 17β-E2 increased serum levels of TGF-β1 in postmenopausal women (251). With the exception of a single study (252), ovariectomized rats were observed to have lower protein and mRNA levels of TGF-β1 in bone tissue compared with sham-operated rats (253–255). Interestingly, in contrast to rats, whose capacity for bone remodeling is limited and in whom the primary effects of estrogen on TGF-β production are likely to predominate, estrogen deficiency in humans is associated with an increase in skeletal TGF-β, and this increase can be prevented by hormone replacement therapy (256). However, this is most likely a consequence of the reactive increases in bone formation and not a direct effect on TGF-β expression.

The stimulatory effects of estrogen on TGF-β activity are also ERE independent and can be mimicked by the SERMs tamoxifen and raloxifene (246, 248, 257). Yang *et al.* (248) demonstrated a direct interaction of the ER with a sequence in the TGF-β3 gene promoter termed "raloxifene response element," which did not require the DNA binding domain of the ER. Astonishingly, activation of TGF-β3 transcription

was also observed with the pure ER antagonist ICI 164,384 (248), but this was not confirmed in another study (245).

B. Effects of estrogen on PG synthesis

COX is the key step in the conversion of arachidonic acid to a series of bioactive prostanoids. Two COX genes have been identified: a constitutive form (COX-1), which is expressed ubiquitously, and a second form (COX 2), which is highly inducible in response to proinflammatory cytokines (258). COX 2 expression is greatly elevated in both acute and chronic inflammation. PGE_2 is one of the most abundant COX metabolites. Estrogen deficiency increased PGE_2 concentrations in *ex vivo* cultures of human and rat bone marrow cells (12, 259) and in organ cultures of calvariae from ovariectomized rats (260). Postmenopausal women also excrete larger amounts of urinary PGE_2 compared with premenopausal women (261). Conversely, E2 inhibited PGE_2 production in stromal cells and monocytes (259, 261–263).

At present, there is no evidence for a direct inhibitory effect of estrogen on COX activity. Rather, estrogen increases the release of another COX product, PGI_2, by vascular endothelium (264–266). It is likely that the increased production of PGE_2 with menopause is predominantly due to the up-regulation of its major cytokine inducers. This is supported by the finding that IL-1 receptor antagonist (IL-1ra) and anti-IL-1α-neutralizing antibody were able to decrease exaggerated PG production and COX-2 expression in cultured neonatal mouse calvariae when stimulated with bone marrow supernatants from ovariectomized mice (259).

C. Effects of estrogen on macrophage colony-stimulating factor (M-CSF) and granulocyte-macrophage-colony stimulating factor (GM-CSF)

GM-CSF and M-CSF control the production, maturation, and function of granulocytes and monocyte-macrophages (267). Increases in GM-CSF activity were observed after surgical menopause in *ex vivo* cultures of peripheral blood mononuclear cells (7). Bone marrow cells from women who had discontinued estrogen replacement shortly before aspiration or who were within 5 yr of natural menopause also secreted more GM-CSF than bone marrow cells from premenopausal women (12). As with PG production, the increases in GM-CSF with estrogen deficiency may be predominantly mediated by IL-1 and TNF-α. However, other mechanisms must be involved as well, as the increase in GM-CSF production in mononuclear cells from ovariectomized women was very rapid and preceded the increase in IL-1 and TNF (7).

Estrogen also down-regulates membrane-bound and soluble M-CSF. Whereas the expression of membrane-bound M-CSF on bone marrow cells is directly inhibited by estrogen (268, 269), down-regulation of soluble M-CSF secretion by estrogen may involve the differentiation of stroma cell precursors toward a mature phenotype characterized by a lower production of soluble M-CSF. *Vice versa*, estrogen deficiency increases the production of soluble M-CSF in bone marrow by an IL-1- and TNF-mediated expansion of a stromal cell population that produces larger amounts of soluble M-CSF (34, 270).

VII. Implications for Postmenopausal Bone Loss (Fig. 4)

A. Impact of cytokine changes with estrogen deficiency on osteoclastogenesis

There is progressive loss of bone tissue after natural or surgical menopause, leading to increased fractures within 15–20 yr from the cessation of ovarian function (271). ERs have been detected in many cells that reside in bone tissue (272–278), suggesting that menopause may have direct consequences on cytokine secretion by cells located within the bone microenvironment. Bone marrow cells of the monocyte/macrophage lineage are believed to be the major source of the postmenopausal increases in TNF-α and IL-1 secretion in bone tissue (279). However, in the past few years it has been increasingly recognized that activated T cells are also an important source of increased TNF-α production in the bone marrow after menopause (195, 196, 209, 280–283). In contrast, stromal cells/osteoblasts are considered to be the major producers of IL-6 in bone tissue (284).

Proinflammatory cytokines are among the most powerful stimulants of bone resorption known. They directly and through the stimulation of other local factors intervene with every single step in osteoclastogenesis that determines the rate of bone resorption, from the proliferation and differentiation of the early osteoclast precursor cell to the resorption capacity and the lifespan of the mature osteoclast (9, 285–301). The first step in osteoclastogenesis that determines the rate of bone resorption is the proliferation of osteoclast precursor cells. In fact, a major consequence of estrogen deficiency is the expansion of the pool of osteoclastic precursor cells in the bone marrow. Loss of ovarian function is permissive for the expression of the major cytokines that directly stimulate early osteoclast precursor proliferation, *i.e.*, M-CSF, GM-CSF, and IL-6 (289, 301–307). Spontaneous increases in these cytokines may be further enhanced by the parallel increases in IL-1 and TNF-α with menopause, which are potent stimulators of M-CSF, GM-CSF (292, 298, 308–311), and IL-6 (64, 286, 306, 312–314). On the response side, these favorable changes in the cytokine environment with respect to osteoclast progenitor proliferation are met by complementary increases in the responsiveness of the osteoblast/stromal cells to IL-6 by up-regulation of the IL-6 receptor and its signal transduction unit gp130 (26).

Because estrogen is a potent stimulator of OPG production (237) and suppresses M-CSF production, loss of estrogen would also be expected to promote the signaling and gene expression cascade that leads to the differentiation of osteoclast progenitors to mature osteoclasts. A decrease in OPG production with estrogen deficiency is bound to increase the ratio of RANKL/OPG activity. It thus facilitates the binding of RANKL-expressing osteoblast/stromal cells and lymphocytes to osteoclast progenitors as the key signal for initiating osteoclast differentiation (227, 315–319). Estrogen deficiency is associated with the loss of inhibitory effects on a closely interrelated network of cytokine stimuli that are suited to tip

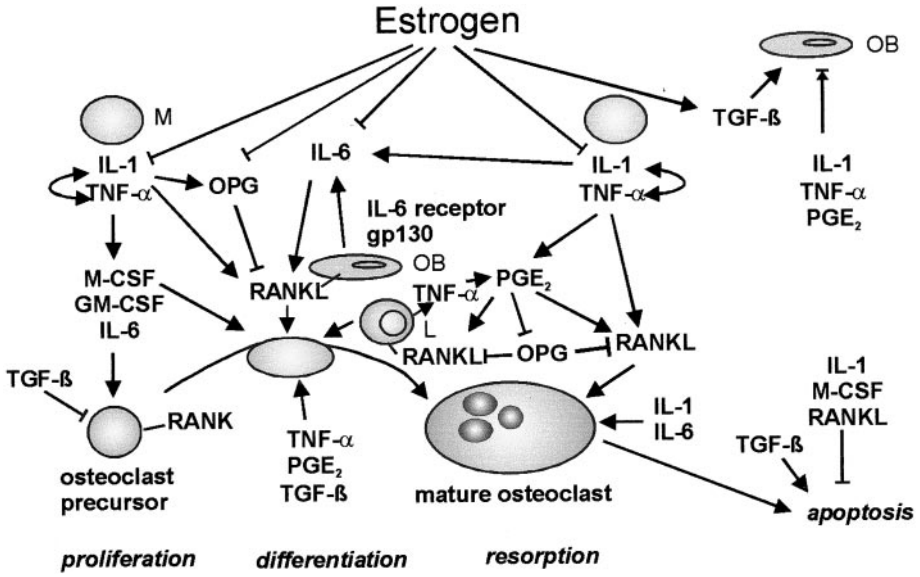

FIG. 4. Overview of the multiple interactions by which cytokines and estrogen regulate bone resorption. The arrows indicate stimulatory (↓) or inhibitory (⊥) effects of a cytokine on the synthesis of another cytokine or on a particular step in osteoclastogenesis. Macrophages (IL-1 and TNF-α), lymphocytes (TNF-α and RANKL), and stromal cells/osteoblasts (IL-6, TGF-β, RANKL, M-CSF, GM-CSF, and PGE_2) are thought to contribute to the increased cytokine production in the bone microenvironment with estrogen deficiency. M-CSF, GM-CSF, and IL-6 facilitate osteoclast precursor proliferation. RANKL, TNF-α, PGE_2, and TGF-β participate in the differentiation of osteoclast precursors into mature osteoclasts. RANKL, IL-1, and IL-6 directly stimulate the bone resorbing activity of the mature osteoclast. IL-1, M-CSF, RANKL, and TGF-β modulate osteoclast apoptosis. IL-1, TNF-α, PGE_2, and TGF-β also have potent effects on osteoblast function. Most cytokines are stimulated or inhibited by other cytokines, thereby creating a network of synergistic cytokine actions, which ultimately lead to increased bone resorption. OB, Osteoblast/stromal cell; L, lymphocyte; M, monocyte/macrophage.

the balance of RANKL-to-OPG production further toward increased RANKL activity. In particular, PGE_2, which increases RANKL expression and decreases OPG expression in osteoblastic/stromal cells (240, 320, 321), may be involved in the increase in the expression of RANKL in bone marrow pre-B cells from ovariectomized mice, as the increase could be mimicked by PGE_2 and was abolished by indomethacin (210, 322). The PGE_2-induced increases in the RANKL/OPG ratio with estrogen deficiency may again only be a consequence of increases in monocyte and T cell-derived IL-1 and TNF-α secretion (238, 318, 323–326). Although the data are still somewhat conflicting (226, 237, 239, 327), IL-6 also appears to contribute to this up-regulation of RANKL activity in estrogen-deficient osteoblastic cells (318, 328).

Both TNF-α (316, 318, 329, 330) and PGE_2 (331) may have a direct, RANKL-independent effect on osteoclast differentiation. This effect may be small compared with that of RANKL, but it may be important for potentiating RANKL-induced osteoclast formation (332). Indeed, it has been proposed that because minuscule amounts of RANKL are sufficient to maximally stimulate osteoclastogenesis, RANKL-independent effects may be the true rate-limiting steps of increased osteoclast differentiation with menopause (332).

Whereas all of the consequences of estrogen deficiency on proinflammatory cytokine activity described so far are clearly synergistic with respect to osteoclast formation and strongly support a major role of these cytokines in postmenopausal bone loss, the role of TGF-β is more ambivalent. Proliferation of osteoclast progenitors may be facilitated by the relative decline in TGF-β activity with estrogen deficiency, as TGF-β has been shown to be a major inhibitor of osteoclast generation (333, 334). Relative decreases in TGF-β with menopause may also contribute to the increases in the RANKL/OPG ratio with estrogen deficiency (236, 326, 335). On the other hand, TGF-β has recently been observed to be essential for the differentiation of osteoclast precursors cells into osteoclasts (336–338). Moreover, TGF-β has been described as both stimulating (339–341) and inhibiting (73, 342, 343) proinflammatory cytokine production.

IL-1, TNF-α (344, 345), and PGE_2 (346) may not only promote osteoclast generation, but they also appear to stimulate mature osteoclasts to perform more resorption cycles via modulation of RANKL activity (347). IL-1 (318, 329, 348, 349) and IL-6 (350) also directly enhance osteoclast activity by RANKL-independent mechanisms. Finally, increases in IL-1 (351, 352), M-CSF (353), RANKL (225), and a relative decrease in TGF-β (246) may directly extend the lifespan of the osteoclast by inhibiting osteoclast apoptosis.

In premenopause, variations in bone resorption are usually compensated by appropriate changes in bone formation. This is thought to be the result of locally released anabolic growth factors, such as TGF-β (354) and IGFs (355). Efforts to compensate the increased bone resorption are also evident with estrogen deficiency (256) but obviously do not suffice to achieve a neutral bone balance. Thus, depending on the perspective, postmenopausal bone loss may also be viewed as resulting from inadequate bone formation. It is well known that proinflammatory cytokines have potent effects on osteoblast function. Both TNF-α and IL-1 inhibit collagen synthesis in osteoblasts (299, 356–361) and stimulate or inhibit bone cell proliferation, depending on the experimental systems employed (325, 357, 362–365). Both TGF-β and PGE_2

are potent stimulators of bone formation (366, 367). However, the relevance of postmenopausal changes in these cytokines with respect to bone formation has been much less explored compared with bone resorption.

B. Evidence for an essential role of proinflammatory cytokines in bone loss with estrogen deficiency

There is now compelling evidence from studies in mice and rats that changes in cytokine activity with estrogen deficiency are indeed essential for the bone loss that is observed in these animals. Mice insensitive to IL-1 due to the lack of IL-1 receptor type I are protected against ovariectomy-induced bone loss (368). Treatment with IL-1ra decreased bone resorption (11) and prevented bone loss in ovariectomized rats (10). The essential role of TNF-α in ovariectomy-associated bone loss is suggested by the finding that treatment of ovariectomized mice with TNF-α binding protein, a potent inhibitor of TNF-α, completely prevented bone loss (11, 369). Likewise, transgenic mice insensitive to TNF-α due to the overexpression of soluble TNF receptor (370), as well as rats receiving an orally active inhibitor of IL-1 and TNF production (371), have been shown to be protected against bone loss with ovariectomy. Increases in osteoclast numbers *in vitro* and *in vivo* in ovariectomized mice were also prevented by an antibody to IL-6 (Refs. 9 and 372 and Fig. 5). In accordance, IL-6-knockout mice are protected against the loss of trabecular bone induced by ovariectomy (373). The critical involvement of PGs is supported by the findings that the nonsteroidal COX inhibitor naproxen inhibited bone loss induced by up to 70% ovariectomy in rats (374). T cell deficiency also effectively prevented bone loss in ovariectomized mice (199).

Of interest is that IL-1β-deficient mice do not appear to be protected against ovariectomy-induced bone loss (375). Thus, simultaneous suppression of both the IL-1α and IL-1β genes may in fact be required for blocking bone loss with estrogen deficiency. Moreover, some of the requirements of cytokines for bone loss, at least in these animal models, appear to change with time after estrogen withdrawal. Whereas simultaneous treatment with IL-1ra and the TNF-binding protein was required to completely prevent the bone loss and increase in bone resorption in the first month after ovariectomy (376), IL-1α alone was sufficient to prevent bone loss in the following months (10).

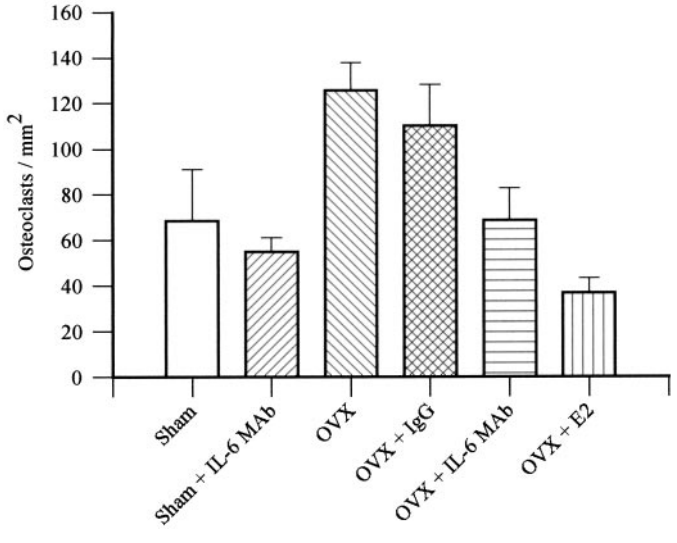

FIG. 5. Inhibitory effect of a neutralizing antibody to IL-6 (IL-6 MAb) or estrogen administration (E2) on osteoclast numbers per millimeter of secondary spongiosa of the tibia of ovariectomized (OVX) female mice. Data are mean ± SEM. In contrast, osteoclast numbers in OVX mice and OVX mice receiving a monoclonal IgG antibody to β-galactosidase (OVX + IgG) were significantly increased compared with those of sham-operated animals (Sham). [Derived from R. L. Jilka *et al.*: *Science* 257:88–91, 1992 (9).]

As outlined above, bone loss with estrogen deficiency involves a large number of interrelated changes in estrogen-dependent regulatory factors (377). In contrast to other proinflammatory conditions such as inflammatory arthritis, in which the deficiency in single proinflammatory cytokines does not fully prevent the inflammatory process (378), deficiency in each of the above cytokines is sufficient to completely block excessive bone resorption with estrogen deficiency. Miyaura *et al.* (379) showed that the endogenous bone-resorbing activity present in bone marrow supernatants from ovariectomized mice was only equaled by the concurrent addition of IL-1, IL-6, sIL-6R, and PGE_2 in those concentrations that were present in bone marrow supernatants, suggesting that the full extent of bone resorption in this *in vitro* system was only achieved by the additive effects of these factors. However, it should be pointed out that the central role of cytokines for bone loss with estrogen deficiency does not exclude the possibility that additional cytokine-independent changes in bone metabolism may also be among the determinants of postmenopausal bone loss. For example, there is an increasing body of evidence supporting direct, cytokine-independent effects of estrogen on osteoclast and osteoblast function (380–384). Some of the postmenopausal changes in bone metabolism may also result from the loss of extraskeletal effects of estrogen on calcium homeostasis (6) and IGF-I synthesis (253, 385–393).

Despite the impressive prevention of ovariectomy-induced bone loss by neutralizing each single cytokine involved in this process, in most of the above animal studies bone resorption was normal in the animals that were not deficient in estrogen, indicating the particular role of the changes in these cytokines and cellular components in bone loss with estrogen deficiency. The redundancy of the function of most of these cytokines for osteoclast formation may compensate the lack of function of each of these components in situations apart from estrogen deficiency. The clear exceptions are M-CSF and the components of the RANKL/OPG/RANK system, whose activity is essential for osteoclast generation (199, 230, 317, 394–396).

C. Evidence for a role of cytokines in human bone loss after menopause

Most of the evidence for a causal involvement of proinflammatory cytokines in bone loss with estrogen deficiency has been obtained in rodents. However, as bone metabolism in rodents differs in many ways from that in humans, it is not certain that these pathways will also prove to be as important in humans. In fact, evidence that proinflammatory cytokines

play a similar decisive role in postmenopausal human bone loss is still very limited. Arguing in favor of a similar mechanism in humans are findings that the enhanced capacity of monocyte cultures from postmenopausal women to stimulate bone resorption *in vitro* correlated with the levels of IL-1, IL-6, and TNF-α in the conditioned medium and could be blocked by the addition of IL-1ra and anti-TNF antibody (397). Ralston *et al.* (33) demonstrated that IL-1, TNF, and IL-6 mRNAs are expressed more frequently in bone cells from untreated postmenopausal women than in those from women on estrogen replacement. There are preliminary reports that serum OPG levels may be associated with bone mineral density [BMD (398)], but these findings have not been confirmed by a subsequent study (235). Further evidence for a specific role of proinflammatory cytokines as a determinant of human postmenopausal bone loss comes from the Heidelberg cohort of the European Vertebral Osteoporosis Study [EVOS (399)]. In this study, serum IL-6 strongly and specifically predicted femoral bone loss in early postmenopausal women. In fact, serum IL-6 was the single most important predictor of early postmenopausal bone loss, accounting for up to 34% of the total variance of change in BMD in the first few years after menopause. This is, however, in contrast to a study of peri- and postmenopausal Danish women, in which high circulating IL-6 levels were associated with slower spinal bone loss (77). Associations in the latter study were weak, but these discrepancies clearly point out the necessity for further studies.

VIII. Potential Implications for Vascular Function (Fig. 4)

The risk of cardiovascular disease strongly increases after menopause (3). Data from the United States show that, by the age of 60 yr, only 1 in 17 women has had a coronary event, as compared with 1 in 5 men. After the age of 60, however, coronary heart disease is the primary cause of death among women in Western countries. Epidemiological and case-controlled studies consistently suggest that postmenopausal women have about a 50% reduction in cardiovascular disease with estrogen administration (400). Ross and Glomset (401) proposed the "response to injury" hypothesis for the pathogenesis of atherosclerosis some 25 yr ago, leading the way to the now widely acknowledged active role of inflammation in this disease. During the last decade, it has indeed become increasingly evident that atherosclerosis manifests many of the characteristics of an inflammatory response (402–404). Inflammatory parameters such as IL-6-dependent C-reactive protein are strong predictors of cardiovascular events and cardiovascular mortality in postmenopausal women (405–409). Many of the cytokines whose activity is altered with menopause are thought to be involved in the process of atherogenesis. Thus, IL-1, TNF-α, and IL-6 are all secreted in the vascular wall by endothelial cells, smooth muscle cells, and monocytes/macrophages (403, 410–412). These cytokines can increase the permeability of the endothelial cell barrier and can induce the expression of surface leukocyte adhesion molecules and, thus, may be acting already very early on in the pathogenesis of atherosclerosis. Estrogen also down-regulates monocyte chemotactic protein-1, which mediates the recruitment of macrophages to the arterial wall (413). IL-1 stimulates the secretion of platelet-derived growth factor, which in turn acts as a mitogen for smooth muscle cells and fibroblasts and may serve as a mediator in the invasion of the damaged vessel intima by hypertrophic smooth muscle (414). The adhesive interactions between endothelial cells and recruited inflammatory cells can again signal the production of other cytokines and growth factors, with all such activities being considered to promote atherosclerosis. Cytokine-induced NF-κB activity mediates the expression of matrix metalloproteinases, which may promote plaque instability and rupture. Expression of IL-6 in atherosclerotic plaques seems to be elevated compared with normal intima (415). Strong evidence for a causal role of proinflammatory cytokines in the pathogenesis of atherosclerosis is also derived from the use of neutralizing antibodies against proinflammatory cytokines in animal models. For example, blocking IL-1 and TNF action by using IL-1ra or TNF-binding protein, respectively, inhibited fatty-streak formation in ovariectomized apolipoprotein E-deficient female mice (Ref. 416 and Fig. 6). Mice that are homozygous for a null mutation of IL-1ra develop lethal arterial inflammation (417). Further evidence for a causal role of IL-6 in the development of atherosclerosis is provided by the findings that C57BL/6 and apolipoprotein-E-deficient mice show a progression of atherosclerotic lesions with weekly injections of IL-6 (418). Down-regulation of OPG and TGF-β with menopause may also negatively affect vascular homeostasis (419–422). In particular, OPG appears to be a critical factor for endothelial cell survival (229), which is clearly supported by the findings that mice deficient in OPG exhibit calcification of the aorta and renal arteries (230, 231).

In summary, there is some reason to believe that changes in proinflammatory cytokines with menopause may also have long-term detrimental effects on vascular function

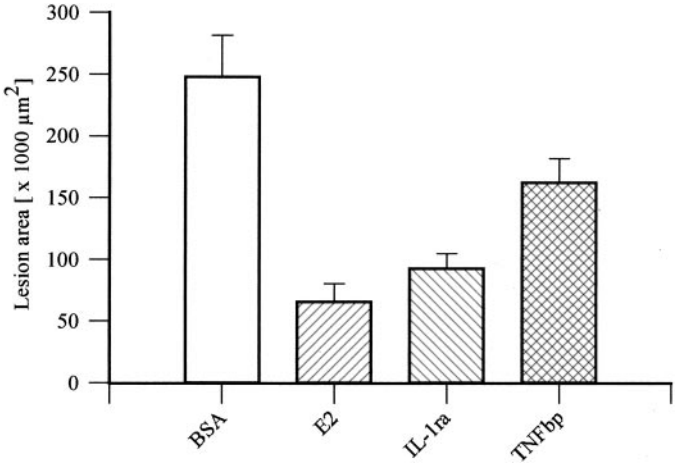

FIG. 6. Effects of treatment with BSA (25 mg/kg body weight·d) and E2 (0.2 mg 60-d time-release pellets), and sc infusion of IL-1ra (25 mg/kg body weight·d) or TNF-binding protein (1 mg/kg body weight·d) on fatty-streak formation in the aorta of ovariectomized apolipoprotein-E-deficient female mice. Areas of fatty-streak formation, an early step in the atherosclerotic process, were significantly reduced in the estrogen- and IL-1ra-treated mice compared with those of the BSA-treated animals. Data are mean ± SEM. [Derived from Ref. 416.]

and may contribute to atherosclerosis in postmenopausal women. A direct relationship between atherosclerosis, IL-6, and estrogen deficiency was revealed by the finding that IL-6 mRNA and protein are overexpressed in the atherosclerotic plaques of apolipoprotein-E-knockout mice and that this IL-6 production was suppressed by 17β-E2 treatment in this apolipoprotein-E-knockout mouse model of atherosclerosis (46). However, in contrast to the changes in bone resorption after menopause, IL-6 does not appear to constitute an essential target for E2 to prevent fatty-streak formation, as estrogen supplementation decreased fatty streak formation in a similar way in apolipoprotein-E-knockout mice with an intact and nonfunctioning IL-6 gene (423). In analogy to bone tissue, part of the anti-inflammatory effects of estrogen may be mediated by an inhibitory effect on lymphocyte maturation. Studies in lymphocyte-deficient atherosclerosis-prone recombination activating gene 1/low-density lipoprotein receptor-double knockout mice suggested that lymphocytes play an important role in lesion initiation and early progression (424). Both lymphocyte depletion and estrogen-substitution reduced lesion size in the aortic root to a similar extent in ovariectomized apolipoprotein-E-deficient mice. Interestingly, no additional atheroprotective effects were provided by combining estrogen replacement and lymphocyte depletion (425), providing further tentative clues for an interrelated pathway of the immune system and estrogen action on vessel function.

Nevertheless, in contrast to bone tissue, the number of studies that have addressed the role of proinflammatory cytokines in vascular dysfunction with menopause are still extremely limited, precluding a more definitive conclusion on the relevance of these effects to the atheroprotective actions of estrogen at the present time.

IX. Individual Susceptibility to Estrogen-Dependent Cytokine Changes

In humans, menopause-related metabolic changes in the skeleton can be easily monitored by the means of sequential BMD measurements. These measurements have revealed that the rate and extent of bone loss after menopause varies widely between individuals, with some women losing bone quickly and others losing it slowly. It is of course attractive to postulate that this may be in part due to individual differences in cytokine activity. The most obvious explanation may be an inverse association of cytokine activity with the declining serum estrogen concentrations. In support of this, Chiu *et al.* (426) have observed a negative correlation between circulating IL-6 and E2 concentrations during the normal menstrual cycle. In contrast, no such association was present in early postmenopausal women who took part in the EVOS study (399), suggesting that once a certain lower threshold of estrogen concentrations has been reached, the residual estrogen concentrations may cease to be a major determinant of individual differences in cytokine activity.

Because most of the currently known interactions between estrogen and cytokines in nonreproductive tissues at the molecular level are indirect, and estrogen therefore appears to play the role of a gatekeeper of cytokine activity, a decline in estrogen may allow for a better efficiency of preexisting proinflammatory stimuli to stimulate cytokine expression. In other words, the magnitude of the postmenopausal increase in proinflammatory cytokine production may depend to a great extent on the proinflammatory stimuli that are present in the cellular environment at the time of menopause. Based on this hypothesis, the consequences of estrogen deficiency may be small, if few such stimuli are present or if the stimuli are so strong as to overcome the mildly suppressive effects of estrogen. It may be the "in-between" situation, in which estrogen might exert its greatest impact on cytokine activity. For example, Rogers and Eastell (38) observed an inhibitory effect of physiological concentrations of E2 on spontaneous proinflammatory cytokine release from whole-blood cultures, but not after cytokine secretion had been stimulated with lipopolysaccharide. The most common physiological stimuli for cytokine expression are oxygen-derived free radicals (427), which are inevitable byproducts of normal biological functions such as respiration or energy generation. Free radical-derived advanced glycation end products and other inflammatory mediators, via binding to the advanced glycation end products receptor (428), hypoxia (429), and chronic subclinical infections (430, 431), are examples of nonphysiological stimuli that may determine cytokine expression. Estrogen withdrawal has been shown to augment PTH-induced IL-6 production *in vitro* and *in vivo* (432). This raises the hypothesis that differences in PTH secretion, which again to a large extent reflect differences in calcium and vitamin D status, may also be a potential and physiologically relevant determinant of the susceptibility to menopause-related increases in IL-6 in bone tissue and perhaps in other tissues as well. In bone cells, early responses to strain and estrogen have been described to share a common pathway, which involves ER-α (433), making it tempting to speculate that differences in ER-α activation by strain may also determine postmenopausal cytokine production. Apart from identifying stimuli that may determine the extent of cytokine changes with menopause, it will, of course, also be of great interest to identify endogenous and environmental factors that may protect against increases in proinflammatory cytokines in postmenopausal women.

On the response side, a number of cytokine genes and genes involved in inflammatory responses are polymorphic and may be important for defining the magnitude of the individual responses to a given environmental stimulus of cytokine production. The list includes genes that affect cytokine expression, binding of cytokines to their receptors, genes involved in the cytokine signaling pathways, and many others. There is a growing number of studies that have examined the effects of these cytokine polymorphisms on postmenopausal bone loss. Tsukamoto *et al.* (434) investigated an association between a CA-repeat polymorphism at the IL-6 gene locus and BMD of radial bone in 472 postmenopausal Japanese women. The 73 women who possessed an A1 allele (134 bp, containing 18 repeats of CA) had significantly lower BMD than those who did not carry an allele of that size. Keen *et al.* (435) examined the relationship between annual rates of change in BMD and an 86-bp variable number tandem-repeat polymorphism of the IL-1ra gene in 108 women without hormonal replacement therapy within 5

yr of menopause. They observed that carriage of at least one copy of the A2 allele was associated with reduced bone loss at the spine. Langdahl *et al.* (436) also showed that genotypes associated with a low IL-1ra production (A1A1/A3) were significantly more frequent in women with osteoporotic fractures compared with normal individuals, but this polymorphism had no effect on bone loss in another study of 487 postmenopausal Danish women (437). Data from our group from the EVOS study have shown a 2-fold increase in circulating IL-6 levels in early postmenopausal women with a CC genotype of the G-to-C polymorphism at position −174 of the IL-6 promoter, compared with women with the GG phenotype. Higher circulating IL-6 levels in this study corresponded to increased rates of bone loss (C. Clanget and J. Pfeilschifter, unpublished data). In contrast, in postmenopausal healthy women, serum levels of C-telopeptide of type I collagen, a marker of bone resorption, were significantly higher in women with the GG phenotype compared with women with the GC and CC genotype. This was associated with a tendency for a higher BMD at the hip and forearm in CC subjects compared with the GG subjects (438). Jones *et al.* (439) again reported a strikingly lower cardiovascular and all-cause mortality for individuals of GG genotype compared with those of GC and CC genotype. There are other studies that have observed associations between cytokine polymorphisms and coronary artery disease (249), although none of these data have been evaluated in the context of early postmenopause.

With respect to TGF-β, a 1-base deletion in intron 4 (713–8delC) of the TGF-β1 gene has been associated with low BMD, increased bone turnover, and an increased rate of fragility fractures in osteoporotic Danish and Italian women (440, 441). In postmenopausal Japanese women, the CC genotype of a T/C polymorphism at nucleotide 29 in the signal sequence of the TGF-β1 gene has been observed to be associated with lower spinal bone loss, fewer vertebral fractures, and lower circulating levels of TGF-β1 compared with women with the TC or TT genotype (442–444), but the latter findings could not be confirmed in postmenopausal Caucasian women by our own group (445).

It should be cautioned that these association studies are subject to a high rate of spurious results. Most of the existent data suffer from small numbers, lack of reproducibility, and lack of knowledge about the functional significance in terms of the effect of the variant on transcriptional activity. Moreover, despite their potential influence on the extent of cytokine changes after menopause, the strength of each of these phenotypic variations as a single determinant of cytokine activity may be rather modest.

X. Time Course of Cytokine Increases after Menopause

A fascinating aspect of metabolic changes with estrogen deficiency is that they may, in fact, be limited in time. The most prominent examples for this are hot flashes, which in most women abate within the first 5–10 yr after menopause (446). It is also well established that the accelerated phase of bone loss is limited to the first 5–10 yr following menopause (271). Of note, this temporal limit in rapid bone loss after menopause appears to be closely related to temporal changes in proinflammatory cytokine activity. In the Heidelberg cohort of the EVOS study (53), the predictive effect of circulating IL-6 levels on postmenopausal femoral bone loss was limited to the first decade past menopause. Consistent with this, Pacifici *et al.* (447) observed that the production of IL-1β, the ratio of IL-1β to IL-1ra, and IL-1 bioactivity in monocyte cultures were all increased in the first years after menopause but declined to within the premenopausal range thereafter. Moreover, levels of IL-1, TNF, and IL-6 in bone marrow cultures from early postmenopausal women were higher compared with those from premenopausal women, but there was no difference in these cytokine levels between late postmenopausal women and premenopausal women, again suggesting confinement of cytokine increases to the fist years after menopause (Ref. 12 and Fig. 7).

As the precise mechanisms that are responsible for cytokine changes with menopause are still a matter of intense investigation, it may not be surprising that the transient nature of these cytokine increases is even less understood. Inflammatory conditions tend to increase age independently from menopause (448, 449), and it is thus possible that age-associated increases in proinflammatory cytokines may simply dilute the impact of menopause on cytokine metabolism. On the contrary, it is also possible that age-related changes in cellular function, and particularly in immune cell function, may compensate for the intracellular pathways that are activated by estrogen deficiency, including estrogen-independent activation of the ER itself (450). Pointing in this direction, Bruunsgaard *et al.* (451) observed an age-related decline in the production of proinflammatory cytokines in whole-blood supernatants in response to lipopolysaccharides. Wang *et al.* (32) observed that 17β-E2 inhibited IL-1β mRNA expression *in vitro* only in osteoblastic cells derived from young mice, but not from old mice, further indicating that aging may directly modulate estrogen-dependent modulation of IL-1. Arguing against this are findings by Bismar *et al.* (12), who observed elevated proinflammatory cytokine production by human bone marrow cells not only immediately after natural menopause, but also upon withdrawal from long-term estrogen replacement therapy (Fig. 7). However, the women in this study were still relatively young when they stopped hormonal replacement therapy, and it remains to be established whether similar cytokine increases might also occur in older women.

XI. Role of Progesterone

With menopause, there is not only a decline in endogenous estrogen, but also in gestagens. Our knowledge about the role of natural progesterone in menopause-related changes in tissue function is, however, far more limited, because most of the clinical studies that have looked at the effects of gestagens on postmenopausal tissue metabolism alone or in combination with estrogen have been conducted with synthetic gestagen compounds, which are known to counteract some of the beneficial effects of estrogens on lipid metabolism (452, 453). With respect to postmenopausal cytokine

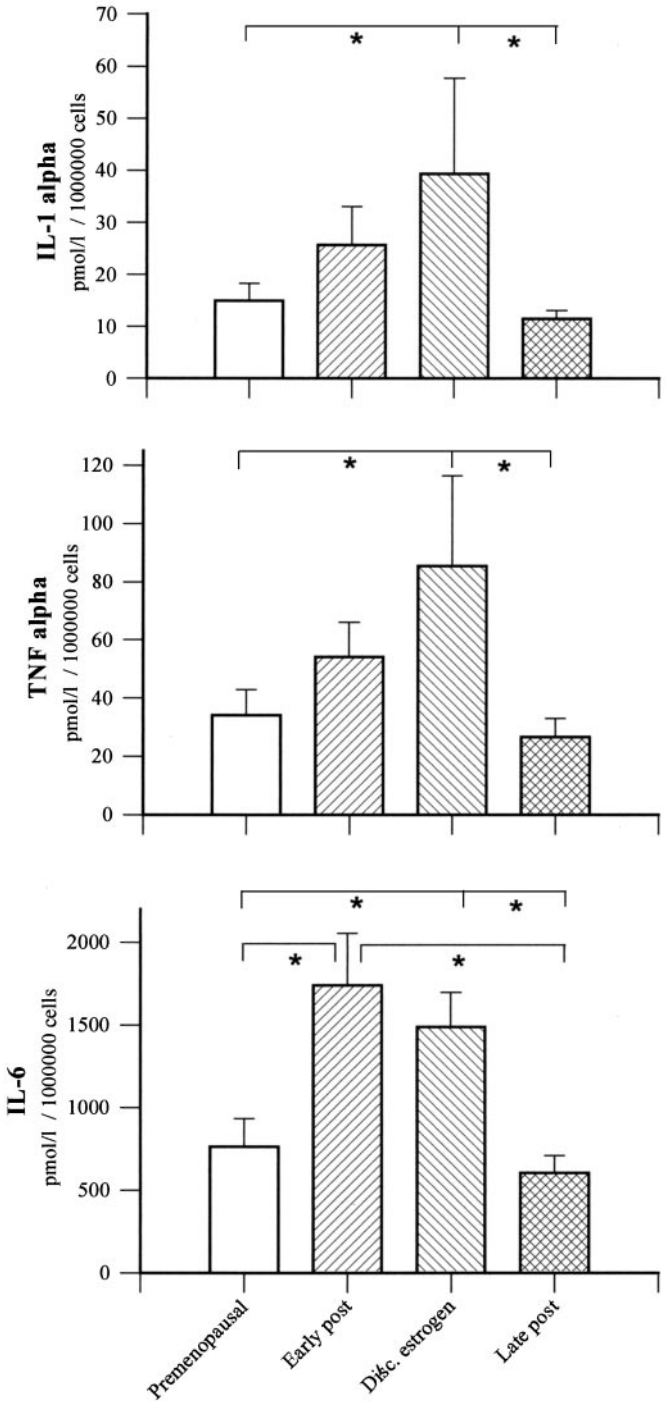

FIG. 7. Differences in the concentrations of IL-1-α, TNF-α, and IL-6 in 48-h-conditioned medium from human bone marrow cultures with time after estrogen withdrawal. Cells were either obtained from premenopausal women (n = 12), early-postmenopausal women less than 5 yr since menopause (n = 5, "Early post"), women who had recently discontinued estrogen replacement (n = 5, "Disc. estrogen"), or late-postmenopausal women more than 8 yr since menopause (n = 18, "Late post"). Data are mean ± SE. *, $P < 0.05$. [Derived from Ref. 12.]

changes, progesterone in many cases appears to act in a similar way to estrogens. Low physiological doses of progesterone have been shown to increase human or rat macrophage and monocyte IL-1 and TNF-α production, whereas higher doses suppressed cytokine release and IL-1 mRNA expression (36, 454). As with the ER, mutually repressive effects of NF-κB and progesterone receptor transactivation have been described (455). Clinical studies using progesterone to prevent bone loss have found variable results, with some studies showing an effect of progesterone in prevention of spinal or cortical bone loss and other studies finding little benefit of progesterone treatment on postmenopausal bone loss (383, 456). With respect to the interactions between progesterone and cytokine metabolism in nonreproductive tissues, many gaps still remain to be filled.

XII. Role of Androgens

The effect of the menopausal transition on circulating androgen levels has been addressed in several studies with variable results. Longitudinal measurements of circulating androgen concentrations in peri- and early-postmenopausal women suggest that the free androgen index, calculated as the ratio of T to sex hormone binding globulin increases by 80% from 4 yr before the final menstrual period to 2 yr after the final menstrual period (457). This may be, in part, due to an estrogen-related decline in sex hormone binding globulin, but also in part to a small decline in T production during the first years after menopause (457–459). The adrenal androgen dehydroepiandrosterone (DHEA) and its sulfate conjugate (DHEAS), which are the major source of circulating and tissue estrogen concentrations after menopause (460, 461), clearly decrease in postmenopause. However, relative to menopause, this is as a function of age rather than of time (457, 462).

An increasing body of evidence suggests that androgen deficiency may up-regulate proinflammatory cytokines in a way similar to estrogen deficiency. T deficiency induced IL-6 protein synthesis and mRNA expression in the bone marrow (463). T inhibited IL-6 expression and/or production by human osteoblastic cells (464), bone marrow stromal cells (42), human gingival fibroblasts *in vitro* (465), prostate carcinoma cells (466), HeLa cells (43), and peripheral blood mononuclear cells (467). T also inhibited the expression of the IL-1 gene in murine bone marrow-derived stromal cells through the AR (468). Orchidectomized IL-6-deficient mice do not lose bone, suggesting that IL-6 is involved in bone loss caused by androgen deficiency in a similar way as in bone loss with estrogen deficiency (464, 468).

With respect to DHEA, there is an inverse relationship between plasma DHEA levels and circulating IL-6 levels (469, 470). Inhibitory effects of DHEA on IL-6 and/or TNF-α production have been observed in spleen cell suspension cultures (469), lymphocytes (471, 472), astrocytes (473), and peripheral blood mononuclear cells (470, 474). DHEA/DHEAS have also been shown to inhibit circulating concentrations of TNF-α (475) and IL-6 (476) in rodents.

The similarity between estrogen and androgen effects on cytokine production is even evident in those experimental designs, in which estrogen stimulated rather than inhibited cytokine production. Thus, both T and E2 synergized with IL-1 in the induction of IL-6 in cultures of human articular chondrocytes (477) and increased IL-1β mRNA levels in hu-

man osteoblastic cells (478). Although androgens and estrogen have many different effects on T and B cell function (195), they appear to share repressive effects of B lymphopoiesis (203, 479). Moreover, like estrogen, androgens block PGE_2 production (480) and stimulate TGF-β activity (481, 482).

Many of the effects of DHEA and T on bone and vascular tissue appear to be mediated by their aromatization to estrogen (483, 484). Part of their inhibitory effects on proinflammatory cytokine concentrations may thus be secondary to the suppressive effects of estrogen on these cytokines. However, suppressive effects of androgens on proinflammatory cytokine activity have also been observed with 5-α-dihydrotestosterone (465, 485), which is a nonaromatizable androgen, suggesting that direct inhibitory effects are also involved. Clinical data by Falahati-Nini *et al.* (484) show that in men, these direct effects of androgens, which are not mediated by aromatization to estrogen, may account for at most 30% of the increase in bone resorption that is observed with combined androgen and estrogen deficiency. Given the homology between the ER and the AR, the molecular mechanisms for the direct suppressive effects of androgens on cytokine expression may be similar to that described for estrogen (86). In support of this hypothesis, inhibitory effects of DHEA on the activation of the IL-6-promoter have been found to involve NF-κB-DNA complex formation (466, 485). The direct effects of the weaker androgens, DHEA and DHEAS, on cytokine expression also appear to be mediated by the AR, as suggested by DHEAS-induced repression of the IL-6 promoter in HeLa cells when transfected with the AR (468).

In view of the remarkable similarity between androgens and estrogen with respect to cytokine suppression, one would predict that these steroids may also be exchangeable in preventing cytokine-induced tissue damage. Indeed, in a model of inflammation-induced cartilage degradation in female rodents, ovariectomy resulted in accelerated cartilage breakdown associated with increased production of IL-1, and the effects on cartilage were reversed by treatment with E2 or T (486). In contrast, plasma levels of IL-6 and TNF-α in male, but not female, mice increased after hemorrhage and subsequent sepsis, and there only was an increased mortality in male mice as compared with sham-treated animals. Thus, there appear to be distinct sex differences in the inflammatory responses, which may either be due to some concentration-dependent function of androgens that cannot be replaced by estrogen and *vice versa* or to sex hormone-independent mechanisms that remain to be identified (487).

The impact of the decrease in adrenal androgens in postmenopausal women on proinflammatory cytokine activity is currently unknown. Nevertheless, inhibition of aromatization in postmenopausal women is associated with an increase in bone resorption, and there is increasing evidence for a protective role of DHEA with respect to elevated bone resorption and osteoporotic fractures in postmenopausal women (488, 489). It will be interesting to find out whether part of these effects may be related to changes in proinflammatory cytokine activity. Decreases in T may be of particular relevance after surgically induced menopause, as the ovaries are an important source of T, and serum T concentrations decline by 50% after bilateral oophorectomy both in pre- and postmenopausal women (458, 490).

XIII. Potential Implications for the Prophylaxis of Menopause-Associated Diseases

Treatment strategies for the use of prophylactic medications for the prevention of menopause-associated diseases may greatly gain in feasibility if it is possible to better predict who will benefit most from such medication, particularly because widespread screening for menopause-associated disorders is problematic in terms of validity and cost. The findings that circulating IL-6 levels predict femoral bone loss in early postmenopausal women (399) and that IL-6-derived C-reactive protein strongly predicts the development of symptomatic coronary artery disease (407) make these measurements attractive parameters for the identification of high-risk groups of early postmenopausal women, although the clinical utility of these measurements clearly needs to be assessed in larger studies.

In line with the increasing shift of medicine away from the treatment of acute symptoms to the prophylaxis of complex chronic disorders, the major emphasis of health care after menopause is also shifting from the immediate relief of postmenopausal symptoms to the prophylaxis of menopause-associated chronic diseases. However, the long interval between the initiation of prophylactic treatment and the occurrence of clinical events that has to be passed until the benefits of treatment can be assessed makes it increasingly important to take into account all cellular and molecular changes that may eventually occur with treatment before embarking on costly long-term studies. In view of the emerging role of inflammation as an important component in the pathogenesis of menopause-related disorders, it may be worthwhile to include the effects of treatment on proinflammatory cytokine activity into these considerations.

Estrogen is not the only compound that may be useful to combat detrimental effects of cytokine increases associated with menopause. Breast- and uterine-related side-effects are principal causes for noncontinuation of conventional hormone replacement therapy. Drugs that avoid the side-effects of natural estrogen may be equally or even better-suited to achieve this goal. The search is therefore on for chemists to create molecules that distinguish between estrogen not at the level of binding but, rather, by the complement of coactivators and corepressors that are recruited to the ligand-bound ER. This goal appears to have been at least in part accomplished with the SERMs, a new and fascinating class of drugs that reverse menopause-associated cytokine changes in a similar way as natural estrogen, but avoid many unwarranted effects that are associated with binding of estrogen to its classical EREs in reproductive tissues.

Compounds that directly interact with cytokine activity may be even more effective in neutralizing menopause-related cytokine changes. Indeed, one might speculate that, because many of the interactions between cytokines and estrogen are mutual (491), potent stimuli of proinflammatory cytokines might render estrogen or SERMs rather insufficient in suppressing cytokine activity. Efforts to develop IL-6-

secretion inhibitors or small molecule antagonists to IL-6 are currently underway and might be a promising way for the systemic amelioration of all postmenopausal dysfunctions in which IL-6 is involved.

There are a number of drugs already in use for the treatment of metabolic disorders, whose clinical response may in fact be partially due to their anti-inflammatory effects (492, 493). For example, this seems to apply to the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) that have been convincingly shown to decrease the risk of coronary heart disease (494). The potent anti-inflammatory actions of these drugs is clearly illustrated by the observation that pretreatment of mice with cerivastatin counteracted lipopolysaccharide-triggered increases in circulating TNF-α and IL-1β and improved survival (495). In men, treatment with pravastatin for 8 wk also significantly reduced circulating TNF-α concentrations (496).

Ligands of PPARα, such as fenofibrate, also inhibit inflammatory responses by repressing NF-κB signaling. As a consequence, hyperlipidemic patients treated with fenofibrate have reduced circulating levels of IL-6, TNF-α, and acute-phase proteins (497, 498). Ligands of PPAR-γ, such as the thiazolidinediones, which have been developed for clinical use because of their capacity to increase sensitivity to insulin (499), decrease the expression of cytokines, such as TNF-α, IL-1, and IL-6, in monocytes and smooth muscle cells (500, 501), a mechanism that is mediated via inhibition of the AP-1 and NF-κB cytokine-inducing pathways outlined above (502). Plasma levels of TNF-α and C-reactive protein significantly declined in obese individuals who were given 400 mg of the thiazolidinedione troglitazone for 4 wk (501, 503). In turn, proinflammatory cytokines such as TNF-α, IL-1, and IL-6 are able to decrease PPAR-γ mRNA expression (504).

In fact, the thiazolidinediones already have a range of actions as broad as that observed with estrogen, because they not only affect glucose metabolism but also appear to be potent inhibitors of cytokine-mediated bone resorption (505, 506) and may have beneficial effects in preventing atherosclerotic lesions (502). It is tempting to speculate that at least part of these systemic effects may be due to their cytokine-antagonistic effects. Indeed, in a recent study, the PPAR-γ-specific agonists rosiglitazone and GW7845 strongly inhibited the development of atherosclerosis in male low-density lipoprotein receptor-deficient mice, and this antiatherogenic effect was correlated with decrease tissue expression of TNF-α (507).

XIV. Summary and Conclusions

Bone metabolism and vascular function are determined throughout life by a multitude of genetic and environmental influences that appear to have little in common. The pathogenesis of chronic disorders of these tissues is complex, but there is increasing support from experimental and clinical studies that the development of these disorders may be in part linked to an increased state of proinflammatory activity. Because estrogen has been shown to modulate proinflammatory cytokine activity, it is tempting to speculate that, for a limited period of time after menopause, women who are susceptible for environmental or genetic reasons may experience a progression or initiation of diverse diseases such as osteoporosis and coronary heart disease, triggered by a systemic change in the balance of proinflammatory cytokine activity. In contrast to the predominantly genomic effects of estrogen on reproductive tissues, most of the actions of estrogen on cytokine activity in nonreproductive tissues appear to be mediated by nongenomic mechanisms. They potentially include antagonistic effects of activated ERs on proinflammatory transcription factors, modulation of NO activity, direct antioxidative effects, and secondary effects due to estrogen actions on the immune system. Assessment of cytokine activity or of related products after menopause may help to identify those women who are at increased risk to experience detrimental metabolic effects in early postmenopause. Understanding the mechanisms and the time course of these interactions may also open new avenues for the prophylaxis and treatment of some of the most prevalent and important disorders in elderly women. Nevertheless, much of the evidence available at this time is suggestive rather than definitive. Continued efforts both at the workbench and in clinical research will be necessary to determine the ultimate impact of these interactions for postmenopausal health.

Acknowledgments

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References

1. **Burger HG, Dudley EC, Hopper JL, Groome N, Guthrie JR, Green A, Dennerstein L** 1999 Prospectively measured levels of serum follicle-stimulating hormone, estradiol, and the dimeric inhibins during the menopausal transition in a population-based cohort of women. J Clin Endocrinol Metab 84:4025–4030
2. **Cooper C** 1997 The crippling consequences of fractures and their impact on quality of life. Am J Med 103:12S–19S
3. **Maxwell SRJ** 1998 Women and heart disease. Basic Res Cardiol 93(Suppl 2):79–84
4. **Farhat MY, Lavigne MC, Ramwell PW** 1996 The vascular protective effects of estrogen. FASEB J 10:615–624
5. **Mendelsohn ME, Karas RH** 1999 The protective effects of estrogen on the cardiovascular system. N Engl J Med 340:1801–1811
6. **Prince RL** 1994 Counterpoint: estrogen effects on calcitropic hormones and calcium homeostasis. Endocr Rev 15:301–309
7. **Pacifici R, Rifas L, McCracken R, Vered I, McMurtry C, Avioli LV, Peck WA** 1989 Ovarian steroid treatment blocks a postmenopausal increase in blood monocyte interleukin 1 release. Proc Natl Acad Sci USA 86:2398–2402
8. **Pacifici R, Brown C, Puscheck E, Friedrich E, Slatopolsky E, Maggio D, McCracken R, Avioli LV** 1991 Effect of surgical menopause and estrogen replacement on cytokine release from human blood mononuclear cells. Proc Natl Acad Sci USA 88:5134–5138
9. **Jilka RL, Hangoc G, Girasole G, Passert G, Williams DC, Abrams JS, Boyce B, Broxmeyer H, Manolagas SC** 1992 Increased osteoclast development after estrogen loss: mediation by interleukin-6. Science 257:88–91
10. **Kimble RB, Vannice JL, Bloedow DC, Thompson RC, Hopfer W, Kung VT, Brownfield C, Pacifici R** 1994 Interleukin-1 receptor

antagonist decreases bone loss and bone resorption in ovariectomized rats. J Clin Invest 93:1959–1967
11. **Kitazawa R, Kimble RB, Vannice JL, Kung VT, Pacifici R** 1994 Interleukin-1 receptor antagonist and tumor necrosis factor binding protein decrease osteoclast formation and bone resorption in ovariectomized mice. J Clin Invest 94:2397–2406
12. **Bismar H, Diel I, Ziegler R, Pfeilschifter J** 1995 Increased cytokine secretion by human bone marrow cells after menopause or discontinuation of estrogen replacement. J Clin Endocrinol Metab 80:3351–3355
13. **Passeri G, Girasole G, Jilka RL, Manolagas SC** 1993 Increased interleukin-6 production by murine bone marrow and bone cells after estrogen withdrawal. Endocrinology 133:822–828
14. **Cohen MC, Cohen S** 1996 Cytokine function: a study in biologic diversity. Am J Clin Pathol 105:589–598
15. **Vargas SJ, Naprta A, Lee SK, Kalinowski J, Kawaguchi H, Pilbeam CC, Raisz LG, Lorenzo JA** 1996 Lack of evidence for an increase in interleukin-6 expression in adult murine bone, bone marrow, and marrow stromal cell cultures after ovariectomy. J Bone Miner Res 11:1926–1934
16. **Van Bezooijen RL, Farih-Sips HC, Papapoulos SE, Lowik CW** 1998 IL-1α, IL-1β, IL-6, and TNF-α steady state mRNA levels analyzed by reverse transcription-competitive PCR in bone marrow of gonadectomized mice. J Bone Miner Res 13:185–194
17. **McKane WR, Khosla S, Peterson JM, Egan K, Riggs BL** 1994 Circulating levels of cytokines that modulate bone resorption: effects of age and menopause in women. J Bone Miner Res 9:1313–1318
18. **Girasole G, Pedrazzoni M, Giuliani N, Passeri G, Passeri M** 1995 Increased serum-soluble interleukin-6 receptor levels are induced by ovariectomy, prevented by estrogen replacement and reversed by alendronate administration. J Bone Miner Res 10:A86
19. **Gregory MS, Duffner LA, Faunce DE, Kovacs EJ** 2000 Estrogen mediates the sex difference in post-burn immunosuppression. J Endocrinol 164:129–138
20. **Martinetti A, Ferrari L, Celio L, Mariani L, Miceli R, Zilembo N, Di Bartolomeo M, Toffolatti L, Pozzi P, Seregni E, Bombardieri E, Bajetta E** 2000 The luteinising hormone-releasing hormone analogue triptorelin with or without the aromatase inhibitor formestane in premenopausal breast cancer: effects on bone metabolism markers. J Steroid Biochem Mol Biol 75:65–73
21. **O'Brien CA, Gubrij I, Roberson PK, Jilka RL, Manolagas SC** 1999 *In vivo* demonstration of the negative transcriptional control of the IL-6 gene by estrogen using IL-6 promoter-luciferase transgenic mice. J Bone Miner Res 14(Suppl 1):S179
22. **Cantatore FP, Loverro G, Ingrosso AM, Lacanna R, Sassanelli E, Selvaggi L, Carrozzo M** 1995 Effect of oestrogen replacement on bone metabolism and cytokines in surgical menopause. Clin Rheumatol 14:157–160
23. **Girasole G, Giuliani N, Modena AB, Passeri G, Pedrazzoni M** 1999 Oestrogens prevent the increase of human serum soluble interleukin-6 receptor induced by ovariectomy *in vivo* and decrease its release in human osteoblastic cells *in vitro*. Clin Endocrinol (Oxf) 51:801–807
24. **Woodward M, Rumley A, Tunstall-Pedoe H, Lowe GD** 1999 Associations of blood rheology and interleukin-6 with cardiovascular risk factors and prevalent cardiovascular disease. Brit J Haematol 104:246–257
25. **Deswal A, Petersen NJ, Feldman AM, Young JB, White BG, Mann DL** 2001 Cytokines and cytokine receptors in advanced heart failure: an analysis of the cytokine database from the Vesnarinone trial (VEST). Circulation 103:2055–2059
26. **Lin SC, Yamate T, Taguchi Y, Borba VZC, Girasole G, O'Brien CA, Bellido T, Abe E, Manolagas SC** 1997 Regulation of the gp80 and gp130 subunits of the IL-6 receptor by sex steroids in the murine bone marrow. J Clin Invest 100:1980–1990
27. **Keller ET, Zhang J, Yao Z, Qi Y** 2001 The impact of chronic estrogen deprivation on immunologic parameters in the ovariectomized rhesus monkey (*Macaca mulatta*) model of menopause. J Reprod Immunol 50:41–55
28. **O'Brien CA, Manolagas SC** 1997 Isolation and characterization of the human gp130 promoter-regulation by STATS. J Biol Chem 272:15003–15010
29. **Yamamoto T, Matsuda T, Junicho A, Kishi H, Saatcioglu F, Muraguchi A** 2000 Cross-talk between signal transducer and activator of transcription 3 and ER signaling. FEBS Lett 486:143–148
30. **Wang LH, Yang XY, Mihalic K, Xiao W, Li D, Farrar WL** 2001 Activation of estrogen receptor blocks IL-6 inducible cell growth of human multiple myeloma involving molecular cross-talk between ER and STAT3 mediated by a co-regulator PIAS3. J Biol Chem 276:31839–31844
31. **Rickard D, Russell G, Gowen M** 1992 Oestradiol inhibits the release of tumour necrosis factor but not interleukin 6 from adult human osteoblasts *in vitro*. Osteoporos Int 2:94–102
32. **Wang X, Schwartz Z, Yaffe P, Ornoy A** 1999 The expression of transforming growth factor-β and interleukin-1β mRNA and the response to 1,25(OH)2D3′ 17β-estradiol, and testosterone is age-dependent in primary cultures of mouse-derived osteoblasts *in vitro*. Endocrine 11:13–22
33. **Ralston SH, Russell RGG, Gowen M** 1990 Estrogen inhibits release of tumor necrosis factor from peripheral blood mononuclear cells in postmenopausal women. J Bone Miner Res 5:983–988
34. **Shanker G, Sorci-Thomas M, Adams MR** 1994 Estrogen modulates the expression of tumor necrosis factor α mRNA in phorbol ester-stimulated human monocytic THP-1 cells. Lymphokine Cytokine Res 13:377–382
35. **Kimble RB, Srivastava S, Ross FP, Matayoshi A, Pacifici R** 1996 Estrogen deficiency increases the ability of stromal cells to support osteoclastogenesis via an interleukin-1 and tumor necrosis factor-mediated stimulation of macrophage colony-stimulating factor production. J Biol Chem 271:28890–28897
36. **Morishita M, Miyagi M, Iwamoto Y** 1999 Effects of sex hormones on production of interleukin-1 by human peripheral monocytes. J Periodontol 70:757–760
37. **Bernard-Poenaru O, Roux C, Blanque R, Gardner C, de Vernejoul MC, Cohen-Solal ME** 2001 Bone-resorbing cytokines from peripheral blood mononuclear cells after hormone replacement therapy: a longitudinal study. Osteoporos Int 12:769–776
38. **Rogers A, Eastell R** 2001 The effect of 17β-estradiol on production of cytokines in cultures of peripheral blood. Bone 29:30–34
39. **Zuckerman SH, Bryan-Poole N, Evans GF, Short L, Glasebrook AL** 1995 *In vivo* modulation of murine serum tumour necrosis factor and interleukin-6 levels during endotoxemia by oestrogen agonists and antagonists. Immunology 86:18–24
40. **Cheleuitte D, Mizuno S, Glowacki J** 1998 *In vitro* secretion of cytokines by human bone marrow: effects of age and estrogen status. J Clin Endocrinol Metab 83:2043–2051
41. **Le Bail J, Liagre B, Vergne P, Bertin P, Beneytout J, Habrioux G** 2001 Aromatase in synovial cells from postmenopausal women. Steroids 66:749–757
42. **Girasole G, Jilka RL, Passeri G, Boswell S, Boder G, Williams DC, Manolagas SC** 1992 17β-Estradiol inhibits interleukin-6 production by bone marrow-derived stromal cells and osteoblasts *in vitro*: a potential mechanism for the antiosteoporotic effect of estrogens. J Clin Invest 89:883–891
43. **Pottratz ST, Bellido T, Mocharla H, Crabb D, Manolagas SC** 1994 17β-Estradiol inhibits expression of human interleukin-6 promoter-reporter constructs by a receptor-dependent mechanism. J Clin Invest 93:944–950
44. **Stein B, Yang MX** 1995 Repression of the interleukin-6 promoter by estrogen receptor is mediated by NF-κ-B and C/EBP-β. Mol Cell Biol 15:4971–4979
45. **Kassem M, Harris SA, Spelsberg TC, Riggs BL** 1996 Estrogen inhibits interleukin-6 production and gene expression in a human osteoblastic cell line with high levels of estrogen receptors. J Bone Miner Res 11:193–199
46. **Sukovich DA, Kauser K, Shirley FD, Del Vecchio V, Halks-Miller M, Rubanyi GM** 1998 Expression of interleukin-6 in atherosclerotic lesions of male ApoE-knockout mice: inhibition by 17β-estradiol. Arterioscler Thromb Vasc Biol 18:1498–1505
47. **Keck C, Herchenbach D, Pfisterer J, Breckwoldt M** 1998 Effects of 17β-estradiol and progesterone on interleukin-6 production and proliferation of human umbilical vein endothelial cells. Exp Clin Endocrinol Diabetes 106:334–339
48. **Miyamoto N, Mandai M, Suzuma I, Suzuma K, Kobayashi K, Honda Y** 1999 Estrogen protects against cellular infiltration by

reducing the expressions of E-selectin and IL-6 in endotoxin-induced uveitis. J Immunol 163:374–379
49. **Knoferl MW, Diodato MD, Angele MK, Ayala A, Cioffi WG, Bland KI, Chaudry IH** 2000 Do female sex steroids adversely or beneficially affect the depressed immune responses in males after trauma-hemorrhage? Arch Surg 135:425–433
50. **Mizushima Y, Wang P, Jarrar D, Cioffi WG, Bland KI, Chaudry IH** 2000 Estradiol administration after trauma-hemorrhage improves cardiovascular and hepatocellular functions in male animals. Ann Surg 232:673–679
51. **Xiao E, Xia-Zhang L, Ferin M, Wardlaw SL** 2001 Differential effects of estradiol on the adrenocorticotropin responses to interleukin-6 and interleukin-1 in the monkey. Endocrinology 142:2736–2741
52. **Puder JJ, Freda PU, Goland RS, Wardlaw SL** 2001 Estrogen modulates the hypothalamic-pituitary-adrenal and inflammatory cytokine responses to endotoxin in women. J Clin Endocrinol Metab 86:2403–2408
53. **Scheidt-Nave CE, Bismar H, Leidig-Bruckner G, Seibel MJ, Ziegler R, Pfeilschifter J** 1999 Serum interleukin-6 is a major predictor of bone loss in women specific to the first decade past menopause. J Bone Miner Res 14(Suppl 1):S147
54. **Straub RH, Hense HW, Andus T, Scholmerich J, Riegger GA, Schunkert H** 2000 Hormone replacement therapy and interrelation between serum interleukin-6 and body mass index in postmenopausal women: a population-based study. J Clin Endocrinol Metab 85:1340–1344
55. **Okubo T, Urabe M, Tsuchiya H, Iwasa K, Yokota K, Kikuchi N, Yamamoto T, Honjo H** 2000 Effect of estrogen and progesterone on gene expression of growth regulatory molecules and proto-oncogene in vascular smooth muscle cells. Endocr J 47:205–214
56. **Wang MJ, Huang HM, Hsieh SJ, Jeng KC, Kuo JS** 2001 Resveratrol inhibits interleukin-6 production in cortical mixed glial cells under hypoxia/hypoglycemia followed by reoxygenation. J Neuroimmunol 112:28–34
57. **Dinarello CA** 2000 The role of the interleukin-1-receptor antagonist in blocking inflammation mediated by interleukin-1. N Engl J Med 343:732–734
58. **Sunyer T, Lewis J, Osdoby P** 1997 Estrogen decreases the steady state levels of the IL-1 signaling receptor (type I) while increasing those of the IL-1 decoy receptor (type II) in human osteoclast-like cells. J Bone Miner Res 12(Suppl 1):131
59. **Kassem M, Khosla S, Spelsberg TC, Riggs BL** 1996 Cytokine production in the bone marrow microenvironment: failure to demonstrate estrogen regulation in early postmenopausal women. J Clin Endocrinol Metab 81:513–518
60. **Miller L, Hunt JS** 1998 Regulation of TNF-α production in activated mouse macrophages by progesterone. J Immunol 160:5098–5104
61. **Woodfork KA, Schuller KC, Huffman LJ** 2001 Cytokine and nitric oxide release by J774A.1 macrophages is not regulated by estradiol. Life Sci 69:2287–2294
62. **Gordon CM, Makhluf H, Blahut E, LeBoff MS, Glowacki J** 1999 Gonadal and adrenal steroids inhibit secretion by human marrow cells. J Bone Miner Res 14 (Suppl 1):S268
63. **Keller ET, Binkley NC, Stebler BA, Hall DM, Johnston GM, Zhang J, Ershler WB** 2000 Ovariectomy does not induce osteopenia through inteleukin-6 in rhesus monkeys (*Macaca mulatta*). Bone 26:55–62
64. **Rifas L, Kenney JS, Marcelli M, Pacifici R, Dawson LL, Cheng S, Avioli LV** 1995 Production of interleukin-6 in human osteoblasts and human bone marrow stromal cells: evidence that induction by interleukin-1 and tumor necrosis factor-α is not regulated by ovarian steroids. Endocrinology 136:4056–4067
65. **Chaudhary LR, Spelsberg TC, Riggs BL** 1992 Production of various cytokines by normal human osteoblast-like cells in response to interleukin-1β and tumor necrosis factor-α: lack of regulation by 17β-estradiol. Endocrinology 130:2528–2534
66. **Dovio A, Sartori ML, Masera RG, Racca S, Angeli A** 2001 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 15:47–52
67. **Maret A, Clamens S, Delrieu I, Elhage R, Arnal JF, Bayard F** 1999 Expression of the interleukin-6 gene is constitutive and not regulated by estrogen in rat vascular smooth muscle cells in culture. Endocrinology 140:2876–2882
68. **Rogers A, Eastell R** 1998 Effects of estrogen therapy of postmenopausal women on cytokines measured in peripheral blood. J Bone Miner Res 13:1577–1586
69. **Zanger D, Yang BK, Ardans J, Waclawiw MA, Csako G, Wahl LM, Cannon III RO** 2000 Divergent effects of hormone therapy on serum markers of inflammation in postmenopausal women with coronary artery disease on appropriate medical management. J Am Coll Cardiol 36:1797–1802
70. **Chao TC, Van Alten PJ, Greager JA, Walter RJ** 1995 Steroid sex hormones regulate the release of tumor necrosis factor by macrophages. Cell Immunol 160:43–49
71. **Hu SK, Mitcho YL, Rath NC** 1998 Effect of oestradiol on interleukin 1 synthesis by macrophages. Int J Immunopharmacol 10:247–252
72. **Stock JL, Coderre JA, McDonald B, Rosenwasser LJ** 1989 Effects of estrogen *in vivo* and *in vitro* on spontaneous interleukin-1 release by monocytes from postmenopausal women. J Clin Endocrinol Metab 68:364–368
73. **Hogasen AK, Nordsletten L, Aasen AO, Falch JA** 1995 There is no difference in spontaneous and 17β-estradiol-induced interleukin-1β release by peripheral blood mononuclear cells from non-osteoporotic women with different rates of early postmenopausal bone loss. J Clin Endocrinol Metab 80:2480–2484
74. **Ruh MF, Bi Y, D'Alonzo R, Bellone CJ** 1998 Effect of estrogens on IL-1β promoter activity. J Steroid Biochem Mol Biol 66:203–210
75. **Li ZG, Danis VA, Brooks PM** 1993 Effect of gonadal steroids on the production of IL-1 and IL-6 by blood mononuclear cells *in vitro*. Clin Exp Rheumatol 11:157–162
76. **Kawasaki T, Ushiyama T, Inoue K, Hukuda S** 2000 Effects of estrogens on interleukin-6 production in rheumatoid fibroblast-like synoviocytes. Clin Exp Rheumatol 18:743–745
77. **Abrahamsen B, Bonnevie-Nielsen V, Ebbesen EN, Gram J, Beck-Nielsen H** 2000 Cytokines and bone loss in a 5-year longitudinal study-hormone replacement therapy suppresses serum soluble interleukin-6 receptor and increases interleukin-1-receptor antagonist: the Danish Osteoporosis Prevention Study. J Bone Miner Res 15:1545–1554
78. **Enmark E, Gustafsson JA** 1999 Oestrogen receptors–an overview. J Intern Med 246:133–138
79. **Beato M** 1989 Gene regulation by steroid hormones. Cell 56:335–344
80. **McDonnell DP** 1999 The molecular pharmacology of SERMS. Trends Endocrinol Metab 10:301–311
81. **Bryant HU, Glasebrook AL, Yang NN, Sato M** 1999 An estrogen receptor basis for raloxifene action in bone. J Steroid Biochem Mol Biol 69:37–44
82. **Paul S, Dean RA, Tracy RP, Cox DA, Walsh BW, Anderson PW** 1998 Effects of raloxifene and hormone replacement therapy on homocysteine and c-reactive protein levels in postmenopausal women. Circulation 17(Suppl 1):I7
83. **Ghosh S, May MJ, Kopp EB** 1998 NF-κB and REL proteins: evolutionary conserved mediators of immune responses. Annu Rev Immunol 16:225–260
84. **Bondeson J, Foxwell B, Brennan F, Feldmann M** 1999 Defining therapeutic targets by using adenovirus: blocking NF-κB inhibits both inflammatory and destructive mechanisms in rheumatoid synovium but spares anti-inflammatory mediators. Proc Natl Acad Sci USA 96:5668–5673
85. **Ray A, Prefontaine KE** 1994 Physical association and functional antagonism between the p65 subunit of transcription factor NF-κB and the glucocorticoid receptor. Proc Natl Acad Sci USA 91:752–756
86. **McKay LI, Cidlowski JA** 1999 Molecular control of immune/inflammatory responses: interactions between nuclear factor-κB and steroid receptor-signaling pathways. Endocr Rev 20:435–459
87. **Palvimo JJ, Reinikainen P, Ikonen T, Kallio PJ, Moilanen A, Janne OA** 1996 Mutual transcriptional interference between RelA and androgen receptor. J Biol Chem 271:24151–24156
88. **Kalkhoven E, Wissink S, van der Saag PT, van der Burg B** 1996 Negative interaction between the RelA(p65) subunit of NF-κB and the progesterone receptor. J Biol Chem 271:6217–6224
89. **Na SY, Kang BY, Chung SW, Han SJ, Ma X, Trinchieri G, Im SY,**

Lee JW, Kim TS 1999 Retinoids inhibit interleukin-12 production in macrophages through physical associations of retinoid X receptor and NFκB. J Biol Chem 274:7674–7680
90. **Staels B, Koenig W, Habib A, Merval R, Lebret M, Torra IP, Delerive P, Fadel A, Chinetti G, Fruchart JC, Najib J, Maclouf J, Tedgui A** 1998 Activation of human aortic smooth-muscle cells is inhibited by PPARα but not by PPARγ activators. Nature 393:790–793
91. **Dodel RC, Du Y, Bales KR, Gao F, Paul SM** 1999 Sodium salicylate and 17β-estradiol attenuate nuclear transcription factor NF-κB translocation in cultured rat astroglial cultures following exposure to amyloid A β (1–40) and lipopolysaccharides. J Neurochem 73: 1453–1460
92. **Cerillo G, Rees A, Manchanda N, Reilly C, Brogan I, White A, Needham M** 1998 The oestrogen receptor regulates NFκB and AP-1 activity in a cell-specific manner. J Steroid Biochem Mol Biol 67: 79–88
93. **Galien R, Evans HF, Garcia T** 1996 Involvement of CCAAT/enhancer-binding protein and nuclear factor-κB binding sites in interleukin-6 promoter inhibition by estrogens. Mol Endocrinol 10:713–722
94. **Deshpande R, Khalili H, Pergolizzi RG, Michael SD, Chang MD** 1997 Estradiol down-regulates LPS-induced cytokine production and NFkB activation in murine macrophages. Am J Reprod Immunol 38:46–54
95. **Harnish DC, Scicchitano MS, Adelman SJ, Lyttle CR, Karathanasis SK** 2000 The role of CBP in estrogen receptor cross-talk with nuclear factor-κB in HepG2 cells. Endocrinology 141:3403–3411
96. **Pelzer T, Neumann M, de Jager T, Jazbutyte V, Neyses L** 2001 Estrogen effects in the myocardium: inhibition of NF-κB DNA binding by estrogen receptor-α and -β. Biochem Biophys Res Commun 286:1153–1157
97. **Quaedackers ME, Van Den Brink CE, Wissink S, Schreurs RH, Gustafsson Jk JA, Van Der Saag PT, Van Der Burg BB** 2001 4-hydroxytamoxifen trans-represses nuclear factor-κB activity in human osteoblastic U2-OS cells through estrogen receptor (ER) α, and not through ERβ. Endocrinology 142:1156–1166
98. **Galien R, Garcia T** 1997 Estrogen receptor impairs interleukin-6 expression by preventing protein binding on the NF-κB site. Nucleic Acids Res 25:2424–2429
99. **Kurebayashi S, Miyashita Y, Hirose T, Kasayama S, Akira S, Kishimoto T** 1997 Characterization of mechanisms of interleukin-6 gene expression by estrogen receptor. J Steroid Biochem Mol Biol 60:11–17
100. **Perkins ND** 2000 The Rel/NF-κB family: friend and foe. Trends Biochem Sci 25:434–441
101. **Sheppard KA, Phelps KM, Williams AJ, Thanos D, Glass CK, Rosenfeld MG, Gerritsen ME, Collins T** 1998 Nuclear integration of glucocorticoid receptor and NF-κB signaling by CREB-binding protein and steroid receptor coactivator-1. J Biol Chem 273:29291–29294
102. **Speir E, Yu ZX, Takeda K, Ferrans VJ, Cannon III RO** 2000 Competition for p300 regulates transcription by estrogen receptors and nuclear factor-κB in human coronary smooth muscle cells. Circ Res 87:1006–1011
103. **Wang C, Fu M, Angeletti RH, Siconolfi-Baez L, Retuens AT, Albanese C, Lisanti MP, Katzenellenbogen BS, Kato S, Hopp T, Fuqua SAW, Lopez GN, Kushner PJ, Pestell RG** 2001 Direct acetylation of the estrogen receptor α hinge region by p300 regulates transactivation and hormone sensitivity. J Biol Chem 276:18375–18383
104. **Sun WH, Keller ET, Stebler BS, Ershler WB** 1998 Estrogen inhibits phorbol ester-induced I κ B α transcription and protein degradation. Biochem Biophys Res Commun 244:691–695
105. **Matsusaka T, Fujikawa K, Nishio Y, Mukaida N, Matsushima K, Kishimoto T, Akira S** 1993 Transcription factors NF-IL6 and NF-κB synergistically activate transcription of the inflammatory cytokines, interleukin 6 and interleukin 8. Proc Natl Acad Sci USA 90:10193–10197
106. **Deb S, Tessier C, Prigent-Tessier A, Barkai U, Ferguson-Gottschall S, Srivastava RK, Faliszek J, Gibori G** 1999 The expression of interleukin-6 (IL-6), IL-6 receptor, and gp130-kilodalton glycoprotein in the rat decidua and a decidual cell line: regulation by 17β-estradiol and prolactin. Endocrinology 140:4442–4450
107. **Bhat RA, Harnish DC, Stevis PE, Lyttle CR, Komm BS** 1998 A novel human estrogen receptor β: identification and functional analysis of additional N-terminal amino acids. J Steroid Biochem Mol Biol 67:233–240
108. **Inadera H, Sekiya T, Yoshimura T, Matsushima K** 2000 Molecular analysis of the inhibition of monocyte chemoattractant protein-1 gene expression by estrogens and xenoestrogens in MCF-7 cells. Endocrinology 141:50–59
109. **Wissink S, van Der Burg B, Katzenellenbogen BS, van Der Saag PT** 2001 Synergistic activation of the serontonin-1a receptor by nuclear factor-κB and estrogen. Mol Endocrinol 15:543–552
110. **Chiu R, Boyle WJ, Meek J, Smeal T, Hunter T, Karin M** 1988 The c-Fos protein interacts with c-Jun/AP-1 to stimulate transcription of AP-1 responsive genes. Cell 54:541–552
111. **Garcia E, Lacasa D, Giudicelli Y** 2000 Estradiol stimulation of c-fos and c-jun expressions and activator protein-1 deoxyribonucleic acid binding activity in rat white adipocyte. Endocrinology 141: 2837–2846
112. **Gaub MP, Bellard M, Scheuer I, Chambon P, Sassone CP** 1990 Activation of the ovalbumin gene by the estrogen receptor involves the fos-jun complex. Cell 63:1267–1276
113. **Philips A, Chalbos D, Rochefort H** 1993 Estradiol increases and anti-estrogens antagonize the growth factor-induced activator protein-1 activity in MCF7 breast cancer cells without affecting c-fos and c-jun synthesis. J Biol Chem 268:14103–14108
114. **Umayahara Y, Kawamori R, Watada H, Imano E, Iwama N, Morishima T, Yamasaki Y, Kajimoto Y, Kamada T** 1994 Estrogen regulation of the insulin-like growth factor I gene transcription involves an AP-1 enhancer. J Biol Chem 269:16433–16442
115. **Webb P, Lopez GN, Uht RM, Kushner PJ** 1995 Tamoxifen activation of the estrogen receptor/AP-1 pathway: potential origin for the cell-specific estrogen-like effects of antiestrogens. Mol Endocrinol 9:443–456
116. **Paech K, Webb P, Kuiper GG, Nilsson S, Gustafsson J, Kushner PJ, Scanlan TS** 1997 Differential ligand activation of estrogen receptors ER α and ER β at AP1 sites. Science 277:1508–1510
117. **Philips A, Teyssier C, Galtier F, Rivier-Covas C, Rey JM, Rochefort H, Chalbos D** 1998 FRA-1 expression level modulates regulation of activator-protein-1 activity by estradiol in breast cancer cells. Mol Endocrinol 12:973–985
118. **Miller CD, Miller WL** 1996 Transcriptional repression of the ovine follicle-stimulating hormone-β gene by 17β-estradiol. Endocrinology 137:3437–3446
119. **Schmitt M, Bausero P, Simoni P, Queuche D, Geoffroy V, Marschal C, Kempf J, Quirin-Stricker C** 1995 Positive and negative effects of nuclear receptors on transcription activation by AP-1 of the human choline acetyltransferase proximal promoter. J Neurosci Res 40:152–164
120. **An J, Ribeiro RCJ, Webb P, Gustafsson J-A, Kushner PJ, Baxter JD, Leitman DC** 1999 Estradiol repression of tumor necrosis factor-α transcription requires estrogen receptor activation function-2 and is enhanced by coactivators. Proc Natl Acad Sci USA 96:15161–15166
121. **An J, Tzagarakis-Foster C, Scharschmidt TC, Lomri N, Leitman DC** 2001 Estrogen receptor β-selective transcriptional activity and recruitment of coregulators by phytoestrogens. J Biol Chem 276: 17808–17814
122. **Jakacka M, Ito M, Weiss J, Chien PY, Gehm BD, Jameson JL** 2001 Estrogen receptor binding to DNA is not required for its activity through the nonclassical AP1 pathway. J Biol Chem 276:13615–13621
123. **Srivastava S, Weitzmann MN, Cenci S, Ross FP, Adler S, Pacifici R** 1999 Estrogen decreases TNF gene expression by blocking JNK activity and the resulting production of c-Jun and JunD. J Clin Invest 104:503–513
124. **Shevde NK, Bendixen AC, Dienger KM, Pike JW** 2000 Estrogens suppress RANK ligand-induced osteoclast differentiation via a stromal cell independent mechanism involving c-Jun repression. Proc Natl Acad Sci USA 97:7829–7834
125. **Wong CW, Komm B, Cheskis BJ** 2001 Structure-function evalu-

ation of ER α and β interplay with SRC family coactivators. ER selective ligands. Biochemistry 40:6756–6765
126. **Tak PP, Firestein GS** 2001 NF-κB: a key role in inflammatory diseases. J Clin Invest 107:7–11
127. **Sachs L, Lotem J** 1994 The network of hematopoietic cytokines. Proc Soc Exp Biol Med 206:170–175
128. **Philip R, Epstein LB** 1986 Tumour necrosis factor as immunomodulator and mediator of monocyte cytotoxicity induced by itself, γ-interferon and interleukin-1. Nature 323:86–89
129. **Feldmann M, Brennan FM, Maini R** 1996 Rheumatoid arthritis. Cell 85:307–310
130. **Beck K-F, Eberhardt W, Frank S, Huwiler A, Messmer UK, Mühl H, Pfeilschifter J** 1999 Inducible NO synthase: role in cellular signaling. J Exp Biol 202:645–653
131. **Zamora R, Vodovotz Y, Billiar TR** 2000 Inducible nitric oxide synthase and inflammatory diseases. Mol Med 6:347–373
132. **Kauser K, Rubanyi GM** 1994 Gender differences in bioassayable endothelium-derived nitric oxide release from isolated rat aortae. Am J Physiol 267:H2311–H2317
133. **Hayashi T, Fukuto JM, Ignarro LJ, Chaudhuri G** 1995 Gender differences in atherosclerosis: possible role of nitric oxide. J Cardiovasc Pharmacol 26:792–802
134. **Armour KE, Ralston SH** 1998 Estrogen upregulates endothelial constitutive nitric oxide synthase expression in human osteoblast-like cells. Endocrinology 139:799–802
135. **Stefano GB, Prevot V, Beauvillain JC, Fimiani C, Welters I, Cadet P, Breton C, Pestel J, Salzet M, Bilfinger TV** 1999 Estradiol coupling to human monocyte nitric oxide release is dependent on intracellular calcium transients: evidence for an estrogen surface receptor. J Immunol 163:3758–3763
136. **Simoncini T, Genazzani AR** 2000 Raloxifene acutely stimulates nitric oxide release from human endothelial cells via an activation of endothelial nitric oxide synthase. J Clin Endocrinol Metab 85: 2966–2969
137. **Rubanyi GM, Freay AD, Kauser K, Sukovich D, Burton G, Lubashn DB, Couse JF, Curtis SW, Korach KS** 1997 Vascular estrogen receptors and endothelial-derived nitric oxide production in the mouse aorta: gender difference and effect of estrogen receptor gene disruption. J Clin Invest 99:2429–2437
138. **Stacey E, Korkia P, Hukkanen MVJ, Polak JM, Rutherford OM** 1998 Decreased nitric oxide levels and bone turnover in amenorrheic athletes with spinal osteopenia. J Clin Endocrinol Metab 83: 3056–3061
139. **Rosselli M, Imthurn B, Keller PJ, Jackson EK, Dubey RK** 1995 Circulating nitric oxide (nitrite/nitrate) levels in postmenopausal women substituted with 17β-estradiol and norethisterone acetate. Hypertension 25:848–853
140. **Jamal S, Browner W, Bauer D, Cummings S** 1998 Intermittent use of nitrates increases bone mineral density: the study of osteoporotic fractures. J Bone Miner Res 13:1755–1759
141. **Konukoglu D, Serin O, Yelke HK** 2000 Effects of hormone replacement therapy on plasma nitric oxide and total thiol levels in postmenopausal women. J Toxicol Environ Health 60:81–87
142. **Chen Z, Yuhanna IS, Galcheva-Gargova Z, Karas RH, Mendelsohn ME, Shaul PW** 1999 Estrogen receptor α mediates the nongenomic activation of endothelial nitric oxide synthase by estrogen. J Clin Invest 103:401–406
143. **Stefano GB, Prevot V, Beauvillain J-C, Cadet P, Fimiani C, Welters I, Fricchione GL, Breton C, Lassalle P, Salzet M, Bilfinger TV** 2000 Cell-surface estrogen receptors mediate calcium-dependent nitric oxide release in human endothelia. Circulation 101:1594–1597
144. **Wyckoff MH, Chambliss KL, Mineo C, Yuhanna IS, Mendelsohn ME, Mumby SM, Shaul PW** 2001 Plasma membrane estrogen receptors are coupled to endothelial nitric-oxide synthase through Gαi. J Biol Chem 276:27071–27076
145. **Haynes MP, Sinha D, Russell KS, Collinge M, Fulton D, Morales-Ruiz M, Sessa WC, Bender JR** 2000 Membrane estrogen receptor engagement activates endothelial nitric oxide synthase via the PI3-kinase-akt pathway in human endothelial cells. Circ Res 87:677–682
146. **Simoncini T, Hafezi-Moghadam A, Brazil DP, Ley K, Chin WW, Liao JK** 2000 Interaction of oestrogen receptor with the regulatory subunit of phosphatidiylinositol-3-OH kinase. Nature 407:538–541
147. **Hayashi T, Yamada K, Esaki T, Muto E, Chaudhuri G, Iguchi A** 1998 Physiological concentrations of 17β-estradiol inhibit the synthesis of nitric oxide synthase in macrophages via a receptor-mediated system. J Cardiovasc Pharamacol 31:292–298
148. **Zancan V, Santagati S, Bolego C, Vegeto E, Maggi A, Puglisi L** 1999 17β-estradiol decreases nitric oxide synthase II synthesis in vascular smooth muscle cells. Endocrinology 140:2004–2009
149. **Cuzzocrea S, Santagati S, Sautebin L, Mazzon E, Calabró G, Serraino I, Caputi AP, Maggi A** 2000 17β-Estradiol antiinflammatory activity in carrageenan-induced pleurisy. Endocrinology 141: 1455–1463
150. **Drew PD, Chavis JA** 2000 Female sex steroids: effects upon microglial cell activation. J Neuroimmunol 111:77–85
151. **De Frutos T, de Miguel LS, Garcia-Duran M, Gonzalez-Fernandez F, Rodriguez-Feo JA, Monotn M, Guerra J, Farre J, Casado S, Lopez-Farre A** 1999 NO from smooth muscle cells decreases NOS expression in endothelial cells: role of TNF-α. Am J Physiol 277: H1317–H1325
152. **Galley HF, Nelson SJ, Dhillon J, Dubbels AM, Webster NR** 1999 Effect of the nitric oxide inhibitor, L-N(G)-monomethylarginine, on accumulation of interleukin-6 and interleukin-8, and nuclear factor-κB activity in a human endothelial cell line. Crit Care Med 27:908–912
153. **Jouzeau JY, Cipolletta C, Presle N, Netter P, Terlain B** 1999 Modulation of IL-1 effects on cartilage by NO synthase inhibitors: pharmacological studies in rats. Osteoarthritis Cartilage 7:382–385
154. **Wang S, Wang W, Wesley RA, Danner RL** 1999 A Sp1 binding site of the tumor necrosis factor α promoter functions as a nitric oxide response element. J Biol Chem 274:33190–33193
155. **Tang Y, Han C, Wang X** 1999 Role of nitric oxide and prostaglandins in the potentiating effects of calcitonin gene-related peptide on lipopolysaccharide-induced interleukin-6 release from mouse peritoneal macrophages. Immunology 96:171–175
156. **Kuo HP, Wang CH, Huang KS, Lin HC, Yu CT, Liu CY, Lu LC** 2000 Nitric oxide modulates interleukin-1β and tumor necrosis factor-α synthesis by alveolar macrophages in pulmonary tuberculosis. Am J Respir Crit Care Med 161:192–199
157. **Yamashita T, Kawashima S, Ohashi Y, Ozaki M, Ueyama T, Ishida T, Inoue N, Hirata K, Akita H, Yokoyama M** 2000 Resistance to endotoxin shock in transgenic mice overexpressing endothelial nitric oxide synthase. Circulation 101:931–937
158. **Peng HB, Libby P, Liao JK** 1995 Induction and stabilization of IκBα by nitric oxide mediates inhibition of NF-κB. J Biol Chem 270: 14214–14219
159. **Zeiher AM, Fisslthaler B, Schray-Utz B, Busse R** 1995 Nitric oxide modulates the expression of monocyte chemoattractant protein 1 in cultured human endothelial cells. Circ Res 76:980–986
160. **Kim YM, Talanian RV, Li J, Billiar TR** 1998 Nitric oxide prevents IL-1β and INF-gamma-inducing factor (IL-18) release from macrophages by inhibiting caspase-1 (IL-1β-converting enzyme). J Immunol 161:4122–4128
161. **Meldrum DR, Shames BD, Meng X, Fullerton DA, McIntyre Jr RC, Grover FL, Harken AH** 1998 Nitric oxide downregulates lung macrophage inflammatory cytokine production. Ann Thorac Surg 66:313–317
162. **Tomita H, Egashira K, Kubo-Inoue M, Usui M, Koyanagi M, Shimokawa H, Takeya M, Yoshimura T, Takeshita A** 1998 Inhibition of NO synthesis induces inflammatory changes and monocyte chemoattractant protein-1 expression in rat hearts and vessels. Arterioscler Thromb Vasc Biol 18:1456–1464
163. **Kallmann BA, Malzkorn R, Kolb H** 1999 Exogenous nitric oxide modulates cytokine production in human leukocytes. Life Sci 65: 1787–1794
164. **Obermeier F, Gross V, Scholmerich J, Falk W** 1999 Interleukin-1 production by mouse macrophages is regulated in a feedback fashion by nitric oxide. J Leukoc Biol 66:829–836
165. **Raychaudhuri B, Dweik R, Connors MJ, Buhrow L, Malur A, Drazba J, Arroliga AC, Erzurum SC, Kavuru MS, Thomassen MJ** 1999 Nitric oxide blocks nuclear factor κB activation in alveolar macrophages. Am J Respir Cell Mol Biol 21:311–316
166. **Schroeder RA, Cai C, Kuo PC** 1999 Endotoxin-mediated nitric oxide synthesis inhibits IL-1 β gene transcription in ANA-1 murine macrophages. Am J Physiol 277:C523–C530

167. **van't Hof RJ, Armour KJ, Smith LM, Armour KE, Wei XQ, Liew FY, Ralston SH** 2000 Requirement of the inducible nitric oxide synthase pathway for IL-1-induced osteoclastic bone resorption. Proc Natl Acad Sci USA 97:7993–7998
168. **Wimalawansa SJ, De Marco G, Gangula P, Yallampalli C** 1996 Nitric oxide donor alleviates ovariectomy-induced bone loss. Bone 18:301–304
169. **Wimalawansa SJ** 2000 Restoration of ovariectomy-induced osteopenia by nitroglycerin. Calcif Tissue Int 66:56–60
170. **Wimalawansa SJ** 2000 Nitroglycerein therapy is as efficacious as standard estrogen replacement therapy (Premarin) in prevention of oophorectomy-induced bone loss: a human pilot clinical study. J Bone Miner Res 15:2240–2244
171. **Subbiah MT** 1998 Mechanisms of cardioprotection by estrogens. Proc Soc Exp Biol Med 217:23–29
172. **Bruce-Keller AJ, Keeling JL, Keller JN, Huang FF, Camondola S, Mattson MP** 2000 Antiinflammatory effects of estrogen on microglial activation. Endocrinology 141:3646–3656
173. **Melton L** 2000 Sex is all in the brain: report of a Novartis Foundation Symposium on the neuronal and cognitive effects of oestrogens, London UK, 7–9 September 1999. Trends Endocrinol Metab 11:69–71
174. **Gurdol F, Oner-Yyidothan Y, Yalcyn O, Genc S, Buyru F** 1997 Changes in enzymatic antioxidant defense system in blood and endometrial tissues of women after menopause. Res Comm Mol Pathol Pharmacol 97:38–46
175. **Massafra C, Buonocore G, Gioia D, Sargentini I, Farina G** 1997 Effects of estradiol and medroxyprogesterone acetate treatment on erythrocyte antioxidant enzyme activities and malondialdehyde plasma levels in amenorrhoic women. J Clin Endocrinol Metab 82:173–175
176. **Massafra C, Gioia D, De Felice C, Picciolini E, De Leo V, Bonifazi M, Bernabei A** 2000 Effects of estrogens and androgens on erythrocyte antioxidant superoxide dismutase, catalase and glutathione peroxidase activities during the menstrual cycle. J Endocrinol 167: 447–452
177. **Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW** 1994 Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. Circ Res 74:1141–1148
178. **Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Griendling KK, Harrsion DG** 1996 Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation: contribution to alterations of vasomotor tone. J Clin Invest 97:1916–1923
179. **Nickenig G, Baumer AT, Grohe C, Kahlert S, Strehlow K, Rosenkranz S, Stablein A, Beckers F, Smith JF, Daemen MJ, Vetter H, Bohm M** 1998 Estrogen modulates AT1 receptor gene expression *in vitro* and *in vivo*. Circulation 97:2197–2201
180. **Shwaery GT, Vita JA, Keaney Jr JF** 1997 Antioxidant protection of LDL by physiological concentrations of 17β-estradiol. Requirement for estradiol modification. Circulation 95:1378–1385
181. **Yoon BK, Oh WJ, Kessel B, Roh CR, Choi D, Lee JH, Kim DK** 2001 17β-estradiol inhibits proliferation of cultured vascular smooth muscle cells induced by lysophosphatidylcholine via a nongenomic antioxidant mechanism. Menopause 8:58–64
182. **Kelly MJ, Levin ER** 2001 Rapid actions of plasma membrane estrogen receptors. Trends Endocrinol Metab 12:152–156
183. **Razandi M, Pedram A, Levin ER** 2000 Estrogen signals to the preservation of endothelial cell form and function. J Biol Chem 275:38540–38546
184. **Wade CB, Robinson S, Shapiro RA, Dorsa DM** 2001 Estrogen receptor (ER)α and ERβ exhibit unique pharmacological properties when coupled to activation of the mitogen-activated protein kinase pathway. Endocrinology 142:2336–2342
185. **De Jager T, Pelzer T, Müller-Botz S, Imam A, Muck J, Neyses L** 2001 Mechanisms of estrogen receptor action in the myocardium. Rapid gene activation via the ERK1/2 pathway and serum response elements. J Biol Chem 276:27873–27880
186. **Kovacs EJ, Radzioch D, Young HA, Varesio L** 1988 Differential inhibition of IL-1 and TNF-α mRNA expression by agents which block second messenger pathways in murine macrophages. J Immunol 141:3101–3105
187. **Zubiaga AM, Munoz E, Merrow M, Huber BT** 1990 Regulation of interleukin 6 production in T helper cells. Int Immunol 2:1047–1054
188. **Chung IY, Kwon J, Benveniste EN** 1992 Role of protein kinase C activity in tumor necrosis factor-α gene expression. Involvement at the transcriptional level. J Immunol 149:3894–3902
189. **Shapira L, Takashiba S, Champagne C, Amar S, Van Dyke TE** 1994 Involvement of protein kinase C and protein tyrosine kinase in lipopolysaccharide-induced TNF-α and IL-1 β production by human monocytes. J Immunol 153:1818–1824
190. **Carter AB, Monick MM, Hunninghake GW** 1999 Both Erk and p38 kinases are necessary for cytokine gene transcription. Am J Respir Cell Mol Biol 20:751–758
191. **Baldassare JJ, Bi Y, Bellone CJ** 1999 The role of p38 mitogen-activated protein kinase in IL-1 β transcription. J Immunol 162: 5367–5373
192. **Leonard M, Ryan MP, Watson AJ, Schramek H, Healy E** 1999 Role of MAP kinase pathways in mediating IL-6 production in human primary mesangial and proximal tubular cells. Kidney Int 56:1366–1377
193. **Rutault K, Hazzalin CA, Mahadevan LC** 2001 Combinations of ERK and p38 MAPK inhibitors ablate tumor necrosis factor-α (TNF-α) mRNA induction. Evidence for selective destabilization of TNF-α transcripts. J Biol Chem 276:6666–6674
194. **Bowie AG, O'Neill LA** 2000 Vitamin C inhibits NF-κB activation by TNF via the activation of p38 mitogen-activated protein kinase. J Immunol 165:7180–7188
195. **Olsen NJ, Kovacs WJ** 1996 Gonadal steroids and immunity. Endocr Rev 17:369–384
196. **Rifas L** 1999 Bone and cytokines: beyond IL-1, IL-6 and TNF-α. Calcif Tissue Int 64:1–7
197. **Fujita T, Matsui T, Nakao Y, Watanabe S** 1984 T lymphocyte subsets in osteoporosis. Effect of 1-α hydroxyvitamin D3. Miner Electrolyte Metab 10:375–378
198. **Imai Y, Tsunenari T, Fukase M, Fujita T** 1990 Quantitative bone histomorphometry and circulating T lymphocyte subsets in postmenopausal osteoporosis. J Bone Miner Res 5:393–399
199. **Cenci S, Weitzmann MN, Roggia C, Namba N, Novack D, Woodring J, Pacifici R** 2000 Estrogen deficiency induces bone loss by enhancing T-cell production of TNF-α. J Clin Invest 106:1229–1237
200. **Ito A, Bebo Jr BF, Matejuk A, Zamora A, Silverman M, Fyfe-Johnson A, Offner H** 2001 Estrogen treatment down-regulates TNF-α production and reduces the severity of experimental autoimmune encephalomyelitis in cytokine knockout mice. J Immunol 167:542–552
201. **Masuzawa T, Miyaura C, Onoe Y, Kusano K, Ohta H, Nozawa S, Suda T** 1994 Estrogen deficiency stimulates B lymphopoiesis in mouse bone marrow. J Clin Invest 94:1090–1097
202. **Danilovich N, Babu PS, Xing W, Gerdes M, Krishnamurthy H, Sairam MR** 2000 Estrogen deficiency, obesity, and skeletal abnormalities in follicle-stimulating hormone receptor knockout (FORKO) female mice. Endocrinology 141:4295–4308
203. **Onoe Y, Miyaura C, Ito M, Ohta H, Nozawa S, Suda T** 2000 Comparative effects of estrogen and raloxifene on B lymphopoiesis and bone loss induced by sex steroid deficiency in mice. J Bone Miner Res 15:541–549
204. **Kincade PW, Medina KL, Smithson G, Scott DC** 1994 Pregnancy: a clue to normal regulation of B lymphopoiesis. Immunol Today 15:539–544
205. **Kouro T, Medina KL, Oritani K, Kincade PW** 2001 Characteristics of early murine B-lymphocyte precursors and their direct sensitivity to negative regulators. Blood 97:2708–2715
206. **Grimaldi CM, Michael DJ, Diamond B** 2001 Cutting edge: expansion and activation of a population of autoreactive marginal zone b cells in a model of estrogen-induced lupus. J Immunol 167:1886–1890
207. **Seo H, Miyaura C, Sato S, Sudo T, Suda T** 1995 Immature B-lymphopoiesis accumulating in estrogen deficiency stimulate IL-6 production by mouse bone marrow stromal cells. J Bone Miner Res 10:S159
208. **Zarvis LJ, LeBien TW** 1995 Stimulation of human bone marrow stromal cell tyrosine kinases and IL-6 production by contact with B lymphocytes. J Immunol 155:2359–2368
209. **Kong YY, Boyle WJ, Penninger JM** 2000 Osteoprotegerin ligand:

a regulator of immune responses and bone physiology. Immunol Today 21:495–502
210. **Kanematsu M, Sato T, Takai H, Watanabe K, Ikeda K, Yamada Y** 2000 Prostaglandin E2 induces expression of receptor activator of nuclear factor-κ B ligand/osteoprotegerin ligand on pre-B cells: implications for accelerated osteoclastogenesis in estrogen deficiency. J Bone Miner Res 15:1321–1329
211. **Jilka RL, Passeri G, Girasole G, Cooper S, Abrams J, Broxmeyer H, Manolagas SC** 1995 Estrogen loss upregulates hematopoiesis in the mouse: a mediating role of IL-6. Exp Hematol 23:500–506
212. **Denhardt DT, Noda M, O'Regan AW, Pavlin D, Berman JS** 2001 Osteopontin as a means to cope with environmental insults: regulation of inflammation, tissue remodeling, and cell survival. J Clin Invest 107:1055–1061
213. **Peeva E, Grimaldi C, Spatz L, Diamond B** 2000 Bromocriptine restores tolerance in estrogen-treated mice. J Clin Invest 106:1373–1379
214. **Grossman CJ** 1991 Immunoendocrinology. In: Greenspan FS, ed. Basic and clinical endocrinology, 3rd Ed. Norwalk, CT: Appleton and Lang; 40–52
215. **Chrousos GP, Torpy DJ, Gold PW** 1998 Interactions between the hypothalamic-pituitary-adrenal axis and the female reproductive system: clinical implications. Ann Intern Med 129:229–240
216. **Elenkov IJ, Papanicolaou DA, Wilder RL, Chrousos GP** 1996 Modulatory effects of glucocorticoids and catecholamines on human interleukin-12 and interleukin-10 production: clinical implications. Proc Assoc Am Physicians 108:374–381
217. **Poehlman ET, Toth MJ, Gardner AW** 1995 Changes in energy balance and body composition at menopause: a controlled longitudinal study. Ann Intern Med 123:673–675
218. **Jones MEE, Thorburn AW, Britt KL, Hewitt KN, Wreford NG, Proietto J, Oz OK, Leury BJ, Robertson KM, Yao S, Simpson ER** 2000 Aromatase-deficient (ArKO) mice have a phenotype of increased adiposity. Proc Natl Acad Sci USA 97:12735–12740
219. **Heine PA, Taylor JA, Iwamoto GA, Lubahn DB, Cooke PS** 2000 Increased adipose tissue in male and female estrogen receptor-α knockout mice. Proc Natl Acad Sci USA 97:12729–12734
220. **Haarbo J, Marslew U, Gotfredsen A, Christiansen C** 1991 Postmenopausal hormone replacement therapy prevents central distribution of body fat after menopause. Metabolism 40:323–326
221. **Bastard JP, Jardel C, Bruckert E, Blondy P, Capeau J, Laville M, Vidal H, Hainque B** 2000 Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. J Clin Endocrinol Metab 85:3338–3342
222. **Ahima RS, Flier JS** 2000 Adipose tissue as an endocrine organ. Trends Endocrinol Metab 11:327–332
223. **Fried SK, Bunkin DA, Greenberg AS** 1998 Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. J Clin Endocrinol Metab 83:847–850
224. **Zhao Y, Nichols JE, Bulun SE, Mendelson CR, Simson ER** 1995 Aromatase P450 gene expression in human adipose tissue. Role of a Jak/STAT pathway in the regulation of the adipose-specific promoter. J Biol Chem 270:16449–16457
225. **Takahashi N, Udagawa N, Suda T** 1999 A new member of tumor necrosis factor ligand familiy, ODF/OPGL/TRANCE/RANKL, regulates osteoclast differentiation and function. Biochem Biophys Res Commun 256:449–455
226. **Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Boyle WJ, Riggs BL** 2000 The roles of osteoprotegerin and osteoprotegerin ligand in the paracrine regulation of bone resorption. J Bone Miner Res 15:2–12
227. **Kong YY, Yoshida H, Sarosi I, Tan HL, Timms E, Capparelli C, Morony S, Oliveria-dos-Santos AJ, Van G, Itie A, Khoo W, Wakeham A, Dunstan CR, Lacey DL, Mak TW, Boyle WJ, Penninger JM** 1999 OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature 397:315–323
228. **Dougall WC, Glaccum M, Charrier K, Rohrbach K, Brasel K, De Smedt T, Daro E, Smith J, Tometsko ME, Maliszewski CR, Armstrong A, Shen V, Bain S, Cosman D, Anderson D, Morrissey PJ, Peschon JJ, Schuh J** 1999 RANK is essential for osteoclast and lymph node development. Genes Dev 13:2412–2424
229. **Malyankar UM, Scatena M, Suchland KL, Yun TJ, Clark EA, Giachelli CM** 2000 Osteoprotegerin is an α vβ 3-induced NF-κB-dependent survival factor for endothelial cells. J Biol Chem 275: 20959–20962
230. **Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, Scully S, Tan HL, Xu W, Lacey DL, Boyle WJ, Simonet WS** 1998 Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev 12:1260–1268
231. **Min H, Morony S, Sarosi I, Dunstan CR, Capparelli C, Scully S, Van G, Kaufman S, Kostenuik PJ, Lacey DL, Boyle WJ, Simonet WS** 2000 Osteoprotegerin reverses osteoporosis by inhibiting endosteal osteoclasts and prevents vascular calcification by blocking a process resembling osteoclastogenesis. J Exp Med 192:463–474
232. **Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Spelsberg TC, Riggs BL** 1999 Estrogen stimulates gene expression and protein production of osteoprotegerin in human osteoblastic cells. Endocrinology 140:4367–4370
233. **Saika M, Inoue D, Kido S, Matsumoto T** 2001 17β-Estradiol stimulates expression of osteoprotegerin by a mouse stromal cell line, ST-2, via estrogen receptor-α. Endocrinology 142:2205–2212
234. **Hofbauer LC, Heufelder AE** 2000 Clinical review 114: hot topic. The role of receptor activator of nuclear factor-κB ligand and osteoprotegerin in the pathogenesis and treatment of metabolic bone diseases. J Clin Endocrinol Metab 85:2355–2363
235. **Browner WS, Lui LY, Cummings SR** 2001 Associations of serum osteoprotegerin levels with diabetes, stroke, bone density, fractures, and mortality in elderly women. J Clin Endocrinol Metab 86:631–637
236. **Nakashima T, Kobayashi Y, Yamasaki S, Kawakami A, Eguchi K, Sasaki H, Sakai H** 2000 Protein expression and functional difference of membrane-bound and soluble receptor activator of NF-κB ligand: modulation of the expression by osteotropic factors and cytokines. Biochem Biophys Res Commun 275:768–775
237. **Hofbauer LC, Dunstan CR, Spelsberg TC, Riggs BL, Khosla S** 1998 Osteoprotegerin production by human osteoblast lineage cells is stimulated by vitamin D, bone morphogenetic protein-2, and cytokines. Biochem Biophys Res Commun 250:776–781
238. **Hofbauer LC, Lacey DL, Dunstan CR, Spelsberg TC, Riggs BL, Khosla S** 1999 Interleukin-1β and tumor necrosis factor-α, but not interleukin-6, stimulate osteoprotegerin ligand gene expression in human osteoblastic cells. Bone 25:255–259
239. **Vidal ON, Sjogren K, Eriksson BI, Ljunggren O, Ohlsson C** 1998 Osteoprotegerin mRNA is increased by interleukin-1 α in the human osteosarcoma cell line MG-63 and in human osteoblast-like cells. Biochem Biophys Res Commun 248:696–700
240. **Brandstrom H, Bjorkman T, Ljunggren O** 2001 Regulation of osteoprotegerin secretion from primary cultures of human bone marrow stromal cells. Biochem Biophys Res Commun 280:831–835
241. **Srivastava S, Toraldo G, Weitzmann MN, Cenci S, Ross FP, Pacifici R** 2001 Estrogen decreases osteoclast formation by down-regulating receptor activator of NF-κB ligand (RANKL)-induced JNK activation. J Biol Chem 276:8836–8840
242. **Letterio JJ, Roberts AB** 1997 TGF-β: a critical modulator of immune cell function. Clin Immunol Immunpathol 84:244–250
243. **Grande JP** 1997 Role of transforming growth factor-β in tissue injury and repair. Proc Soc Exp Biol Med 214:27–40
244. **Blobe GC, Schiemann WP, Lodish HF** 2000 Role of transforming growth factor β in human disease. N Engl J Med 342:1350–1358
245. **Robinson A, Riggs BL, Spelsberg TC, Oursler MJ** 1996 Osteoclasts and transforming growth factor-β: estrogen-mediated isoform-specific regulation of production. Endocrinology 137:615–621
246. **Hughes DE, Dai A, Tiffee JC, Li HH, Mundy GR, Boyce BF** 1996 Estrogen promotes apoptosis of murine osteoclasts mediated by TGF-β. Nat Med 2:1132–1136
247. **Oursler MJ, Cortese C, Keeting P, Anderson MA, Bonde SK, Riggs BL, Spelsberg TC** 1991 Modulation of transforming growth factor-β production in normal human osteoblast-like cells by 17 β-estradiol and parathyroid hormone. Endocrinology 129:3313–3320
248. **Yang NN, Bryant HU, Hardikar S, Sato M, Galvin RJ, Glasebrook AL, Termine JD** 1996 Estrogen and raloxifene stimulate transforming growth factor-β 3 gene expression in rat bone: a potential mechanism for estrogen- or raloxifene-mediated bone maintenance. Endocrinology 137:2075–2084

249. **Selzman CH, Gaynor JS, Turner AS, Whitehill TA, Horwitz LD, Harken AH** 1998 Estrogen replacement inhibits intimal hyperplasia and the accumulation and effects of transforming growth factor β 1. J Surg Res 80:380–385
250. **Ashcroft GS, Dodsworth J, van Boxtel E, Tarnuzzer RW, Horan MA, Schultz GS, Ferguson MW** 1997 Estrogen accelerates cutaneous wound healing associated with an increase in TGF-β1 levels. Nat Med 3:1209–1215
251. **Djurovic S, Os I, Hofstad AE, Abdelnoor M, Westheim A, Berg K** 2000 Increased plasma concentrations of TGF-β1 after hormone replacement therapy. J Intern Med 247:279–285
252. **Westerlind KC, Wronski TJ, Evans GL, Turner RT** 1994 The effect of long-term ovarian hormone deficiency on transforming growth factor-β and bone matrix protein mRNA expression in rat femora. Biochem Biophys Res Commun 200:283–289
253. **Finkelman RD, Bell NH, Strong DD, Demers LM, Baylink DJ** 1992 Ovariectomy selectively reduces the concentration of transforming growth factor β in rat bone: implications for estrogen deficiency-associated bone loss. Proc Natl Acad Sci USA 89:12190–12193
254. **Ikeda T, Shigeno C, Kasai R, Kohno H, Ohta S, Okumura H, Konishi J, Yamamuro T** 1993 Ovariectomy decreases the mRNA levels of transforming growth factor-β1 and increases the mRNA levels of osteocalcin in rat bone *in vivo*. Biochem Biophys Res Commun 194:1228–1233
255. **Yang NN, Venugopalan M, Hardikar S, Glasebrook A** 1996 Identification of an estrogen response element activated by metabolites of 17β-estradiol and raloxifene. Science 273:1222–1225
256. **Pfeilschifter J, Diel I, Scheppach B, Bretz A, Krempien R, Erdmann J, Schmid G, Reske N, Bismar H, Seck T, Krempien B, Ziegler R** 1998 Concentration of transforming growth factor β in human bone tissue: relationship to age, menopause, bone turnover, and bone volume. J Bone Miner Res 13:716–730
257. **Grainger DJ, Witchell CM, Metcalfe JC** 1995 Tamoxifen elevates transforming growth factor-β and suppresses diet-induced formation of lipid lesions in mouse aorta. Nat Med 1:1067–1073
258. **Simon LS** 1999 Role and regulation of cyclooxygenase-2 during inflammation. Am J Med 106:37S–42S
259. **Kawaguchi H, Pilbeam CC, Vargas SJ, Morse EE, Lorenzo JA, Raisz LG** 1995 Ovariectomy enhances and estrogen replacement inhibits the activity of bone marrow factors that stimulate prostaglandin production in cultured mouse calvariae. J Clin Invest 96:539–548
260. **Feyen JH, Raisz LG** 1987 Prostaglandin production by calvariae from sham operated and oophorectomized rats: effect of 17 β-estradiol *in vivo*. Endocrinology 121:819–821
261. **Akgul C, Canbaz M, Vural P, Yildirim A, Geren N** 1998 Hormone replacement therapy and urinary prostaglandins in postmenopausal women. Maturitas 30:79–83
262. **Pilbeam CC, Klein-Nulend J, Raisz LG** 1989 Inhibition by 17 β-estradiol of PTH stimulated resorption and prostaglandin production in cultured neonatal mouse calvariae. Biochem Biophys Res Commun 163:1319–1324
263. **Miyagi M, Morishita M, Iwamoto Y** 1993 Effects of sex hormones on production of prostaglandin E2 by human peripheral monocytes. J Periodontol 64:1075–178
264. **Seeger H, Mueck AO, Lippert TH** 1999 Effect of estradiol metabolites on prostacyclin synthesis in human endothelial cell cultures. Life Sci 65:PL167–PL170
265. **Akarasereenont P, Techatraisak K, Thaworn A, Chotewuttakorn S** 2000 The induction of cyclooxygenase-2 by 17β-estradiol in endothelial cells is mediated through protein kinase C. Inflamm Res 49:460–465
266. **Mikkola T, Viinikka L, Ylikorkala O** 2000 Administration of transdermal estrogen without progestin increases the capacity of plasma and serum to stimulate prostacyclin production in human vascular endothelial cells. Fertil Steril 73:72–74
267. **Baldwin GC** 1992 The biology of granulocyte-macrophage colony-stimulating factor: effects on hematopoietic and nonhemoatopoietic cells. Dev Biol 151:352–367
268. **Sarma U, Edwards M, Motoyoshi K, Flanagan AM** 1998 Inhibition of bone resorption by 17β-estradiol in human bone marrow cultures. J Cell Physiol 175:99–108
269. **Lea CK, Sarma U, Flanagan AM** 1999 Macrophage colony stimulating factor transcripts are differentially regulated in rat bone-marrow by gender hormones. Endocrinology 140:273–279
270. **Srivastava S, Weitzmann MN, Kimble RB, Rizzo M, Zahner M, Milbrandt J, Ross FP, Pacifici R** 1998 Estrogen blocks M-CSF gene expression and osteoclast formation by regulating phosphorylation of Egr-1 and its interaction with Sp-1. J Clin Invest 102:1850–1859
271. **Riggs BL, Khosla S, Melton III LJ** 1998 A unitary model for involutional osteoporosis: estrogen deficiency causes both type I and type II osteoporosis in postmenopausal women and contributes to bone loss in aging men. J Bone Miner Res 13:763–773
272. **Eriksen EF, Colvard DS, Berg NJ, Graham ML, Mann KG, Spelsberg TC, Riggs BL** 1988 Evidence of estrogen receptors in normal human osteoblast-like cells. Science 241:84–86
273. **Komm BS, Terpening CM, Benz DJ, Graeme KA, Gallegos A, Korc M, Greene GL, O'Malley BW, Haussler MR** 1988 Estrogen binding, receptor mRNA, and biological response in osteoblast-like osteosarcoma cells. Science 241:81–84
274. **Oursler MJ, Osdoby P, Pyfferoen J, Riggs BL, Spelsberg TC** 1991 Avian osteoclasts as estrogen target cells. Proc Natl Acad Sci USA 88:6613–6617
275. **Westerlind KC, Sarker G, Bolander ME, Turner RT** 1995 Estrogen receptor mRNA is expressed *in vivo* in rat calvarial periosteum. Steroids 60:484–487
276. **Hoyland JA, Mee AP, Baird P, Braidman IP, Mawer EB, Freemont AJ** 1997 Demonstration of estrogen receptor mRNA in bone using *in situ* reverse-transcriptase polymerase chain reaction. Bone 20:87–92
277. **Onoe Y, Miyaura C, Ohta H, Nozawa S, Suda T** 1997 Expression of estrogen receptor β in rat bone. Endocrinology 138:4509–4512
278. **Bellido T, Girasole G, Passeri G, Yu XP, Mocharla H, Jilka RL, Notides A, Manolagas SC** 1993 Demonstration of estrogen and vitamin D receptors in bone marrow-dervied stromal cells: up-regulation of the estrogen receptor by 1,25-dihydroxyvitamin-D_3. Endocrinology 133:553–562
279. **Horowitz MC** 1993 Cytokines and estrogen in bone: antiosteoporotic effects. Science 260:626–627
280. **Bebo Jr BF, Schuster JC, Vandenbark AA, Offner H** 1999 Androgens alter the cytokine profile and produce encephathogenicity of myelin-reactive T cells. J Immunol 162:35–40
281. **Benten WP, Lieberherr M, Giese G, Wunderlich F** 1998 Estradiol binding to cell surface raises cytosolic free calcium in T cells. FEBS Lett 422:349–353
282. **Gilmore W, Weiner LP, Correale J** 1997 Effect of estradiol on cytokine secretion by proteolipid protein-specific T cell clones isolated from multiple sclerosis patients and normal control subjects. J Immunol 158:446–451
283. **Cenci S, Weitzmann MN, Gentile MA, Aisa MC, Pacifici R** 2000 M-CSF neutralization and egr-1 deficiency prevent ovariectomy-induced bone loss. J Clin Invest 105:1279–1287
284. **Manolagas SC, Jilka RL** 1995 Bone marrow, cytokines, and bone remodeling. Emerging insights into the pathophysiology of osteoporosis. N Engl J Med 332:305–311
285. **Mundy GR** 1993 Cytokines and growth factors in the regulation of bone remodeling. J Bone Miner Res 8(Suppl 1):S505–S510
286. **Passeri G, Girasole G, Manolagas SC, Jilka RL** 1994 Endogenous production of tumor necrosis factor by primary cultures of murine calvarial cells: influence on IL-6 production and osteoclast development. Bone Miner 24:109–126
287. **Suda T, Nakamura I, Jimi E, Takahashi N** 1997 Regulation of osteoclast function. J Bone Miner Res 12:869–879
288. **Kanatani M, Sugimoto T, Fukase M, Chihara K** 1994 Role of interleukin-6 and prostaglandins in the effect of monocyte-conditioned medium on osteoclast formation. Am J Physiol 267:E868–E876
289. **Kurihara N, Bertolini D, Suda T, Akiyama Y, Roodman GD** 1990 IL-6 stimulates osteoclast-like multinucleated cell formation in long-term human marrow cultures by inducing IL-1 release. J Immunol 144:4226–4230
290. **O'Keefe RJ, Teot LA, Singh D, Puzas JE, Rosier RN, Hicks DG** 1997 Osteoclasts constitutively express regulators of bone resorption: an immunohistochemical and *in situ* hybridization study. Lab Invest 76:457–465

291. **Pacifici R** 1995 Cytokines and osteoclast activity. Calcif Tissue Int 56(Suppl 1):S27–S28
292. **Pfeilschifter J, Chenu C, Bird A, Mundy GR, Roodman GD** 1989 Interleukin-1 and tumor necrosis factor stimulate the formation of human osteoclast-like cells *in vitro*. J Bone Miner Res 4:113–118
293. **Suda T, Udagawa N, Nakamura I., Miyaura C, Takahashi N** 1995 Modulation of osteoclast differentiation by local factors. Bone 17: 87S–91S
294. **Gowen M, Wood DD, Ihrie EJ, McGuire MK, Russell RG** 1983 An interleukin-1-like factor stimulates bone resorption *in vitro*. Nature 306:378–380
295. **Sabatini M, Boyce B, Aufdemorte T, Bonewald L, Mundy GR** 1988 Infusions of recombinant human interleukins 1 α and 1 β cause hypercalcemia in normal mice. Proc Natl Acad Sci USA 85:5235–5239
296. **Boyce BF, Aufdemorte TB, Garrett IR, Yates AJ, Mundy GR** 1989 Effects of interleukin-1 on bone turnover in normal mice. Endocrinology 125:1142–1150
297. **Gowen M, Mundy GR** 1986 Actions of interleukin-1, interleukin-2, and interferon γ on bone resorption *in vitro*. J Immunol 136:2478–2482
298. **Lorenzo JA, Sousa SL, Fonseca JM, Hock JM, Medlock ES** 1987 Colony-stimulating factors regulate the development of multinucleated osteoclasts from recently replicated cells *in vitro*. J Clin Invest 80:160–164
299. **Bertolini DR, Nedwin GE, Bringman TS, Smith DD, Mundy GR** 1986 Stimulation of bone resorption and inhibition of bone formation *in vitro* by human tumour necrosis factors. Nature 319:516–518
300. **Johnson RA, Boyce BF, Mundy GR, Roodman GD** 1989 Tumors producing human tumor necrosis factor induced hypercalcemia and osteoclastic bone resoprtion in nude mice. Endocrinology 124: 1424–1427
301. **Roodman GD** 1992 Interleukin-6: an osteotropic factor? J Bone Miner Res 7:475–478
302. **Tanaka S, Takahashi N, Udagawa N, Tamura T, Akatsu T, Stnley ER, Kurokawa T, Suda T** 1993 Macrophage colony-stimulating factor is indispensable for both proliferation and differentiation of osteoclast progenitors. J Clin Invest 91:257–263
303. **Jilka RL** 1998 Cytokines, bone remodeling, and estrogen deficiency: a 1998 update. Bone 23:75–81
304. **Kurihara N, Civin C, Roodman GD** 1991 Osteotropic factor responsiveness of highly purified populations of early and late precursors for human multinucleated cells expressing the osteoclast phenotype. J Bone Miner Res 6:257–261
305. **Lowik CW, van der Pluijm G, Bloys H, Hoekman K, Bijvoet OL, Aarden LA, Papapoulos SE** 1989 Parathyroid hormone (PTH) and PTH-like protein (PLP) stimulate interleukin-6 production by osteogenic cells: a possible role of interleukin-6 in osteoclastogenesis. Biochem Biophys Res Commun 162:1546–1552
306. **Ishimi Y, Miyaura C, Jin CH, Akatsu T, Abe E, Nakamura Y, Yamaguchi A, Yoshiki S, Matsuda T, Hirano T, Kishimoto T, Suda T** 1990 IL-6 is produced by osteoblasts and induces bone resorption. J Immunol 145:3297–3303
307. **Pacifici R** 1999 Aging and cytokine production. Calcif Tissue Int 65:345–351
308. **Dexter TM, Allen TD, Lajtha LG** 1977 Conditions controlling the proliferation of hematopoietic cells *in vitro*. J Cell Physiol 91:335–344
309. **Kaplan DL, Eielson CM, Horowitz MC, Insogna KL, Weir EC** 1996 Tumor necrosis factor-α induces transcription of the colony-stimulating factor-1 gene in murine osteoblasts. J Cell Physiol 168:199–208
310. **Felix R, Fleisch H, Elford PR** 1989 Bone-resorbing cytokines enhance release of macrophage colony-stimulating activity by osteoblastic cell MC3T3–E1. Calcif Tissue Int 44:356–360
311. **Sherman ML, Weber BL, Datta R, Kufe DW** 1990 Transcriptional and posttranscriptional regulation of macrophage-specific colony stimulating factor gene expression by tumor necrosis factor. Involvement of arachidonic acid metabolites. J Clin Invest 85:442–447
312. **Littlewood AJ, Russell J, Harvey GR, Hughes DE, Russell RG, Gowen M** 1991 The modulation of the expression of IL-6 and its receptor in human osteoblasts *in vitro*. Endocrinology 129:1513–1520
313. **Elias JA, Lentz V** 1990 IL-1 and tumor necrosis factor synergistically stimulate fibroblast IL-6 production and stabilize IL-6 messenger RNA. J Immunol 145:161–166
314. **Linkhart TA, Linkhart SG, MacCharles DC, Long DL, Strong DD** 1991 Interleukin-6 messenger RNA expression and interleukin-6 protein secretion in cells isolated from normal human bone: regulation by interleukin-1. J Bone Miner Res 6:1285–1294
315. **Aubin JE, Bonnelye E** 2000 Osteoprotegerin and its ligand: a new paradigm for regulation of osteoclastogenesis and bone resorption. Osteoporosis Int 11:905–913
316. **Li J, Sarosi I, Yan XQ, Morony S, Capparelli C, Tan HL, McCabe S, Elliott R, Scully S, Van G, Kaufman S, Juan SC, Sun Y, Tarpley J, Martin L, Christensen K, McCabe J, Kostenuik P, Hsu H, Fletcher F, Dusntan CR, Lacey DL, Boyle WJ** 2000 RANK is the intrinsic hematopoietic cell surface receptor that controls osteoclastogenesis and regulation of bone mass and calcium metabolism. Proc Natl Acad Sci USA 97:1566–1571
317. **Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, Elliot R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian YX, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J, Boyle WJ** 1998 Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 93:165–176
318. **Suda T, Takahashi N, Udagawa N, Jimi E, Gillespie MT, Martin TJ** 1999 Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocr Rev 20:345–357
319. **Amano H, Yamada S, Felix R** 1998 Colony-stimulating factor-1 stimulates the fusion process in osteoclasts. J Bone Miner Res 13: 846–853
320. **Brandstrom H, Jonsson KB, Ohlsson C, Vidal O, Ljunghall S, Ljunggren O** 1998 Regulation of osteoprotegerin mRNA levels by prostaglandin E2 in human bone marrow stroma cells. Biochem Biophys Res Commun 247:338–341
321. **Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T** 1998 Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci USA 95:3597–3602
322. **Tsukii K, Shima N, Mochizuki S, Yamaguchi K, Kinosaki M, Yano K, Shibata O, Udagawa N, Yasuda H, Suda T, Higashio K** 1998 Osteoclast differentiation factor mediates an essential signal for bone resorption induced by 1α,25-dihydroxyvitamin D3, prostaglandin E2 or parathyroid hormone in the microenvironment of bone. Biochem Biophys Res Commun 246:337–341
323. **Lader NS, Flanagan AM** 1998 Prostaglandin E2, interleukin 1α, and tumor necrosis factor-α increase human osteoclast formation and bone resorption *in vitro*. Endocrinology 139:3157–3164
324. **Akatsu T, Takahashi N, Udagawa N, Imamura K, Yamaguchi A, Sato K, Nagata N, Suda T** 1991 Role of prostaglandins in interleukin-1-induced bone resorption in mice *in vitro*. J Bone Miner Res 6:183–190
325. **Gowen M, MacDonald BR, Russell RG** 1988 Actions of recombinant human γ-interferon and tumor necrosis factor α on the proliferation and osteoblastic characteristics of human trabecular bone cells *in vitro*. Arthritis Rheum 31:1500–1507
326. **Takai H, Kanematsu M, Yano K, Tsuda E, Higashio K, Ikeda K, Watanabe K, Yamada Y** 1998 Transforming growth factor-β stimulates the production of osteoprotegerin/osteoclastogenesis inhibitory factor by bone marrow stromal cells. J Biol Chem 273:27091–27096
327. **Brandstrom H, Jonsson KB, Vidal O, Ljunghall S, Ohlsson C, Ljunggren O** 1998 Tumor necrosis factor-α and -β upregulate the levels of osteoprotegerin mRNA in human osteosarcoma MG-63 cells. Biochem Biophys Res Commun 248:454–457
328. **O'Brien CA, Gubrij I, Lin SC, Saylors RL, Manolagas SC** 1999 STAT3 activation in stromal/osteoblastic cells is required for induction of the receptor activator of NF-κB ligand and stimulation of osteoclastogenesis by gp130-utilizing cytokines or interleukin-1 but not 1,25-dihydroxyvitamin D3 or parathyroid hormone. J Biol Chem 274:19301–19308
329. **Kobayashi K, Takahashi N, Jimi E, Udagawa N, Takami M, Ko-**

take S, Nakagawa N, Kinosaki M, Yamaguchi K, Shima N, Yasuda H, Morinaga T, Higashio K, Martin TJ, Suda T 2000 Tumor necrosis factor α stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL-RANK interaction. J Exp Med 191:275–286

330. **Azuma Y, Kaji K, Katogi R, Takeshita S, Kudo A** 2000 Tumor necrosis factor-α induces differentiation of and bone resorption by osteoclasts. J Biol Chem 275:4858–4864
331. **Wani MR, Fuller K, Kim NS, Choi Y, Chambers T** 1999 Prostaglandin E2 cooperates with TRANCE in osteoclast induction from hemopoietic precursors: synergistic activation of differentiation, cell spreading, and fusion. Endocrinology 140:1927–1935
332. **Lam J, Takeshita S, Barker JE, Kanagawa O, Ross FP, Teitelbaum SL** 2000 TNF-α induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand. J Clin Invest 106:1481–1488
333. **Pfeilschifter J, Seyedin SM, Mundy GR** 1988 Transforming growth factor β inhibits bone resorption in fetal rat long bone cultures. J Clin Invest 82:680–685
334. **Chenu C, Pfeilschifter J, Mundy GR, Roodman GD** 1988 Transforming growth factor β inhibits formation of osteoclast-like cells in long-term human marrow cultures. Proc Natl Acad Sci USA 85:5683–5687
335. **Murakami T, Yamamoto M, Ono K, Nishikawa M, Nagata N, Motoyoshi K, Akatsu T** 1998 Transforming growth factor-β1 increases mRNA levels of osteoclastogenesis inhibitory factor in osteoblastic/stromal cells and inhibits the survival of murine osteoclast-like cells. Biochem Biophys Res Commun 252:747–752
336. **Kaneda T, Nojima T, Nakagawa M, Ogasawara A, Kaneko H, Sato T, Mano H, Kumegawa M, Hakeda Y** 2000 Endogenous production of TGF-β is essential for osteoclastogenesis induced by a combination of receptor activator of NF-κB ligand and macrophage-colony-stimulating factor. J Immunol 165:4254–4263
337. **Sells Galvin J, Gatlin CL, Horn JW, Fuson TR** 1999 TGF-β enhances osteoclast differentiation in hematopoietic cell cultures stimulated with RANKL and M-CSF. Biochem Biophys Res Commun 265:233–239
338. **Horwood NJ, Kartsogiannis V, Quinn JM, Romas E, Martin TJ, Gillespie MT** 1999 Activated T lymphocytes support osteoclast formation *in vitro*. Biochem Biophys Res Commun 265:144–150
339. **Franchimont N, Rydziel S, Canalis E** 2000 Transforming growth factor-β increases interleukin-6 transcripts in osteoblasts. Bone 26: 249–253
340. **Gimble JM, Hudson J, Henthorn J, Hua XX, Burstein SA** 1991 Regulation of interleukin 6 expression in murine bone marrow stromal cells. Exp Hematol 19:1055–1060
341. **Marusic A, Kalinowski JF, Harrison JR, Centrella M, Raisz LG, Lorenzo JA** 1991 Effects of transforming growth factor-β and IL-1 α on prostaglandin synthesis in serum-deprived osteoblastic cells. J Immunol 146:2633–2638
342. **Lotem J, Sachs L** 1990 Selective regulation of the activity of different hematopoietic regulatory proteins by transforming growth factor β 1 in normal and leukemic myeloid cels. Blood 76:1315–1322
343. **Musso T, Espinoza-Delgado I, Pulkki K, Gusella GL, Longo DL, Varesio L** 1992 IL-2 induces IL-6 production in human monocytes. J Immunol 148:795–800
344. **Thomson BM, Mundy GR, Chambers TJ** 1987 Tumor necrosis factor α and β induce osteoblastic cells to stimulate osteoclastic bone resorption. J Immunol 138:775–779
345. **Thomson BM, Saklatvala J, Chambers TJ** 1986 Osteoblasts mediated interleukin 1 stimulation of bone resorption by rat osteoclasts. J Exp Med 164:104–112
346. **Tashjian Jr AH, Voelkel EF, Lazzaro M, Goad D, Bosma T, Levine L** 1987 Tumor necrosis factor-α (cachectin) stimulates bone resorption in mouse calvaria via a prostaglandin-mediated mechanism. Endocrinology 120:2029–2036
347. **Burgess TL, Qian Y, Kaufman S, Ring BD, Van G, Capparelli C, Kelley M, Hsu H, Boyle WJ, Dunstan CR, Hu S, Lacey DL** 1999 The ligand for osteoprotegerin (OPGL) directly activates mature osteoclasts. J Cell Biol 145:527–538
348. **Van der Pluijm G, Most W, van der Wee-Pals L, de Groot H, Papapoulos S, Lowik C** 1991 Two distinct effects of recombinant human tumor necrosis factor-α on osteoclast development and subsequent resorption of mineralized matrix. Endocrinology 129: 1596–1604
349. **Jimi E, Nakamura I, Ikebe T, Akiyama S, Takahashi N, Suda T** 1998 Activation of NF-κB is involved in the survival of osteoclasts promoted by interleukin-1. J Biol Chem 273:8799–8805
350. **Adebanjo OA, Moonga BS, Yamate T, Sun L, Minkin C, Abe E, Zaidi M** 1998 Mode of action of interleukin-6 on mature osteoclasts. Novel interactions with extracellular Ca^{2+} sensing in the regulation of osteoclastic bone resorption. J Cell Biol 142:1347–1356
351. **Jimi E, Shuto T, Koga T** 1995 Macrophage colony-stimulating factor and interleukin-1 α maintain the survival of osteoclast-like cells. Endocrinology 136:808–811
352. **Jimi E, Ikebe T, Takahashi N, Hirata N, Suda T, Koga T** 1996 Interleukin-1 α activates an NF-κB-like factor in osteoclast-like cells. J Biol Chem 271:4605–4608
353. **Fuller K, Owens JM, Jagger CJ, Wilson A, Moss R, Chambers TJ** 1993 Macrophage colony-stimulating factor stimulates survival and chemotactic behavior in isolated osteoclasts. J Exp Med 178: 1733–1744
354. **Pfeilschifter J, Mundy GR** 1987 Modulation of type β transforming growth factor activity in bone cultures by osteotropic hormones. Proc Natl Acad Sci USA 84:2024–2028
355. **Mohan S, Jennings JC, Linkhart TA, Baylink DJ** 1988 Primary structure of human skeletal growth factor: homology with human insulin-like growth factor-II. Biochim Biophys Acta 966:44–55
356. **Centrella M, McCarthy TL, Canalis E** 1988 Tumor necrosis factor-α inhibits collagen synthesis and alkaline phosphatase activity independently of its effect on deoxyribonucleic acid synthesis in osteoblast-enriched bone cell cultures. Endocrinology 123:1442–1448
357. **Smith DD, Gowen M, Mundy GR** 1987 Effects of interferon-γ and other cytokines on collagen synthesis in fetal rat bone cultures. Endocrinology 120:2494–2499
358. **Harrison JR, Vargas SJ, Petersen DN, Lorenzo JA, Kream BE** 1990 Interleukin-1 α and phorbol ester inhibit collagen synthesis in osteoblastic MC3T3–E1 cells by a transcriptional mechanism. Mol Endocrinol 4:184–190
359. **Stashenko P, Dewhirst FE, Rooney ML, Desjardins LA, Heeley JD** 1987 Interleukin-1 β is a potent inhibitor of bone formation *in vitro*. J Bone Miner Res 2:559–565
360. **Nguyen L, Dewhirst FE, Hauschka PV, Stashenko P** 1991 Interleukin-1 β stimulates bone resorption and inhibits bone formation *in vivo*. Lymphokine Cytokine Res 10:15–21
361. **Gilbert L, He X, Farmer P, Boden S, Kozlowski M, Rubin J, Nanes MS** 2000 Inhibition of osteoblast differentiation by tumor necrosis factor-α. Endocrinology 141:3956–3964
362. **Gowen M, Wood DD, Russell RG** 1985 Stimulation of the proliferation of human bone cells *in vitro* by human monocyte products with interleukin-1 activity. J Clin Invest 75:1223–1229
363. **Canalis E** 1986 Interleukin-1 has independent effects on deoxyribonucleic acid and collagen synthesis in cultures of rat calvariae. Endocrinology 118:74–81
364. **Modrowski G, Godet D, Marie PJ** 1995 Involvement of interleukin 1 and tumour necrosis factor α as endogenous growth factors in human osteoblastic cells. Cytokine 7:720–726
365. **Hanazawa S, Ohmori Y, Amano S, Hirose K, Miyohsi T, Kumegawa M, Kitano S** 1986 Human purified interleukin-1 inhibits DNA synthesis and cell growth of osteoblastic cell line (MC3T3–E1), but enhances alkaline phosphatase activity in the cells. FEBS Lett 203:279–284
366. **Centrella M, Horowitz MC, Wozney JM, McCarthy TL** 1994 Transforming growth factor-β gene family members and bone. Endocr Rev 15:27–39
367. **Raisz LG** 2001 Potential impact of selective cyclooxygenase-2 inhibitors on bone metabolism in health and disease. Am J Med 110:43–45
368. **Lorenzo JA, Naprta A, Rao Y, Alander C, Glaccum M, Widmer M, Gronowicz G, Kalinowski J, Pilbeam CC** 1998 Mice lacking the type I interleukin-1 receptor do not lose bone mass after ovariectomy. Endocrinology 139:3022–3025
369. **Kimble RB, Bain S, Pacifici R** 1997 The functional block of TNF but not of IL-6 prevents bone loss in ovariectomized mice. J Bone Miner Res 12:935–941
370. **Ammann P, Rizzoli R, Bonjour JP, Bourrin S, Meyer JM, Vassalli**

P, Garcia I 1997 Transgenic mice expressing soluble tumor necrosis factor-receptor are protected against bone loss caused by estrogen deficiency. J Clin Invest 99:1699–1703
371. **Bradbeer JN, Stroup SJ, Hoffman JC, Gowen M** 1996 An orally active inhibitor of cytokine synthesis prevents bone loss in the ovariectomized rat. J Bone Miner Res 11(Suppl 1):123
372. **Most W, Schot L, Ederveen A, van der Wee-Pals L, Papapoulos S, Löwik C** 1995 *In vitro* and *ex vivo* evidence that estrogens suppress increased bone resorption induced by ovariectomy or PTH stimulation through an effect on osteoclastogenesis. J Bone Miner Res 10:1523–1530
373. **Poli V, Balena R, Fattori E, Markatos A, Yamamoto M, Tanaka H, Ciliberto G, Rodan GA, Constantini F** 1994 Interleukin-6 deficient mice are protected from bone loss caused by estrogen depletion. EMBO J 13:1189–1196
374. **Lane N, Coble T, Kimmel DB** 1990 Effect of naproxen on cancellous bone in ovariectomized rats. J Bone Miner Res 5:1029–1035
375. **Dinarello CA** 1996 Biological basis for interleukin-1 in disease. Blood 87:2095–2147
376. **Kimble RB, Matayoshi AB, Vannice JL, Kung VT, Williams C, Pacifici R** 1995 Simultaneous block of interleukin 1 and tumor necrosis factor is required to completely prevent bone loss in the early post-ovariectomy period. Endocrinology 136:3054–3061
377. **Riggs BL** 2000 The mechanisms of estrogen regulation of bone resorption. J Clin Invest 106:1203–1204
378. **Campbell IK, O'Donnell K, Lawlor KE, Wicks IP** 2001 Severe inflammatory arthritis and lympadenopathy in the absence of TNF. J Clin Invest 107:1519–1527
379. **Miyaura C, Kusano K, Masuzawa T, Chaki O, Onoe Y, Aoyagi M, Sasaki T, Tamura T, Koishihara Y, Ohsugi Y, Suda T** 1995 Endogenous bone-resorbing factors in estrogen deficiency: cooperative effects of IL-1 and IL-6. J Bone Miner Res 10:1365–1373
380. **Sato T, Shibata T, Ikeda K, Watanabe K** 2001 Generation of bone-resorbing osteoclasts from B220+ cells: its role in accelerated osteoclastogenesis due to estrogen deficiency. J Bone Miner Res 16:2215–2221
381. **Oursler MJ, Pederson L, Pyfferoen J, Osdoby P, Fitzpatrick L, Spelsberg TC** 1993 Estrogen modulation of avian osteoclast lysosomal gene expression. Endocrinology 132:1373–1380
382. **Kameda T, Mano H, Yuasa T, Mori Y, Miyazawa K, Shiokawa M, Nakamaru Y, Hiroi E, Hiura K, Kameda A, Yang NN, Hakeda Y, Kumegawa M** 1997 Estrogen inhibits bone resorption by directly inducing apoptosis of the bone-resorbing osteoclasts. J Exp Med 186:6613–6617
383. **Spelsberg TC, Subramaniam M, Riggs BL, Khosla S** 1999 The actions and interactions of sex steroids and growth factors/cytokines on the skeleton. Mol Endocrinol 13:819–828
384. **Di Gregorio GB, Yamamoto M, Ali AA, Abe E, Roberon P, Manolagas SC, Jilka RL** 2001 Attenuation of the self-renewal of transit-amplifying osteoblast progenitors in the murine bone marrow by 17 β-estradiol. J Clin Invest 107:803–812
385. **Rosen CJ, Donahue LR, Hunter SJ** 1994 Insulin-like growth factors and bone: the osteoporosis connection. Proc Soc Exp Biol Med 206:83–102
386. **Ernst M, Heath JK, Rodan GA** 1989 Estradiol effects on proliferation, messenger ribonucleic acid for collagen and insulin-like growth factor-I, and parathyroid hormone-stimulated adenylate cyclase activity in osteoblastic cells from calvaria and long bones. Endocrinology 125:825–833
387. **Bankson DD, Rifai N, Williams ME, Silverman LM, Gray TK** 1989 Biochemical effects of 17β-estradiol on UMR106 cells. Bone Miner 6:55–63
388. **Schmid C, Ernst M, Zapf J, Froesch ER** 1989 Release of insulin-like growth factor carrier proteins by osteoblasts: stimulation by estradiol and growth hormone. Biochem Biophys Res Commun 160: 788–794
389. **Slater M, Patava J, Kingham K, Bason RS** 1994 Modulation of growth factor incorporation into ECM of human osteoblast-like cells *in vitro* by 17 β estradiol. Am J Physiol 267:E990–E1001
390. **Gray TK, Mohan S, Linkhart TA, Baylink DJ** 1989 Estradiol stimulates *in vitro* the secretion of insulin-like groth factors by the clonal osteoblastic cell line, UMR106. Biochem Biophys Res Commun 158:407–412
391. **Kassem M, Okazaki R, Harris SA, Spelsberg TC, Conover CA, Riggs BL** 1998 Estrogen effects on insulin-like growth factor gene expression in a human osteoblastic cell line with high levels of estrogen receptor. Calcif Tissue Int 62:60–66
392. **Erdmann J, Storch S, Pfeilschifter J, Ochlich P, Ziegler R, Bauss F** 1998 Effects of estrogen on the concentration of insulin-like growth factor-I in rat bone matrix. Bone 22:503–507
393. **Aerssens J, van Audekercke R, Geusens P, Schot LP, Osman AA, Dequeker J** 1993 Mechanical properties, bone mineral content, and bone composition (collagen, osteocalcin, IGF-I) of the rat femur: influence of ovariectomy and nandrolone decanoate (anabolic steroid) treatment. Calcif Tissue Int 53:269–277
394. **Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R, Amgen EST Program, Boyle WJ** 1997 Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 89:309–319
395. **Bekker PJ, Holloway D, Nakanishi A, Arrighi M, Leese PT, Dunstan CR** 2001 The effect of a single dose of osteoprotegerin in postmenopausal women. J Bone Miner Res 16:348–360
396. **Kong YY, Feige U, Sarosi I, Bolon B, Tafuri A, Morony S, Capparelli C, Li J, Elliott R, McCabe S, Wong T, Campagnuolo G, Moran E, Bogoch ER, Van G, Nguyen LT, Ohashi PS, Lacey DL, Fish E, Boyle WJ, Penninger JM** 1999 Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature 402:304–309
397. **Cohen-Solal ME, Graulet AM, Denne MA, Gueris J, Baylink D, De Vernejoul MC** 1993 Peripheral monocyte culture supernatants of menopausal women can induce bone resorption: involvement of cytokines. J Clin Endocrinol Metab 77:1648–1653
398. **Yano K, Tsuda E, Washida N, Kobayashi F, Goto M, Harada A, Ikeda K, Higashio K, Yamada Y** 1999 Immunological characterization of circulating osteoprotegerin/osteoclastogenesis inhibitory factor: increased serum concentrations in postmenopausal women with osteoporosis. J Bone Miner Res 14:518–527
399. **Scheidt-Nave CE, Bismar H, Leidig-Bruckner G, Woitge H, Seibel MJ, Ziegler R, Pfeilschifter J** 2001 Serum interleukin 6 is a major predictor of bone loss in women specific to the first decade past menopause. J Clin Endocrinol Metab 86:2032–2042
400. **Stampfer MJ, Colditz GA, Willett WC, Manson JE, Rosner B, Speizer FE, Hennekens CH** 1991 Postmenopausal estrogen therapy and cardiovascular disease. Ten-year follow-up from the nurses' health study. N Engl J Med 325:756–662
401. **Ross R, Glomset JA** 1976 The pathogenesis of atherosclerosis (second of two parts). N Engl J Med 295:420–425
402. **Ross R** 1999 Atherosclerosis–an inflammatory disorder. N Engl J Med 340:115–126
403. **Krishnaswamy G, Kelley J, Yerra L, Smith JK, Chi DS** 1999 Human endothelium as a source of multifunctional cytokines: molecular regulation and possible role in human disease. J Interferon Cytokine Res 19:91–104
404. **Ludewig B, Freigang S, Jaggi M, Kurrer MO, Pei YC, Vlk L, Odermatt B, Zinkernagel RM, Hengartner H** 2000 Linking immune-mediated arterial inflammation and cholesterol-induced atherosclerosis in a transgenic mouse model. Proc Natl Acad Sci USA 97:12752–12757
405. **Whicher J, Biasucci L, Rifai N** 1999 Inflammation, the acute phase response and atherosclerosis. Clin Chem Lab Med 37:495–503
406. **Harris TB, Ferrucci L, Tracy RP, Corti MC, Wacholder S, Ettinger Jr WH, Heimovitz H, Cohen HJ, Wallace R** 1999 Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. Am J Med 106:506–512
407. **Ridker PM, Hennekens CH, Buring JE, Rifai N** 2000 C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 342:836–843
408. **Gabay C, Kushner I** 1999 Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 340:448–454
409. **Ridker PM** 2001 High-sensitivity C-reactive protein. Potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. Circulation 103:1813–1818

410. **Sironi M, Breviario F, Proserpio P, Biondi A, Vecchi A, Van Damme J, Dejana E, Mantovani A** 1989 IL-1 stimulates IL-6 production in endothelial cells. J Immunol 142:549–553
411. **Ikeda U, Ikeda M, Seino Y, Takahashi M, Kano S, Shimada K** 1992 Interleukin 6 transcripts are expressed in atherosclerotic lesions of genetically hyperlipidemic rabbits. Atherosclerosis 92:213–218
412. **Belmin J, Bernard C, Corman B, Meval R, Esposito B, Tedgui A** 1995 Increased production of tumor necrosis factor and interleukin-6 by arterial wall of aged rats. Am J Physiol 268:H2288–H2293
413. **Seli E, Selam B, Mor G, Kayisli UA, Pehlivan T, Arici A** 2001 Estradiol regulates monocyte chemotactic protein-1 in human coronary artery smooth muscle cells: a mechanism for its antiatherogenic effect. Menopause 8:296–301
414. **Raines EW, Dower SK, Ross R** 1989 Interleukin-1 mitogenic activity for fibroblasts and smooth-muscle cells is due to PDGF-AA. Science 243:393–396
415. **Seino Y, Ikeda U, Ikeda M, Yamamoto K, Misawa Y, Hasegawa T, Kano S, Shimada K** 1994 Interleukin 6 gene transcripts are expressed in human atherosclerotic lesions. Cytokine 6:87–91
416. **Elhage R, Maret A, Pieraggi MT, thieres JC, Arnal JF, Bayard F** 1998 Differential effects of interleukin-1 receptor antagonist and tumor necrosis factor binding protein on fatty-streak formation in apolipoprotein E-deficient mice. Circulation 97:242–244
417. **Nicklin MJ, Hughes DE, Barton JL, Ure JM, Duff GW** 191 2000 Arterial inflammation in mice lacking the interleukin 1 receptor antagonist gene. J Exp Med 303–311
418. **Huber SA, Sakkinen P, Conze D, Hardin N, Tracy R** 1999 Interleukin-6 exacerbates early atherosclerosis in mice. Arterioscler Thromb Vasc Biol 19:2364–2367
419. **Prud'homme GJ, Piccirillo CA** 2000 The inhibitory effects of transforming growth factor-β-1 (TGF-β1) in autoimmune diseases. J Autoimmun 14:23–42
420. **Grainger DJ, Kemp PR, Liu AC, Lawn RM, Metcalfe JC** 1994 Activation of transforming growth factor-β is inhibited in transgenic apolipoprotein(a) mice. Nature 362:460–462
421. **Grainger DJ, Kemp PR, Metcalfe JC, Liu AC, Lawn RM, Williams NR, Grace AA, Schofield PM, Chauhan A** 1995 The serum concentration of active transforming growth factor-β is severely depressed in advanced atherosclerosis. Nat Med 1:74–79
422. **Borkowski P, Robinson MJ, Kusiak JW, Borkowski A, Brathwaite C, Mergner WJ** 1995 Studies on TGF-β 1 gene expression in the intima of the human aorta in regions with high and low probability of developing atherosclerotic lesions. Mod Pathol 8:478–482
423. **Elhage R, Clamens S, Besnard S, Mallat Z, Tedgui A, Arnal J, Maret A, Bayard F** 2001 Involvement of interleukin-6 in atherosclerosis but not in the prevention of fatty streak formation by 17β-estradiol in apolipoprotein E-deficient mice. Atherosclerosis 156:315–320
424. **Song L, Leung C, Schindler C** 2001 Lymphocytes are important in early atherosclerosis. J Clin Invest 108:251–259
425. **Elhage R, Clamens S, Reardon-Alulis C, Getz GS, Fievet C, Maret A, Arnal JF, Bayard F** 2000 Loss of atheroprotective effect of estradiol in immunodeficient mice. Endocrinology 141:462–465
426. **Chiu KM, Arnaud CD, Ju J, Mayes D, Bacchetti P, Weitz S, Keller ET** 2000 Correlation of estradiol, parathyroid hormone, interleukin-6, and soluble interleukin-6 receptor during the normal menstrual cycle. Bone 26:79–85
427. **Stadtman ER, Berlett BS** 1998 Reactive oxygen-mediated protein oxidation in aging and disease. Drug Metab Rev 30:225–243
428. **Schmidt AM, Yan SD, Wautier J-L, Stern D** 1999 Activation of receptor for advanced glycation end products. A mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. Circulation 84:489–497
429. **Yan SF, Tritto I, Pinsky D, Liao H, Huang J, Fuller G, Brett J, May L, Stern D** 1995 Induction of interleukin-6 (IL-6) by hypoxia in vascular cells. Central role of the binding site for nuclear factor IL-6. J Biol Chem 270:11463–11471
430. **Wong Y-K, Gallagher PJ, Ward ME** 1999 Chlamydia pneumoniae and atherosclerosis. Heart 81:232–238
431. **Schussheim AE, Fuster V** 1999 Antibiotics for myocardial infarction? A possible role of infection in atherogenesis and acute coronary syndromes. Drugs 75:283–291
432. **Masiukiewicz US, Mitnick M, Grey AB, Insogna KL** 2000 Estrogen modulates parathyroid hormone-induced interleukin-6 production *in vivo* and *in vitro*. Endocrinology 141:2526–2531
433. **Jessop HL, Sjoberg M, Cheng MZ, Zaman G, Wheeler-Jones CP, Lanyon LE** 2001 Mechanical strain and estrogen activate estrogen receptor α in bone cells. J Bone Miner Res 16:1045–1055
434. **Tsukamoto K, Yoshida H, Watanabe S, Suzuki T, Miyao M, Hosoi T, Orimo H, Emi M** 1999 Association of radial bone mineral density with CA repeat polymorphism at the interleukin 6 locus in postmenopausal Japanese women. J Hum Genet 44:148–151
435. **Keen RW, Woodford-Richens KL, Lanchbury JS, Spector TD** 1998 Allelic variation at the interleukin-1 receptor antagonist gene is associated with early postmenopausal bone loss at the spine. Bone 23:367–371
436. **Langdahl BL, Lokke E, Carstens M, Stenkjaer LL, Eriksen EF** 2000 Osteoporotic fractures are associated with an 86-base pair repeat polymorphism in the interleukin-1-receptor antagonist gene but not with polymorphisms in the interleukin-1β gene. J Bone Miner Res 15:402–414
437. **Langdahl BL, Abrahamsen B, Hermann AP, Brot C, Eiken P, Soerensen OH, Nielsen SP, Beck-Nielsen H, Charles P, Mosekilde L** 1999 An 86 basepair repeat in the interleukin 1 receptor antagonist gene is weakly associated with peak bone mass, but not with early postmenopausal bone loss or the response to HRT. J Bone Miner Res 14(Suppl 1):S334
438. **Ferrari SL, Garnero P, Emond S, Montgomery H, Humphries SE, Greenspan SL** 2001 A functional polymorphic variant in the interleukin-6 gene promoter associated with low bone resorption in postmenopausal women. Arthritis Rheum 44:196–201
439. **Jones KG, Brull DJ, Brown LC, Sian M, Greenhalgh RM, Humphries SE, Powell JT** 2001 Interleukin-6 (IL-6) and the prognosis of abdominal aortic aneurysms. Circulation 103:2260–2265
440. **Langdahl BL, Knudsen JY, Jensen HK, Gregersen N, Eriksen EF** 1997 A sequence variation: 713–8delC in the transforming growth factor-β1 gene has higher prevalence in osteoporotic women than in normal women and is associated with very low bone mass in osteoporotic women and increased bone turnover in both osteoporotic and normal women. Bone 20:289–294
441. **Bertoldo F, D'Agruma L, Furlan F, Colapietro F, Lorenzi MT, Maiorano N, Iolascon A, Zelante L, Locascio V, Gasparinin P** 2000 Transforming growth factor-β1 gene polymorphism, bone turnover, and bone mass in Italian postmenopausal women. J Bone Miner Res 15:634–639
442. **Yamada Y, Miyauchi A, Goto J, Takagi Y, Okuizumi H, Kanematsu M, Hase M, Takai H, Harada A, Ikeda K** 1998 Association of a polymorphism of the transforming growth factor β-1 gene with genetic susceptibility to osteoporosis in postmenopausal Japanese women. J Bone Miner Res 13:1569–1576
443. **Yamada Y, Harada A, Hosoi T, Miyauchi A, Ikeda K, Ohta H, Shiraki M** 2000 Association of transforming growth factor β-1 genotype with therapeutic response to active vitamin D for postmenopausal osteoporosis. J Bone Miner Res 15:415–420
444. **Yamada Y, Miyauchi A, Takagi Y, Nakauchi K, Miki N, Mizuno M, Harada A** 2000 Association of a polymorphism of the transforming growth factor β-1 gene with prevalent vertebral fractures in Japanese women. Am J Med 109:244–247
445. **Hinke V, Seck T, Clanget C, Scheidt-Nave C, Ziegler R, Pfeilschifter J** Association of transforming growth factor-β1 (TGFβ1) $T^{29}\rightarrow C$ gene polymorphism with bone mineral density (BMD), changes in BMD, and serum concentrations of TGF-β1 in a population-based sample of postmenopausal German women. Calcif Tissue Int, in press
446. **Kronenberg F** 1990 Hot flashes: epidemiology and physiology. In: Flint M, Kronenberg F, Utian W, eds. Annals of the New York Academy of Sciences. New York: The New York Academy of Sciences; vol 592:52–86
447. **Pacifici R, Vannice JL, Rifas L, Kimble RB** 1993 Monocytic secretion of interleukin-1 receptor antagonist in normal and osteoporotic women: effects of menopause and estrogen/progesterone therapy. J Clin Endocrinol Metab 77:1135–1141
448. **Fagiolo U, Cossarizza A, Scala E** 1993 Increased cytokine production in mononuclear cells of healthy elderly people. Eur J Immunol 23:2375–2378
449. **Roubenoff R, Harris TB, Abad LW, Wilson PWF, Dallal GE,**

Dinarello CA 1998 Monocyte cytokine production in an elderly population: effect of age and inflammation. J Gerontol 53A: M20–M26
450. **Miller RA** 1996 The aging immune system: primer and prospectus. Science 273:70–74
451. **Bruunsgaard H, Pedersen AN, Schroll M, Skinhoj P, Pedersen BK** 1999 Impaired production of proinflammatory cytokines in response to lipopolysaccharide (LPS) stimulation in elderly humans. Clin Exp Immunol 118:235–241
452. **Godsland IF** 1996 The influence of female sex steroids on glucose metabolism and insulin action. J Intern Med Suppl 738:1–60
453. **Kuhl H** 1996 Comparative pharmacology of newer progestogens. Drugs 51:188–215
454. **Miller L, Hunt JS** 1996 Sex steroid hormones and macrophage function. Life Sci 59:1–14
455. **Kelly RW, King AE, Critchley HO** 2001 Cytokine control in human endometrium. Reproduction 121:3–19
456. **Prior JC** 1990 Progesterone as a bone-trophic hormone. Endocr Rev 11:386–398
457. **Burger HG, Dudley EC, Cui J, Dennerstein L, Hopper JL** 2000 A prospective longitudinal study of serum testosterone, dehydroepiandrosterone sulfate, and sex hormone-binding globulin levels through the menopause transition. J Clin Endocrinol Metab 85: 2832–2838
458. **Snyder PJ** 2001 The role of androgens in women. J Clin Endocrinol Metab 86:1006–1007
459. **Liu Y, Ding J, Bush TL, Longenecker JC, Nieto FJ, Golden SH, Szklo M** 2001 Relative androgen excess and increased cardiovascular risk after menopause: a hypothesized relation. Am J Epidemiol 154:489–494
460. **Bulun SE, Zeitoun K, Sasano H, Simpson ER** 1999 Aromatase in aging women. Semin Reprod Endocrinol 17:349–358
461. **Harper AJ, Buster JE, Casson PR** 1999 Changes in adrenocortical function with aging and therapeutic implications. Semin Reprod Endocrinol 17:327–338
462. **Longcope C, Franz C, Morello C, Baker R, Johnston Jr CC** 1986 Steroid and gonadotropin levels in women during the peri-menopausal years. Maturitas 8:189–196
463. **Zhang J, Pugh TD, Stebler B, Ershler WB, Keller ET** 1998 Orchidectomy increases bone marrow interleukin-6 levels in mice. Calcif Tissue Int 62:219–226
464. **Hofbauer LC, Khosla S** 1999 Androgen effects on bone metabolism: recent progress and controversies. Eur J Endocrinol 140:271–286
465. **Gornstein RA, Lapp CA, Bustos-Valdes SM, Zamorano P** 1999 Androgens modulate interleukin-6 production by gingival fibroblasts *in vitro*. J Periodontol 70:604–609
466. **Keller ET, Chang C, Ershler WB** 1996 Inhibition of NFκB activity through maintenance of IκBα levels contributes to dihydrotestosterone-mediated repression of the interleukin-6 promoter. J Biol Chem 271:26267–26275
467. **Kanda N, Tsuchida T, Tamaki K** 1996 Testosterone inhibits immunoglobulin production by human peripheal blood mononuclear cells. Clin Exp Immunol 106:410–415
468. **Bellido T, Jilka RL, Boyce BF, Girasole G, Broxmeyer H, Dalrymple SA, Murray R, Manolagas SC** 1995 Regulation of interleukin-6, osteoclastogenesis, and bone mass by androgens: the role of the androgen receptor. J Clin Invest 95:2886–2895
469. **James K, Premchand N, Skibinska A, Skibinski G, Nicol M, Mason JI** 1997 IL-6, DHEA and the ageing process. Mech Ageing Dev 93:15–24
470. **Straub RH, Konecna L, Hrach S, Rothe G, Kreutz M, Scholmerich J, Falk W, Lang B** 1998 Serum dehydroepiandrosterone (DHEA) and DHEA sulfate are negatively correlated with serum interleukin-6 (IL-6), and DHEA inhibits IL-6 secretion from mononuclear cells in man *in vitro*: possible link between endocrinosenescence and immunosenescence. J Clin Endocrinol Metab 83:2012–2017
471. **Casson PR, Andersen RN, Herrod HG, Stentz FB, Straughn AB, Abraham GE, Buster JE** 1993 Oral dehyroepiandrosterone in physiologic doses modulates immune function in postmenopausal women. Am J Obestet Gynecol 169:1536–1539
472. **Kim HR, Ryu SY, Kim HS, Choi BM, Lee EJ, Kim HM, Chung HT** 1995 Administration of dehydroepiandrosterone reverses the immune suppression induced by high dose antigen in mice. Immunol Invest 24:583–593
473. **Kipper-Galperin M, Galilly R, Danenberg HD, Brenner T** 1999 Dehydroepiandrosterone selectively inhibits production of tumor necrosis factor α and interleukin-6 in astrocytes. Int J Dev Neurosci 17:765–775
474. **Padgett DA, Loria RM** 1998 Endocrine regulation of murine macrophage function: effects of dehydroepiandrosterone, androstenediol, and androstenetriol. J Neuroimmunol 84:61–68
475. **Kimura M, Tanaka S, Yamada Y, Kiuchi Y, Yamakawa T, Sekihara H** 1998 Dehydroepiandrosterone decreases serum tumor necrosis factor-α and restores insulin sensitivity: independent effect from secondary weight reduction in genetically obese Zucker fatty rats. Endocrinology 139:3249–3253
476. **Daynes RA, Araneo BA, Ershler WB, Maloney C, Li GZ, Ryu SY** 1993 Altered regulation of IL-6 production with normal aging. Possible linkage to the age-associated decline in dehydroepiandrosterone and its sulfated derivative. J Immunol 150:5219–5230
477. **Guerne PA, Carson DA, Lotz M** 1990 IL-6 production by human articular chondrocytes. Modulation of its synthesis by cytokines, growth factors, and hormones *in vitro*. J Immunol 144:499–505
478. **Pivirotto LA, Cissel DS, Keeting PE** 1995 Sex hormones mediate interleukin-1 β production by human osteoblastic HOBIT cells. Mol Cell Endocrinol 111:67–74
479. **Wilson CA, Mrose SA, Thomas DW** 1995 Enhanced production of B lymphocytes after castration. Blood 85:1535–1539
480. **Pilbeam CC, Raisz LG** 1990 Effects of androgens on parathyroid hormone and interleukin-1-stimulated prostaglandin production in cultured neonatal mouse calvariae. J Bone Miner Res 5:1183–1188
481. **Bodine PV, Riggs BL, Spelsberg TC** 1995 Regulation of c-fos expression and TGF-β production by gonadal and adrenal androgens in normal human osteoblastic cells. J Steroid Biochem Mol Biol 52:149–158
482. **Pederson L, Kremer M, Judd J, Pascoe D, Spelsberg TC, Riggs BL, Oursler MJ** 1999 Androgens regulate bone resorption activity of isolated osteoclasts *in vitro*. Proc Natl Acad Sci USA 96:505–510
483. **Bilezikian JP, Morishima A, Bell J, Grumbach MM** 1998 Increased bone mass as a result of estrogen therapy in a man with aromatase deficiency. N Engl J Med 339:599–603
484. **Falahati-Nini A, Riggs BL, Atkinson EJ, O'Fallon WM, Eastell R, Khosla S** 2000 Relative contribution of testosterone and estrogen in regulating bone resorption and formation in normal elderly men. J Clin Invest 106:1553–1560
485. **Hofbauer LC, Ten RM, Khosla S** 1999 The anti-androgen hydroxyflutamide and androgens inhibit interleukin-6 production by an androgen-responsive human osteoblastic cell line. J Bone Miner Res 14:1330–1337
486. **da Silva JA, Colville-Nash P, Spector TD, Scott DL, Willoughby DA** 1993 Inflammation-induced cartilage degradation in female rodents. Protective role of sex hormones. Arthritis Rheum 36:1007–1013
487. **Diodato MD, Knoferl MW, Schwacha MG, Bland KI, Chaudry IH** 2001 Gender differences in the inflammatory response and survival following haemorrhage and subsequent sepsis. Cytokine 14:162–169
488. **Baulieu EE, Thomas G, Legrain S, Lahlou N, Roger M, Debuire B, Faucounau V, Girard L, Hervy MP, Latour F, Leaud MC, Mokrane A, Pitti-Ferrandi H, Trivalle C, de Lacharriere O, Nouveau S, Rakoto-Arison B, Souberbielle JC, Raison J, Le Bouc Y, Raynaud A, Girerd X, Forette F** 2000 Dehydroepidandrosterone (DHEA), DHEA sulfate, and aging: contribution of the DHEAge study to a sociobiomedical issue. Proc Natl Acad Sci USA 97:4279–4284
489. **Garnero P, Sornay-Rendu E, Claustrat B, Delmas PD** 2000 Biochemical markers of bone turnover, endogenous hormones and the risk of fractures in postmenopausal women: the OFELY study. J Bone Miner Res 15:1526–1536
490. **Davis SR, Burger HG** 1996 Clinical review 82: androgens and the postmenopausal women. J Clin Endocrinol Metab 81:2759–2763
491. **Bodine PVN, Harris HA, Komm BS** 1999 Suppression of ligand-dependent estrogen receptor activity by bone-resorbing cytokines in human osteoblasts. Endocrinology 140:2439–2451
492. **Kluft C, de Maat MPM, Gevers Leuven JA, Potter van Loon BJ,**

Mohrschladt MF 1999 Statins and C-reactive protein. Lancet 353: 1274
493. **Ridker PM, Rifai N, Pfeffer MA, Sacks F, Braunwald E, for the Cholesterol and Recurrent Events (CARE) Investigators** 1999 Long-term effects of pravastatin on plasma concentration of C-reactive protein. Circulation 100:230–235
494. **LaRosa JC, He J, Vupputuir S** 1999 Effect of statins on risk of coronary disease. JAMA 282:2340–2346
495. **Ando H, Takamura T, Ota T, Nagai Y, Kobayashi K** 2000 Cerivastatin improves survival of mice with lipopolysaccharide-induced sepsis. J Pharmacol Exp Ther 294:1043–1046
496. **Solheim S, Seljeflot I, Arnesen H, Eritsland J, Eikvar L** 2001 Reduced levels of TNFαlpha in hypercholesterolemic individuals after treatment with pravastatin for 8 weeks. Atherosclerosis 157: 411–415
497. **Xu X, Otsuki M, Saito H, Sumitani S, Yamamoto H, Asanuma N, Kouhara H, Kasayama S** 2001 PPARα and GR differentially downregulate the expression of nuclear factor-κB-responsive genes in vascular endothelial cells. Endocrinology 142:3332–3339
498. **Madej A, Okopien B, Kowalski J, Zielinski M, Wysocki J, Szygula B, Kalina Z, Herman ZS** 1998 Effects of fenofibrate on plasma cytokine concentrations in patients with atherosclerosis and hyperlipoproteinemia IIb. Int J Clin Pharmacol Ther 36:345–349
499. **Nolan JJ, Ludvik B, Beerdsen P, Joyce M, Olefsky J** 1994 Improvement of glucose tolerance and insulin resistance in obese subjects treated with troglitazone. N Engl J Med 331:1188–1193
500. **Jiang C, Ting AT, Seed B** 1998 PPAR-γ agonists inhibit production of monocyte inflammatory cytokines. Nature 391:82–86
501. **Ghanim H, Garg R, Aljada A, Mohanty P, Kumbkarni Y, Assian E, Hamouda W, Dandona P** 2001 Suppression of nuclear factor-κB and stimulation of inhibitor κB by troglitazone: evidence for an anti-inflammatory effect and a potential antiatherosclerotic effect in the obese. J Clin Endocrinol Metab 86:1306–1312
502. **Desvergne B, Wahli W** 1999 Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev 20:649–688
503. **Aljada A, Garg R, Ghanim H, Mohanty P, Hamouda W, Assian E, Dandona P** 2001 Nuclear factor-κB suppressive and inhibitor-κB stimulatory effects of troglitazone in obese patients with type 2 diabetes: evidence of an antiinflammatory action? J Clin Endocrinol Metab 86:3250–3256
504. **Tanaka T, Itoh H, Doi K, Fukunaga Y, Hosoda K, Shintani M, Yamashita J, Chun TH, Inoue M, Masatsugu K, Sawada N, Saito T, Inoue G, Nishimura H, Yoshimasa Y, Nakao K** 1999 Down-regulation of peroxisome proliferator-activated receptorγ expression by inflammatory cytokines and its reversal by thiazolidinediones. Diabetologia 42:702–710
505. **Okazaki R, Toriumi M, Fukumoto S, Miyamoto M, Fujita T, Tanaka K, Takeuchi Y** 1999 Thiazolidinediones inhibit osteoclast-like cell formation and bone resorption *in vitro*. Endocrinology 140:5060–5065
506. **Mbalaviele G, Meng A, Jaiswal R, Jaiswal N, Beck S, Pittenger M, Thiede M** 1999 Blockade of cytokine-mediated human osteoclastogenesis by activation of peroxisome proliferator-activated receptor-γ pathway. J Bone Miner Res 14(Suppl 1):S358
507. **Li AC, Brown KK, Silvestre MJ, Willson TM, Palinski W, Glass CK** 2000 Peroxisome proliferator-activated receptor gamma ligands inhibit development of atherosclerosis in LDL receptor-deficient mice. J Clin Invest 106:523–531