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The Complex Role of Estrogens in Inflammation

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There is still an unresolved paradox with respect to the immunomodulating role of estrogens. On one side, we recognize inhibition of bone resorption and suppression of inflammation in several animal models of chronic inflammatory diseases. On the other hand, we realize the immunosupportive role of estrogens in trauma/sepsis and the proinflammatory effects in some chronic autoimmune diseases in humans. This review examines possible causes for this paradox.

This review delineates how the effects of estrogens are dependent on criteria such as: 1) the immune stimulus (foreign antigens or autoantigens) and subsequent antigen-specific immune responses (*e.g.*, T cell inhibited by estrogens *vs.* activation of B cell); 2) the cell types involved during different phases of the disease; 3) the target organ with its specific microenvironment; 4) timing of 17β-estradiol administration in relation to the disease course (and the reproductive status of a woman); 5) the concentration of estrogens; 6) the variability in expression of estrogen receptor α and β depending on the microenvironment and the cell type; and 7) intracellular metabolism of estrogens leading to important biologically active metabolites with quite different anti- and proinflammatory function. Also mentioned are systemic supersystems such as the hypothalamic-pituitary-adrenal axis, the sensory nervous system, and the sympathetic nervous system and how they are influenced by estrogens.

This review reinforces the concept that estrogens have antiinflammatory but also proinflammatory roles depending on above-mentioned criteria. It also explains that a uniform concept as to the action of estrogens cannot be found for all inflammatory diseases due to the enormous variable responses of immune and repair systems. (*Endocrine Reviews* 28: 521–574, 2007)

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Abbreviations: AP-1, Activator protein-1; CEE, conjugated equine estrogens; CI, confidence interval; E1, estrone; E2, 17β-estradiol; E3, estriol; EAE, experimental autoimmune encephalitis; ER, estrogen receptor; ERT, estrogen replacement therapy; FasL, Fas ligand; FoxP3, forkhead box P3; HPA, hypothalamic-pituitary-adrenal; HRT, hormone replacement therapy; HUVEC, human umbilical vein endothelial cell; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; IκB, inhibitor of NF-κB; IL-1ra, IL-1 receptor antagonist; iNOS, inducible NOS; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein; MMP, matrix metalloproteinase; MS, multiple sclerosis; NF-κB, nuclear factor κB; NO, nitric oxide; NOS, NO synthase; OC, oral contraceptives; OR, odds ratio; PBMCs, peripheral blood mononuclear cells; RA, rheumatoid arthritis; RANK, receptor activator of NF-κB; RANKL, RANK ligand; RANTES, regulated on activation normally T cell expressed and secreted; ROS, reactive oxygen species; RR, relative risk; SLE, systemic lupus erythematosus; SNS, sympathetic nervous system; Th1, T helper type 1; Th2, T helper type 2; Th17, T helper type 17; TIMP, tissue inhibitor of MMPs; VCAM-1, vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factor.

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I. Introduction

IT IS WIDELY ACCEPTED that females of all ages experience significantly lower rates of infection and resultant mortality than men. This significant difference in the inflammatory response of women compared with that of men has long been noted (1, 2). This heightened inflammatory response is advantageous in response to infection and sepsis but is unfavorable in immune responses against self, leading to an overall increased rate of autoimmune diseases in women compared to men (2, 3). Epidemiological and immunological evidence has suggested that female sex hormones play a role in the etiology and course of chronic inflammatory diseases because the menstrual cycle, pregnancy, and menopausal status are important influencing factors (2, 4, 5).

In a previous review, the role of the menopause on proinflammatory cytokine activity has been extensively studied (6). This review focused on the increase of proinflammatory cytokines with the menopause (the fall of estrogens and other gonadal steroids), but this review only marginally touched chronic inflammatory diseases. Another review on gonadal steroids and T and B cell immunity was presented 10 yr ago, but since then, a lot of new information has been generated (2). This is particularly true with respect to chronic diseases that formerly have not been allocated to "inflammatory diseases" such as bone resorption. This is important because incidence rates over age for osteoporosis match incidence rates over age for, *e.g.*, rheumatoid arthritis (RA). Furthermore, the progress in animal models of chronic inflammation was immense, and a good portion of this information has been published after 1996.

There is still the unresolved paradox with respect to the immunomodulating role of estrogens. On one side, we recognize inhibition of bone resorption and suppression of inflammation in several animal models of chronic inflammatory diseases. On the other side, we realize the immune supportive role in trauma/sepsis and the proinflammatory effects in some chronic autoimmune diseases in humans (as an initiating or perpetuating factor). This review examines possible causes for this paradox and suggests a solution in the *Discussion* (*Section XV*).

Because the author has been confronted by an enormous quantity of literature (approximately 5200 references in the primary retrieval), this review only focuses on estrogens. It is evident that androgens and progesterone are important in inflammation, but presentation of these subjects was not possible due to space constraints. Throughout the text, tables, and figures, the Système International unit for the concentration is given as moles/liter (abbreviated with M).

II. Estrogen Receptors in Inflammation and Hypoxia

The presence of estrogen receptors (ERs), ERα and ERβ, is of outstanding importance because a preponderance of one ER subtype over the other might change estrogen effects (7, 8). For instance, in synovial tissue of patients with RA, macrophage-like and fibroblast-like synoviocytes were positive for ERα (9–11) and ERβ (12). Importantly, one study demonstrated higher density of ERβ+ cells than of ERα+ cells (13). Others demonstrated a higher density of ERβ+ cells in relation to ERα+ cells in RA synovial tissue compared with controls (12). ERβ preponderance was observed in all three synovial compartments investigated: in the lining cell layer, in fibroblasts, and in inflammatory cells (12). Similarly, the amount of ERα was lower in T cells of patients with systemic lupus erythematosus (SLE) than controls, but the quantity of ERβ was similar, which indicates a relative increase of ERβ in relation to ERα in SLE patients (14). In animals subjected to trauma and hemorrhage, a general inflammatory condition, ERβ mRNA expression was increased, whereas ERα expression was decreased (15). These studies suggest inflammation-dependent up-regulation of ERβ relative to ERα.

It was further demonstrated that hypoxia, which typically accompanies inflammatory conditions, reduced expression of ERα (16, 17), and oxidative stress increased the expression of ERβ (18). In endothelial cells (E304), oxidative stress increased ERβ relative to ERα. In activated macrophages, lipopolysaccharide (LPS) plus interferon (IFN)-γ in the presence of hypoxia increased expression of ERβ but not of ERα (18). These studies support a concept of up-regulation of ERβ relative to ERα under hypoxic conditions, which might lead to a preponderance of signaling through ERβ pathways.

The preponderance of ERβ relative to ERα under inflammatory and hypoxic conditions might influence estrogen effects. One might hypothesize that this depends on the time point of estrogen application in relation to the state of an inflammatory disease. A situation before disease outbreak or at the beginning of a disease with slight tissue inflammation and little hypoxia might be governed by a balance of ERα and ERβ, whereas in the chronic phase of a disease with much inflammation and a higher degree of hypoxia, ERβ is increased relative to ERα. In such a situation, ERβ-mediated cross-modulation of ERα, *e.g.*, demonstrated as ERβ-mediated inhibition of ERα-stimulated IL-1 secretion (8), might play an important role in the chronic disease course.

III. Estrogen Modulation of Specific Target Cells

This section delineates effects of estrogens on cells relevant to inflammation. In most situations, this reflects investigation

of estrogens *in vitro* (in culture medium without phenol red; see Ref. 19). In addition, animal experiments are mentioned, in which 17β-estradiol (E2) was applied at different doses leading to various E2 serum levels reflecting metestrus (postmenopausal), diestrus (early follicular), proestrus (periovulatory), and pregnancy levels. Throughout this review, the expected or measured serum levels of estrogens are given in the subsequent text.

A. Estrogens and human peripheral blood mononuclear cells or whole cell cultures

In human peripheral blood mononuclear cells (PBMCs) in the presence of LPS (at 1 ng/ml ≈ EC_{50} of LPS), E2 inhibited TNF at concentrations of 10^{-10} to 10^{-7} M in male subjects and at 10^{-8} to 10^{-7} M in female subjects, but E2 had a stimulating effect in the absence of LPS (20). In whole human blood cultures, E2 at 10^{-10} to 10^{-8} M decreased spontaneous secretion of IL-6, TNF, IL-1 receptor antagonist (IL-1ra), IL-1β, and the ratio of IL-1β/IL-1ra compared with control, but E2 did not strongly change LPS-stimulated cytokine release (LPS at 500 ng/ml, which is largely higher than the EC_{50} of LPS) (21). In PBMCs, E2 inhibited TNF release in postmenopausal women with fractures in a dose-dependent manner between 10^{-12} and 10^{-6} M but had no consistent effect on PBMCs derived from men or premenopausal women (22).

From these data, it seems that E2 at periovulatory to pregnancy levels inhibited proinflammatory cytokines from PBMCs, which is not unchallenged (23). The contrasting results might be due to divergent effects of E2 on different subtypes of immune cells. Necessarily, it is important to examine the effects of E2 on immune cell subtypes, which is demonstrated in the subsequent sections.

B. Estrogens, the B cell, and antibodies

Mitogen stimulation of T and B lymphocytes resulted in accumulation of IgM-containing and IgM-secreting cells, which was further enhanced by E2 (late follicular to pregnancy levels). This E2 effect was mediated by inhibiting T cell-mediated suppression of B cells (24). A similar phenomenon was observed with human PBMCs stimulated with sheep red blood cells, whereby addition of 10^{-9} to 10^{-7} M E2 (maximum at 2×10^{-9} M) augments the antigen-specific immune response by inhibiting CD8+ T cell-mediated suppression of B cells (blocked by tamoxifen, no effect at 5×10^{-10} M) (25). E2 increased antibody-forming cells against sheep erythrocytes (maximum at 1.8×10^{-9} M, no effect at 3.7×10^{-11} M) (26). In PBMCs from normal donors, E2 at pregnancy levels enhanced mitogen-induced generation of antibody-producing cells (27). In human PBMCs, E2 at 10^{-10} to 10^{-8} M (IC_{50}, 10^{-9} M) enhanced IgG and IgM production without altering cell viability and proliferation, and anti-IL-10 antibody partially blocked the E2 effect (28). A similarly enhanced production of anti-double-stranded DNA antibodies was observed with E2 at 10^{-10} to 10^{-8} M (IC_{50}, 10^{-9} M), and E2 increased the B cell-stimulating cytokine IL-10 from monocytes (29). In PBMCs of female rhesus macaques, E2 at 3.7×10^{-9} M increased the frequency of Ig-secreting cells (30).

In women on oral contraceptives (OC), serum levels of IgA and IgG were increased (31). In cycling women, the largest quantities of Ig were detected before ovulation (31). In female rhesus macaques, the frequency of Ig-secreting cells was high in tissues collected from animals in the periovulatory period of the menstrual cycle (30).

In mice, administration of E2 (pregnancy levels) markedly augments the ability of CD5+ B cells to express their autoimmune potential (32). In male MRL lpr/lpr mice (a lupus model), administration of E2 (pregnancy levels) for 31 wk increased the frequency of IgG- and IgM-producing cells (33). In Swiss male mice, administration of E2 (pregnancy levels) for 4 wk increased serum levels of IgG1. In female C3H/N mice, the same treatment increased serum IgG2 levels (33), which was confirmed by others in nonautoimmune C57BL/6J mice with respect to anti-double-stranded DNA antibodies (34). In the collagen-induced arthritis model, E2-treated mice (pregnancy levels) demonstrated an increase in the levels of IgG1 anti-collagen type II antibodies (35).

In Balb/c mice transgenic for the H chain of anti-double-stranded DNA antibodies, E2 (pregnancy levels) rescued naive autoreactive B cells that normally are deleted and caused them to mature to a marginal zone phenotype (36). E2 (at pregnancy levels) further led to the activation of this population, causing an elevation of serum anti-double-stranded DNA antibody titers and renal disease (36). E2 enhanced transitional B cell resistance to apoptosis and expanded the population of marginal zone B cells (36). Estriol (E3) treatment at high pregnancy doses increased IgG1 antibodies in the experimental autoimmune encephalitis (EAE) model in mice most probably by an E3-induced increase of IL-10 production from T cells (37).

From above-mentioned studies, it is clear that E2 can stimulate antibody production by B cells, probably by inhibiting T cell suppression of B cells. In contrast, E2 at high concentrations leads to a suppression of B-lymphocyte lineage precursors (38). In addition, IL-7-responsive B lineage precursors were greatly expanded in genetically hypogonadal female mice. Estrogen replacement in these mice resulted in a dose-dependent reduction in B cell precursors (39). Similarly, pregnancy led to a clear decline in B cell precursors in the bone marrow, which was blocked by ICI 182,780, and an increase was observed in hypogonadal mice (40). E2 at pregnancy levels induced a down-regulation of B lymphopoietic cells in bone marrow of young ovariectomized mice, which is mediated through both ERα and ERβ (41).

In conclusion, E2 at periovulatory to pregnancy serum levels is able to stimulate antibody secretion under healthy conditions but also in autoimmune diseases, whereas similar serum levels of E2 lead to a suppression of bone marrow B cell lineage precursors. Today we know that autoimmune diseases are not uniform, and sometimes B cells play a major role (as substantiated by the success of anti-CD20 antibody treatment in humans, Rituximab). In chronic inflammatory disorders, where B cells play a decisive role, E2 would promote the disease when autoaggressive B cells are already present, whereas chronically elevated E2 would inhibit initiation of an autoimmune disease when no such B cells are available. This might be a good reason why particularly B cell-dependent diseases such as SLE, mixed connective tissue disease (Sharp syndrome), IgA nephropathy, dermatitis her-

petiformis, gluten sensitive enteropathy, myasthenia gravis, and thyroiditis appear in women in the reproductive years, predominantly, in the third or fourth decades of life. It also explains why sometimes these particular diseases are started during or after pregnancy. These ideas are further developed at the end of this review.

C. Estrogens and the T cell

For more than 20 yr, the so-called T helper type 1 (Th1) immune response by CD4-positive T cells was thought to drive cell-mediated immunity leading to tissue damage as in chronic inflammatory autoimmune diseases (42). In contrast, T helper type 2 (Th2) CD4-positive cells drive certain antibody-mediated responses, particularly those that are involved in allergy (42). It is also known that these two pathways reciprocally inhibit each other. Cytokines such as IFN-γ, IL-12, and TNF were allocated to Th1 reactions, and IL-4, IL-5, and IL-10 to Th2 responses. In recent years, this viewpoint has changed due to the appearance of antiinflammatory T regulatory cells producing TGF-β and proinflammatory T helper type 17 cells (Th17) producing IL-17 (43). Today, Th17 cells are thought to be the main responsible cells for chronic inflammatory tissue destruction in autoimmune diseases (43). Importantly, this clarifies some unresolved questions such as the ameliorating effects of IFN-γ in several autoimmune diseases, because IFN-γ inhibits Th17 cells, which is opposite for TNF that stimulates Th17 cells (Fig. 1) (43). In addition, IL-4 from Th2-positive cells also inhibits Th17 cells, leading to amelioration of chronic inflammatory autoimmune diseases (Fig. 1) (43). The interested reader is referred to the important recent revision of this subject by L. Steinman (43). With this information in mind, effects of estrogens on T cells need to be revised (Fig. 1). Unfortunately, no direct effects of estrogens on Th17 cells or IL-17 secretion have been described until now. Thus, an influence of estrogens on Th17 cells is deduced from estrogen effects on other T cell cytokines (Fig. 1).

Some important aspects concerning the role of estrogens on T cells has been generated in multiple sclerosis (MS) research. In the presence of E2 at pregnancy levels, the majority of the tested neuroantigen-specific human CD4+ T cell clones isolated from normal control subjects and patients with MS showed a dose-dependent enhancement of antigen-stimulated IL-10 secretion (44). The secretion of IFN-γ was also increased at the same dose. In contrast, the effect of E2 on antigen-stimulated secretion of TNF was biphasic, with enhancement occurring at lower doses at 3.6×10^{-9} and 1.8×10^{-8} M, and inhibition present at high concentrations of $3.6 \times$

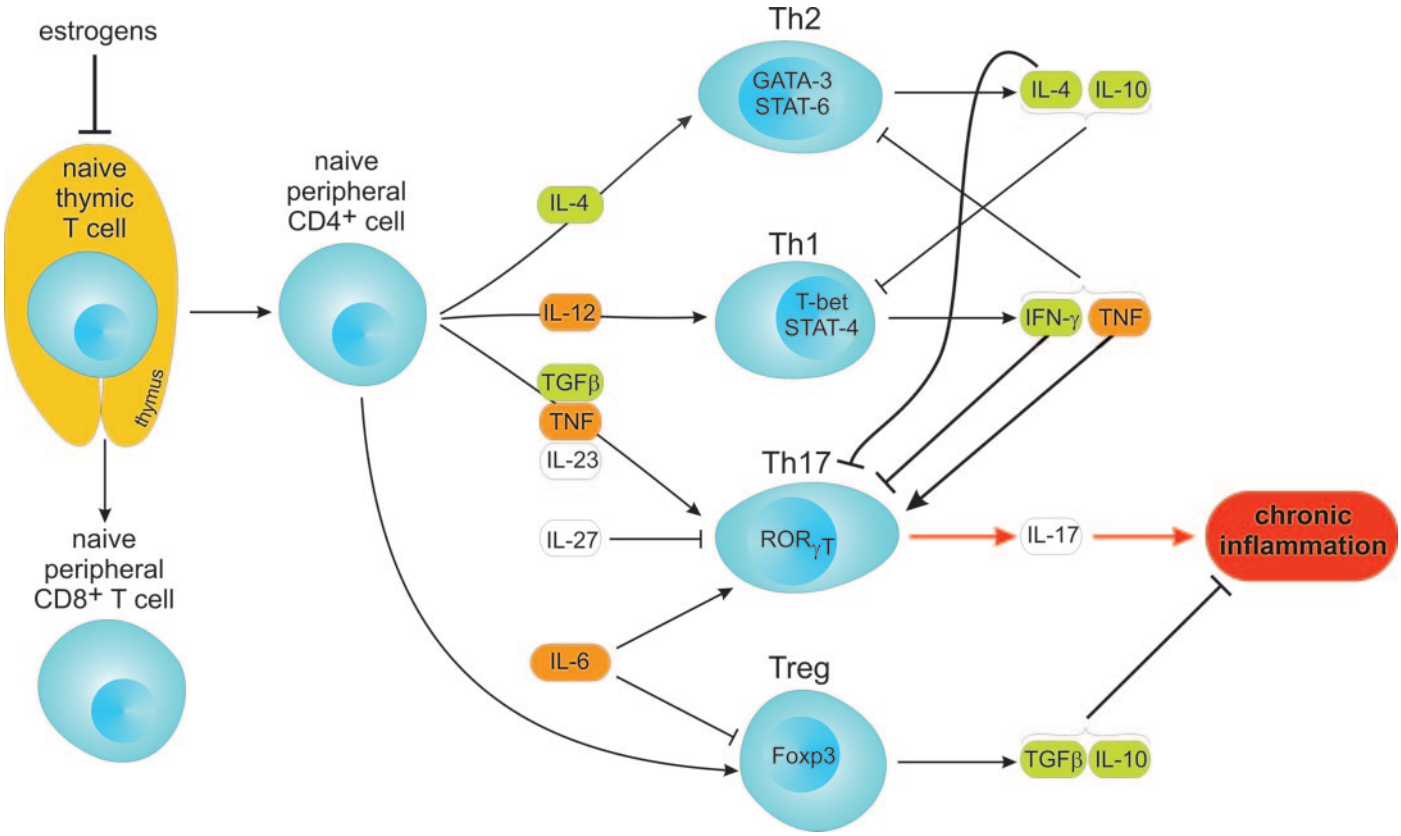

FIG. 1. Influence of estrogens on T cells. This diagram depicts the main T cell pathways (modified according to Ref. 43). *Lines with an arrow* indicate a stimulatory effect, and *lines with a bar at the end* represent an inhibitory effect. Cytokines in *green boxes* are stimulated by ovulatory to pregnancy doses of estrogens, whereas cytokines in *orange boxes* are inhibited by estrogens at the same dose. The role of estrogens for secretion and production of cytokines in the *white boxes* is not known. So-called Th17 cells producing IL-17 are the main T cells responsible for chronic inflammation. Th17 cells are inhibited by IL-4, IFN-γ, and IL-27. The generation of Th17 cells from naive Th cells is stimulated by TGFβ, TNF, and IL-23. Th17 cells are stimulated by the proinflammatory cytokines TNF and IL-6, whereas the latter cytokine inhibits T regulatory cells (Treg). The factors given in the cell nucleus are transcription factors relevant for development of T cells into the respective T cell subtype.

10^{-8} to 3.6×10^{-7} M (44). The same group reported that estrone (E1) and E3 at pregnancy levels enhance secretion of antigen-induced or anti-CD3-stimulated IL-10 and IFN-γ from CD4+ cells of MS patients, almost identical to that of E2 (45). In addition, E1 and E3, like E2, had a biphasic effect on TNF secretion, with lower concentrations stimulatory and high doses inhibitory (45). In T cells of patients with MS, E3 at pregnancy levels stimulated secretion of IL-10 and inhibited TNF [via inhibition of nuclear factor-κB (NF-κB)] (46). As compared with the pretreatment situation, relapsing remitting MS patients treated with oral E3 demonstrated a marked decrease in T cell-dependent delayed type hypersensitivity responses to tetanus (47).

These findings were corroborated by studies in mice. In ovariectomized mice, E2 replacement (pregnancy levels) suppressed the T cell-mediated delayed type hypersensitivity reaction (48–50). Treatment of CD4+ T cells from nonobese diabetic mice with E2 at pregnancy levels increased secretion of IFN-γ (51). E2 at pregnancy levels increased α-galactosylceramide-induced IFN-γ synthesis by invariant natural killer T cells (52). Ovariectomized C57BL/6 mice were given E2 replacement (pregnancy levels), which increased IFN-γ mRNA in concanavalin-A-activated enriched splenic T cells but there was no effect on IL-4 mRNA (53). In ovariectomized mice, E2 replacement (proestrus to early pregnancy levels) results in the selective development of IFN-γ-producing cells (via ERα) (54). All these experiments clearly demonstrate the positive effect of E2 at pregnancy levels on IFN-γ production in T cells. Because IFN-γ has been allocated a Th17-inhibiting role (Fig. 1), its increase by E2 at pregnancy doses and the E2-mediated inhibition of TNF must be viewed as a favorable effect in chronic inflammation.

Other important indications for the estrogen-meditated inhibition of the proinflammatory TNF came from bone research. Ovariectomy enhanced T cell production of TNF and subsequent bone loss, which was abolished by E2 replacement therapy (proestrus or early pregnancy levels) (55). Ovariectomy-induced bone loss, which was absent in T cell-deficient nude mice, was restored by adoptive transfer of wild-type T cells but not by reconstitution with T cells from TNF-deficient mice (56).

In contrast to the inhibitory effects of E2 on TNF, E2 can increase antiinflammatory T cell responses. In C57BL/6 mice, E2 at pregnancy levels (not at 10^{-10} or 10^{-11} M) increased IL-4 secretion and GATA-3 mRNA levels from ERα+ CD4+ T cells, whereas this effect was abrogated in ERα-deficient T cells (57). Forkhead box P3 (FoxP3) in CD4+CD25+ cells is an important indicator of T regulatory cells (Fig. 1). In C57Bl/6 mice, E2 (pregnancy levels) is capable of augmenting FoxP3 expression *in vitro* and *in vivo* and increases CD25+ cell number, which is reduced in ERα-deficient animals. Pregnancy stimulated the expression of FoxP3 protein to a similar degree (58), but progesterone might also play a supportive role.

In addition to effects of estrogens on peripheral CD4+ T cells, estrogens have a strong influence on the development and maintenance of thymic function and, thus, on generation of naive CD4+ and CD8+ T cells. ERα-deficient mice have smaller thymi than their wild-type littermates; and E2-induced thymus atrophy was reduced in ERα-deficient mice (59, 60). In one study, the CD4+/CD8+ phenotype was not changed in ERα-deficient mice (59), but a higher frequency of CD4+CD8+ thymocytes was found in another study (60). In ER-intact animals, E2 (pregnancy levels) induced profound thymus involution in normal and adrenalectomized mice (49, 61). However, E2 did not induce lymphocytopenia in the peripheral organs but stimulated extrathymic T cell numbers in the liver and other organs (61). E2 treatment (pregnancy levels) for 4 wk decreased thymus cellularity and the percentage of CD4+ cells in the spleen, which was not observed in double ER-deficient mice (62). Whether this E2-induced thymus involution is positive or negative in chronic inflammation remains to be established because autoaggressive but also autotolerant T cells might be eliminated.

In conclusion, in humans and mice, E2 at periovulatory to pregnancy levels stimulates IL-4, IL-10, and IFN-γ but inhibits TNF from CD4+ T cells. In humans and mice, E3 and E2, respectively, at pregnancy levels inhibit T cell-dependent delayed type hypersensitivity. In mice, E2 at pregnancy levels increases the Th2-relevant GATA-3 and the T regulatory cell-relevant FoxP3, which might shift the immune response toward tolerance. Because we know that increased IL-4, IL-10, and IFN-γ in the presence of low TNF support an anti-aggressive immune response (Fig. 1), collectively, these data suggest that E2 at periovulatory to pregnancy levels might be a favorable hormone leading to down-regulation of T cell-dependent immunity. The question appears as to what would happen if E2 falls to postmenopausal levels (metestrus) before initiation or during the course of an autoimmune disease. One might assume that the protecting effects of E2 are getting lost under these conditions.

D. Estrogens and the monocyte/macrophage/dendritic cell

In human promonocytic cells (U937), E2 (late pregnancy levels) inhibited LPS-stimulated IL-6 secretion (63). In human monocytic cells (THP-1), the absence of E2 led to an increase of surface CD16 (stimulatory Fc γ-receptor III), which after cross-linking stimulated secretion of TNF, IL-1β, and IL-6 (64). This indicates that E2 normally suppresses these cytokines stimulated via cross-linking of CD16.

In mouse splenic macrophages, pretreatment for 16 h with E2 between 3.6×10^{-11} and 1.8×10^{-9} M decreased LPS (1000 ng/ml, EC_{50} of LPS in mice and rats)-induced TNF production, which was associated with a decreased NF-κB-binding activity (65). In murine bone marrow-derived macrophages, preincubation with E2 at pregnancy levels (maximum at 10^{-8} M) inhibited LPS-induced TNF release (no effect on cells in the absence of LPS) (66). In murine peritoneal macrophages and RAW 264.7 cells, E2 at pregnancy levels inhibited LPS-stimulated nitrite production and TNF secretion (67). In the mouse macrophage cell line RAW 264.7, preincubation with E2 (proestrus levels) and subsequently activated with LPS inhibited nitric oxide (NO) and TNF release (68).

E2 treatment (pregnancy levels) decreased TNF and IL-12 production in mature mouse dendritic cells (69). This study demonstrated an interesting shift toward cytokines such as IL-4 and IL-10. Transfer of E2 (pregnancy levels)-exposed splenic dendritic cells from Lewis rats obtained on d 12 after immunization with myelin basic protein prevented the ex-

pansion of CD4+ T cells and increased proportions of regulatory T cells producing IL-10 and CD4+CD28- suppressor T cells, accompanied with increased IL-10 and IFN-γ (see explanation in Fig. 1), and reduced TNF production (70). This tolerating effect might be mediated by up-regulation of indoleamine 2,3-dioxygenase (70, 71). These inhibitory activities of E2 might be different at lower levels.

Indeed, in human peripheral monocytes, E2 (early follicular to periovulatory levels) resulted in maximal IL-1 stimulation. At higher concentrations of E2 (late pregnancy levels), a significant reduction in IL-1 activity was observed (72). In LPS-stimulated human monocytic cells, E2 (periovulatory levels) increased IL-1β and IL-1α mRNA expression (73). The mouse macrophage cell line RAW 264.7 was stably transfected with the human ER and an IL-1β promoter reporter construct. Pretreatment with E2 at 1.8×10^{-8} to 1.8×10^{-6} M (pregnancy levels, no effect at 1.8×10^{-10} M) markedly enhanced LPS-induced IL-1β promoter-driven activity (blocked by ICI 182,780) (74). In adherent rat peritoneal macrophages, E2 at proestrus levels (little effect at 3.7×10^{-11} M) stimulated secretion of IL-1 with and without LPS, and IL-1 secretion was higher in cells from female compared with male rats (75). These studies demonstrate that lower levels of E2 particularly increase IL-1β secretion.

In conclusion, in human and mouse/rat monocyte/macrophage-like cells, secretion of IL-1β is increased at periovulatory/proestrus to early pregnancy levels, whereas IL-1 secretion is inhibited at high pregnancy levels. It is further obvious that proestrus to pregnancy levels of E2 decreased LPS-stimulated TNF secretion in mouse/rat cells. Thus, with respect to TNF, E2 at pregnancy levels exerts similar effects in macrophages compared with T cells, which in both cell types is most probably due to inhibition of NF-κB. The dichotomous effect of E2 on IL-1β and TNF at high and low concentrations is most probably due to inhibition of NF-κB at high concentrations (see *Section IV.A*).

E. Estrogens and macroglial/microglial cells

In primary rat astrocytes, pretreatment with high doses of E2 at 10^{-6} M reduced the amyloid β-peptide and LPS-induced activation of NF-κB (76). In primary rat astrocytes, E2 at pregnancy levels inhibited the receptor for activated C kinases-1 (RACK-1, the anchoring protein involved in protein kinase C shuttling), protein kinase C activation, LPS-stimulated TNF mRNA, and inducible NO synthase (iNOS) expression (77). In primary rat cortical astrocyte cultures, E2 (proestrus to pregnancy levels) increased neuronal cell survival and both the expression and release of the neuroprotective cytokines TGF-β1 and TGF-β2 (blocked by ICI 182,780), which is mediated by a membrane-bound ER (78).

In primary rat microglia and a microglial cell line (N9), pretreatment with E2 at proestrus to pregnancy levels attenuated phorbol-12-myristate-13-acetate-stimulated superoxide release, LPS-stimulated phagocytic activity, and iNOS protein expression, but did not alter NF-κB activation (blocked by ICI 182,780). E2 at similar concentrations induced rapid phosphorylation of the p42/p44 MAPK, and the MAPK inhibitor PD 98059 blocked the antiinflammatory effects of E2 (79). In LPS-stimulated microglial cells (N9), E2 at pregnancy levels increased IL-10 while decreasing release of TNF (similar as in T cells). In the same cell type, E2 at pregnancy levels attenuated the LPS-induced increase of membrane major histocompatibility complex class I molecules, CD40, and CD86, and E2 also decreased the percentage of nonstimulated cells positive for major histocompatibility complex class I and II, CD40, and CD152, Fas, and Fas ligand (FasL), whereas CD80 cell surface staining was increased (80).

In primary rat microglia cells, E2 at 10^{-10} to 10^{-8} M (proestrus levels, maximum at 10^{-9} M, minimum at 10^{-11} M) inhibited iNOS expression and reduced the accumulation of nitrites and nitrates upon various inflammatory stimuli. Under the same conditions, production of prostaglandin E2 and matrix metalloproteinase (MMP)-9 were also inhibited (blocked by ICI 182,780) (81). In murine microglial cells (BV-2), which naturally express only ERβ, E2 at 10^{-13} to 10^{-9} M (also E3 at 10^{-10} to 10^{-8} M) decreased LPS-stimulated NO production and iNOS expression and expression of cyclooxygenase-2 (82).

In conclusion, experiments with mouse and rat macroglial and microglial cells demonstrate that E2 at proestrus to pregnancy levels exerts neuroprotective effects by increasing TGF-β and by inhibiting iNOS and NO release, and reducing expression of proinflammatory cytokines and prostaglandin E2 production.

F. Estrogens and natural killer cells

An early study in BALB/c mice described that E2 treatment (proestrus to pregnancy levels) led to unresponsiveness of natural killer cells to poly I.C., which was not mediated by changes of IFN (83). Others demonstrated that E2 treatment (pregnancy levels) inhibits IFN-α-stimulated natural killer cell activity (84). This was corroborated by a study in different mouse strains, which demonstrated that E2 (pregnancy levels) reduced natural killer cell cytotoxicity. A high degree of suppression was observed in C3H/N, DBA/1, and NZB/W mice, whereas other strains displayed a low degree of suppression (C57BL/6 and MRL lpr/lpr mice) (33). In rhesus monkeys, E2 deprivation by ovariectomy (serum levels, 3.7×10^{-11} M, postmenopausal) reduced natural killer cell activity (85). These studies indicate that E2 has bimodal effects inhibiting natural killer cells at pregnancy levels but stimulating these cells at diestrus to proestrus levels.

G. Estrogens and fibroblast

In human uterine fibroblasts, E2 at 10^{-10}, 10^{-8}, and 10^{-6} M (no effect at 10^{-12} M) increases the concentration of basic fibroblast growth factor (86). In human cultured fibroblast-like synoviocytes from patients with RA, E2 at pregnancy levels had no effect on constitutive production of IL-6, but E2 increased IL-1β-induced IL-6 production (87). Because E2 has some stimulatory effects on IL-1β secretion of monocyte/macrophages at low levels (see *Section III.D*), the E2-stimulated IL-1β-induced IL-6 secretion might be a proinflammatory signal.

In primary human adipose fibroblasts, E2 at pregnancy levels increased TNF receptor 1 mRNA and protein levels (no

effect at 10^{-11} to 10^{-9} M), whereas E2 at the same concentration decreased TNF receptor 2 mRNA (increase at 10^{-11} to 10^{-10} M) (88). Assuming that a similar modulation of TNF receptor 1 and TNF receptor 2 also happens in other cell types, and because the TNF receptor 1 is mainly responsible for tissue-specific autoimmunity and the TNF receptor 2 is mainly responsible for proproliferative effects and tissue damage (89), E2 at pregnancy levels might initiate an autoimmune disease, whereas E2 at postmenopausal levels might support tissue damage. This needs to be investigated in B/T cells and macrophages/dendritic cells.

In human endometrial stromal cells (fibroblasts), E2 at pregnancy levels inhibited the secretion of monocyte chemoattractant protein (MCP)-1 with an IC_{50} of 10^{-8} M (slight increase at 10^{-12} M, no inhibition at 10^{-11} M and 10^{-10} M, maximum inhibition at 10^{-6} M) (90). In murine fibroblasts, E2 (maximum at 3.7×10^{-12} M and 9.9×10^{-10} M, no effect in the middle range of 3.7×10^{-11} M) inhibited serum-stimulated MCP-1 (91). In a mouse stromal cell line (ST-2), E2 (pregnancy levels) induced up-regulation of osteoprotegerin, an osteoprotective factor. The effect was dependent on ERα, but not of ERβ. Moreover, estrogen withdrawal after 5-d pretreatment, mimicking the event occurring *in vivo* at menopause, dramatically diminished the expression of osteoprotegerin (92). In rabbit uterine cervical fibroblasts, E2 at pregnancy levels (not at 10^{-10} M) decreased secretion of procollagenase (precursor of MMP-1) and prostromelysin (precursor of MMP-3), whereas the natural antagonists, the tissue inhibitor of MMPs (TIMP), increased in the presence of E2 (93).

In conclusion, in normal human and mouse/rat fibroblasts, E2 exerts tissue-protective effects by up-regulation of basic fibroblast growth factor, osteoprotegerin, and TIMP, and by down-regulation of the proforms of MMP-1 and MMP-3. In contrast, in synovial fibroblast of patients with RA, E2 stimulates the proinflammatory cytokine IL-6 in the presence of IL-1β, the latter is most probably not derived from fibroblasts. This demonstrates that a change of the environmental context with an increase of IL-1β from macrophages might render E2 a proinflammatory factor at lower levels.

H. Estrogens and vascular smooth muscle cells

In human vascular smooth muscle cells, E2 at 10^{-10} to 10^{-8} M (also E3) inhibited mRNA expression of platelet-derived growth factor-A, IL-1, and IL-6 but not of TGF-β (94). Additionally, E1 sulfate at 10^{-10} to 10^{-8} M inhibited mRNA expression of platelet-derived growth factor-A, IL-1, and IL-6 (94). These antiinflammatory effects of E1 sulfate are to be expected in the presence of steroid sulfatase (leading to estrogen activation). In human vascular smooth muscle cells, steroid sulfatase expression levels were found to be higher in female aortas with mild atherosclerotic changes than in those with severe atherosclerotic changes and in male aortas regardless of atherosclerotic changes (95). In contrast, expression levels of estrogen sulfotransferase (leading to estrogen inactivation) were higher in female aortas with severe atherosclerotic changes and in male aortas than in female aortas with mild atherosclerotic changes (95). Furthermore, IL-1β markedly inhibited the expression of the sulfatase but stimulated the expression of estrogen sulfotransferase. This was accompanied by IL-1β-induced inhibition of E2 production from E1 sulfate (95). This confirms a similar effect of the proinflammatory cytokine TNF on the sulfatase in macrophages and fibroblasts (96, 97). By way of inhibiting the sulfatase and stimulating the sulfotransferase, these cytokines add to a proinflammatory milieu.

In human coronary artery smooth muscle cells (express ERα and ERβ), E2 at 10^{-9} to 10^{-7} M (not at 10^{-11} and 10^{-10} M) inhibited MCP-1 mRNA expression and protein production (98). In primary aortic smooth cells, E2 at 10^{-9} M (no other concentration tested) inhibited iNOS expression and reduced the accumulation of nitrites and nitrates consequent to various inflammatory stimuli (99).

In rat vascular smooth muscle cells, E2 did not influence stimulated IL-6 production (100). In rat vascular smooth muscle cells, E2 at pregnancy levels inhibited cell proliferation and both constitutive and IL-1β-stimulated NF-κB activation (101).

In conclusion, E2 at pregnancy levels by way of several pathways contributes to an antiinflammatory milieu in vascular smooth muscle cells. However, presence of a proinflammatory cofactor such as IL-1β might change availability of E2 by inhibiting E2 allocation and stimulating E2 degradation.

I. Summary

Figure 2 summarizes the most important effects of high and low concentrations of estrogens on different types of cells involved in chronic inflammation. At pregnancy levels, E2 inhibits important proinflammatory pathways such as TNF, IL-1β, IL-6, MCP-1, iNOS expression, production of MMPs, and activity of natural killer cells, whereas E2 at the same concentration stimulates antiinflammatory pathways such as IL-4, IL-10, TGFβ, TIMP, and osteoprotegerin. At lower concentrations, E2 stimulates TNF, IFN-γ, IL-1β, and activity of natural killer cells. This dichotomy of E2 at high *vs.* low concentrations is not observed for B cells, because antibody production is stimulated throughout the concentration range (Fig. 2). Consequences of these E2 effects for chronic inflammation are summarized in the discussion (*Section XV*).

IV. Estrogens and Inflammatory Factors

A. Nuclear factor κB

NF-κB is an important factor in proinflammatory signaling that can interact with ER pathways (summarized in Ref. 102). Since the overview of McKay and Cidlowski (102), a substantial amount of new data have been generated in cells relevant for inflammation.

In mouse monocytic cells (RAW 264.7), E2 (pregnancy level) blocked LPS-induced DNA binding and transcriptional activity of the p65 subunit of NF-κB by preventing its nuclear translocation (103). Similarly, in primary rat astrocytes, pretreatment with high doses of E2 at 10^{-6} M reduced the LPS-induced activation of NF-κB (76). In lymphocytic

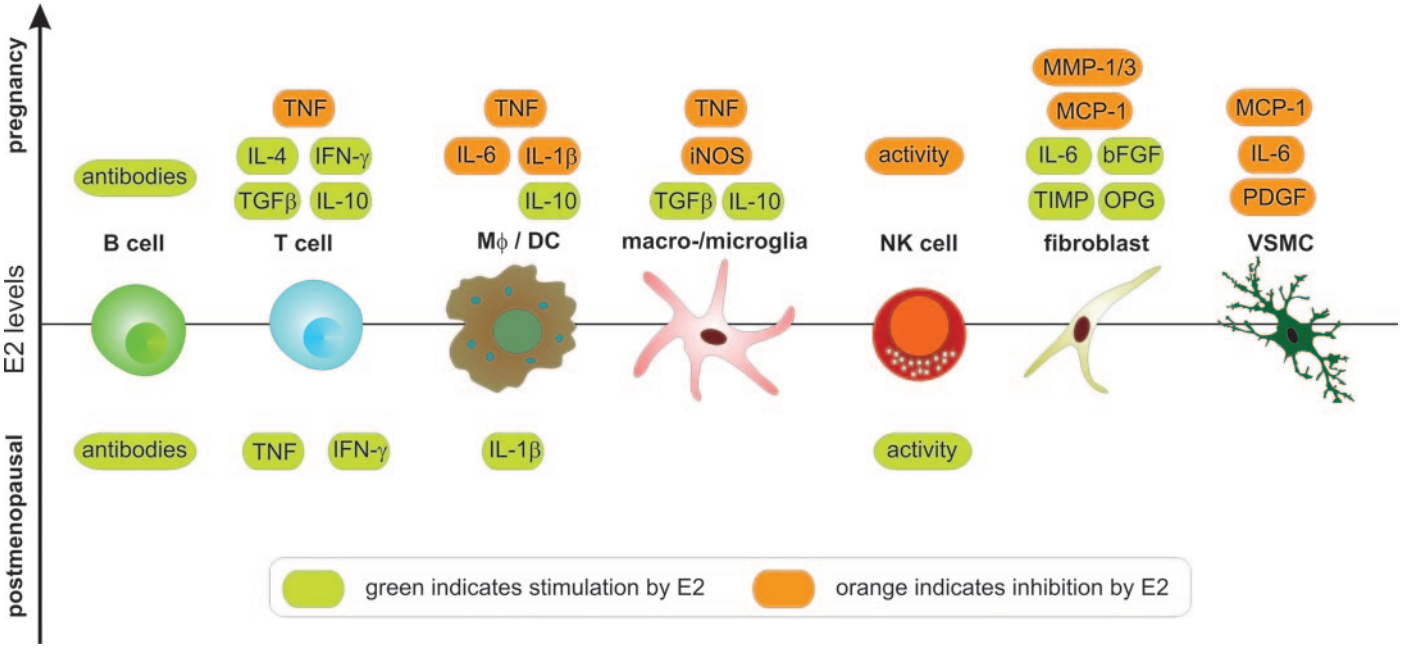

FIG. 2. Influence of estrogens on important pro- and antiinflammatory pathways in different cell types. On the y-axis, the concentration of estrogens is given. Depending on the concentration of estrogens, factors in *green boxes* are stimulated, and factors in *orange boxes* are inhibited by estrogens. DC, Dendritic cell; MΦ, monocyte/macrophage; NK cell, natural killer cell; OPG, osteoprotegerin; PDGF, platelet-derived growth factor; VSMC, vascular smooth muscle cell.

HeLa cells, E2 (pregnancy levels) almost completely inhibited phorbol-12-myristate-13-acetate-induced NF-κB activation and inhibitor of NF-κB (IκB) protein degradation (104). In rat vascular smooth muscle cells, E2 (pregnancy levels) inhibited both constitutive and IL-1β-stimulated NF-κB activation (101). In human aorta endothelial cells, E2 at 10^{-10} to 10^{-7} M (IC_{50}, 10^{-9} M) inhibited NF-κB-mediated luciferase reporter activity and IL-6 secretion (105). In different cell types, it was demonstrated that ERα impairs TNF-induction of IL-6 by preventing binding of c-rel and, to a lesser extent, RelA proteins to the NF-κB site on the IL-6 promoter (106). This was corroborated by others who demonstrated that E2 inhibited IL-6 production by interfering with the function of NF-κB at the IL-6 promoter (107). In macrophages, E2 blocked LPS-induced DNA binding and transcriptional activity of c-rel (103). All these studies clearly show reciprocal inhibition of ER and NF-κB pathways in different cell types. In most occasions, periovulatory to pregnancy levels of E2 have been used. However, other important proinflammatory pathways might be linked to ERs as well.

NF-κB activity is suppressed in PBMCs from pregnant women compared with age-matched nonpregnant women (108). In a burn injury model *in vivo*, E2 treatment resulted in a decrease in splenic NF-κB activation (109). In rats receiving transient middle cerebral artery occlusion *in vivo*, substantial apoptosis and inflammatory responses were observed, including IκB phosphorylation, NF-κB activation, and iNOS overexpression (110). In this model, E2 treatment (short-term pregnancy levels) produced strong protective effects by reducing infarct volume and neuronal apoptosis and by inhibiting NF-κB activation and iNOS overexpression (110). In cerebral arteries of ovariectomized rats, E2 treatment at pregnancy levels reduced cerebrovascular NF-κB activity (111).

In conclusion, E2 at periovulatory to pregnancy levels inhibits NF-κB activation, which must be viewed as an antiinflammatory signal. The necessary E2 levels needed for NF-κB inhibition have been analyzed in human cells infected with an adenoviral NF-κB luciferase reporter. It was shown that E2 concentrations equal to or above 10^{-10} M are necessary to inhibit NF-κB activation. At 10^{-11} M or below (postmenopausal level), no effects were observed (112). Necessarily, only at these higher E2 concentrations is the antiinflammatory effect to be expected.

B. Adhesion molecules

Adhesion molecules are important factors during inflammation because they enable inflammatory cells to migrate to inflamed compartments. Many studies with human and mice/rat cells *in vitro* demonstrated that estrogens inhibited expression of adhesion molecules at pregnancy levels, which seems to be opposite at low estrogen levels.

For instance, cultured human umbilical vein endothelial cells (HUVECs) were propagated in steroid hormone-free medium and were pretreated with E2 (pregnancy levels) for 48 h before IL-1 activation. In these cells, E2 strongly inhibited IL-1-induced expression of membrane E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) (113). In HUVECs, E2 at pregnancy levels decreased the expression of TNF-induced VCAM-1 (114). The E2 metabolite 17-epiestriol, which can be generated *in vivo*, is much more potent than E2 in inhibiting VCAM-1 expression in these cells (U-shaped response curve,

no effect at 10^{-11} and 10^{-8} M, maximum at 3×10^{-10} M) (attenuated by ICI 182,780) (114).

In an ERα overexpressing endothelial cell line (ECV-304), E2 at pregnancy levels inhibited the effects of TNF on VCAM-1 expression and U937 cell adhesion (115). In normal human female iliac artery endothelial cells, E2 at pregnancy levels decreased IL-1β-stimulated VCAM-1 and ICAM-1 membrane expression (116). In human umbilical cord endothelial cells (ECV-304), the E-selectin promoter is down-regulated by E2 (no effect at 10^{-11} M, increasing effect between 10^{-10} and 10^{-5} M) (117). In cultured brain endothelial cells, E2 at suprapregnancy levels inhibited the basal and IL-1β-stimulated expression of ICAM-1 (118).

In a functional assay with human monocytic THP-1 cells, 48-h pretreatment with E2 at suprapregnancy doses inhibited nonstimulated and cytokine-stimulated adhesion to human aortic endothelial cells (119). E2 at pregnancy levels decreased adhesion of U937 promonocytes to TNF-stimulated HUVECs (120). In an *in vitro* model of the vasculature (parallel plate laminar flow chamber), E2 at pregnancy levels potently inhibited monocyte adhesion, and, in parallel, E2 down-regulated Rac1 GTPase activity in monocytes (121). Transfection of monocytic cells with dominant-negative Rac1 significantly decreases adhesion to human endothelial cells (121).

In a rat myocardial ischemia reperfusion injury model, E2 treatment (proestrus levels) lowered myocardial staining of ICAM-1 (122). In a colitis model in mice, E2 treatment (pregnancy levels) decreased expression of ICAM-1 (123). In a rat model of carrageenan-induced pleurisy, E2 (pregnancy levels) inhibited expression of P-selectin and ICAM-1 (124). These studies clearly demonstrate the inhibitory effect of E2 on expression of various adhesion molecules, which appears at proestrus/ovulatory to pregnancy levels. However, the situation might be different at low concentrations of estrogens.

For instance, E2 (diestrus levels) has been shown to up-regulate L-selectin and α4-integrin dependent adhesion pathways in endothelium and lymphocytes (125). Postmenopausal women with coronary artery disease demonstrated increased serum levels of soluble E-selectin, ICAM-1, and VCAM-1, which is an indication of up-regulated membrane-bound adhesion molecules. An increase of E2 serum levels from approximately 3×10^{-11} to 1.5×10^{-10} M decreased serum levels of these soluble adhesion molecules (126). A similar effect of hormone replacement therapy was corroborated by others (127–132). In addition, treatment of ovariectomized monkeys with conjugated equine estrogens (CEE) decreased serum levels of soluble VCAM-1 but not of soluble E-selectin (133).

In conclusion, E2 at periovulatory/proestrus to pregnancy levels decreased membrane expression of adhesion molecules and cell adhesion to endothelial cells. Some studies indicate that diestrus to ovariectomy/postmenopausal levels of E2 even increased these adhesion molecules. It is interesting that the small increase of E2 from 3×10^{-11} to 1.5×10^{-10} M under estrogen replacement therapy (ERT) in postmenopausal

TABLE 1. Effects of estrogens on IL-1, IL-8, and IL-12

Cell type and species	Concentration of estrogen	Ref.
IL-1 is inhibited by estrogens		
Human whole blood cultures	E2 at 10^{-10} to 10^{-8} M	21
Human monocytes and macrophages	E2 at 10^{-7} to 10^{-5} M (opposite at 10^{-10} to 10^{-9} M)	135
Human vascular smooth muscle cells	E1, E2, E1 sulfate at 10^{-10} and 10^{-8} M	94
Human vascular smooth muscle cells	E1 sulfate at 10^{-10} to 10^{-6} M	138
Mouse Th2 cells (D10)	E2 at 10^{-7} to 10^{-5} M (opposite at 10^{-10} to 10^{-9} M)	72
Osteoblast cultures together with osteoclasts of 3-d-old NMRI mice	E2 at 10^{-10} and 10^{-9} M (not at 10^{-11} M)	134
Osteoblast cell line (HCC1)	E2 at 10^{-8} to 10^{-6} M (max. 10^{-6} M)	139
IL-1 is stimulated by estrogens		
Human monocytes and macrophages	E2 at 10^{-10} to 10^{-9} M (opposite at 10^{-7} to 10^{-5} M)	135
Monocytes obtained before estrogen treatment from postmenopausal women	E2 at 10^{-10} to 10^{-7} M	140
PBMCs from postmenopausal women	E2 at 10^{-8} M	141
Human *Streptococci*-stimulated monocytes from donors with lower control levels of IL-1β but not from donors with higher IL-1β	E2 at 10^{-10} M	142
TPA or LPS-stimulated human monocytic cells (THP-1)	E2 at 10^{-9} M	73
Mouse Th2 cells (D10)	E2 at 10^{-10} to 10^{-9} M (opposite at 10^{-7} to 10^{-5} M)	72
Peritoneal rat macrophages	E2 at 3.7×10^{-7} M (not at 10^{-11} M)	75
Macrophage cell line (RAW264.7)	E2 at 1.8×10^{-8} to 1.8×10^{-6} M (no effect at 1.8×10^{-10} M)	74
IL-8 is inhibited by estrogens		
HUVECs	E2 at 10^{-9} to 10^{-8} M	120
Human osteoclasts	E2 at 10^{-7} M (not at 10^{-8} M)	143
Human uterine epithelial cells	E2 at 10^{-7} to 10^{-10} M (not at 10^{-11} and 10^{-12} M)	144
Human breast cancer cells	Expression of ERα reduces IL-8 secretion	145
Melanoma cells	E2 at 10^{-9} M (was the IC_{50} of all experiments)	146
IL-12 is inhibited by estrogens		
Murine splenic dendritic cells followed by coincubation of MBP1–11-specific T cells	E2 pretreatment for 2 d at 7.3×10^{-9} M	69
IL-12 is stimulated by estrogens		
CD4+ T cells from NOD mice	E2 at 10^{-7} M, only one single concentration tested	51

MBP, Myelin basic protein; NOD mice, nonobese diabetic mice; TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate.

women already reduced soluble forms of adhesion molecules, which most probably indicates also down-regulation of membrane-bound adhesion molecules. Again, a dichotomy of estrogen effects at low and high levels can be observed.

C. IL-1, IL-2, IL-6, IL-8, IL-12, TNF, IFN-γ—studies in vitro

E2 effects on cytokine secretion depend on cell types, conditions in the milieu, and estrogen concentrations. Tables 1–4 delineate the effects of estrogens on proinflammatory cytokines (particularly of E2 *in vitro*). Aspects in humans *in vivo* are extensively demonstrated in *Section XII*.

E2 effects on IL-1 secretion are heterogeneous, and a unifying concept as to an inhibitory or stimulatory influence does not exist (Table 1). Generally, E2 effects are only observed at midfollicular (diestrus) to pregnancy levels. Five studies definitely mention that estrogens at low doses (postmenopausal/metestrus levels) did not lead to marked effects (74, 75, 134) or even exert stimulatory effects on IL-1 (72, 135). Particularly, the latter two studies indicate that E2 at low concentrations might stimulate IL-1β secretion, whereas it inhibits its secretion at pregnancy levels. This indicates that the well-known dichotomy of estrogen effects is also present here. Only two of 15 studies demonstrate no effects of E2 on IL-1 secretion at all (23, 136). With respect to IL-8, E2 at periovulatory to pregnancy levels inhibited secretion of this chemotactic IL as demonstrated in five studies (Table 1), and no opposite results were reported.

The situation is relatively homogenous with respect to IL-6, where most studies indicate a suppressive effect of E2 at periovulatory to pregnancy levels but little or no effects at early follicular or postmenopausal levels (Table 2). This is substantiated by the fact that in human osteoblastic cells the IL-6 promoter is inhibited by E2 in the absence of a functional ER binding site, which is mediated via down-regulation of NF-κB and C/EBP (137). Interestingly, E2 had stimulatory effects on IL-6 secretion in synoviocytes (normal or RA), which happens at pregnancy concentrations (Table 2). Only two of 20 studies demonstrated no effects of E2 on IL-6 secretion at all (23, 136).

Similar to IL-6, most studies demonstrated inhibitory effects of estrogens on TNF secretion at midfollicular to pregnancy levels (Table 3). Of the five studies that demonstrate stimulatory effects of E2 on TNF secretion, two studies delineate that stimulation appears only at lower levels of E2, whereas high pregnancy levels inhibit TNF secretion (44, 45). The latter two studies have often been cited to explain the dual role of estrogens at low *vs.* high concentrations. Only one of 17 studies demonstrated no effect of E2 at all (23).

E2 effects on IFN-γ secretion are heterogeneous. A unifying concept seems to exist because E2 stimulates IFN-γ from T cells but inhibits IFN-γ from macrophages and dendritic cells (Table 4). This separation might be important because quite different effects of estrogens are to be expected depending on the immune cell involved.

In conclusion, important proinflammatory cytokines are typically inhibited at periovulatory (proestrus) to pregnancy levels of E2, which is evident for IL-6, IL-8, and TNF. Nevertheless, low E2 concentrations were demonstrated to have no or even stimulatory effects. This renders a woman in the postmenopausal phase to a more proinflammatory situation, which might well contribute to the manifestation of chronic inflammatory diseases after the menopause.

TABLE 2. Effects of estrogens on IL-6

Cell type and species	Concentration of estrogen	Ref.
IL-6 is inhibited by estrogens		
Human whole blood cultures	E2 at 10^{-10} to 10^{-8} M	21
Nonstimulated human PBMCs	E2 at concentrations of 10^{-9} and 10^{-10} M (U-shaped not at 10^{-11} and 10^{-8} M)	147
Primary human bone marrow cells of postmenopausal women	E2 at 10^{-8} M	148
Osteoarthritis synoviocytes from postmenopausal women	E2 at 10^{-9} M	149
Human promonocytic cell line (U937)	Normal glucose (7 mM); E2 at 10^{-7} and 10^{-6} M high glucose (30 mM); E2 at 10^{-9} to 10^{-6} M	63
Human mast cell line	E2 at 10^{-8} and 10^{-7} M (not at 10^{-9} M)	150
Human fetal osteoblast cell line	E2 at 10^{-10} to 10^{-8} M (IC_{50}, 10^{-9} M)	151
Osteoblast (MG-63)	E2 at 3.7×10^{-10} and 3.7×10^{-7} M	152
Osteoblast cell line	E2 between 10^{-10} and 10^{-6} M (maximum 10^{-6} M)	153
Osteoblast, primary	E2 at 10^{-8} to 10^{-6} M (max. 10^{-6} M)	139
HUVECs	E2 at 10^{-12} to 10^{-6} M	154
Human aorta endothelial cells expressing ERα	E2 between 10^{-10} and 10^{-7} M (IC_{50}, 10^{-9} M, maximum 10^{-7} M)	105
Human vascular smooth muscle cells	E1, E2, E1 sulfate at 10^{-10} and 10^{-8} M	94
Human vascular smooth muscle cells	E1 sulfate at 10^{-10} to 10^{-6} M	138
Mouse bone marrow primary cultures	E2 at 10^{-8} M	155
Osteoblast cultures together with osteoclasts of 3-d-old NMRI mice	E2 at 10^{-10} and 10^{-9} M (not at 10^{-11} M)	134
Murine bone marrow-derived stromal cell lines, normal human bone-derived cells, and nontransformed osteoblast cell lines from mice and rats	E2 at 10^{-8} and 10^{-7} M (when stimulated with IL-1) E2 at 10^{-9} and 10^{-8} M (when stimulated with TNF) E2 at 10^{-9} and 10^{-8} M (when stimulated with IL-1 and TNF) E2 had no effect at 10^{-10} M	156
IL-6 is stimulated by estrogens		
Normal synoviocytes of women	E2 at 10^{-7} M (not at 10^{-8} M)	157
Primary human synovial fibroblast of RA	E2 at 10^{-8} M and 10^{-6} M	87
Female mice, macrophages	E2 at 10^{-9} M	50

TABLE 3. Effects of estrogens on TNF

Cell type and species	Concentration of estrogen	Ref.
TNF is inhibited by estrogens		
Human whole blood cultures	E2 at 10^{-10} to 10^{-8} M	21
Antigen-specific human CD4+ T cells	E2 at 3.6×10^{-8} M and particularly at 3.6×10^{-7} M (low doses stimulatory)	44
Primary CD4+ T cells from patients with MS	E2 at 3.5×10^{-7} to 3.5×10^{-5} M (low doses stimulatory)	45
T cells of patients with MS	E3 at 7.3×10^{-8} M	46
Human mast cell line	E2 at 10^{-7} M for TNF (not at lower conc.)	150
Murine splenic macrophages	E2 16 h preincubation between 3.6×10^{-11} and 1.8×10^{-9} M (followed by LPS)	65
Murine macrophages (RAW264.7)	E2 at 4.6 to 3.6×10^{-9} M. Effect was U-shaped with a maximum at 9.2×10^{-11} M (no effect at 3.7×10^{-9} M).	68
Murine splenic dendritic cells	E2 pretreatment for 2 d at 7.3×10^{-9} M followed by coincubation of MBP1–11-specific T cells	69
Murine macrophages	E2 at 10^{-9} and 10^{-8} M	67
Murine microglial cell (N9)	E2 at 10^{-10} M and 10^{-9} M, not at 10^{-11} M	80
Mouse uterine epithelial and stromal cells	E2 at 10^{-8} M	158
Rat uterine epithelial cells	E2 at 10^{-8} M	159
TNF is stimulated by estrogens		
Human *Streptococci*-stimulated monocytes from donors with lower control levels of TNF but not from donors with higher TNF	E2 at 10^{-10} M	142
Antigen-specific human CD4+ T cells	E2 at 3.6×10^{-9} and 1.8×10^{-8} M (high doses inhibitory)	44
Primary CD4+ T cells from patients with MS	E2 at 3.5×10^{-9} to 1.8×10^{-8} M (high doses inhibitory)	45
PMA-stimulated macrophage-like cell (U939)	E2 at 10^{-9} to 10^{-6} M (maximum 10^{-7} M)	160
PMA-stimulated murine microglia cells	E2 at 10^{-9} M	82

MBP, Myelin basic protein; PMA, phorbol-12-myristate-13-acetate.

D. IL-4, IL-10, TGF-β—studies in vitro

The interleukins IL-4, IL-10, and TGF-β are assumed to exert antiinflammatory effects as long as tissue-specific autoimmune diseases are considered. However, these cytokines might have a proinflammatory role in B cell-oriented autoantibody-driven autoimmunity, allergic diseases, or diseases with an overshooting fibrotic repair process (TGF-β).

It is important to mention that some chronic inflammatory disease can demonstrate different types of immune or inflammatory reactions, whereby T cells, B cells, fibroblasts, or macrophages might play a decisive role depending on the time point of inspection or the subtype of the disease. This has been nicely demonstrated in patients with RA and MS (164–167). With these prerequisites, we might better understand the role that IL-4, IL-10, and TGF-β play in chronic inflammatory diseases. The respective immune reaction at a certain time point, at a certain location, and in a given patient defines the set of cytokines used.

With respect to IL-4, all available studies demonstrated that E2 stimulated IL-4 secretion (Table 5). Of the seven studies on IL-10, five demonstrated a stimulating effect of E2 at midfollicular to pregnancy levels. The two studies that report an inhibitory effect on IL-10 (at pregnancy levels) also delineated that E2 at periovulatory doses had no effect (Table 5). All studies devoted to TGF-β demonstrated that E2 at pregnancy levels stimulated secretion of this cytokine (Table 5).

In conclusion, most *in vitro* studies demonstrated a stimulatory effect of E2 on secretion of IL-4, IL-10, and TGF-β typically at periovulatory to pregnancy levels. In conjunction with the information given in the previous section, E2 at periovulatory to pregnancy levels has an ameliorating effect on chronic inflammatory diseases as long as B cell-dependent immunity or an overshooting fibrotic tissue repair process do not play a crucial pathogenic role. However, when the B cell plays an important role, E2 might even stimulate the disease process as substantiated by flare-ups in SLE during pregnancy.

TABLE 4. Effects of estrogens on IFN-γ

Cell type and species	Concentration of estrogen	Ref.
IFN-γ is inhibited by estrogens		
Splenic dendritic cells from Lewis rats with EAE	E2 at 1.8×10^{-9} to 1.8×10^{-7} M	71
Dendritic cells derived from spleen monocytes of EAE rat	E2 at 7.3×10^{-6} to 1.8×10^{-4} M	161
Murine splenic dendritic cells	E2 pretreatment for 2 d at 7.3×10^{-9} M followed by coincubation of MBP1–11-specific T cells	69
Murine microglial cell (N9)	E2 at 10^{-9} M, not at 10^{-11} M or 10^{-10} M	80
LPS-stimulated splenocytes from C57BL/6N mice	E2 between 10^{-8} and 10^{-6} M	162
IFN-γ is stimulated by estrogens		
Primary CD4+ cells from patients with MS	E2 at 3.5×10^{-9} to 3.5×10^{-7} M	45
Antigen-specific human CD4+ cells	E2 at 3.6×10^{-9} to 3.6×10^{-7} M	44
Mouse invariant natural killer T cells	E2 at 10^{-8} M	52
CD4+ T cells from NOD mice	E2 at 10^{-7} M	51
Mice splenocytes	E2 at 10^{-9} M	163

MBP, Myelin basic protein; NOD, nondiabetic.

TABLE 5. Effects of estrogens on IL-4, IL-10, and TGF-β

Cell type and species	Concentration of estrogen	Ref.
IL-4 is stimulated by estrogens		
Postmenopausal women	0.625 mg CEE plus 2.5 mg medroxyprogesterone	168
Postmenopausal women (postmenopausal by oophorectomy)	Transdermal E2 patches, 50 μg/d once a week over 30 d	169
Murine splenic dendritic cells	E2 pretreatment for 2 d at 7.3×10^{-9} M followed by coincubation of MBP1–11-specific T cells	69
IL-10 is inhibited by estrogens		
Macrophage-like cell (U939)	E2 at 10^{-8} to 10^{-6} M (not at 10^{-9} M)	160
Murine keratinocytes	E2 at 10^{-8} and 10^{-7} M (not at 10^{-9} M)	170
IL-10 is stimulated by estrogens		
Human monocytes	E2 at 10^{-10} to 10^{-8} M (IC_{50}, 10^{-9} M)	28
PBMCs from SLE patients	E2 at 10^{-10} to 10^{-8} M (IC_{50}, 10^{-9} M)	29
Antigen-specific human CD4+ cells	E2 at 3.6×10^{-8} and 3.6×10^{-7} M	44
Primary CD4+ cells from patients with MS	E2 at 3.5×10^{-9} to 3.5×10^{-5} M	45
Murine microglial cells (N9)	E2 at 10^{-9} M, not at 10^{-11} or 10^{-10} M	80
TGF-β is stimulated by estrogens		
Human osteoblast-like cell	E2 at 10^{-12} to 10^{-8} M (max. at 10^{-8} M)	171
Human myometrium smooth muscle cells	TGF-β1, E2 at 10^{-7} M (not at 10^{-8} M) TGF-β receptor, E2 at 10^{-7} M (not at 10^{-8} M)	172
Breast cancer cells (MCF7)	E2 at 10^{-9} M	173
Neonatal rat cardiac fibroblasts	E2 at 10^{-7} M	174
Primary rat cortical astrocyte	E2 at 10^{-9} and 10^{-8} M, not at 5×10^{-10} M E2-BSA at 10^{-7} M	78

MBP, Myelin basic protein.

E. Estrogens and growth factors

Studies on TGF-β mentioned in the previous section are not listed here (78, 171, 172, 174). Similar stimulatory effects of E2 at pregnancy levels were demonstrated for TGF-β3 in osteosarcoma cells (175, 176) and TGF-β in human kidney carcinoma cells (177). E2 at follicular to pregnancy levels (not at 10^{-12} M) increased the concentration of basic fibroblast growth factor (86) and keratinocyte growth factor (178). E2 treatment (diestrus to pregnancy levels) caused a dose- and time-dependent increase in keratinocyte growth factor levels in peripubertal (5-wk-old) and mature (11-wk-old) mice (179). In mouse mesenchymal stem cells (C3H10T1/2), E2 at pregnancy levels activated bone morphogenic protein-2 promoter mainly through ERα and only little via ERβ, which requires a classical ER element site, but not activator protein (AP)-1 or Sp-1 sites (180). In human osteoblast cell lines, E2 at pregnancy concentration increased steady-state levels of bone morphogenic protein-6 (181).

In human macrophages, E2 at follicular to pregnancy levels and membrane-impermeable BSA-conjugated E2 enhanced nerve growth factor secretion and mRNA expression (via AP-1 binding sites on the promoter) (182).

In conclusion, most of these studies indicate that E2 at periovulatory to pregnancy levels increased important growth factors such as TGF-β, basic fibroblast growth factor, keratinocyte growth factor, and bone morphogenic proteins. This is in agreement with the important growth-supporting role of E2 during pregnancy. However, several studies also demonstrated that E2 at postmenopausal levels had no effect. In inflammatory diseases, in which the overshooting fibrotic repair response plays a decisive pathogenic role, an E2-stimulated increase of these growth factors might be harmful, whereas it can be positive for tissue repair.

F. Estrogens and nitric oxide

These aspects have been extensively covered in previous reviews from 1997 (183) and 2002 (184). In general, it has been identified that estrogens enhance NO production by the endothelial isoform of NO synthase (NOS) due to increases in expression and level of activation (184). These effects are mediated via ERs, but they are independent of ER element action (nongenomic) (184). Furthermore, it has been demonstrated that E2 increases other NOS isoforms (185–189). This presentation here only examines relevant aspects with respect to inflammatory diseases.

The known stimulatory effect of E2 on NO production at pregnancy levels (little at 10^{-10} M) was also observed in human granulocytes and monocytes via the activation of an estrogen surface receptor that is coupled to increases in intracellular calcium (190, 191). In these studies, results were independent of a proinflammatory stimulus. This might be largely different in a situation when a proinflammatory stimulus is added to the system.

For instance, in LPS-stimulated mouse macrophages (RAW 264.7), preincubation with E2 at proestrus to pregnancy levels inhibited NO and TNF release (68). Similarly, E2 at pregnancy levels inhibited IL-1β-induced NO production in rabbit lacrimal gland acinar cells (192). In primary microglia, aortic smooth cells, and in pleural cells after induction of carrageenan pleurisy, E2 at pregnancy levels inhibited iNOS expression and reduced the accumulation of NO consequent to various inflammatory stimuli (99). E2 and E3 at pregnancy levels inhibit LPS-induced TNF and NO production in primary rat microglia and mouse N9 microglial cells via iNOS inhibition (193). Transient middle cerebral artery occlusion in rats led to a strong inflammatory response with iNOS overexpression, which was strongly inhibited by E2 injection 2 h before occlusion (pregnancy levels) (110). In a

pleurisy model, the close interrelation between TNF and IL-1β and iNOS has been demonstrated, and E2 treatment (pregnancy levels) inhibited production of all three molecules (124). This was also demonstrated in rat primary astrocytes (77).

From this point of view, E2 exerts quite different effects depending on absence or presence of additional inflammatory stimuli such LPS or IL-1β. In the absence of inflammatory stimuli, E2 increases NO production by stimulating the expression and activity of different isoforms of NOS. However, in the presence of an inflammatory stimulus, E2 at pregnancy levels typically inhibits NO production elicited by cytokine-stimulated iNOS activity. In the latter situation, this might reflect the direct effect of E2 on NF-κB activation and proinflammatory cytokine secretion.

G. Estrogens and oxygen radicals

The generation of reactive oxygen species (ROS) is an important proinflammatory factor evolutionarily conserved to overcome infections. However, the generation of ROS is also found in proinflammatory diseases. Thus, it is important to know the effects of E2 on ROS generation.

Several studies using different cell types or species have described that ovariectomy levels of E2 or ovariectomy increased ROS production, whereas treatment with E2 at pregnancy levels decreased ROS production (194–199). E2 at pregnancy levels protected cardiac cells expressing ERβ from H_2O_2-induced apoptosis concomitant with an increase in the activity of Akt. E2 increased the expression of glutaredoxin as well as γ-glutamylcysteine synthase, a rate-limiting enzyme for the synthesis of glutathione (blocked by ICI 182,780) (200). In primary rat microglia and a microglial cell line (N9), pretreatment with E2 at proestrus to pregnancy levels attenuated phorbol-12-myristate-13-acetate-stimulated superoxide release (79). A relevant factor for the generation of oxygen radicals is the NADPH oxidase. In different cell lines, E2 at pregnancy levels decreased expression of the NADPH oxidase, which significantly decreased their capacity to generate ROS (201, 202). Another important factor in generating oxygen radicals is the myeloperoxidase. E2 at pregnancy levels blocked stimulated myeloperoxidase expression (203).

In conclusion, the overwhelming majority of studies demonstrate that E2 at proestrus to pregnancy levels inhibits ROS formation. It might well be that this inhibitory activity of E2 might be reverted depending on the cell type and the microenvironmental conditions leading to catechol estrogen formation (204). In addition, ROS formation might well happen below periovulatory/proestrus to pregnancy levels, which lead to a more proinflammatory situation at low concentrations of E2 in the postmenopausal state.

V. Estrogens and Apoptosis

Under consideration of a chronic inflammatory disease, apoptosis can have favorable effects by inhibiting clonal expansion of autoaggressive lymphocytes, but apoptosis can be deleterious because a tissue repair process can be largely hampered. Thus, apoptosis of different cell types involved in the proinflammatory process might be positive, whereas apoptosis of tissue-regenerating cells such as epithelial cells, fibroblasts, neurons, *etc*., might be unfavorable.

In human peripheral T lymphocytes, E2 at periovulatory to pregnancy levels for 24 h reduced TNF-induced lactate dehydrogenase release and caspase 3/7 activities, which must be viewed as an antiapoptotic effect (205). In human promonocytic cells (U937), E2 at 10^{-10} to 10^{-7} M (not at 10^{-13} to 10^{-11} M) increased survival and prevented apoptosis induced by TNF in both undifferentiated and macrophage-like phorbol-12-myristate-13-acetate differentiated cells (206). In human monocytic cells (THP-1), E2 at pregnancy levels prevented apoptosis (207). In Balb/c mice transgenic for the H chain of anti-dsDNA antibodies, E2 (at pregnancy levels) rescues naive autoreactive B lymphocytes normally deleted (36). Support of survival of these proinflammatory cell types is most probably an unfavorable signal in autoimmune diseases.

In chronic brain inflammation, the effect of E2 on neuronal or macroglial/microglial cell apoptosis might be important. In rat primary cortical neurons, 24-h pretreatment with E2 and E3 at pregnancy levels reduced *N*-methyl-D-aspartate-induced toxicity and the *N*-methyl-D-aspartate-induced apoptotic changes by enhancing Bcl-2 levels (208). In primary cortical neurons, E2 at pregnancy levels reduced the number of apoptotic cells and reduced the number of neurons containing active caspase-3 (blocked by ICI 182,780) (209). In rat primary cortical neurons after glutamate exposure, pretreatment with E2 at pregnancy levels prevented glutamate-induced apoptosis, attenuated calpain up-regulation, shifted the Bax:Bcl-2 ratio toward Bcl-2, and decreased caspase 3 activation (210). In murine microglial cells (N9), E2 at 10^{-9} M decreased the basal percentage of cells positive for Fas and FasL (80). All these effects in brain cells are of antiapoptotic nature and might protect the tissue from inflammation-induced destruction.

Similar antiapoptotic effects are observed *in vivo*; *e.g*., in immature hypophysectomized rats, E2 leading to pregnancy levels prevented apoptosis of granulosa cells, and testosterone had exactly opposite effects (211). In ovariectomized female mice, after myocardial infarction, E2 replacement (pregnancy levels) increased activation of the prosurvival kinase, Akt, and decreased cardiomyocyte apoptosis (212). In cultured neonatal rat cardiomyocytes *in vitro*, E2 at 10^{-10} to 10^{-9} M caused a rapid 2.7-fold increase in Akt phosphorylation and a decrease in apoptosis (reversed by ICI 182,780) (212). Thus, most of these studies demonstrated the antiapoptotic effects of E2.

Nevertheless, some cell types such as osteoclasts behave completely different. In human osteoclasts, E2 at 10^{-7} M (not at 10^{-8} M) inhibited IL-1-promoted cell survival, *i.e*., E2 increased apoptosis (143). In contrast, E2 did not alter IL-1 responsiveness in primary human osteoblasts or bone marrow stromal cells (143). In osteoclast precursors derived from a monoblastic leukemia cell line (FLG29.1), E2 at 10^{-8} and 10^{-7} M (not at 10^{-9} M) induced cell apoptosis (accompanied by caspase 3 activation and DNA fragmentation) (213). This clearly demonstrates that E2 effects must be dependent on additional factors present in different cell types.

An interesting solution for this paradox might be given by a study of Acconcia *et al*. (214): in different epithelial cell lines,

the E2-ERα complex rapidly activates multiple signaling pathways (*i.e.*, ERK/MAPK, phosphotidylinositol 3-kinase/Akt) committed to both cell cycle progression and prevention of apoptotic cascades. On the other hand, the E2-ERβ complex induced the rapid and persistent phosphorylation of p38/MAPK, which in turn was involved in caspase 3 activation and cleavage of poly(ADP-ribose) polymerase, driving cells into the apoptotic cycle. E2 at 10^{-8} M promotes cell survival through ERα nongenomic signaling and cell death through ERβ nongenomic signaling (214). Thus, the influence of E2 would be dependent on the abundance of either ERα or ERβ (see *Section II*).

In conclusion, most of these studies demonstrated the antiapoptotic effects of E2. However, E2 at pregnancy levels can also have proapoptotic effects (osteoclasts) and cytopenic effects on hematopoiesis (41, 61, 215–220). Given the fact that E2 decreases apoptosis of immune cells, this particular aspect of estrogens must be considered a proinflammatory effect.

VI. Estrogens and Migration of Leukocytes

This subject has never been reviewed before, but its presentation is important because migration of inflammatory cells plays an outstanding role in chronic inflammatory diseases. In *Section IV.B*, it was demonstrated that E2 at periovulatory/proestrus to pregnancy levels decreased membrane expression of various adhesion molecules and cell adhesion to endothelial cells. From these data, one might expect that E2 at these elevated levels also inhibits migration of leukocytes.

Indeed, in T cells of patients with MS, E3 at pregnancy levels inhibited T cell migration in a transwell model (46). RANTES (regulated on activation normally T cell expressed and secreted) attracts T lymphocytes and macrophages, and its production is enhanced in human keratinocytes. E2 (IC_{50}, 10^{-9} M) inhibited TNF or IL-1β-induced RANTES secretion (221). In human keratinocytes, E2 (IC_{50}, 10^{-9} M) inhibited constitutive and stimulated secretion of MCP-1 and its promoter activity (222). The Sp-1 element and the AP-1 element on the MCP-1 promoter are required for transcription, and they are involved in transrepression by E2. The results further suggest that E2-bound ERβ may inhibit MCP-1 gene expression by inhibiting Sp-1 and AP-1 transcriptional activities in keratinocytes (needs the activation function 2 of ERβ) (222).

E2 at 10^{-12} to 10^{-6} M (maximum at 10^{-10} M) inhibited the migration of human monocytes exposed to MCP-1 (blocked by tamoxifen and clomiphene) (223). E2 inhibited the secretion of MCP-1 from endometrial stromal cells (fibroblasts) with a IC_{50} of 10^{-8} M (slight increase at 10^{-12} M) (90). In IL-1α-stimulated human breast cancer cells (MCF-7), addition of E2 at 10^{-12} M to 10^{-9} M (IC_{50}, 5×10^{-11} M) inhibited MCP-1 production in a dose-dependent manner (224). Treatment with E2 at 10^{-10} and 10^{-11} M decreased the IL-1α-inducible MCP-1 promoter luciferase activity (224). In human coronary artery endothelial cells, E2 at pregnancy levels inhibited MCP-1 (225). In murine macrophages, E2 at 10^{-10} and 10^{-9} M (not at 10^{-11} M) suppressed LPS-stimulated expression of MCP-1 mRNA (226). In murine fibroblasts, E2 inhibited serum-stimulated MCP-1 (bell-shaped curve, maximum at 3.7×10^{-12} and 9.9×10^{-10} M, no effect in the middle range of 3.7×10^{-11} M) (91).

Postmenopausal women were randomized to receive either no hormone replacement therapy (HRT) or 1 mg E2 continuously plus sequential progestin over 1 yr (227). MCP-1 levels in serum gradually decreased after 3, 6, and 12 months with HRT. At 18 months, in women discontinuing HRT, MCP-1 levels rose again, but remained lowered in women continuing HRT (227).

At this point the question appears whether or not a similar inhibitory effect of E2 on migration can be observed *in vivo*. In ovariectomized rats, after sc implantation of perforated Teflon cylinders in a model of experimental inflammation, E2 pretreatment (proestrus levels) decreased accumulation of exudate, inflammatory cells, and formation of granulation tissue (228). A local skin inflammation was induced by intradermal injection of olive oil and cholera toxin in several strains of castrated mice (induces a fast inflammatory response independent of T and B cells) (229). Treatment with a single injection of E2 (proestrus to pregnancy levels) given up to 4 d before the induction of inflammation suppressed the inflammatory response, as measured by footpad swelling and documented by the infiltration of PBMCs into the inflamed area (229). In a rat model of ischemia and reperfusion injury of the myocardium by occlusion of the left coronary artery, E2 at proestrus to pregnancy levels lowered myocardial necrosis and myeloperoxidase activity, a marker of polymorphonuclear leukocyte accumulation (122). In the model of balloon injury of the right carotid artery in ovariectomized rats, E2 (proestrus to pregnancy levels) reduced granulocyte and monocyte/macrophage migration to the adventitial and periadventitial domains of injured vessels but increased T cells (230). In the same model, E2 (proestrus to pregnancy levels) inhibited expression of mRNA for cytokine-induced neutrophil chemoattractant-2β and MCP-1 (231).

In carrageenan-induced pleurisy in the ovariectomized rat, prior exposure to E2 leading to proestrus to pregnancy levels induced a decrease in neutrophil migration as shown by cell counting and myeloperoxidase measurement (232). In a model of LPS-induced uveitis in adult male, female, and ovariectomized Lewis rats, cellular infiltration was more marked in male than in female rats, and ovariectomy increased cellular infiltration (233). Treatment with E2 at proestrus to pregnancy levels reduced the cell number in male and ovariectomized female rats, which was accompanied by inhibition of E-selectin in the iris-ciliary body (233).

Apart from effects on MCP-1, these antimigratory effects might depend on changes of chemokines and their receptors. E2 (pregnancy levels) decreased disease-mediating chemokine receptors such as CCR1, CCR2, CXCR2, CCR3, CCR4, CCR5, CCR7, CCR8, and CXCR3 in the central nervous system in a model of EAE (234). E2 treatment of mice for 3 d (pregnancy levels) reduced expression of CCR2 and CXCR3 on murine blood monocytes (235). All above-mentioned studies indicate that E2 at periovulatory/proestrus to pregnancy levels inhibits migration, which was related to a decrease in inflammation.

Interestingly, E2 had completely opposite effects in the uterus, which shows that effects of E2 are specific for the

location. Numbers of macrophages increase in the uterus during proestrus in mice, and macrophages migrate into the uterus after implantation (236). Treatment with E2 (pregnancy levels) for 4 d induced a strong recruitment of macrophages and neutrophils into the mouse uterus (not B lymphocytes) (237). This interesting prochemotactic effect in the uterus might depend on E2-induced up-regulation of RANTES, MCP-1, and IL-8 in endometrium cells (238, 239). The total uterine leukocyte population increased significantly when women received oral E2 (periovulatory levels), which was due to a significant increase in both the uterine natural killer cells and the macrophage populations (not T cells) (240).

A possible explanation for increased migration into the uterus might come from E2 regulation of chemokines in uterine cells. In an *in vitro* organ culture system with human endometrium, E2 at 10^{-9} M (not at 10^{-10} or 10^{-11} M) induces expression of chemokines CXCL10 and/or CXCL11 (blocked by ICI 182,780) (241). In rat uterine epithelial cells, E2 pretreatment at pregnancy levels enhanced stimulated LPS-secretion of CCL20 (blocked by ICI 182,780) but E2 inhibited its constitutive secretion (159). Another explanation might come from the fact that E2 at pregnancy levels increased uterine microvascular endothelial cell monolayer permeability and transiently redistributes interendothelial junction-forming proteins in endothelial cells. Concomitantly, adherens junction proteins are disconnected from the cytoskeleton (blocked by ICI 182,780 and provoked by E2-BSA) (242).

In conclusion, E2 has a strong inhibitory effect on MCP-1 as demonstrated in many nonuterine cell types. In addition, E2 *in vivo* demonstrated a marked inhibitory effect on migration of leukocytes into inflamed areas, particularly neutrophils and monocytes (innate immune cells). E2-mediated inhibition of leukocyte migration was accompanied by less severe inflammation. Interestingly, E2 had completely opposite effects in the uterus, which might be necessary for normal implantation. The reasons for these site-specific effects are presently not known but might be found in regulation of chemotactic factors/receptors. This section demonstrates that E2 at high levels inhibits migration of innate immune cells to the site of inflammation. This phenomenon probably inhibits the immune/inflammatory response to a tissue autoantigen in women with high levels of estrogens.

VII. Estrogens and Angiogenesis

Neoangiogenesis is an important element in inflammation, and the role of E2 on angiogenesis has been reviewed in 1996 (243), 2001 (244), and 2002 (245). The concept appeared that E2 at proestrus to pregnancy levels stimulates angiogenesis, especially collateral vessel formation in ischemic tissues (245). Furthermore, E2 at proestrus to pregnancy levels enhances secretion of vascular endothelial growth factor (VEGF) in different cell types, E2 increases endothelial cell attachment, proliferation, migration, and organization into capillary-like structures *in vitro*, and E2 augments experimental angiogenesis *in vivo* (243). The following text adds to the knowledge by describing new facets on the role of E2 in angiogenesis.

Whereas it is obvious that E2 at periovulatory/proestrus to pregnancy levels enhances VEGF under normoxia conditions *in vitro*, it was demonstrated in bovine retinal vascular endothelial cells that E2 under hypoxia conditions inhibited VEGF (246). Others have demonstrated that E2 preincubation at ovariectomy levels (not at pregnancy levels) increased LPS-stimulated VEGF secretion from the mouse macrophage cell line RAW 264.7 (68). These studies demonstrate the dual behavior of E2 in different microenvironments and concentration ranges.

It is important to mention that endogenous downstream conversion products of E2 can have opposite effects on angiogenesis. Indeed, 2-methoxy-E2 is a potent inhibitor of endothelial cell proliferation and migration as well as angiogenesis *in vitro* (IC_{50}, 1×10^{-8} M; Ref. 247) (248–250). Moreover, when administered orally in mice, it strongly inhibits the neovascularization of solid tumors and suppresses their growth (247–250). Thus, the role of E2 is also regulated by possible conversion to downstream metabolites, which might be largely regulated by environmental factors. These concepts are further developed in *Section X*.

In conclusion, recent studies confirmed the proangiogenic effect of E2, but it was also shown that the role of E2 is determined by environmental factors such as oxygen tension, E2 concentration, and endogenous E2 conversion to metabolites.

VIII. Estrogens Modulate Inflammatory Processes: Diseases and Models

At this point, the reader might have obtained the impression that E2 at periovulatory/proestrus to pregnancy levels has most often antiinflammatory activities by inhibiting many proinflammatory pathways of innate immunity, adaptive immunity, and inflammatory tissue responses. Arguments in favor of a proinflammatory response are the antiapoptotic effects on immune cells, promotion of neoangiogenesis, and stimulation of B cells, which has been delineated to be an unfavorable factor in B cell-driven diseases such as SLE. In 1989, Holmdahl *et al*. (251) stated that "estrogens stimulate SLE by enhancing B cell immunity and suppressing T cell-mediated inflammatory reactions (arthritis, thyroiditis, experimental allergic neuritis, and experimental autoimmune encephalomyelitis)." The question appears to be whether this holds true considering experimental diseases and human diseases.

A. Arthritis

In arthritis, effects of estrogens have been extensively studied, and several important reviews have been presented (4, 251–255). When investigating effects of estrogens on arthritis, three important aspects need to be mentioned primarily.

First, in experimental studies in arthritic animals, most often E2 at proestrus to pregnancy levels has been used. Second, when immune cells of animals or patients were investigated, most often E2 at periovulatory/proestrus to pregnancy levels was used as the sole estrogen (no combi-

nations were applied). Third, and in contrast to items one and two, in observational studies in humans the focus is not solely on E2; *e.g.*, in pregnancy or during the reproductive period, not only E2 increases, and in the postmenopausal phase, not only E2 decreases. Furthermore, in experimental studies in women or men with arthritis, most often mixtures of estrogens have been applied (E2 was only a minor part, and often a gestagen plays an additional role; Ref. 256). Additionally, E2 serum levels were not markedly increased by these therapies. For example, HRT in postmenopausal women increases E2 serum levels from approximately 3×10^{-11} to 1.5×10^{-10} M (early follicular levels) (126), which is not a strong increase in relation to periovulatory and pregnancy levels (1×10^{-9} to 1×10^{-7} M). Thus, the type of estrogen, coadministration of a gestagen, and the concentration of estrogens were largely different in animal experiments compared with studies in humans.

1. Experimental arthritis in animals. Ovariectomy of female DBA/1 mice increased arthritis induced with type II collagen (257). Ovariectomy-induced loss of endogenous estrogens and inflammation contributed concertedly to osteoporosis in this model (220). In this model, E2 treatment before immunization (proestrus levels) decreased levels of IgG anti-type II collagen antibodies and T cell proliferation, whereas levels of IgM antibodies are increased (257), and it is independent of the thymus (258). This was similar in a rat model of arthritis, which was also independent of the thymus and the CD8+ T cell subpopulation (259, 260). Other groups confirmed the positive effects of E2 at proestrus to pregnancy levels in experimental arthritis (35, 261–264), and the favorable effect of E2 was also confirmed in male animals with experimental arthritis (265). Collagen type II-induced arthritis can also be elicited by transfer of collagen type II-specific monoclonal antibodies, and E2 treatment (proestrus levels) starting before immunization decreased the severity of arthritis (266). Furthermore, E3 treatment leading to pregnancy levels has an even stronger ameliorating effect on experimental arthritis than E2 (267).

Treatment with the ERα/ERβ antagonist ICI 182,780 triggered an earlier onset of arthritis during the period when the proestrus cycle was blocked (268). The arthritis-modulating effect of ICI 182,780 was even obtained at doses that were insufficient to block proestrus cycling (268), which indicates that relatively low levels of E2 (diestrus levels) can induce an ameliorating effect. Interestingly, a highly specific agonist of the ERβ (called ERB-041) had a strong beneficial effect in the Lewis rat adjuvant-induced arthritis model (269).

In addition, the endogenous metabolite of E2, 2-methoxy-E2, also showed an antiarthritic effect in the collagen type II model in DBA/1 mice but it did not show a feminizing effect on the reproductive tract or inhibition of leukocyte development in hematopoietic or lymphoid organs (270). Neither the T cell-dependent delayed type hypersensitivity nor the B cell-dependent production of anti-type II collagen antibodies was suppressed by 2-methoxy-E2 (270). This indicates that 2-methoxy-E2 exerts its effects directly in inflamed tissue by affecting bystander inflammation.

Female mice similar to women are sheltered from RA flares during pregnancy (see *Section XI*). Interestingly, an E2 maintenance dose (pregnancy levels) during a short period immediately after parturition protects the mouse from a postpartum flare of the disease, but progesterone and hydrocortisone had no clinical effect (271). A similar postpartum effect of E2 was confirmed by others (272).

In another model of joint disease independent of T cells and B cells and most probably dependent on monocyte/neutrophil invasion, da Silva *et al.* (273) demonstrated that female mice showed a higher ability than males to degrade cartilage in the cotton-pellet cartilage implant model, which leads to granuloma formation. Gonadectomy resulted in a significant acceleration of cartilage damage in both sexes, which was reverted by E2 replacement (pregnancy levels) (273). This demonstrates a biphasic effect where low to normal levels of E2 exerted an unfavorable effect, whereas pregnancy levels inhibited granuloma formation. It is expected that the granuloma mainly exists of neutrophils and monocytes/macrophages, which are suppressed by E2 at high levels.

Another model makes use of a very fast plasma extravasation response into the synovial cavity within minutes and hours (274). The magnitude of bradykinin-induced synovial plasma extravasation is markedly less in female rats. Ovariectomy of female rats increased bradykinin-induced synovial plasma extravasation, and administration of E2 (proestrus to pregnancy levels) to ovariectomized female rats reconstituted the female phenotype (274).

In conclusion, most studies reported a beneficial effect of E2 at proestrus to pregnancy levels in experimental arthritis.

2. Arthritis in humans. Female sex has a deteriorating effect on RA disease activity, but the menopausal state with its lower steroid hormone levels is responsible for the major part of the differences in outcome between men and women (275). RA women had a higher disease activity score and health assessment questionnaire scores than RA men (276). RA women below 50 yr of age at study entry had milder disease than older women (close to that of men). At 2-yr follow-up, RA women still had higher disease activity scores and health assessment questionnaire scores compared with men, who had achieved remission in a higher frequency. A radiology damage score showed no sex difference either at study entry or after 2 yr (276).

Not only the biological sex but also the number of gestations had an influence on arthritis. An interesting study was carried out by Jorgensen *et al.* (277), who demonstrated that having more than three children increased the risk of developing severe disease 4.8-fold when adjusted for age and use of OC. One might speculate that higher fertility, which is most probably also dependent on higher estrogens levels, is linked to a more autoaggressive immune response. However, the increased risk may also be related to the higher probability of immunization with allogenic or semiallogenic material and, thus, repeated triggering of clonal expansion of B cells (278). Nevertheless, these results point to a possible importance of estrogen levels in patients with RA.

The question appears to be whether or not serum or synovial fluid levels of estrogens are different in healthy subjects compared with patients with RA. Most studies indicate that serum levels of E2 and E1 are similar in patients with RA

compared with controls (Table 6 and Ref. 279). In addition, 12 wk of anti- TNF antibody treatment did not change serum levels of E2 (280). One study in premenopausal women with RA demonstrated higher E2 serum levels in patients with than without anticardiolipin antibodies (3.6×10^{-10} *vs.* 2.0×10^{-10} M), but the difference was marginal (281). In male RA patients, E2 levels were higher compared with healthy men, and E2 levels were positively correlated with indices of inflammation (Table 6) (282). Interestingly, this relatively uniform situation is largely different for levels of androgens such as dehydroepiandrosterone sulfate and testosterone, which are markedly lower in inflammatory diseases. This loss of androgens was thought to play an unfavorable role in chronic inflammatory diseases (not subject of this review) (summarized in Ref. 5).

Urinary levels of estrogens represent a time integral of estrogen production and might, thus, be more relevant than single measurements of serum levels in time. One early study in the 1950s detected lower levels of urinary estrogens in patients with RA compared with controls, but the exact type of estrogen is not given (283). Recently, one study reported in patients with RA high levels of 16α-hydroxylated estrogens and, in addition, low levels of 2-hydroxy-estrogens (284). The 16-hydroxylated estrogens serve as mitogenic and proliferative estrogens, and 2-hydroxylated forms might play an opposite role due to conversion to *O*-methylated estrogens (*Section X*).

Importantly, a similar increase of 4- and 16α-hydroxylated forms was detected in the synovial fluid of patients with RA compared with trauma controls (Table 6) (285). This later study also found an increased molar ratio of estrogens to androgens in synovial fluid of patients with RA compared with trauma controls. In conclusion, whereas serum levels of estrogens in patients with RA are similar to levels in healthy controls, local levels of estrogens in inflamed synovial tissue and synovial fluid are markedly higher, particularly in relation to androgens. In addition, conversion of E2 to downstream metabolites provides mitogenic estrogens (see *Section X*). Both factors must be considered as proinflammatory phenomena in RA.

Another important determining factor of arthritis might be a polymorphism in the ER. For instance, one study demonstrated an influence of an ERα polymorphism on the course of RA in Japanese patients: female patients with the ERα genotype PPxx (homozygote of Px) tended to have developed RA at a younger age, whereas those with PPXX and ppxx (lack of Px haplotype) developed RA at an older age (no association in men) (293). Others studied ER microsatellites in Japanese patients with RA (294). The ER genotype was classified according to number of dinucleotide (thymine-adenine) repeats between 10 and 27. The frequency of allele 14 (14 repeats) was significantly increased in patients *vs.* controls but not with disease severity (294). Presently, the functional consequence of these genetic variations is not fully understood. The distribution of ERα and ERβ in patients with RA was already reported in *Section II* (an increase of ERβ+ cells in the synovial tissue was mentioned).

Effects of OC or HRT on the risk to develop arthritis and on the course of arthritis are extensively demonstrated in *Section XII*, and the conclusions are shortly summarized here. Almost all studies in women with *vs.* without OC demonstrated no increased risk to develop RA. In addition, OC use was not associated with changes in the disease course in patients with RA. During OC administration, serum levels of estrogens and progestins only increase to a small extent, and, thus, no change of risk and disease course is to be expected. Prior HRT did not increase the risk to develop RA. HRT or sole ERT markedly improved bone mineral density and reduced bone resorption markers in RA. In addition, HRT had marginal beneficial effects on the articular index, pain score, and morning stiffness, particularly when patients with higher E2 treatment levels were separately analyzed (increase of serum E2 from 5×10^{-11} to 2×10^{-10} M) (295). Because women under HRT do not have serum levels of E2 in the periovulatory or pregnancy range, a strong E2 effect as in animal models is not to be expected. In addition, HRT also includes therapy with gestagens, which might contribute to the observed effects by neutralizing or supporting effects of estrogens.

TABLE 6. E2 serum levels in patients with RA

Patients	Hormone and level	Ref.
Serum levels		
Pre- and postmenopausal women with RA	E2 in premenopausal women with RA, 1.8×10^{-10} M E2 in postmenopausal women with RA, 9×10^{-11} M (both similar compared to controls)	286
Premenopausal RA women	No significant differences *vs.* healthy controls	287
Postmenopausal RA female siblings	E2, 5×10^{-11} M (similar in the unaffected sibling and controls)	288
Male patients with RA	E2, 7.2×10^{-11} M (similar compared to controls)	289
Male patients with RA	E2, 6.8×10^{-11} M (similar compared to controls); trend toward increased FSH and LH	290
Male patients with RA	E2, 9.3×10^{-11} M *vs.* healthy controls 7.4×10^{-11} M (higher compared to controls) E1, 1.4×10^{-10} M *vs.* healthy controls 2.2×10^{-10} M	282
Female patients with RA	E1, 1.8×10^{-11} M in RA *vs.* 4.9×10^{-11} M in controls	291
Tissue levels		
Synovial fluid levels in patients with RA compared to trauma controls	Free E2, 2.5×10^{-8} M	285
Level in the superfusate of synovial tissue	Free E2, 1.8×10^{-10} M Free E3, 7.5×10^{-10} M	292

B. Systemic lupus erythematosus

This subject has been reviewed several times by different groups (296–301). The general concept is summarized as follows:

The prevalence of SLE is far higher in females than in males, particularly during the reproductive phase. Animal models demonstrated that E2 treatment accelerates the disease process (3, 302, 303). However, it is important to mention that not all lupus pathologies are accelerated in a similar way within the same animal (304). Pregnancy doses of E2 accelerate immune-complex glomerulonephritis but ameliorate focal sialadenitis, renal vasculitis, and periarticular inflammation (304). One can concur with the authors that E2 stimulates antibody production and immune complex phenomena but inhibits T cell-mediated organ pathologies.

In the B cell compartment, E2 is an immune stimulator that affect maturation, selection, and antibody secretion (see also *Section III.B*) (299, 301). Its impact may be based on the capacity to allow autoreactive B cells to escape the normal mechanisms of tolerance and to accumulate in sufficient numbers to cause clinically apparent disease (36, 299, 301). This outstanding research has demonstrated that E2 leads to the survival and activation of autoreactive B cells eliciting T cell-independent autoreactive marginal zone B cells (36, 299, 301). BALB/c mouse transgenic for the heavy chain of an anti-double-stranded DNA antibody are prone to develop lupus after exposure to exogenous sex hormones (301). These are very good arguments that an E2-enriched environment can stimulate a B cell-dependent autoimmune milieu.

In addition, important studies supported a direct effect of E2 on SLE immune cells, which is most probably unfavorable during the course of the disease. E2 at 10^{-10} M to 10^{-8} M (IC_{50}, 10^{-9} M) enhanced production of IgG anti-double-stranded DNA antibodies as well as total IgG in PBMCs from SLE patients (29). E2 also enhanced total IgG, but not anti-double-stranded DNA, production in the PBMCs of normal donors. E2 increased the B cell-stimulating IL-10 production by monocytes (29). In cultured T cells from female SLE patients, E2 at periovulatory to pregnancy levels increased calcineurin, which was not observed in T cells from control women (305). Calcineurin expression in T cells from patients with vasculitis and RA taking similar medications was unaffected by E2 (305). In peripheral blood T cells from patients with SLE, 2-fluoro-E2 at 10^{-7} M showed a significant increase in the amount of CD40 ligand (CD40L) on the cell surface (blocked by ICI 182,780), which was not observed on the surface of T cells in normal women (306). These are excellent arguments that E2 not only generates an environment to propagate autoimmune B cells, but also supports several key pathways of already generated autoimmune B cells. It seems that these effects are particularly stimulated at periovulatory to pregnancy levels of E2.

At this point, the question appears to be whether or not estrogen serum or urinary levels of patients with SLE are altered (Table 7). The conclusion is that estrogen serum levels are not different in healthy controls compared with SLE patients, which is different with respect to urinary levels of estrogens. In early studies of the 1980s, increased levels of 16α-hydroxylated estrogens were detected in the urine of patients with SLE compared with healthy controls (307, 308). Recently, these studies were confirmed in patients with SLE and, in addition, low levels of 2-hydroxy-estrogens were detected (284). Because 16α-hydroxylated estrogens serve as mitogenic and proliferative estrogens and 2-hydroxylated forms might play an opposite role, this altered hormone pattern indicates a proinflammatory milieu.

Effects of OC or HRT on the risk of developing SLE and on the course of SLE are demonstrated in *Section XII*. In summary, all studies in women with *vs.* without OC demonstrated no increased risk to develop SLE, but HRT in postmenopausal women seems to increase the risk of developing SLE. OC use was not associated with changes in the disease course in premenopausal women with SLE, but HRT increased the risk of mild flares in postmenopausal patients. This information indicates that the positive effect of estrogens on B cells does not play a role in premenopausal women with normal menstrual cycles, but estrogens mildly stimulate SLE in women with postmenopausal levels.

C. Brain inflammation

The role of estrogens in chronic inflammatory brain diseases such as EAE and MS was reviewed in 2004 (311). This review emphasized the antiinflammatory effects of E2 and E3. The subsequent text adds new information to this earlier discussion.

As early as 1994, it was reported that ovariectomy led to an earlier EAE onset (267). Long-term treatment with proestrus levels of E2 delayed onset of disease. In contrast, treatment with E3 (achieving proestrus levels) delayed the disease onset for a longer time. Five times higher doses of E2, compared with those seen during pregnancy, were required to obtain similar effects as the low E3 dose (267). Although E3-treated mice had higher levels of serum antibodies of the IgG1 specific for myelin basic protein, E3 reduced the severity of EAE, and antigen-specific T lymphocyte produced higher amounts of IL-10 (37). E2 at pregnancy levels decreased EAE incidence, which was not demonstrated for lower serum levels ($<1 \times 10^{-9}$ M) (312). Higher E2 levels were accompanied by lower ratios of IFN-γ/IL-10. This was confirmed in several other studies with E2 or E3 at proestrus to pregnancy levels (312–315). Others demonstrated that E2 suppressed EAE in mice deficient in IL-4, IL-10, and IFN-γ, and there was a profound decrease in the frequency of TNF-

TABLE 7. E2 serum levels in patients with SLE

Patients	Hormone and serum level	Ref.
Boys with SLE	E2, 2.5×10^{-11} M (similar compared to controls)	309
Girls with SLE	E2, 2×10^{-10} M (similar compared to controls)	309
Premenopausal SLE women	E2, $5 \times 10^{-10} \pm 3 \times 10^{-10}$ M (similar compared to controls)	310
Male SLE patients	E2, 9.4×10^{-11} M (similar compared to controls); trend toward increased FSH and LH	290

producing cells in the central nervous system and the periphery (316).

Additionally, E2 treatment (pregnancy levels) drastically decreased the recruitment of total inflammatory cells as well as TNF-positive macrophages and T cells into the central nervous system at disease onset (317). E2 treatment (pregnancy levels) decreased frequency of CD11b+/CD11c+ dendritic cells in the brain of EAE mice (69). E2 decreased the frequency of CD11c+/CD8α+ dendritic cells in the spleen in mice with EAE, which produced TNF and IFN-γ. E2 pretreatment (pregnancy levels) was found to suppress the ability of cultured dendritic cells bearing a mature phenotype to present antigen to specific T cells. E2 decreased TNF, IFN-γ, and IL-12 production in mature dendritic cells (shift toward production of Th2 cytokines IL-4 and IL-10) (69).

Transfer of E2-exposed (3.7×10^{-8} M) splenic dendritic cells from Lewis rats obtained on d 12 after immunization with myelin basic protein prevented the expansion of CD4+ T cells and increased the proportions of regulatory T cells producing IL-10 and CD4+CD28- suppressor T cells, accompanied with increased IL-10 and IFN-γ, and reduced TNF production (70). E2 up-regulated the expression of indoleamine 2,3-dioxygenase, which promotes tolerogenic properties of dendritic cells (70).

Importantly, the protective effect of E2 in animals with EAE was abrogated in ERα-deficient mice but not in ERβ-deficient mice (318, 319). This was supported in a brain injury model of stroke (320). Additionally, treatment of mice with EAE, with an ERα agonist (10 mg propyl pyrazole triol/kg body weight·d) before the induction of disease resulted in suppression of clinical symptoms of disease, whereas treatment with an ERβ-selective agonist (WAY-202041, 10 mg/kg body weight·d) had no effect (321). However, others demonstrated that ERα was dispensable for the E2-induced amelioration in an EAE model (322).

In a recent important study in patients with relapsing remitting MS, the favorable effects of E3 (pregnancy levels, 8 mg/d for 6 months) were demonstrated (47). E3 treatment decreased delayed type hypersensitivity responses to tetanus, IFN-γ levels in PBMCs, and the number and volume of inflammatory lesions on monthly cerebral magnetic resonance images (47). Oral E3 treatment in patients with MS (pregnancy levels) led to increased levels of IL-5 and IL-10 and decreased TNF in stimulated PBMCs (323). These changes in cytokines correlated with reductions of inflammatory lesions on magnetic resonance imaging in relapsing remitting MS. The increase in IL-5 was primarily due to an increase in CD4+ and CD8+ T cells, and the increase in IL-10 was primarily due to an increase in CD64+ monocytes/macrophages, whereas the decrease in TNF was primarily due to a decrease in CD8+ T cells (323). In summary, almost all studies indicate that E2 or E3 at periovulatory/proestrus to pregnancy levels ameliorates EAE or relapsing remitting MS. The question appears to be whether or not this is similar in other inflammatory models of brain inflammation.

In an inflammatory/injury model of the brain, E2 treatment (pregnancy levels) decreased IL-1β expression in the olfactory bulb in young adult females but paradoxically enhanced its expression in reproductive senescent females (324). In an LPS model, systemic administration of E2 (proestrus levels) 6 h before LPS injection prevents the activation of microglia and the recruitment of peripheral monocytes (325). This effect occurs by limiting the expression of neuroinflammatory mediators, such as the MMP-9, MCP-1, CXCL2, TNF, lysosomal enzymes, and complement C3 receptor (via ERα and not via ERβ) (325, 326).

Using the APP23 mice, an animal model of Alzheimer's disease with chronic neuroinflammation, ovariectomy increased microglia activation at β-amyloid deposits (326). Long-term administration of E2 at proestrus to pregnancy levels reverted the effects of ovariectomy and decreased microglia reactivity (326). Rats receiving transient middle cerebral artery occlusion demonstrated substantial apoptosis and inflammatory responses, including IκB phosphorylation, NF-κB activation, and iNOS overexpression (110). In ovariectomized rats, E2 treatment leading to proestrus levels (one injection 2 h before occlusion) produced strong protective effects by reducing infarct volume, neuronal apoptosis, and inflammatory responses (110). Thus, also in other models of brain inflammation, E2 at proestrus to pregnancy levels exerts beneficial effects. The question appears to be how this translates into brain cell cultures.

In primary cultures of rat microglia, E2 at proestrus to pregnancy levels (maximum 10^{-9} M, minimum 10^{-11} M) inhibited LPS-induced production of iNOS, prostaglandin E2, and MMP-9 (blocked by ICI 182,780) (81). E2 at pregnancy levels inhibited the basal and IL-1β-mediated expression of ICAM-1 and NF-κB activation in cultured brain endothelial cells. Authors concluded that decreased expression of adhesion molecules may account for the capacity of E2 to reduce adhesion of leukocytes in cerebral endothelium *in vivo* (118). E2 administration at 1×10^{-7} M (only one concentration) to serum-deprived cortical neurons of rats induced neuroprotection (327). In rat primary cortical neurons after glutamate exposure, pretreatment with 10^{-8} M E2 prevented apoptosis (210). E2 decreased the neurotoxicity of gp120 in mixed neuronal/glial cultures (328).

In conclusion, E2 and E3 at periovulatory/proestrus to pregnancy levels demonstrated many beneficial effects in MS, models of brain inflammation, and cell culture assays. Most of the models are T cell mediated or innate immunity mediated. As such, an important similarity to experimental arthritis exists, where E2 and E3 at higher levels also exerts favorable antiinflammatory effects. The situation might be opposite in brain inflammation dominated by the B cell.

D. Thyroiditis

From the above-mentioned effects of E2 on SLE, it seems obvious that other B cell-dependent diseases are stimulated by E2 in a very similar way. This might also apply for thyroiditis, which appears much more frequent in women in the reproductive years compared with men. However, in thyroiditis, E2 effects have not been studied in great detail.

Interestingly, ERβ is a candidate locus in a linkage analysis study in 45 multiplex families with thyroiditis, but the functional significance remains unclear (329). In patients already affected by thyroiditis, estrogen use was associated with a lower rate of hyperthyroidism [relative risk (RR), 0.169; 95% confidence interval (CI), 0.06–0.52], whereas having been

pregnant was associated with a higher RR for hyperthyroidism (RR, 6.88; 95% CI, 1.50–30.96) (330). In a fibrotic form of thyroiditis with little inflammation, treatment with tamoxifen was demonstrated to have favorable effects, which might demonstrate the growth-promoting effects of E2 on fibroblasts (331). In experimental autoimmune thyroiditis, administration of E2 at pregnancy levels increased antibody titers in male but not in female mice and in male castrated mice (332). Ovariectomized female animals have a higher incidence of experimental thyroiditis, and testosterone reduces the incidence (333). Female PVG/c rats are more susceptible to the induction of autoimmune thyroiditis initiated by thymectomy and irradiation than similarly treated males (334). Most of these studies demonstrate a supportive effect of E2 on thyroiditis.

Because 2-methoxy-E2 appeared to be an antiinflammatory endogenous metabolite of E2 in other diseases, it might also be an interesting therapeutic principle in thyroiditis. One study has demonstrated that 2-methoxy-E2 decreased the viability of thyroid follicular cells (335). Flow cytometric analysis showed that 2-methoxy-E2 halted cell proliferation by arresting the cells in the G_2/M cell-cycle phase, and prolonged exposure to 2-methoxy-E2 led to apoptosis (not blocked by ER antagonist) (335).

E. Intestinal and liver inflammation

1. Intestinal inflammation. Irritable bowel syndrome, a situation with low-grade intestinal inflammation, is more prevalent in women than in men (336), but we do not know whether E2 directly affects intestinal inflammation in these women. Ovariectomized rats treated with E2 (diestrus levels) exhibited visceral hypersensitivity after partial restraint stress (337). The authors concluded that stress-induced visceral hypersensitivity in female rats is E2-dependent and mediated through neurokinin 1 receptor activation (337). Thus, it seems that pain pathways are up-regulated in women, a subject that is further developed in *Section XIII.B*. The role of E2 in human inflammatory bowel diseases has not been studied.

In dinitrobenzene sulfonic acid colitis, E2 (pregnancy levels), but not E2 plus tamoxifen or 17α-E2, reduced macroscopic and histological scores, myeloperoxidase activity, malondialdehyde levels, and expression of ICAM-1, IFN-γ, and IL-13 mRNA compared with placebo (123). HLA-B27 transgenic rats with chronic inflammation were treated with ethinyl E2 (proestrus levels) for 5 d (338). E2 treatment improved stool scores, histological scores of the degree of ulceration, inflammatory cell infiltration, fibrosis, and lesion depth of the colon, local myeloperoxidase levels, and mast cell proteases 1, 3, and 4 mRNA (338). In the same model of inflammatory bowel disease, a highly selective ERβ agonist (ERB-041) has a good beneficial effect (269), which was confirmed using another ERβ agonist (105). In contrast, E2 increased the macroscopic and histological scores compared with placebo in mice with acute dextrane sodium sulfate colitis (123). In this latter model, bacteria influence the disease severity and E2 might up-regulate LPS-induced antiinfectious proinflammatory pathways (see also *Section VIII.L*).

These results demonstrate antiinflammatory and proinflammatory effects of E2 in three different models of experimental colitis. The reasons for these discrepant results are presently not known but may be related to the different immune cells responsible or the different triggering factors.

2. Liver inflammation. In a model of hepatic fibrosis with a single dose of dimethylnitrosamine, the fibrotic response of the male liver was stronger than that of the female liver (339). In the male dimethylnitrosamine model, E2 (proestrus or pregnancy levels) reduced hepatic mRNA for type I and III procollagens, TIMP-1, deposition of type I and III collagen protein, total hepatic collagen, and malondialdehyde, a product of lipid peroxidation (339). Ovariectomy in the female dimethylnitrosamine model had a fibrogenic effect. In rat hepatic stellate, E2 at high pregnancy levels reduced cell number, type I collagen production, and α-smooth muscle actin expression (339).

In tetrachloric acid-induced liver fibrosis, E2 treatment for 8 wk (proestrus levels) reduced aspartate aminotransferase, alanine aminotransferase, hyaluronic acid, and type IV collagen in sera; suppressed hepatic collagen content; decreased the areas of hepatic stellate cells positive for α-smooth muscle actin; and lowered the synthesis of hepatic type I collagen significantly in both sexes (blocked by tamoxifen) (340).

In a liver ischemia/reperfusion injury model, E2 treatment (pregnancy levels) significantly reduced liver necrosis, disintegration of hepatic cords, neutrophil infiltration, and serum TNF (blocked by ICI 182,780) (341). Female C57BL/6 mice are protected to a much greater extent from ischemia/reperfusion injury to the liver than male mice (342). Pretreatment with E2 (pregnancy levels) of C57BL/6 mice subjected to 45 min of warm ischemia decreased liver injury and apoptosis in male mice (partially reversed by ICI 182,780) (342). E2 down-regulated c-Jun N-terminal kinase and p38α MAPK activities, whereas ischemia/reperfusion injury promoted p38β MAPK and ERK 1/2 activation (342). These results suggest that the cytoprotective effects of E2 are associated with selective modulation of MAPK.

Ovariectomized mice fed an atherogenic diet had increased hepatic levels of active NF-κB and numerous up-regulated inflammatory genes, including VCAM-1, TNF, and RANTES (343). Treatment with ethinyl E2 (proestrus levels) strongly blocked induction of these genes but had no effect on their basal expression levels (blocked by ICI 182,780) (343). In hepatocytes, the oxidative stress-induced activation of NF-κB was inhibited by E2 at pregnancy levels through the attenuation of hepatocyte oxidative bursts, which led to decreased extracellular levels of lactate dehydrogenase (344). IL-1β treatment induced the expression of approximately 75 genes in the liver of ovariectomized mice (345). Ethinyl E2 pretreatment for 5 d (pregnancy levels) reduced the IL-1β induction (after 1 h) of approximately one third of these genes (proinflammatory in nature). ERα was required for this inhibitory activity, because E2 inhibition of IL-1β-stimulated gene expression occurred in ERβ knockout mice but not in ERα knockout mice (345).

Above-mentioned studies obviously demonstrate an antiinflammatory role of E2 on inflammatory models of liver disease including fibrosis models. Interestingly, quite opposite effects of E2 were observed in the enteral ethanol feeding

model. Ethanol increased serum alanine transaminase levels, liver weight, fat accumulation, and infiltrating leukocytes, which was blocked by ovariectomy and reversed by E2 replacement (proestrus levels) (346). Blood endotoxin and Kupffer cell TNF were increased by ethanol, which was blocked in ovariectomized rats and elevated by estrogen replacement (346). This indicates that E2 at proestrus levels might sensitize Kupffer cells to LPS. Similarly, in rats given E3 (proestrus to pregnancy levels), authors found that E3 treatment *in vivo* sensitizes Kupffer cells to LPS via mechanisms dependent on increases in CD14 (347). This is most likely due to elevated portal blood LPS caused by increased gut permeability. This largely increased mortality and liver inflammation (347). Toremifene (ER antagonist) treatment did not affect ethanol-increased steatosis, but significantly reduced inflammation and necrosis (348). Ethanol increased the expression of CD14 and TNF mRNA and also the production of TNF by isolated Kupffer cells, but toremifene had no significant counteracting effect. However, toremifene significantly alleviated both ethanol induction of the prooxidant enzyme CYP2E1 and ethanol reduction of the oxidant-protective enzyme selenium-dependent-glutathione peroxidase (348).

In conclusion, these experiments in liver inflammation models demonstrate that E2 has antifibrotic and antiinflammatory effects, which is similar in renal inflammation (see next section). However, it also demonstrates that Kupffer cells in the presence of ethanol are sensitized to LPS, which would be an unfavorable signal in LPS-dependent liver inflammation.

F. Renal inflammation

It is well known that men with chronic renal failure show more rapid decline in renal function with time than do women (349). In salt-sensitive rats (Dahl), ovariectomy increased the severity of glomerulosclerosis and cortical tubulointerstitial fibrosis after 12 months (350), which was attenuated by E2 replacement (proestrus levels). Ovariectomy was also associated with increased deposition and expression of laminin and TGF-β1 and decreased activity of cortical MMP-9, and E2 replacement (proestrus levels) opposed these effects in the glomerulum (350).

In a model of chronic allograft nephropathy (351), animals were investigated 24 wk after transplantation for histological and immunohistological studies as well as for molecular analysis. E2 treatment (proestrus levels) improved graft function, reduced glomerulosclerosis, and diminished cellular infiltration. Renal allograft damage paralleled intragraft mRNA expression of TGF-β1, which was reduced in E2-treated animals (351). In mesangial cells, selective ER modulators such as tamoxifen and LY-117018 (an analog of raloxifene) suppressed mesangial type IV and type I collagen protein synthesis (10^{-7} to 10^{-10} M, not 10^{-11} M) with a potency identical to that of E2 (tested at 10^{-9} M, periovulatory level) (352).

Because TGF-β1 stimulates extracellular matrix of the mesangium in several renal diseases, the beneficial influence of female gender on the progression of chronic renal diseases may be explained by an inhibitory effect of E2 on secretion of TGF-β1 and on generation of extracellular matrix (353). In transgenic mice expressing active TGF-β1 in the glomerulum, E2 treatment (pregnancy levels) attenuated interstitial fibrosis (353), which was confirmed in another model. In transgenic mice, which overexpress TGF-β1 exclusively in the liver (with high circulating concentrations of TGF-β1) and develop proteinuria and progressive glomerulosclerosis, E2 at pregnancy levels attenuated mesangial expansion with less glomerular deposition of type I collagen, type IV collagen, and TIMP-2. E2 also prevented proteinuria (349). However, TGF-β levels were higher in E2-treated mice compared with placebo-treated mice, which underlines that TGF-β1 is not the primary target of E2 (349). The primary target is most probably a direct influence of E2 on protein kinase CK2 (349). This confirms the stimulatory influence of E2 on TGF-β, which was also demonstrated in several other cell types (*Section IV.D*), but this E2-stimulated increase of TGF-β is not decisive for disease outcome in these glomerulosclerosis models.

In a model of chronic spontaneous inflammation in the MRL lpr/lpr mouse, pregnancy doses of E2 accelerated immune-complex glomerulonephritis but ameliorated renal vasculitis and periarticular inflammation (304). This demonstrates that the same hormone can exert quite different effects depending on the major pathways switched on during chronic inflammation.

In conclusion, male gender is associated with a more rapid progression of chronic renal disease. In various experimental models of renal injury or inflammation, manipulation of the hormonal milieu can replicate the effects of gender on the course of renal disease. Although TGF-β is an important factor in glomerulosclerosis, the direct stimulating effect of E2 on TGF-β does not explain the positive effects of E2 in this disease. Positive effects of E2 are mediated by a direct antifibrotic and antiinflammatory activity, which has been described in liver fibrosis in a similar way (*Section VIII.E*).

G. Inflammation of the lung and heart

Ovariectomy enhanced the carrageenan-induced degree of pleural exudation and polymorphonuclear leukocyte migration, lung myeloperoxidase activity, lipid peroxidation, and activity/expression of P-selectin, ICAM-1, nitrotyrosine, poly (ADP-ribose) synthase, and iNOS in rats subjected to carrageenan-induced pleurisy (124). The increase of iNOS activity was correlated with a marked enhancement in the production of TNF and IL-1β. E2 replacement (pregnancy level) 1 h before challenge counteracted lung inflammation (124). This was confirmed by another group in the same model (354): Ovariectomy amplified inflammation, whereas E2 (proestrus levels) attenuated inflammation, tissue damage associated with pleurisy, polymorphonuclear cell infiltration, and cyclooxygenase-2 and iNOS expression in inflamed areas (354).

LPS-induced lung injury was increased in male and ovariectomized mice compared with normal female mice (355). Bronchoalveolar lavage fluids demonstrated enhanced levels of IL-1β in ovariectomized and male mice. A similar increase for levels of IL-6 and ICAM-1 was observed in lung homog-

enates from ovariectomized and male mice compared with intact female mice (355). E2 treatment of ovariectomized mice (pregnancy levels, for 6 h) reduced numbers of polymorphonuclear cells, which was accompanied by reduced IL-1β, IL-6, and ICAM-1 levels (355).

In rats, myocardial ischemia plus reperfusion produced marked myocardial necrosis, increased serum creatinine phosphokinase activity, enhanced cardiac myeloperoxidase activity, increased macrophage and serum TNF, and increased ICAM-1 staining in the myocardium (122). E2 (proestrus to pregnancy levels), injected im 5 min after induction of myocardial ischemia-reperfusion injury, lowered myocardial necrosis and myeloperoxidase activity, reduced serum and macrophage TNF, and decreased serum creatinine phosphokinase activity and staining of ICAM-1 in the injured myocardium (122). E2 added *in vitro* to peritoneal macrophages collected from untreated rats subjected to myocardial ischemia-reperfusion injury reduced TNF production (122).

In conclusion, in all available inflammatory models of the lung and heart, E2 was demonstrated to have an antiinflammatory effect at proestrus to pregnancy levels.

H. Eye inflammation

Concerning the role of E2 in Sjögren syndrome, this was reviewed in 1997 (356). This review reports about the proinflammatory role of estrogens in Sjögren syndrome, and it also mentioned the antiinflammatory role of androgens.

In a model of endotoxin-induced uveitis in adult male, female, and ovariectomized Lewis rats, cellular infiltration was more marked in male than in female rats, and ovariectomy increased cellular infiltration (233). Treatment with E2 (proestrus to pregnancy levels) reduced the cell number in male and ovariectomized female rats, and E2 inhibited E-selectin and IL-6 expression in the iris-ciliary body (233).

Lewis rats were implanted with E2 pellets (pregnancy levels), and 4 d later were immunized with the retinal antigen interphotoreceptor retinoid binding protein peptide (357). However, E2 therapy in female and male rats did not ameliorate this autoimmune form of uveitis (357). In retinal soluble antigen-induced experimental autoimmune uveoretinitis, incorporated tamoxifen into polyethylene glycol-coated nanoparticles injected 1–2 d before expected onset of the disease resulted in significant inhibition of inflammation, which was reversed by coinjection of E2 (358). Diminished infiltration by major histocompatibility complex class II-positive inflammatory cells and low expression of TNF, IL-1β, and RANTES mRNA were noted in eyes treated with the tamoxifen nanoparticles (358). Interestingly, autoimmune uveitis was related to the menstrual cycle because disease severity increased in the premenstrual phase (359). In women with uveitis, disease activity decreased after the first pregnancy trimester but flared in the early postpartum period (360).

I. Inflammation in the pancreas

Type 2 diabetes is characterized by loss of β-cell mass and concomitant deposition of amyloid derived from islet amyloid polypeptide. In human islet amyloid polypeptide transgenic mice, only the males routinely develop diabetes (361). E2 treatment (pregnancy levels) of young prediabetic transgenic mice blocked the progression to hyperglycemia with normal islet morphology with no apparent deposition of islet amyloid. E2 treatment (pregnancy levels) of 1-yr-old diabetic males rapidly reverses obesity and hyperglycemia (361).

Although inflammation in the pancreas has not been studied in humans with type 2 diabetes mellitus on HRT, one study demonstrated that HRT (2 months of 0.625 mg CEE/d, followed by 4 months 0.625 mg CEE/d and medroxyprogesterone 5 mg/d) resulted in improved glycemic control (glycosylated hemoglobin), which was accompanied by reductions in waist-to-hip ratio and central abdominal fat (362). However, this has not been confirmed in other studies (363, 364). Because we do not have pancreas tissue of HRT-treated and -untreated type 1 or type 2 diabetic patients, the animal studies were not confirmed in humans.

In isolated human pancreatic islets stimulated with a cocktail of IFN-γ, IL-1β, and TNF, E2 at pregnancy levels decreased cytokine-induced cell death, cell apoptosis, caspase-9 activity, and NF-κB activity (365). Euglycemia was achieved in six of 12 animals that received E2-treated islets compared with one of 12 control animals (365). In conclusion, the studies in animals show a beneficial effect of E2 on experimental pancreas inflammation.

J. Prostatitis

In most of the above-mentioned inflammatory diseases, E2 at proestrus to pregnancy levels exerts beneficial effects. Interestingly, this is completely opposite in the prostate. Short-term administration of E2 at pregnancy levels was shown to induce an inflammatory response specific to the lateral prostate of the castrated male rat (366). These changes are histologically similar to a spontaneously arising nonbacterial prostatitis. When the same hormones were administered on a chronic basis, the intensity of inflammation increased with time and was accompanied by a fibromuscular proliferation that consisted of fibroblasts, smooth muscle cells, and collagen (366). Others have demonstrated that this treatment stimulated transcript levels of IL-1β, IL-6, macrophage inflammatory protein-2, and iNOS in the lateral prostate leading to a proinflammatory reaction (367). Under the same treatment, others confirmed E2-induced lateral prostate inflammation and observed an up-regulation of MMP-2 and MMP-9 and strong leukocyte infiltration (368). These MMP activities were strongly attenuated by cotreatment with testosterone (368).

It can be summarized that the lateral prostate lobe might be a very specific organ in which pregnancy levels of E2 in male castrated rats induce a proinflammatory reaction. This model is used to study prostatitis, and it seems that effects of E2 are highly tissue-specific.

K. Endometriosis

A striking influence of E2 on inflammation can be observed in women with endometriosis (369–371). In a proportion of women (1–7%), refluxing endometrial cells are not

destroyed, although retrograde menstruation is a common phenomenon in menstruating women (>90%). These endometrial cells colonize the peritoneal cavity but also other organs in the abdomen such as the gut, the bladder, and others. These endometrial cells produce abnormal quantities of chemotactic and angiogenic factors, which attract monocytes, macrophages, T cells, B cells, and other immune cells and induce neovascularization (369–371). In addition, these lesions produce E2, which is locally converted from precusor hormones as demonstrated in *Section X*.

Once the endometrial cells have taken up residence in ectopic areas, growth factors establish a micromilieu that allows the colonization of these cells in a mesenchymal environment (369–371). The established dysbalance induces local inflammation and pain. Because inflammation and pain are highly linked to the different phases of the menstrual cycle with a peak during ovulation, the influence of E2 can be directly observed. Necessarily, therapeutic interventions need to suppress production of E2 by the ovary and inhibit E2 synthesis by the lesions. These interventions include androgens, GnRH agonists, progestins, OC, and aromatase inhibitors (372). Endometrial cellular components are responsive to E2 throughout the cycle, and interestingly, there is resistance to progesterone action on these cells in the postovulatory phase (373). Thus E2 has a dual role—as a mitogen for endometrial cells and as a stimulator of proinflammatory cytokines. Hence, therapies are also tailored to the inflammatory component of the disorder and include nonsteroidal antiinflammatory drugs, danazol, and inhibitors of prostaglandin synthesis (372).

Although we have to keep in mind that endometrial cells are particularly responsive to E2 (and this is not the same in other tissues), endometriosis is an example where the proinflammatory role of E2 at follicular to periovulatory levels on immune and other bystander cells is outstandingly visible.

L. Injury: wound repair, trauma, and shock

This subject was reviewed in 2002 (374). These authors concluded that there is a naturally occurring gender difference in immune responses that persists after traumatic injury. Physiological levels of E2 are immunostimulatory, whereas high pregnancy and superphysiological levels are immunosuppressive (374). Evidence suggest that the gender difference in immune responses after injury is mediated in part by alterations in the circulating levels of gonadal steroid hormones through modulation of production of inflammatory and immunoregulatory cytokines (375).

1. Wound repair. In an excellent review, Kanda and Watanabe (376) demonstrated that estrogens have the potential to accelerate cutaneous wound healing stimulating nerve growth factor and VEGF production in macrophages, granulocyte macrophage colony-stimulating factor in keratinocytes, basic fibroblast growth factor and TGF-β1 in fibroblasts, which leads to improved wound reinnervation, reepithelialization, and granulation tissue formation.

An age-related decline in wound healing in healthy females was corrected by HRT (377, 378). Topical administration of E2 to the wound in rodents accelerated wound healing by up-regulation of TGF-β1. Ovariectomized mice have large wound areas, which was not observed in mice deficient for the macrophage migration inhibitory factor (378). LPS-stimulated macrophage migration inhibitory factor from murine peritoneal macrophages was inhibited by E2 at 10^{-11} to 10^{-8} M (maximum 10^{-8} M) (378). These studies support the growth-promoting effects of E2 at proestrus to pregnancy levels, which leads to an acceleration of wound healing (compare also *Section IV.E*).

2. Trauma and hemorrhage. One study in humans with severe trauma demonstrated that male patients were at increased risk to develop injury-related sepsis and other sequelae compared with female patients (379). This indicates that similar to sepsis in noninjured patients, men are more prone to sepsis than women. However, no systematic studies that demonstrate the positive effects of E2, ERT, or HRT in these patients have been carried out.

In a review on animal experiments of the year 2002, Chaudry and colleagues (380) demonstrated the beneficial effects of proestrus levels of estrogens on trauma and hemorrhage (380). They noted that several studies have shown marked immunosuppression in males after trauma and hemorrhage, which was not evident in diestrus to proestrus female rats.

Male C3H/HeN mice were castrated and treated with pellets containing either vehicle or E2 (proestrus levels) for 14 d before soft tissue trauma (*i.e.*, laparotomy) and hemorrhagic shock were performed (381). In Kupffer cell and splenic/peritoneal macrophage cultures, E2 treatment reduced IL-10 secretion but did not change IL-6 (381). In ovariectomized mice, trauma plus hemorrhage markedly inhibited splenocyte proliferation, splenocyte release of IFN-γ, IL-2, and IL-3, peritoneal macrophage IL-1β and IL-6 release, and splenic macrophage IL-6 and IL-12 release (382). Trauma-hemorrhage-induced suppression of these factors was reverted by a single injection of E2 (proestrus levels) (382). Two hours after trauma plus hemorrhage, splenocyte proliferation, IL-2, IL-3, and IFN-γ release, and splenic macrophage IL-6 release were maintained in control animals but not in animals receiving an ER antagonist (EM-800) (383). In addition, Kupffer cell TNF release and circulating TNF were increased only in ER antagonist-treated females (383).

Male mice were pretreated with E2 (proestrus levels) or vehicle for 3 d before induction of hypoxemia and again immediately before induction of hypoxia (5% O_2) (384). In vehicle-treated mice, splenocyte proliferation, IL-2 and IL-3 production, and splenic macrophage IL-6 and IL-10 production were depressed after hypoxemia. E2-pretreated animals displayed no such depression in splenic cell parameters after hypoxemia (384).

In a model of burn-induced remote organ injury in castrated male rats, E2 treatment (pregnancy levels) inhibited burn-induced elevation in serum TNF levels and the increase of myeloperoxidase activity in liver and lung (385). In male mice after combined ethanol and burn injuries, suppression of cellular immunity was observed (109). E2 (diestrus levels) restored the delayed-type hypersensitivity and splenocyte-proliferative responses, reduced macrophage IL-6, and increased survival after bacterial challenge (109). E2 treatment

resulted in a decrease in splenic NF-κB activation in injured mice (109). After burn injury, in placebo-treated, aged mice, there was a greater than 75% suppression in the delayed-type hypersensitivity response relative to placebo-treated, sham-injured, aged mice (375). E2 supplementation (diestrus levels) before injury yielded a partial recovery in this response. The increase in circulating levels of IL-6 in burn-injured, aged mice was lowered by nearly 50% in aged, E2-treated mice (and E2 increased survival from 42 to 70% in aged, burn-injured mice) (375). In burn-injured mice, E2 restoration (diestrus levels) is paralleled by a recovery in IFN-γ production by splenocytes, but not IL-4 production (386).

In conclusion, injury-induced suppression of various immune responses such as delayed-type hypersensitivity, splenocyte proliferation, and cytokine secretion were normalized by E2 treatment at diestrus to proestrus levels. In contrast, pregnancy levels decreased TNF secretion and NF-κB activation after injury. These studies demonstrate a bimodal role of E2 because at lower concentrations (diestrus to proestrus levels) E2 stimulates suppressed immune responses, whereas at high levels (pregnancy levels) inhibitory effects were observed. It is interesting that these important studies in animals never triggered HRT or ERT studies in human subjects with trauma and hemorrhage.

3. Endotoxin shock. Rats given E2 (proestrus levels) ip 24 h before an injection of a sublethal dose of LPS (5 mg/kg) died within 24 h, whereas none of the control rats died (387). Peak serum TNF, plasma nitrite, CD14 expression on Kupffer cells, and mRNA for TNF, iNOS, LPS binding protein in the liver were increased after LPS in the estrogen-treated group compared with controls, which was interpreted as Kupffer cell sensitization (347, 387). In mice administered a sublethal dose of LPS, pretreatment with E3 for 7 d (pregnancy levels) increased serum TNF in both control and autoimmune MRL lpr/lpr mice (blocked by tamoxifen) (388). E3-treated mice also exhibited a rapid elevation in serum IL-6 levels after LPS challenge occurring 1 h after LPS, whereas in the placebo group maximal serum IL-6 levels were detected at 3 h post-challenge (blocked by tamoxifen) (388).

In conclusion, these studies demonstrate an endotoxin-sensitizing effect of E2 and E3 at proestrus to pregnancy levels when given shortly before the challenge.

M. Bone resorption

E2 inhibits bone resorption via changes in cytokine and growth factor levels, T cell activation, and oxygen radical production (389–394), all of which have already been discussed (*Sections III.C* and *IV.C*, *IV.D*, *IV.E*, *and IV.G*). These factors play an important role in chronic inflammatory diseases. Thus, it is likely that studies on E2 and bone resorption will unravel important effects of E2 in a concentration range of E2 between postmenopausal and follicular to periovulatory levels. It is accepted that the activation of inflammatory pathways during bone resorption is much smaller than in chronic inflammatory diseases. Nevertheless, studies on bone resorption demonstrate that the fall of estrogen levels from 3×10^{-10} to 3×10^{-11} M is already sufficient to stimulate local inflammation in the bone. Because some chronic inflammatory diseases start in the postmenopausal phase, bone resorption might also be a good model to demonstrate important pathogenetic aspects for chronic inflammatory diseases (*e.g.*, the postmenopausal activation of T cells!).

Most of the estrogenic effects of E2 on bone cells are mediated via ERα (7, 269), and E2 induces osteoclast apoptosis and inhibits osteoblast apoptosis (see also *Section V*) (394, 395). E2 deficiency leads to up-regulation of cytokines such as IL-1, IL-7, TNF, IFN-γ, and IL-6, which contribute to the activation of osteoclasts (394, 396). Particularly, TNF from T cells is an important cofactor in bone resorption because this cytokine supports osteoclast activation mediated by the system of receptor activator of NF-κB (RANK)/RANK ligand (RANKL) and c-Fms/macrophage colony stimulating factor (55, 56, 394).

Another important factor in osteoporosis is TGF-β. Because E2 at 10^{-12} to 10^{-8} M stimulates TGF-β secretion from osteoblasts (see *Section IV.D*) (171, 397, 398), the inhibitory influence of this cytokine on T cells is lost during menopause or after ovariectomy (394). Ovariectomy up-regulates IFN-γ-induced class II transactivator, a multitarget immune modulator, resulting in increased antigen presentation by macrophages, enhanced T cell activation, and prolonged lifespan of active T cells (399). The resulting T cell expansion and bone loss are prevented *in vivo* by both blockade of antigen presenting cell-induced T cell activation and silencing of IFN-γ receptor signaling (399). A further important factor is IL-7 because this cytokine is important to stimulate T cell pools, which are necessary to elicit bone-resorbing activities (400, 401). Because E2 decreases IL-7-dependent T cell pools in the thymus, increased availability of T cells after ovariectomy can lead to a higher degree of osteoclastogenesis. In addition, IL-7 suppresses bone-forming osteoblasts, while stimulating formation and function of osteoclasts (402).

Additionally, ROS are suppressed by E2 (see *Section IV.G*), and ROS activate osteoclastogenesis (403–405). Thus, E2 deficiency necessarily increases ROS-stimulated osteoclastogenesis. An additional factor for postmenopausal bone resorption is the loss of E2-stimulated growth factors such as bone morphogenic proteins and matrix proteins (180, 181, 406).

It is obvious that most of these experiments have been carried out in animals undergoing ovariectomy, but many studies have also been carried out using cells *in vitro*. E2 inhibits important bone-resorbing factors at periovulatory/proestrus to pregnancy levels, which is true for the inhibiting effect on TNF, IL-1β, soluble IL-1 receptor type I, and IL-6 (139, 143, 151, 152, 407). Similarly, treatment of postmenopausal or oophorectomized women with HRT resulted in decreased *ex vivo* secretion of IL-1β or decreased serum levels of IL-1 and IL-6 (408, 409). These favorable effects of hormone replacement were also observed in patients with chronic inflammatory diseases such as RA (410, 411).

In conclusion, during the last two decades, the study of postmenopausal or ovariectomy-induced E2 deficiency has revealed important effects on bone formation and resorption. Importantly, this involves many aspects of an activated immune system. An activated immune system is the hallmark of chronic inflammatory disease, which is visible in the form of activated immune cells, circulating and locally produced

proinflammatory cytokines, and generation of ROS. In comparison to the postmenopausal situation in healthy women, these bone-resorbing factors are increased to a much higher extent in chronic inflammatory diseases. This necessarily leads to accelerated bone resorption independent of glucocorticoid therapy. These studies further demonstrate that E2 at follicular to periovulatory levels is an outstandingly important factor to inhibit a gently activated immune system responsible for an increased postmenopausal bone resorption. However, after initiation of a chronic inflammatory disease, E2 at follicular to periovulatory levels is insufficient to inhibit a strongly activated immune system. Only pregnancy levels of E2/E3 exert strong enough antiinflammatory activities to inhibit activated monocytes, macrophages, dendritic cells, T cells, and neutrophils (47, 323).

N. Summary

Depending on the prevailing factors and dominant cell types involved, different roles of estrogens are to be expected in a given disease. Figure 3 summarizes the role of estrogens in inflammatory disease. If B cells are dominant in an inflammatory disease, E2 at low to high levels stimulates the disease process (Fig. 3). This is opposite in chronic inflammatory disease where monocytes, macrophages, dendritic cells, T cells, fibroblasts, and neutrophils play a dominant role (Fig. 3). This figure also demonstrates the dual role of estrogens depending on the concentration.

IX. Timing of Estrogen Administration in Relation to the Time Point of an Inflammatory Disease

It was recently demonstrated that treatment with the same compound before immunization or during the chronic inflammatory disease can elicit completely different effects on inflammation (412, 413). This is important because contrasting effects of exogenous compounds or endogenous factors described in the literature might be explained by the time point of treatment in relation to the disease course. This section will summarize whether or not estrogens show a similar dual role on inflammatory diseases depending on the time point of administration.

In Alzheimer's disease, HRT only demonstrates beneficial effects when treatment is started before disease outbreak (414–416), whereas it had no effects in women already affected by the disease (417). Very similar concepts were developed in atherosclerosis research. Observational studies demonstrate that endogenous E2 in women has beneficial effects on development of atherosclerosis by delaying the disease onset (256). In contrast, HRT or ERT increased the frequency of atherosclerotic events in elderly women (256). In ovariectomized primates, a 70% reduction of atherosclerosis was observed when HRT was initiated simultaneously with an atherosclerotic diet (418), but when HRT was started 2 yr after initiation of an atherosclerotic diet, no protection was observed (418). Similarly, administration of E2 before or during, but not 7 d after, balloon injury inhibited neointima formation in rats (419).

In a model with EAE, treatment of mice with E2 (proestrus to pregnancy levels) at immunization markedly delayed disease onset and reduced disease severity (312). However, when E2 treatment (proestrus to pregnancy levels) was started at disease onset, severity of the established disease was not changed (and E3 treatment had a mild but nonsignificant beneficial effect) (312). Interestingly, ethinyl E2 (pregnancy levels) inhibited disease severity when given before immunization and also at disease onset, but the reasons for these contrasting effects are not known (234). One might speculate that ethinyl E2 other than E2 can be converted to an important favorable metabolite.

In ovariectomized DBA/1 mice with collagen type II arthritis, E2 treatment (proestrus levels) from 4 d before to 8 d after immunization and between d 21 and d 33 (before disease outbreak) delayed arthritis onset, but severity was not markedly changed. When mice were treated beginning at disease onset, severity was markedly reduced (420). This also demonstrates a time-dependent effect of E2 administration.

In the antibody-dependent myasthenia gravis model in mice, E2 treatment for 3 wk before immunization (pregnancy levels) was necessary and sufficient to promote acetylcholine receptor-specific T cell expansion *in vivo* and to increase disease severity (421). In contrast, when E2 treatment was started at immunization, no increase in antigen-specific T cells was observed (421).

In conclusion, E2 most probably has time-dependent effects on inflammatory diseases. In some diseases, E2 administration delays the onset of the disease but has no significant effect after onset or vice versa. This subject has not been extensively studied, and thus, no firm conclusions can be drawn.

X. The Role of Estrogen Metabolites in Inflammation

In premenopausal women, the ovaries are the major source of E2. This situation changes in postmenopausal women when ovaries undergo gradual involution, and an endocrine milieu appears that is similar to men. Then, E2 is produced in a number of extragonadal sites and acts locally as a paracrine or even intracrine factor (422). In peripheral tissue such as the normal macrophage or in inflamed tissue, estrogens are mainly derived from androgen precursors, which can happen in macrophages or fibroblasts (149, 292, 423). Human undifferentiated monocytes do not express the aromatase, whereas monocyte-derived macrophages express the enzyme (423). This has been confirmed in differentiated monocytic cells (424). A number of factors, including IL-1, IL-6, TNF, prostaglandin E2, and granulocyte macrophage colony-stimulating factor secreted by macrophages and lymphocytes can stimulate aromatase activity (425–428), which leads to elevated levels of E1 and E2. Locally elevated levels of estrogens in relation to androgens have been described in inflammatory compartments (285, 292).

In the last decade, the possible role of downstream metabolites of E1 and E2 were shown to have important effects relevant to inflammation. Particularly, the growth-inhibiting effects of 2-hydroxy-E2 and 2-methoxy-E2 came into the focus of recent research. Interestingly, several of these effects are independent of ERs, and relatively high concentrations of hormones between 10^{-8} and 10^{-6} M were used (429–431).

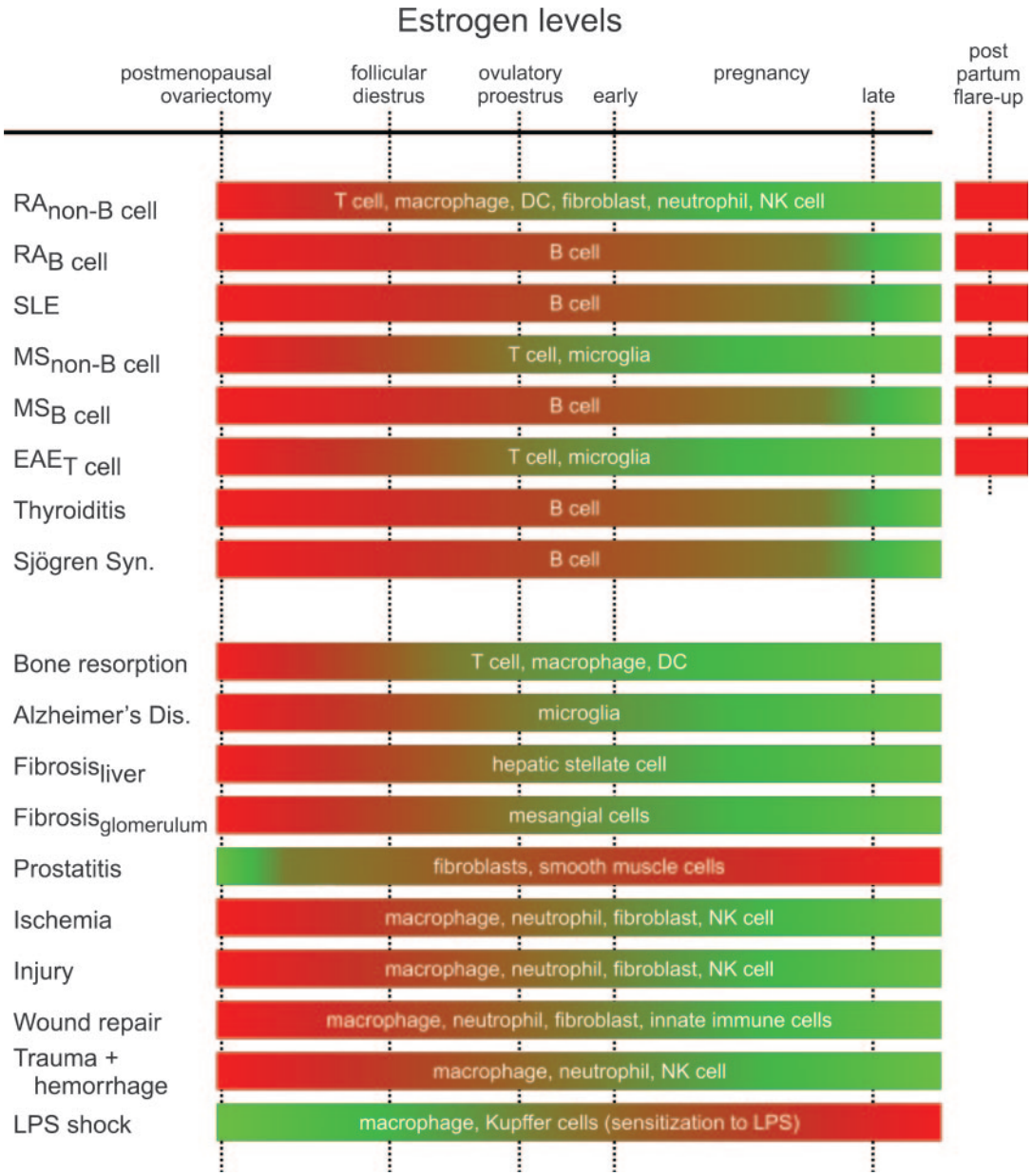

FIG. 3. Relation between estrogen levels and inflammatory diseases. *Red* indicates that estrogens at respective concentrations exert proinflammatory effects, whereas *green* demonstrates estrogen levels with an antiinflammatory effect. In this summary, RA and MS (*upper block*) are divided according to the underlying dominant cell type involved. Today, we know that in a fraction of patients B cells play a dominant role, whereas in another group of patients macrophages, dendritic cells, T cells, and other non-B cells are predominant (164–167). In diseases with a B cell predominance, estrogens at all levels stimulate the proinflammatory process, whereas in disease without a strong B cell involvement, estrogens demonstrate a dual role: at low concentrations estrogens stimulate, and at high levels, estrogens inhibit the disease process. A somewhat different picture appears for prostatitis and LPS-induced Kupffer cell-dependent shock where estrogens seem to sensitize the inflammatory response. DC, Dendritic cell; NK cell, natural killer cell.

In normal cycling women, plasma concentrations of 2-hydroxy-E1 plus 2-hydroxy-E2 are 8×10^{-10} to 1.5×10^{-9} M, and circulating levels of 2-methoxy-E1 plus 2-methoxy-E2 are 9.4×10^{-10} to 1.2×10^{-9} M (430). In pregnant women, plasma levels of the latter two hormones can increase up to 3.9×10^{-8} M. Binding affinity of 2-methoxy-E1 plus 2-methoxy-E2 to classical ERs is lower compared with E2, but there is a higher binding affinity to SHBG compared with E2 (430). These latter findings suggest that these compounds have a longer half-life than E2. The question that arises is whether or not these metabolites exert pro- or antiinflammatory activities. In this respect, cytostatic or proapoptotic effects might be considered as antiinflammatory.

The mitogenic and growth-arresting effects of 2-methoxy-E2 were demonstrated in maturing oocytes (432). In breast cancer cells (MCF-7), 2-hydroxy-E2 at 1×10^{-8} M enhanced cell growth more than did E2, whereas 2-methoxy-E2 inhibited cell growth (433). 2-Methoxy-E2 inhibited DNA synthesis and mitosis, increased cAMP concentration in early S-phase, and decreased it during mitosis. The results

of this study indicate that 2-hydroxy-E2 may be the more potent mitogen, whereas 2-methoxy-E2 acts as a cytostatin (433). In the same breast cancer cells (MCF-7), others demonstrated that 2-methoxy-E2 at 10^{-8} M inhibited the growth-promoting activities of E2 and 16α-hydroxy-E1 at 10^{-8} M (434). These results further show that 2-methoxy-E2 can down-regulate cyclin D1 and thereby cell cycle progression by a mechanism involving the disruption of ATF-2 binding to the cyclin D1 promoter (434).

In rat aortic smooth muscle cells, endogenous metabolites of E2 differentially inhibit serum-induced DNA synthesis, proliferation, and collagen synthesis in the following order of potency: 2-methoxy-E2 > 2-hydroxy-E2 > E2 ≥ 4-methoxy-E2 (430). In contrast, E1, E3, 16α-hydroxy-E1, 2-hydoxy-E1, and 4-methoxy-E1 are significantly less potent (only at high concentrations above 10^{-6} M) (430). These antimitogenic effects of 2-hydroxy-E2 and 2-methoxy-E2 are not blocked by ICI 182,780 (430). In addition, 2-methoxy-E2 inhibited the platelet-derived growth factor-BB-induced MAPK activity in both rat and human aortic smooth muscle cells, and 2-hydroxy-E2 and 2-methoxy-E2 inhibited the mitogenic effects of IGF-I (430). Importantly, catecholamines such as norepinephrine, epinephrine, and isoproterenol at 10^{-9} to 10^{-7} M abrogated the inhibitory effects of 2-hydroxy-E2 on cell number, DNA synthesis, collagen synthesis, and cell migration (not blocked by α- and β-adrenergic receptor antagonists!). This is most probably due to competition of catecholamines and 2-hydroxy-E2 for the catechol-*O*-methyltransferase leading to lower 2-methoxy-E2 levels (435).

In adult dermal fibroblasts, 10^{-7} M 2-methoxy-E2 bis-sulfamate but not 2-methoxy-E2 caused a reversible morphology change and induced G_2/M arrest but no subsequent apoptosis (436). In contrast, treatment of HUVECs did not induce morphology change or G_2/M arrest (436). Others demonstrated that 2-methoxy-E2 bis-sulfamate was a potent inhibitor of HUVEC proliferation (IC_{50}, 5×10^{-8} M) (247). Similar results were observed in a coculture system with HUVECs and human dermal fibroblasts leading to an inhibition of tube formation. 2-Methoxy-E2 bis-sulfamate induced Bcl-2 phosphorylation, p53 protein expression, and apoptosis in HUVECs (247).

In three cultured osteoclast model systems (RAW 264.7 cells cultured with RANKL, bone marrow cells cocultured with stromal support cells, and spleen cells cultured without support cells in media supplemented with RANKL and macrophage colony-stimulating factor), 2-methoxy-E2 at 2×10^{-6} M reduced osteoclast number by more than 95% and induced apoptosis (437). 2-Hydroxy-E2, the immediate precursor to 2-methoxy-E2, exhibited less cytotoxicity, and 2-methoxy-E1, the E1 analog of 2-methoxy-E2, was not cytotoxic (437). Interestingly, the 2- methoxy-E2-mediated decrease in cell survival was partially inhibited by antilymphotoxin β-antibodies, suggesting that 2-methoxy-E2-dependent effects involve lymphotoxin β (437).

In multiple myeloma cells, 2-methoxy-E2 triggered an early transient induction of genes known to stimulate apoptosis and repression of growth/survival-related genes (438). In rat sarcoma cells, 2-methoxy-E2 induced translocation of the proapoptotic protein Bax to mitochondria as an initial apoptotic event that was accompanied by a decrease in mitochondrial transmembrane potential and the formation of ROS followed by mitochondrial release of apoptosis-inducing factor and endonuclease G (439). In addition, 2-methoxy-E2 treatment caused up-regulation of death receptor ligands FasL and TNF and induced caspase 8 activation. A caspase inhibitor did not suppress apoptotic cell death, indicating that the major proapoptotic effect of 2-methoxy-E2 is mediated by a caspase-independent mechanism (439). From these data, it seems that 2-methoxy-E2 has strong proapoptotic and cytostatic activities, but does this also imply that this endogenous E2 metabolite has any effects on relevant pathways in inflammation?

2-Methoxy-E2 inhibited endothelial cell proliferation and migration as well as angiogenesis *in vitro* (248). Moreover, when administered orally in mice, it strongly inhibits the neovascularization of solid tumors and suppresses their growth (248). Because neoangiogenesis is an important mechanism in inflammation, 2-methoxy-E2 can exert anti-inflammatory activities by inhibiting vessel formation. An antiangiogenic effect of 2-methoxy-E2 was confirmed in highly neoangiogenic prolactin-secreting tumors (440).

In the disease model of type II collagen-induced arthritis in ovariectomized DBA/1 mice, 2-methoxy-E2 suppressed development of arthritis (270). In contrast to E2 (proestrus levels), 2-methoxy-E2 exerted neither detectable feminizing effects on the reproductive tract nor inhibition of leukocyte development in hematopoietic organs or lymphoid organs. In contrast, E2 induced a clear decrease of CD4+, CD8+, Ig+ cells in the spleen, and a decrease of CD4+, CD8+, and CD4+CD8+ cells in the thymus. Neither the T cell-dependent delayed type hypersensitivity nor the B cell-dependent production of anti-type II collagen antibodies was suppressed by 2-methoxy-E2 (270). These results are highly interesting because they demonstrate that 2-methoxy-E2 might be a safe antiinflammatory drug without strong immunosuppressive activities.

In chronic puromycin aminonucleoside-induced nephropathy in rats (441), 2-hydroxy-E2 improved glomerular filtration, reduced proteinuria, and attenuated elevated blood pressure. 2-Hydroxy-E2 had no effect on renal tubular cell TGF-β, but it reduced glomerular proliferating cell nuclear antigen and collagen IV, and glomerular and interstitial macrophage infiltration (not blocked by ICI 182,780) (441). The question arises whether or not proapoptotic or cytostatic effects play a role in human disease.

A case-control analysis using pretreatment urine samples suggested that a lower 2-hydroxy-estrogen/16-hydroxyestrogen ratio was associated with an increased risk of premenopausal and postmenopausal breast cancer diagnosis among Chinese women (442). This might indicate that the cytostatic effect of 2-hydroxy-estrogen-derived 2-methoxy-E2 is missing. Because inflammatory signals are unpleasant in breast cancer development, the loss of 2-methoxy-E2 is thought to play an important unfavorable role (443). This might be similar in inflammatory diseases.

Urinary concentration and total urinary loss of 2-hydroxyestrogens was 10 times higher in healthy subjects compared with patients with either SLE or RA, irrespective of prior prednisolone treatment or sex (284). The urinary con-

centration and loss of 16α-hydroxy-E1 did not differ between healthy subjects and patients with RA/SLE. The ratio of urinary 16α-hydroxy-E1/2-hydroxyestrogens was more than 20 times higher in RA and SLE than healthy subjects, irrespective of prior glucocorticoid treatment or sex, which indicates that production of 2-hydroxyestrogens is largely down-regulated in these patients (284). Interestingly, women who developed knee osteoarthritis, which is also an inflammatory disease, were more likely to have a ratio of 16α-hydroxy-E1 to 2-hydroxy-E1 in the highest tertile and 2-hydroxy-E1 in the lowest tertile (444). The loss of 2-hydroxyestrogens is obviously a proinflammatory signal, which might be unfavorable in breast cancer development and inflammatory diseases.

An unwanted shift from 2-hydroxyestrogens to 4-hydroxyestrogens might be an additional proinflammatory signal because these endogenous metabolites can be converted to 3,4-quinones, which lead to depurination and mutation *in vivo* (summarized in Ref. 431). The 3,4-quinones produce ROS, which stimulate inflammation, whereas methylation and sulfation are detoxification pathways, which can inhibit these negative tissue-destroying sequelae (431). Others confirmed that 4-hydroxy-E2 undergoes 2-electron oxidation to quinone via semiquinone, and during this process, ROS can be generated to cause DNA damage and cell death (204). A similar effect of E2 conversion was confirmed in hepatocytes (445). In contrast to the prooxidant activities of 4-hydroxy-E2, 2-hydroxy-E2 is a potent antioxidant (more potent than vitamin E) and thereby protects membrane phospholipids and cells against peroxidation (446). Thus, 4-hydroxy-E2 in contrast to 2-hydroxy-E2 induces carcinogenic and proinflammatory effects (430).

Importantly, cytochrome P4501B1 (CYP1B1) is a dioxin-inducible gene that is active in the formation of 4-hydroxy-E2 (447). In human peripheral blood lymphocytes, absolute CYP1B1 mRNA levels varied more than 30-fold in uncultured mononuclear cells obtained from 10 individuals. *In vitro* treatment of mitogen-stimulated lymphocytes with dioxin for 1–5 d of culture resulted in a peak induction of CYP1B1 after 3 d. These observations indicate that CYP1B1 exhibits variable constitutive expression and is inducible *in vitro* by dioxin in human lymphocytes (447). Presently, we do not know whether proinflammatory mediators induce a shift from 2-hydroxyestrogens to 4- and 16-hydroxylated estrogens.

In conclusion, there is clear evidence that 2-methoxy-E2 is a proapoptotic and cytostatic endogenous compound, which can inhibit neoangiogenesis and can attenuate inflammation in animal models. These effects are not mediated via the classical ERs, and they do not lead to feminizing effects. In contrast, 4-hydroxylated estrogens might exert proinflammatory roles by inducing ROS and DNA damage. Thus, stimulation of one or the other pathway is possibly decisive in chronic inflammation.

XI. Pregnancy and Inflammation

As delineated in the sections above, pregnancy levels of E2 suppress many cytotoxic and innate immune responses, whereas antibody production, neoangiogenesis, and growth-associated phenomena can be stimulated. One of the most important immunological modifications during pregnancy is the increase of B cell responses due to the progressive increase of progesterone and estrogens during pregnancy, which reach their peak level in the third trimester of gestation (300). It is important to mention that during pregnancy several steroid hormones increase, such as cortisol, estrogens, and progesterone. The role of cortisol as an antiinflammatory hormone is well known, and the importance of progesterone is briefly summarized in Table 8. The role of progesterone is particularly mentioned because a separate effect of one single steroid hormone cannot be derived from studies with pregnant women. Thus, the following discussion must take into account these particular circumstances during pregnancy.

This section briefly summarizes present knowledge in exemplary diseases, namely SLE, RA, and MS.

SLE tends to flare during pregnancy, and the puerperium and, not rarely, SLE starts during pregnancy. Most flares are mild, and cutaneous and joint disease are the most common manifestations (455, 456). SLE flares are associated with increased prematurity (457), and active nephritis is an independent factor for fetal mortality (458). SLE disease activity scores varied during pregnancy, being increased in the second trimester and decreased in the third trimester (459). Similarly, pregnancy also affects the disease course in mice. In female MRL lpr/lpr mice, one observes a postpartum exacerbation of their mild spontaneous arthritis (272). Administration of E2 (pregnancy levels) for 2 wk after Freund's complete adjuvant injection prevented adjuvant-enhanced arthritis, reducing the incidence from 67% to the baseline level of 21% (272). In contrast, the same dose increased proteinuria and mortality rate, which demonstrates completely different effects on joint disease compared with nephritis (272).

Observations in the 19th century (Trousseau 1871, Charcot 1881, Bannatyne 1896) indicated that pregnancy is favorable in RA as summarized in the Nobel Prize Lecture of the rheumatologist Philip S. Hench. He wrote: ". . . after 1931, records of these cases [of cases with RA] were more carefully made and assembled . . . because of my growing belief that this phenomenon of relief [from arthritic disability] was analogous to, if not identical with, that which may occur during

TABLE 8. Immunomodulating effects of progesterone

Effect	Ref.
Antiinflammatory effects	
Inhibits phagocytosis of rat macrophages	448
Inhibits IL-1β secretion from human peripheral blood lymphocytes	449
Inhibits IL-6 from decidual stroma cells	450
Inhibits IL-12 secretion from mouse mitogen-activated lymphocytes	451
Inhibits IFN-γ secretion of concanavalin A-stimulated lymphocytes	452
Inhibits NK cell activity	453
Inhibits stimulated proliferation of human lymphocytes	454
Th2 stimulating effects	
Stimulates IL-4 secretion from lymphocytes	45, 451
Stimulates IL-5 secretion from lymphocytes	449
Stimulates IL-10 secretion from lymphocytes	451

NK cells, Natural killer cells.

jaundice, and that the same agent might be responsible for the relief both during pregnancy and jaundice, although the mechanism . . . might be different." (460). A pregnancy-induced remission is experienced by 75% of patients with RA, but a flare-up occurs in more than 60% after delivery (461).

The first indication that T cell pathways are suppressed in pregnant patients with RA was provided by Russell *et al.* in 1997 (462). LPS-stimulated whole blood cultures were demonstrated to release less IL-2 but more soluble TNF receptor p55 and p75 during pregnancy. In this study, serum TNF and IL-1β concentrations were unchanged (462). Others demonstrated decreased IFN-γ and IL-12 production by lymphocytes after phytohemagglutinin stimulation in third-trimester pregnant women with RA compared with healthy pregnant women (463). This study was complemented by data in RA patients (third-trimester pregnancy) that demonstrated that *ex vivo* monocytic IL-12 production was about 3-fold and TNF production was approximately 40% lower than postpartum values (464). In a prospective longitudinal study, elevated IL-10 levels were found in RA and SLE pregnant women compared with healthy pregnant controls (465), which also fits to the stimulating effects of E2 on this cytokine (*Section IV.D*). In addition, the concomitantly increasing progesterone has very similar effects (Table 8).

Significantly higher concentrations of soluble TNF receptor 2 and IL-1ra were measured in pregnant compared with nonpregnant women (466). An increase of IL-1ra from the second to the third trimester correlated with improvement of disease activity in both RA and ankylosing spondylitis patients. Compared with nonpregnant patients and other pregnant women, RA patients showed markedly elevated levels of soluble CD30 during pregnancy (466). CD30 (Ki-1) antigen has been considered to be expressed on hematopoietic cells (*e.g.*, Reed-Sternberg cells of Hodgkin's disease) but also in nonhematopoietic cells such as human decidual cells. It is thought that CD30 is a relatively specific marker for Th2 lymphocytes (449). All these data in pregnant women with inflammatory arthritis demonstrate a Th2 dominance that might be even more pronounced compared with healthy pregnant women. These studies also demonstrate that important antiinflammatory factors such as soluble TNF receptors and IL-1ra are additionally up-regulated (462, 466). Furthermore, these studies indicate the favorable changes during pregnancy to reverse after delivery leading to increased disease activity. These effects are most probably mediated by estrogens and progesterone together.

Others studied 254 women with MS during 269 pregnancies in 12 European countries (467). The women were followed during their pregnancies and for up to 12 months after delivery. In women with MS, the rate of relapses declined during pregnancy, especially in the third trimester, and increased during the first 3 months postpartum before returning to the prepregnancy rate (467).

In conclusion, on the basis of effects of E2 and progesterone on innate and adaptive immune responses (see sections above and Table 8), it is expected that B cell/antibody-driven diseases exacerbate, whereas T cell-driven diseases with cytotoxic and innate immune responses improve. In some diseases, both aspects of immune responses might be present at the same time (272, 304). In such a situation, antibody-dominated pathways are supported, and cytotoxic and innate immunity pathways are inhibited.

XII. The Contraceptive Pill, Hormone Replacement Therapy, and Inflammation

Similar to the situation during pregnancy, treatment with OC and HRT is often accompanied by concomitant administration of estrogens and progestins. Thus, effects of these two hormones cannot be separated. Because progesterone has strong influences on several inflammatory pathways (Table 8), the discussion below must be viewed with this premise.

A. The contraceptive pill

The influence of OC on RA was studied in population-based samples in which the relative risk to develop RA is investigated in women with *vs.* without OC treatment before disease outbreak. When compared with women who had never used OC, no increase of the age-adjusted relative risk was found for past users in several independent studies (277, 468–476). There were only three studies that found a decreased risk for RA in women who had been administered OC before disease outbreak compared with women without OC treatment (477–479), but the effects were not very marked.

As early as 1972, the influence of OC on women with RA was investigated (480). RA women under OC compared with RA women using other contraceptive methods did not demonstrate changes in levels of antinuclear antibodies, rheumatoid factor, and erythrocyte sedimentation rate as markers of disease activity (480). Only two studies reported improvements by OC treatment with respect to swollen joint count and severity (277, 481), but the long-term effects of OC treatment are not marked (482).

In three case-control studies, OC use before diagnosis was not associated with an increased disease risk in patients with SLE (483–485). Global disease activity, maximum SLE disease activity index, incidence of flares, time to first flare, and incidence of adverse events were similar among women with SLE independent of OC use (486). In a recent study, it was confirmed that OC did not influence the course of SLE (487).

Similarly, prior use of OC did not change the risk for MS (488–490)

In conclusion, almost all studies in women with *vs.* without OC demonstrated no increased risk to develop a chronic inflammatory disease such as RA, SLE, or MS. In addition, OC use was not associated with changes in the disease course in patients with RA and SLE (it is not known for MS). During OC administration, serum levels of estrogens and progestins only increase to a small extent, and, thus, no change of risk is to be expected in a woman with premenopausal estrogen levels.

B. Hormone replacement therapy

HRT is a combination therapy of naturally occurring or synthetic estrogens together with progestins or synthetic progestins. Thus, often a separate analysis of estrogen and progestin effects is impossible to perform, and the effects

observed must be assigned to the combination of estrogens and progestins. In addition, HRT makes use of different types of estrogens, and most often CEE or synthetic E2 derivatives were used. CEE are a mixture of many different types of conjugated estrogens, which might have different effects and different pharmacodynamics and pharmacokinetics compared with E2 (256). In contrast, in animal studies most often E2 was used, but rarely a synthetic E2 derivative (ERT) was administered in women as the sole therapy.

Because the sections above mentioned exact E2 concentrations, it is important to point out that serum levels of E2 during hormonal therapy only increase to a relatively small extent; for instance, serum levels of E2 increased from 7×10^{-11} M to 2.5–4.3 $\times 10^{-10}$ M in orally treated women (2 mg/d E2 for 22 d, 1 mg/d E2 for 6 d combined with 1 mg/d norethisterone during the last 10 d of the cycle) and from 7×10^{-11} M to 1.5–1.6 $\times 10^{-10}$ M in transdermally treated women (continuously 50 μg/d E2 combined with 10 mg/d oral medroxyprogesterone during the last 10 d of the cycle) (491). With this information in mind, the following text should be carefully studied.

1. TNF, IL-1β, IL-6, and C-reactive protein. In whole blood assays, prior HRT (E2 + progestins) decreased the levels of TNF and thromboxane B2, and of tissue factor from LPS-stimulated monocytes (491). In supernatants of PBMCs, prior HRT (E2 + progestins) induced a decrease in levels of IL-1β, IL-6, soluble IL-6 receptor, and TNF (23). In LPS-stimulated whole-blood cells, supernatant levels of IL-1β and TNF were reduced after prior HRT (CEE + progestin) compared with untreated postmenopausal women (492). In a prospective study, HRT decreased serum levels of IL-1β and TNF (492). In postmenopausal women, treatment with transdermal E2 (50 μg/d) for 12 months decreased spontaneous IL-6 production by PBMCs (147). This inhibiting effect on IL-6 was confirmed by others, but the effect was abrogated by coadministration of medroxyprogesterone (493). In 196 healthy postmenopausal women, 6 months of HRT (oral E2 plus progestin) did not change serum levels of IL-6 and TNF (494). In healthy postmenopausal women, 12 wk of oral E2 treatment did not change serum levels of IL-6 (495). In postmenopausal women, after a low dose of endotoxin, transdermal E2 (0.1 mg, serum levels 3.7×10^{-10} M compared with 2.7×10^{-11} M in controls) attenuated the endotoxin-induced release of TNF, IL-1ra, and IL-6 (496). In postmenopausal women, HRT (no exact type is given) had lower serum IL-6 levels compared with subjects without HRT, which was independent of age, antihypertensive therapy, smoking habits, and blood pressure (497). Thus, most studies with E2 alone or E2 plus progestins showed an inhibiting effect or no effect on proinflammatory cytokines such as TNF, IL-1β, and IL-6. This situation might be different when CEE with/without progestins are used.

In postmenopausal women, prior HRT (different preparations with CEE and progestins) induced a higher mitogen-induced T cell proliferation, and higher levels of induced TNF from PBMCs (498). CEE plus progestin increased the stimulated secretion of TNF and IL-6 (499). Similarly, in healthy postmenopausal women, HRT (CEE only during 6 wk) resulted in increased levels of IL-6 (130). Others demonstrated that CEE increased TNF secretion from LPS-stimulated PBMCs but did not change IL-6 secretion (499).

In healthy postmenopausal women (with baseline E2 below 9×10^{-11} M, mean = 7.9×10^{-11} M), HRT (CEE only, during 6 wk) resulted in increased levels of C-reactive protein (130). Similarly, CEE increased plasma levels of C-reactive protein (499). In postmenopausal women, HRT (CEE + progestin) increased C-reactive protein levels (131). In healthy postmenopausal women, 6 months of oral HRT (oral E2 plus progestin) increased C-reactive protein serum levels compared with both placebo and transdermal E2 plus progestin (494).

In conclusion, most studies with E2 alone or E2 plus progestins showed an inhibiting effect or no effect on proinflammatory cytokines, but in contrast, studies with CEE with/without progestin most often demonstrated stimulating effects. The different effects of E2 with/without progestins *vs.* CEE with/without progestins may be due to the mixture of many different types of conjugated estrogens in drugs with CEE, which do not contain the pure E2 (256). As demonstrated above, this mixture of conjugated estrogens might even have unwanted proinflammatory effects. The comparison of E2 with/without progestins *vs.* CEE with/without progestins also demonstrates that other estrogens than E2 might well have a proinflammatory potential. Furthermore, the dose and route of estrogens might play an additionally important role because only oral but not transdermal therapy leads to an increase of C-reactive protein. This likely demonstrates that hepatic conversion of E2 to downstream estrogens produces proinflammatory metabolites.

2. MCP-1 and adhesion molecules. In postmenopausal women on 1 mg E2 continuously plus sequential progestin over 1 yr, serum levels of MCP-1, soluble ICAM-1, soluble VCAM-1, and soluble E-selectin gradually decreased after 3, 6, and 12 months (227). HRT (CEE + progestin) for 2 months decreased serum levels of MCP-1, soluble ICAM-1, soluble VCAM-1, soluble E-selectin, tissue factor antigen, and plasminogen activator inhibitor type 1, but increased tissue factor activity (129). In postmenopausal women, HRT (CEE + progestin) decreased soluble ICAM-1, soluble VCAM-1, soluble E-selectin, and soluble thrombomodulin (131). In healthy postmenopausal women, HRT (CEE only during 6 wk) resulted in decreased levels of soluble E-selectin (130). In ovariectomized rhesus macaques, oral CEE decreased serum levels of soluble VCAM-1, MCP-1, and soluble E-selectin (133). However, others demonstrated no effects of oral E2 treatment for 12 wk on serum levels of soluble ICAM-1 (495). In postmenopausal women with coronary artery disease, ERT (CEE only) increased E2 serum levels from approximately 3×10^{-11} M to 1.5×10^{-10} M and decreased serum levels of soluble E-selectin, soluble ICAM-1, and soluble VCAM-1 (126).

In conclusion, most of the studies demonstrated that E2 plus progestins or CEE with/without progestins decrease serum levels of MCP-1 and important soluble adhesion molecules, which is thought to be a favorable effect on atherosclerosis, and probably on other chronic inflammatory diseases. This thinking is based on the idea that soluble adhesion molecules reflect levels on the cell surface, and that lower cell

surface levels are related to decreased migration of leukocytes into inflamed tissue (see also *Sections IV.B and VI*).

3. T and B cells. HRT in postmenopausal women reduced the number of circulating natural killer cells, CD4+ memory T cells, and CD8+CD11b+ T cells (500). Similarly, circulating numbers of CD4+ T cells and CD4+CD69+ T cells decreased in E2-treated ovariectomized rhesus macaques, but B cells did not change (E2 serum levels 5×10^{-10} M) (501). In healthy women with surgical menopause (myoma operation), transdermal E2 treatment decreased numbers of circulating CD8+ T cells and increased CD19+ B cells (169). Similarly, HRT (different preparations with CEE and progestins) increased the number of circulating CD19+ B cells (498).

These studies demonstrate that HRT particularly decreased T cells numbers, but B cell numbers are unaffected or up-regulated. This confirms the stimulating effects of estrogens on B cells (see also *Section III.B*).

4. Arthritis. The influence of HRT on RA initiation was studied in population-based samples. The relative risk to develop RA was investigated in women with *vs.* without HRT before disease outbreak. An important disadvantage is the fact that almost all observational studies did not mention the type of HRT used. Above-mentioned data demonstrate that the type of HRT, whether CEE or E2 with/without progestins, can be an important decisive factor.

Compared with postmenopausal women who never used HRT, current users had an age-adjusted RR of 1.3 (95% CI, 0.9–2.0), past users had an age-adjusted RR of 0.7 (95% CI, 0.5–1.2), and ever-users had a RR of 1.0 (95% CI, 0.7–1.4) (473). This study nicely summarized the findings of other groups, which confirmed that HRT did not increase the relative risk to develop RA (468, 479, 502–505). In addition, it might be interesting to study the influence of HRT or ERT on the course of RA because many antiinflammatory effects of E2 have been described (see sections above).

As early as 1964, the influence of HRT on disease course of RA was investigated, but this treatment had little effect (506). Others demonstrated a beneficial effect of the same HRT (0.45 mg/d mestranol, 30 mg/d norethynodrel) in 33 postmenopausal women, with a complete remission in seven patients and an objective improvement in 11 patients (507). In a prospective double-blind crossover study in RA with ethinyl E2 as the sole therapy (12.5 μg/d), small beneficial effects of E2 were observed as a decrease in 30-m walking time and thrombocytosis, and an increase of hemoglobin concentrations (508). No differences between E2 and placebo therapy were observed for erythrocyte sedimentation rate, C-reactive protein, grip strength, number of swollen joints, and joint tenderness score (508).

HRT with 2 mg E2 valerate plus progesterone for 52 wk has been studied in placebo-controlled investigations in patients with RA (509, 510). No significant difference was found in and between both treatment groups with regard to articular indices, pain score by visual analog scale, erythrocyte sedimentation rate, and health questionnaire score on daily activities before, during, and at the end of the study. However, bone mineral density increased in the lumbar vertebral spine and femoral neck in the E2 group compared with the placebo group (509, 510). Similarly, HRT (50 μg E2, twice weekly, plus 1 mg norethisterone for 10 d on a cyclical basis, for 48 wk) increased spine bone mineral density, but bone mineral density at the femoral neck and distal radius did not change (511). In the HRT group, there was improvement in well-being as assessed by the Nottingham Health Care Profile, but pain score, morning stiffness, erythrocyte sedimentation rate, and C-reactive protein did not change (511).

HRT (50 μg/d transdermal E2 plus 1 mg norethisterone/d for 12 d per month) increased serum E2 in only 58.4% of patients with RA (responders, mean E2 levels, $\sim 2 \times 10^{-10}$ M) but failed to achieve serum E2 levels lower than 10^{-10} M in 41.6% of patients (nonresponders) (295). Only in the responders, HRT for 6 months decreased the Ritchie articular index, pain score, morning stiffness, and erythrocyte sedimentation rate (295). In the same study, the authors observed an increase in spinal bone mineral density and a stabilization of femoral bone mineral density (295). HRT or ERT was accompanied by a reduction in bone resorption markers in RA irrespective of steroid usage (410).

In postmenopausal women with RA, HRT with E2 (2 mg E2 with/without 2 mg norethisterone) increased E2 serum levels (from 1.5×10^{-10} M to 6.5×10^{-10} M) and improved bone mineral density in the hip and lumbar spine (accompanied by an increase of IGF-I and osteoprotegerin serum levels), decreased erythrocyte sedimentation rate and disease activity score, and delayed progression of joint destruction among patients with radiological progressive disease (512, 513). Furthermore, this treatment resulted in a decrease of serum levels of type I collagen, C-terminal telopeptide of type I collagen, bone sialoprotein, and C-terminal propeptide of type I procollagen, and in a down-regulation of urinary levels of collagen type II C-telopeptide degradation fragments and cartilage oligomeric matrix protein at 2 yr, and this was associated with improved bone mineral density (411).

In conclusion, prior HRT did not increase the risk to develop RA. HRT or ERT therapy markedly improved bone mineral density and reduced bone resorption markers. In addition, in some studies HRT had small beneficial effects on the articular index, pain score, and morning stiffness when patients with high treatment levels were separately analyzed (295).

5. SLE. Because estrogens and progesterone can stimulate B cell activities, the importance of HRT on initiation and disease course of SLE was studied. In a prospective cohort study (Nurses' Health Study) in postmenopausal women, women were classified as never-users or ever-users (current and past) of HRT (514). With never-users of postmenopausal hormones as the reference group, age-adjusted RR for SLE was 2.1 (95% CI, 1.1–4.0) for ever-users, 2.5 (1.2–5.0) for current users, and 1.8 (0.8–4.1) for past users (514). A proportional increase in the risk for SLE was observed related to the duration of HRT (514). In postmenopausal women, short-term estrogen exposure was not associated with increased risk to develop SLE (515). The adjusted odds ratio (OR) comparing longer term estrogen users and nonusers for all cases (SLE and discoid lupus combined) was 2.8 (95% CI, 1.3–5.8) (515). Interestingly, a difference in risk was found between long-term users of estrogens alone (OR 5.3; 1.5–18.6)

and those who used estrogens combined with progestins (OR 2.0; 0.8–5.0) compared with nonusers (515). The authors suggest that progestins may reduce the effect of estrogens on SLE and discoid lupus erythematosus.

In postmenopausal women with SLE, the use of HRT (estrogens alone and estrogens plus progestins combined) does not appear to increase the rate of lupus flares over a 1-yr follow-up (516). In a study with 351 postmenopausal patients with SLE, patients were treated for 12 months with HRT (CEE + progestin) or placebo (517). Severe flares were rare in both treatment groups (not different between groups) (517). Mild to moderate flares were significantly increased in the HRT group: 1.14 flares/person-year for HRT, and 0.86 flare/person-year for placebo (RR, 1.34; $P = 0.01$) (517).

In conclusion, postmenopausal HRT seems to increase the risk of developing SLE or discoid lupus erythematosus. In addition, HRT increases the risk of mild flares in postmenopausal patients already diagnosed with SLE. These data indicate that the stimulating effects of estrogens (and also progestins) on B cells stimulate SLE in postmenopausal women with low levels of estrogens.

6. MS. Approximately 40% of women with MS reported worsening of symptoms related to the menopause (518). However, studies that systematically analyzed the effect of HRT on risk and course of MS are rare. One study with a small number of MS patients reported beneficial effects in 75% of patients with HRT (type not given) (519). In another larger retrospective study in women with MS, 87% reported no change after administration of HRT, but only 7% described an improvement after commencement of therapy (518). Both studies have no strong impact due to the retrospective design of the study and the low number of patients.

In a recent important study in patients with relapsing remitting MS, the favorable effects of pregnancy doses of E3 were demonstrated (47, 323). Although this investigation was not designed as a HRT study, it nicely demonstrates the positive effects of estrogen treatment.

XIII. Estrogens and Systemic Response Systems

Systemic response systems such as the hypothalamic-pituitary-adrenal (HPA) axis, the sensory nervous system, and the sympathetic nervous system (SNS) influence immune mechanisms in chronic inflammatory diseases (520). In the following sections, it is explained that E2 can alter these response systems in an unfavorable proinflammatory manner.

A. The hypothalamic-pituitary-adrenal axis

The HPA axis is an important regulating element during acute and chronic inflammation because it can down-regulate several proinflammatory pathways by releasing glucocorticoids and adrenal androgens (520–525). In the 1960s, the stimulating influence of estrogens on the HPA axis was described (526). Treatment with OC increased plasma cortisol 2.5-fold (526). In transsexual men, estrogens (ethinyl E2 100 μg/d) plus antiandrogens increased free cortisol levels in 24-h urine samples, which was opposite in transsexual women on androgens (527). In addition, transdermal E2 activated the HPA axis in men during a stress test (528). Because the CRH promoter contains two estrogen-responsive elements, this might constitute a basis of sexual dimorphism in the expression of the CRH gene in the central nervous system (529–531). This is the situation under normal noninflamed conditions where cytokine levels are generally low. But what happens during chronic inflammation with elevated levels of circulating cytokines?

Indeed, the situation is markedly different when individuals are challenged with proinflammatory cytokines such as IL-1β or with LPS. In this situation, the inhibitory effect of E2 on proinflammatory cytokines plays a dominant role. In postmenopausal women, a low dose of LPS in the presence of transdermal E2 (0.1 mg, serum levels 3.7×10^{-10} M compared with 2.7×10^{-11} M in controls) attenuated the release of ACTH and cortisol (496). Because E2 also attenuated the endotoxin-induced release of IL-6 and TNF, the authors concluded that E2 inhibited secretion of proinflammatory cytokines and, thus, E2 suppressed cytokine-induced stimulation of the HPA axis (496). Similarly, in ovariectomized rhesus monkeys, E2 (early-mid-follicular levels) blunted the cortisol response to intracerebroventricular infusion of IL-1α (532). In ovariectomized rhesus monkeys, 3 wk of E2 replacement therapy decreased IL-1β-induced secretion of ACTH (533).

In conclusion, E2 stimulates the HPA axis in individuals without a proinflammatory stimulus, but E2 inhibits the HPA axis under proinflammatory stimuli such as IL-1 or LPS (Fig. 4). The latter effect is in part mediated by an inhibitory influence of E2 on secretion of proinflammatory cytokines. One might speculate that in chronic inflammatory diseases the cytokine-induced activation of the HPA axis might be more inhibited in women than men. This is unfavorable for women due to the lower levels of antiinflammatory glucocorticoids. These effects of E2 on the HPA axis might contribute to the sexual dimorphism in chronic inflammatory diseases.

B. The sensory nervous system

The sensory nervous system plays an important role in neurogenic inflammation because the secreted neuropeptide substance P is a strong inflammatory stimulus (534). In addition, substance P is a chemotactic factor for many leukocytes, particularly of the innate immune system (neutrophils, monocytes) (535, 536). Upon stimulation of sensory nerve fibers by painful or inflammatory stimuli, substance P is locally released into the vicinity of the nerve terminal within inflamed tissue (efferent action of the afferent sensory nervous system). Thus, a possible modulation of the sensory nervous system by estrogens will play an important immunomodulating role due to changes of substance P release.

To change the activity of the sensory nervous system, receptors for estrogens must be present in respective neurons. ERα and ERβ immunoreactivity was located in sensory neurons of dorsal root ganglia (537). Both receptor subtypes were present in neurons of the dorsal horn (537). On the basis of these findings, one would expect an influence of estrogens on neurogenic inflammatory pathways.

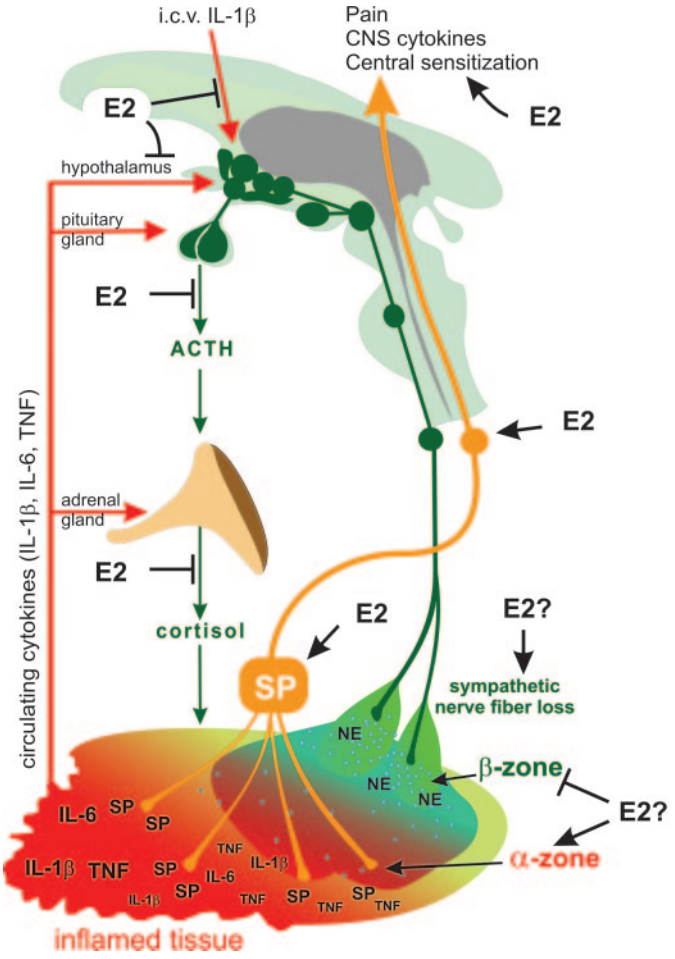

FIG. 4. Influence of estrogens on systemic response systems. *Red* and *orange* indicate proinflammatory pathways, whereas *green* and *blue* represent antiinflammatory pathways. A *line with an arrow* indicates a stimulating effect, and a *line with a bar at the end* marks an inhibitory effect. On the *left side*, the HPA axis is depicted. E2 inhibits the activity of the HPA axis in the presence of intracerebroventricular (i.c.v.) or circulating cytokines, which is a condition present in chronic inflammatory diseases. The consequence is a lowered secretion of adrenal cortisol. On the *left side*, the two portions of the nervous system are depicted in *orange* (the afferent sensory nervous system) and in *green* (the efferent SNS). Usually, the densities of sensory and sympathetic nerve fibers are similar (balance = 1:1) (559). In inflamed tissue, sensory nerve fibers sprout into the tissue and sympathetic nerve fibers get lost (559). The balance is shifted to the proinflammatory substance P (SP), away from antiinflammatory norepinephrine (NE). The affinity of NE is much higher for α-adrenergic receptors than for β-adrenergic receptors. In the direct vicinity of sympathetic nerve endings, the concentration of NE is high enough to exert antiinflammatory effects (via β-adrenergic receptors, β-zone), whereas distant to sympathetic nerve endings only little amounts of NE are present, which leads to proinflammatory effects (via α-adrenergic receptors, α-zone) (546). E2 stimulates SP and its receptor and contributes to peripheral and central sensitization of pain pathways. E2 might contribute to the loss of sympathetic nerve fibers, to an inhibition of β-adrenergic signaling while increasing signaling through α-adrenergic receptors (the *question mark* indicates that this is presently not known in inflamed tissue).

Estrogen treatment of ovariectomized animals stimulated increased expression of the substance P precursor (538, 539). Compared with control rats, mRNA of the substance P receptor (neurokinin 1 receptor) was increased by 2-fold from rats treated with E2 (540). Similarly, E2 at pregnancy levels (not at proestrus levels) led to a 2.5-fold increase in neurokinin 1 receptor mRNA levels (blocked by tamoxifen) (541). Increased neurokinin 1 receptor mRNA levels in response to E2 were related to increased binding of substance P to its receptor (541). Thus, estrogens might increase neurogenic inflammation and sensitization to painful stimuli by upregulating substance P and its receptor.

In the 1970s, the enlargement of the genital and pudendal receptive field was demonstrated after estrogen treatment (542, 543), which is a sign of increased sensitization of sensory pathways. With respect to the reproductive tract, the stimulating role of estrogens on sensory neurons and substance P production has been summarized (544). In rats, E2 treatment (proestrus to pregnancy levels) increased sensory innervation of mesenteric arterioles after 7 d (545). E2 increased neurite area per sensory dorsal root ganglion neuron, and therefore it was concluded that E2 acts directly on the sensory neuron by increasing the neurite outgrowth in the absence of other exogenous growth factors (546). These findings suggest that E2 increases sensitization of sensory pathways, which possibly leads to an increase of neurogenic inflammation (Fig. 4).

In addition, female rats experience more pain than male rats upon different stimuli (547), which has been demonstrated in humans, too (548). Most patients with painful bladder disorders are women (549). Similarly, irritable bowel syndrome is more prevalent in women than in men (336). Ovariectomized rats treated with E2 (diestrus levels) exhibited visceral hypersensitivity after partial restraint stress (337). The authors concluded that stress-induced visceral hypersensitivity in female rats is E2-dependent and mediated through neurokinin 1 receptor activation (337). In the rat, E2-stimulated hyperalgesia was antagonized by inhibitors of the cyclooxygenase pathway (550). In rats, E2 (proestrus levels) increased the excitability of the temporomandibular joint sensory neurons (551). E2 plus Freund's adjuvant demonstrated an additive effect on excitability of the temporomandibular joint sensory neurons (551). Intracerebroventricular injection of E2 increased the amount of licking of the formalin-injected painful paw (inhibited by ICI 182,780). The results demonstrate that centrally acting estrogens influence pain processing (552). Numerous studies of sex differences in opioid analgesia in rodents reported that opioids are more potent in males than in females (summarized in Refs. 553 and 554). Aromatase is expressed and is enzymatically active in pain-sensitive neurons in the dorsal horn (555). Treatment with intrathecal aromatase inhibitors increased pain thresholds, which indicates that estrogens sensitized pain perception (555).

From the above-mentioned studies, it is probable that E2 can enhance pain perception on different levels (in the periphery, in the spinal cord, and in the central nervous system). However, one has to be careful because some studies clearly demonstrated dose-dependent effects of estrogens on pain perception; *e.g.*, in ovariectomized rats, E2 administration (diestrus levels) restored opioid analgesia, but further E2 (proestrus to early pregnancy levels) suppressed opioid analgesia (556). In ovariectomized rats, proestrus levels of E2 increased morphine-induced analgesia, whereas E2 at the

pregnancy level had no effect on morphine-induced analgesia (557). Similarly, it is known that pain thresholds increase throughout late pregnancy, which appears to involve spinal opioid receptors (558). This indicates that E2 at high pregnancy levels inhibits sensory neuronal pathways.

In conclusion, estrogens can enhance pain perception and most probably neurogenic inflammation leading to lower pain thresholds in female rats and women. It appears that E2 affects pain perception according to the concentration of this hormone because E2 might stimulate pain sensitization at metestrus to early pregnancy levels, whereas E2 at high pregnancy levels inhibits pain perception.

C. The sympathetic nervous system

The SNS with its most important neurotransmitter norepinephrine elicits proinflammatory effects via α1- and α2-adrenoceptors but has antiinflammatory effects via β2-adrenoceptors by directly inhibiting TNF secretion and other proinflammatory pathways (559, 560). Thus, a possible influence of estrogens on the SNS might change the influence of the SNS on inflammation.

The question that remains is how the activity of the SNS can be modulated by estrogens. This would imply that ERα and/or ERβ receptors are present in neurons of the SNS. Indeed, sympathetic neurons express ERα and ERβ in different anatomical compartments (561). Similarly, one might ask whether estrogens can modulate adrenergic receptors or signaling pathways. It seems as if there are differences with respect to regulation of the α2-adrenoceptor because administration of clonidine, an α2-adrenergic agonist, resulted in a stronger decrease of plasma norepinephrine in women than men (562). This might demonstrate an elevated α2-adrenergic activity in women.

An increase in α2-adrenoceptor activity may be related to a greater number of α2-adrenoceptors in females than in males as found in the urethra (563). Similar findings of an increased α2-adrenoceptor density were reported from platelets of women compared with men (564). With respect to α2-adrenergic stimulation, E2 replacement enhanced vasoconstriction induced by smooth muscle α2-adrenoceptor activation (565). In ewes, E2 treatment increased α2-adrenergic receptor density in myometrial membranes (566). In rabbit uterine smooth muscle, estrogen treatment increased uterine α1- and α2 adrenoceptor density (567). It was reported that E2 pretreatment increases the responsiveness to norepinephrine and clonidine in isolated rat female aorta, and the authors concluded that E2 pretreatment increases the number of α1-adrenoceptors (568). E2 pretreatment of rats (pregnancy levels) increased α1-adrenoceptor numbers in the nucleus tractus solitarius relative to control (569). If such an estrogen-dependent increase of α1- and α2-adrenergic receptor activity would play a role in immune cells, which is not known, it might be a clear proinflammatory signal because these receptors serve many proinflammatory pathways, which is opposite for β-adrenergic receptors.

Importantly, E2 at pregnancy levels was able to downregulate β1-adrenergic receptors on cardiomyocytes (570). In hypothalamic and preoptic brain slices of rats, E2 treatment reduced the β-adrenergically mediated increase of cAMP (571). Similarly, in rabbit myometrium, E2 inhibited the β-adrenoceptor pathway (572). However, the endometrium of the ovariectomized rat was devoid of any β-adrenoceptors, whereas that of the E2-treated rats was increased. After ovariectomy, there was an increase in β-adrenoceptor density in the myometrium, but this was not observed in the animals given E2 replacement (573). Particularly, these latter results indicate a site-specific modulation of β-adrenergic receptors by estrogens. As with α-adrenoceptors, we do not know whether β-adrenergic receptors are modulated on immune cells. A potential estrogen-induced decrease in β-adrenoceptor density on immune cells would be a proinflammatory signal.

Another question remains whether estrogens influence sympathetic innervation, because a low density of sympathetic nerve fibers must be regarded as a proinflammatory signal (559). Since the late 1970s, it has been known that uterine sympathetic nerve fibers disappear during pregnancy (574, 575). The estrogenic status largely affects sympathetic innervation, which has been demonstrated in the ERα-deficient mouse; ERα-deficient mice demonstrate a marked hyperinnervation of the uterus, which was not demonstrated for sensory or cholinergic nerve fibers (576). The effect of estrogens on sympathetic innervation was also observed in other tissue; ovariectomized rats showed a 59% elevation in vaginal sympathetic nerve fiber density. E2 treatment for 7 d (proestrus level) reduced nerve fiber density to an extent comparable to the situation during estrus (577). However, only urogenital tissues have been investigated, so a general conclusion cannot be drawn. It might be that under the influence of estrogens, similar to the site-specific regulation of β-adrenoceptors, a location-specific change of tissue innervation appears.

In conclusion, estrogens enhance α1- and α2-adrenergic receptor signaling but can decrease β-adrenoceptor signaling. Assuming that estrogens would contribute to the loss of sympathetic innervation in inflamed areas similar to the pregnant uterus, this would be an additional proinflammatory signal due to decreased neurotransmitter concentrations (559). Although these concepts are intriguing, it is important that they are verified in inflammatory diseases.

D. Summary

Estrogens influence systemic response systems by reducing the cytokine-stimulated ACTH and cortisol release, by increasing substance P signaling and sensitization to painful stimuli (increase of neurogenic inflammation), and by increasing signaling through proinflammatory α-adrenergic pathways (Fig. 4). These estrogen effects can partly explain the sexual dimorphism in chronic inflammatory diseases.

XIV. The Dual Role of Estrogens and Consequences for Autoimmune Disease

In 2001, Calabrese (578) presented a thoughtful review on bimodal effects of estrogens on inflammatory pathways. It was delineated that E2 at high doses inhibited many inflammatory mechanisms, whereas at low concentrations no effects or even opposite effects were observed (578). By con-

sidering the concentrations given in the literature examined in this review, the statements of this earlier review can be corroborated. Periovulatory/proestrus to pregnancy levels of E2 inhibit important proinflammatory pathways (exceptions are the B cell, apoptosis, neoangiogenesis, and the above-mentioned systemic responses systems). This is particularly evident at third-trimester pregnancy levels. It is important to mention that the decrease of E2 below the early follicular/diestrus level leading to postmenopausal/metestrus levels most probably leads to a milieu shift toward a proinflammatory direction. Table 9 summarizes the bimodal effects of E2.

Sometimes there is a functional antagonism, which was demonstrated in primary human adipose fibroblasts (88). E2 at pregnancy levels increased TNF receptor 1 mRNA and protein levels (no effect at 10^{-11} to 10^{-9} M), whereas E2 at the same concentration decreased TNF receptor 2 mRNA (increase at 10^{-11} to 10^{-10} M) (88). Because these receptors play quite different roles, a functional antagonism is installed.

Other functional antagonisms were demonstrated with respect to TGF-β (172), VEGF secretion (246), and IL-1RI and IL-1RII (143). Another functional antagonism is dependent on the presence of ERα and ERβ. In different epithelial cell lines, the E2-ERα complex rapidly activates multiple signaling pathways committed to both cell cycle progression and prevention of apoptotic cascades (214). On the other hand, the E2-ERβ complex induced the rapid and persistent phosphorylation of p38 MAPK, which in turn was involved in caspase-3 activation and cleavage of poly(ADP-ribose) polymerase, driving cells into the apoptotic cycle. Thus, E2 promotes cell survival through ERα nongenomic signaling and cell death through ERβ nongenomic signaling (214). It is also a functional antagonism when E2 stimulates many aspects of B cell immunity but inhibits generation of B cell precursors in the bone marrow (*Section III.B*).

These bimodal effects of E2 need to be taken into account when discussing proinflammatory diseases in pre- and postmenopausal women. These bimodal effects are relevant in many chronic inflammatory diseases as outlined in Fig. 3. Some concepts are developed in the following section.

XV. Discussion

In the sections above, many conclusions have been presented at the end of the respective section. In addition, summary sections outlined effects of estrogens on different immune cell subtypes and cytokine secretion (Figs. 1 and 2, *Section III.I*), on various chronic inflammatory diseases (Fig. 3, *Section VIII.N*), and on systemic response systems (Fig. 4, *Section XIII.D*). The following part presents afterthoughts of the author, which are synthesizing the information given above. In some parts, the presented ideas have a hypothetical character, which includes the main aspects "estrogen targets," "estrogen concentration," and "estrogen timing." The discussion is given with the particular focus on chronic inflammatory diseases.

TABLE 9. Bimodal effects of E2 on inflammatory pathways

Cell type or model	Effect	Ref.
Thymocytes (D10)	E2 stimulates IL-1 at 10^{-10} and 10^{-9} M, inhibition at 10^{-7} to 10^{-5} M	72
Primary CD4+ cells from patients with MS	E1 and E2 stimulate antigen- or anti-CD3-stimulated TNF at 3.5×10^{-9} to 1.8×10^{-8} M, inhibition at 3.5×10^{-7} to 3.5×10^{-5} M	45
Antigen-specific human CD4+ T cells	E2 stimulated TNF at 3.6×10^{-9} and 1.8×10^{-8} M, inhibition at 3.6×10^{-8} M and particularly at 3.6×10^{-7} M	44
Human monocytes and macrophages	E2 stimulates IL-1 at 10^{-10} and 10^{-9} M, inhibition at 10^{-7} to 10^{-5} M	135
Human peripheral monocytes	E2 stimulates IL-1 at 10^{-10} to 10^{-9} M, inhibition at 10^{-7} M	72
U937 monocytic cells	Inhibition of the TNF promotor in a concentration range of E2 between 10^{-11} M (no inhibition) and 10^{-9} M (full inhibition)	579
Murine monocytic cell line RAW264.7	E2 at 10^{-8} to 10^{-6} M (but not at 10^{-10} or 10^{-9} M) inhibited RANKL-induced IκB degradation and NF-κB nuclear localization	580
Peritoneal rat macrophage	E2 increased phagocytosis of opsonized sheep erythrocytes at 10^{-12} M to 10^{-8} M but not at higher concentrations of 10^{-6} M	448
Endothelial cells of human umbilical cord veins	E2 at 10^{-8} M and 10^{-7} M stimulated proliferation of HUVECs, but at 10^{-6} M E2 had no effect	249
Capillary endothelial cell line from bovine adrenal medulla	Proliferation and PCNA expression was stimulated at 10^{-9} and 10^{-8} M but inhibited at 10^{-6} and 10^{-5} M	581
Bovine aortic endothelial cells	E2 exhibited biphasic dose-response effects on secretion of plasminogen activator: stimulation at 10^{-12} M and inhibition at 10^{-8} and 10^{-7} M	582
Fetal bovine aortic endothelial cells	E2 stimulated proliferation between 10^{-11} and 10^{-8} M (no effect at 10^{-7} M)	583
Endometrial stromal cells (fibroblasts)	E2 inhibited the secretion of MCP-1 from endometrial stromal cells (fibroblasts) with a IC_{50} of 10^{-8} M (slight increase at 10^{-12} M, no inhibition at 10^{-11} and 10^{-10} M, maximum inhibition at 10^{-6} M)	90
Hybridoma cells	E2 increased antibody production by hybridoma cells at 10^{-10} to 10^{-8} M but not at 10^{-7} or 10^{-6} M	584
Primary human breast adipose fibroblasts	E2 stimulated TNFR2 mRNA levels at 10^{-11} M to 10^{-10} M, had no effect at 10^{-9} M, and inhibited TNFR2 mRNA at 10^{-8} and 10^{-7} M	88
Primary rabbit chondrocytes	E2 at 10^{-10} M counteracts the IL-1β-induced decrease in sulfated proteoglycans, whereas at 10^{-8} M E2 enhanced the IL-1β effects	585
Model of EAE	E2 at pregnancy levels (2×10^{-7} M) decreased EAE incidence, which was not demonstrated for lower serum levels ($<1 \times 10^{-9}$ M)	312

PCNA, Proliferating cell nuclear antigen; TNFR, TNF receptor.

A. Autoimmune diseases: the initiation phase

B cells, T cells, and antigen-presenting cells (macrophages/dendritic cells, B cells) are decisive for the initiation of autoimmune diseases. One might separate two clinically important phases of an autoimmune disease: 1) an asymptomatic phase, and 2) a symptomatic phase (after disease outbreak). Disease outbreak means involvement of a target organ/target tissue leading to the classical signs of visible and functionally relevant inflammation.

Recent important work in the field of RA delineated that autoimmune phenomena are already present more than 10 yr before disease outbreak (586, 587). In the asymptomatic phase, T cells, B cells, and antigen-presenting cells play an important hidden role because clonal expansion of autoreactive T cells and B cells happens without overt disease symptoms. Because the influence of estrogens on these secret players is different from other cell types (Figs. 1 and 2), estrogens can have quite opposite roles depending on involved cells.

If B cells play a center role by antigen presentation, autoantibody production, and/or bystander cytokine secretion, E2 will probably speed up the outbreak of the disease in the early reproductive years (Fig. 5, *upper part*). One might speculate that pregnancy with high levels of E2 and progesterone will prepare the outbreak of the disease because, during this period, autoreactive B cells, which are normally deleted in the bone marrow, are rescued and come to maturity in the marginal zone of the spleen or in other secondary lymphoid organs (36). Because pregnancy with high levels of estrogens but also progesterone has a tremendously inhibitory influence on most inflammatory pathways, women might be sheltered until parturition but might be prone to disease outbreak thereafter in the vulnerable postpartum phase. Because men never experience these high estrogen or progesterone levels, the apparent gender dimorphism of autoimmune disease during the reproductive period of women can be explained.

If tissue-destructive T cells and B cells play an equally important role, the onset of disease in a woman will be delayed because E2 inhibits T cell autoimmunity but stimulates B cell autoimmunity. In such a situation, the onset of the disease might be shifted to the late reproductive phase or postmenopausal period (Fig. 5, *lower part*). Nevertheless, clonal expansion of autoreactive B cells might happen during the reproductive period, but the outbreak is delayed. Preceding pregnancies might have a similar preparing role as mentioned above. This might be the reason why autoantibody production is observed more than 10 yr before disease outbreak in the late reproductive years but the disease breaks out in the postmenopausal period (371, 586). Similarly, if tissue-destructive T cells play the most important role, the onset of the disease is expected in the postmenopausal phase when E2 but also progesterone declines (Fig. 5, *lower part*). Thus, ovariectomy in animal models or the menopause in women is stimulatory for autoimmune arthritis and other autoimmune diseases of late onset. In such a situation, previous pregnancies might even shelter women from the disease.

Today, we know that different subtypes of a given autoimmune disease exist. This has been nicely demonstrated in patients with RA and MS (164–167). One group of patients demonstrates B cell-dependent pathologies, and another group shows mainly tissue-destructive T cell-dependent autoimmunity. Presently, we do not classify these diseases according to the prevailing autoimmune reaction. In addition, we do not classify the diseases according to the success of anti-B cell therapy, but this might be future thinking. It is suggested that such a classification would lead to a better understanding of the role of estrogens in these diseases (251). Classification in this sense might also explain some confusing results obtained in studies in patients with autoimmune diseases.

B. Autoimmune diseases: the symptomatic phase

The location of tissue inflammation in an autoimmune disease is defined by the major autoantigen (or autoantigens). Once a disease has entered the highly inflammatory symptomatic phase, many other cell types get involved depending on the major location of the autoimmune disease (in the brain, in the joint, in the kidney, *etc.*), *e.g.*, in the joint, macrophages, fibroblasts, osteoclasts, osteoblasts, adipocytes, endothelial cells; and many more get gradually involved in the inflammatory process. This does not similarly happen in the brain in MS or in the thyroid gland in thyroiditis due to another environment. Depending on the predominant cell types involved, E2 (and also progesterone) might have quite different effects on these bystander inflammatory activities of participating cells (Fig. 2, and *Section III*). Even during the course of an autoimmune disease the different players might get more or less important so that the character of the disease progressively changes. For example, neoangiogenesis plays an important role in early but not in late RA, which necessarily means that E2 influences this factor more in the early than in the late phase of the disease. These changes of the underlying players are probably important for understanding the different roles of administered E2 at different time points as outlined in *Section IX*.

All players (including B cells, T cells, and antigen-presenting cells) might have very different capacities to take up and to metabolize estrogens. Metabolism of estrogens depends on transport into cells, desulfation of sulfated estrogens, sulfation of nonsulfated estrogens, androgen aromatization, and estrogen conversion to downstream hydroxylated or methylated estrogens, the role of which is slowly being discovered (*Section X*). In addition, up- or down-regulation of ERα and ERβ (in cells or on the cell surface), of coactivators, and of corepressors might well depend on involved cells and microenvironmental conditions such as accompanying hypoxia and the surrounding cytokine cocktail (*Section II*).

In addition, it has been nicely demonstrated in one animal model that E2 accelerates immune-complex glomerulonephritis but ameliorates focal sialadenitis, renal vasculitis, and periarticular inflammation (304). This indicates that quite different pathologies might even be present in the same individual so that estrogens have beneficial effects on one aspect of the disease but a deleterious influence on other mechanisms. This depends most probably on the involved cell types.

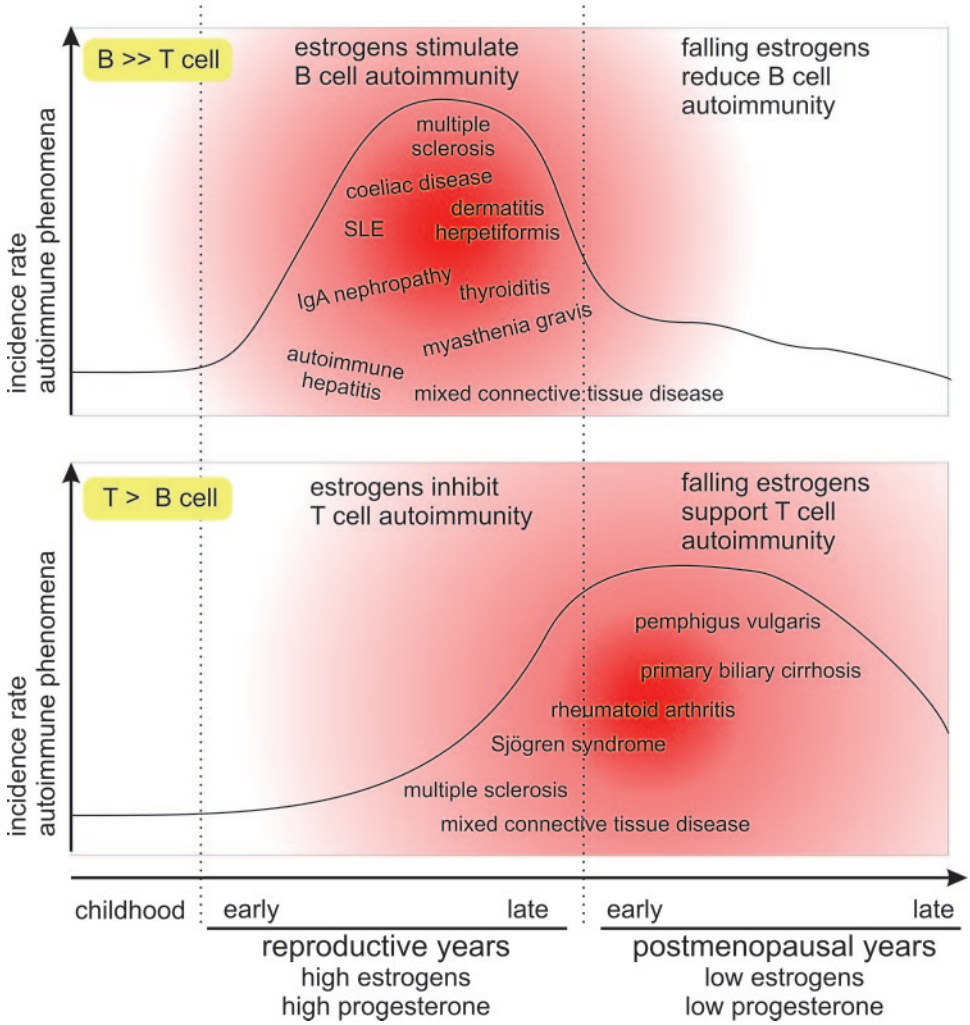

FIG. 5. Relationship between reproductive status in a woman and incidence rate (extent of autoimmune phenomena) in a given autoimmune disease. In the *upper panel*, B cell-dependent autoimmunity plays the key role in the mentioned diseases because autoantibody production is an important phenomenon. In the *lower panel*, T cells play an equal or more important role than B cells. Depending on the influence of estrogens and other hormones in the different phases (separated by *dotted lines*), the time point of the disease outbreak is located either in the reproductive phase or in the postmenopausal phase.

From this point of view, one begins to understand that very high E2 levels (and also other steroid hormones such as progesterone) in pregnancy can be beneficial because so many different aspects of the inflammatory process are inhibited. Even patients with SLE profit from the third trimester of pregnancy most probably due to strong immunosuppression during this time (459). Thus, patients with highly proinflammatory autoimmune diseases would only benefit from pregnancy doses of estrogens as recently demonstrated in MS (47). Studies in the last decade also demonstrated that the small change of estrogen or progesterone levels during therapy with OC or HRT does not have the power to increase the risk or to inhibit an ongoing autoimmune disease (no marked effects).

One also understands that loss of estrogens (but also progesterone) in the late reproductive phase and after the menopause can lead to proinflammatory conditions (see bone resorption and activated T cells outlined in *Section VIII.M*). This is substantiated by the important fact that estrogens can even stimulate several immune mechanisms at postmenopausal levels due to their bimodal role described (Figs. 3 and 5 and Table 9).

C. Inflammatory diseases with involvement of infectious agents

In diseases with infectious agents, particularly cells of the innate immune system (neutrophils, monocytes, macrophages, natural killer cells, mast cells, *etc.*) and bystander cells of the tissue such as fibroblasts, myofibroblasts, endothelial cells, smooth muscle cells, osteoclasts/osteoblasts, macroglia, microglia, adipocytes, and others play a decisive role in the early phase.

As outlined in Fig. 2, estrogens can stimulate several important pathways of innate immunity at follicular to periovulatory levels such as IL-1β from monocytes, the respiratory burst and phagocytosis of monocytes, and the activity of natural killer cells. In addition, estrogens inhibit the LPS-

stimulated activation of the HPA axis (inhibition of antiinflammatory cortisol) and estrogens stimulate sensory nerve fibers with the proinflammatory substance P. All these mechanisms help to overcome critical periods during bacterial infections after trauma and shock. Finally, E2 sensitizes Kupffer cells to LPS, which stimulates a strong response. This is most likely a reason why women have a better outcome during bacterial infections and why women have a lower risk to develop sepsis compared with men.

The stimulatory role of estrogens and progesterone on B cells must be particularly important because pregnant women do not often develop an infectious disease. It is hypothesized that, in the presence of many immunosuppressive effects of estrogens at pregnancy levels, estrogen-dependent stimulation of B cells is the most important compensatory mechanism to overcome infection. This might also be true during the normal menstrual cycle, when women experience most infections in the late luteal phase, and during menses when E2 and progesterone levels are lowest. Based on these concepts of estrogen-dependent stimulation of innate immunity (at follicular to ovulatory levels), women are better protected from infections than men. This also suggests that infectious stimuli such as LPS from Gram-negative bacteria or peptidoglycan from Gram-positive bacteria (and others) must play a decisive role in how estrogens influence secretion of proinflammatory cytokines. Indeed, it has been demonstrated that E2 at 10^{-10} to 10^{-9} M increased IL-1β secretion from human *Streptococci*-stimulated or LPS-stimulated monocytes (73, 142). However, no systematic study is available that has demonstrated the effect of E2 on IL-1β in the absence or presence of LPS or peptidoglycan in the same cell type. It might well be that these important agonists to Toll-like receptors can switch important estrogen signaling pathways or may differentially regulate ERs.

D. Inflammatory diseases with mild inflammation without infectious agents

Usually bone resorption is not accompanied by overt infection or sepsis, neither is it accompanied by a huge inflammation. It is obvious that estrogen levels of healthy women with a regular menstrual cycle protect from bone resorption. The postmenopausal phase with a marked reduction of estrogen (and progesterone) levels constitutes a proinflammatory milieu in the bone.

This long-lasting process with a slow progression and mild local inflammation cannot be compared with autoimmunity and short-lasting trauma/endotoxin shock. In these diseases, the menopause seems to be a transition point, at which mild proinflammatory conditions start to prevail. In this mild inflammation, HRT inducing a little shift of E2 and progesterone concentrations from postmenopausal to follicular levels can prevent inflammation. Without a doubt, HRT is beneficial to hinder bone resorption. Because we know that T cells play an important role in bone resorption, the T cell has attracted recent attention. Similar to above-mentioned autoimmune diseases of late onset, women of reproductive age are protected due to E2-mediated suppression of T cells. With the loss of E2 (but probably also of progesterone), T cells might be important starters of bone resorption. However, after initiation of the disease other cells get involved in the local inflammatory process and the disease course gradually changes (see above). In such a situation, administered E2 can have new roles depending on involved players and metabolism of E2.

E. Inflammatory fibrogenesis in liver and kidney

In these diseases, E2 exerts beneficial effects by decreasing extracellular matrix. Although E2 is a growth-promoting and antiapoptotic hormone, the effects on inflammation-induced fibrogenesis are obviously favorable. By reducing inflammation, E2 is inhibiting other pathways that are important for production of extracellular matrix. Thus, the growth-promoting effect of E2, *e.g.*, on TGF-β1, can be compensated for by inhibition of inflammation, finally leading to favorable effects of this hormone. Interestingly, these antifibrotic effects of E2 have been described in organs with a high content of epithelial cells (liver and kidney). The question arises whether regulation of E2 metabolism has specific characteristics in epithelial cells.

XVI. Conclusions

In conclusion, immune stimuli (foreign antigens or autoantigens) and respective immune responses, the cell types involved (not only immune cells), the target organ, the reproductive status in a woman and timing of E2 administration in relation to the disease process, concentration of estrogens (dual effects), expression of ERα and ERβ (and their isoforms) depending on the microenvironment and the cell type, and intracellular metabolism of estrogens all play important roles in inflammatory diseases. In addition, systemic supersystems such as the HPA axis, the sensory nervous system, and the SNS can be influenced by estrogens to establish a proinflammatory milieu.

This review reinforces the concept that estrogens have antiinflammatory but also proinflammatory roles depending on above-mentioned influencing factors. This review also explains that a uniform concept for the action of estrogens cannot be found for all known chronic inflammatory diseases (Figs. 3 and 5). Nevertheless, for strictly B cell-dependent diseases such as those shown in Fig. 5, the female to male preponderance can be explained by the propagating effects of estrogens (but possibly also of progesterone). The smaller the influence of B cells and the bigger the weight of T cells and other cells, the less evident is the sex dimorphism in chronic inflammatory diseases (Fig. 5). In addition, because men never experience high estrogen (or progesterone) levels like women during pregnancy, the apparent gender dimorphism of chronic inflammatory diseases during the reproductive period of women can be explained. In addition, and this was not reported here, higher androgen levels in men most often exert inhibitory effects on many immune phenomena, which is an other important argument for why women with low androgen levels are protected from infectious diseases but more prone to B cell-dependent autoimmunity. Finally, we should not forget that sexual dimor-

phism of diseases may also depend on factors independent of sex hormones (588).

This review stimulated some important questions for future research (see below). These questions reflect the attitude of the author. It is hoped that the reader may derive some important new ideas, which are necessary to unravel further the complexity of estrogen actions in chronic inflammatory diseases.

Questions and future research directions

- *How are ERα and ERβ and membrane ERs regulated by E2 and in which cells from inflamed tissue does it happen? Do membrane ERs play a role in inflammatory diseases? Do these membrane receptors switch-on different signaling pathways compared with classical ERs?*
- *Is there inflammation-dependent up-regulation of ERβ relative to ERα, and what does it mean? Is there cross-regulation of ERα and ERβ in cells from inflamed tissue? Does up-regulation of ERβ inhibit signaling through ERα?*
- *Which coactivators and corepressors of ERs are present in which cells from inflamed tissue? Does the expression pattern of coactivators and corepressors predict proinflammatory events?*
- *Is E2 stimulating or inhibiting Th17 pathways in chronic inflammatory diseases?*
- *How are estrogen sulfatase and sulfotransferase regulated in cells of inflamed tissue? Which are the major pathways of E1 and E2 metabolism in cells from inflamed tissue? Is there a specific defect of enzymes needed for conversion to 2-hydroxyestrogens or 2-methoxyestrogens?*
- *Is a B cell-dominated form of an autoimmune disease most often appearing in the reproductive phase, and does a T cell-dominated form of the same disease appear in the postmenopausal phase?*
- *Do systemic response systems provide a more proinflammatory environment in women than men with chronic inflammatory diseases, and would high doses of estrogens change this situation?*
- *Do estrogens play a role in the loss of sympathetic nerve fibers in inflamed tissue?*
- *How does E2 influence proinflammatory cytokine secretion in the presence and absence of Toll-like receptor stimuli? Does the expression or ERα and ERβ change upon Toll-like receptor stimuli?*
- *Are E2 metabolism and ER signaling specific in epithelial cells (see the extreme example of prostatitis)?*

Acknowledgments

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References

1. **Grossman CJ** 1985 Interactions between the gonadal steroids and the immune system. Science 227:257–261
2. **Olsen NJ, Kovacs WJ** 1996 Gonadal steroids and immunity. Endocr Rev 17:369–384
3. **Talal N** 1981 Sex steroid hormones and systemic lupus erythematosus. Arthritis Rheum 24:1054–1056
4. **Bijlsma JW, van den Brink HR** 1992 Estrogens and rheumatoid arthritis. Am J Reprod Immunol 28:231–234
5. **Cutolo M, Wilder RL** 2000 Different roles for androgens and estrogens in the susceptibility to autoimmune rheumatic diseases. Rheum Dis Clin North Am 26:825–839
6. **Pfeilschifter J, Koditz R, Pfohl M, Schatz H** 2002 Changes in proinflammatory cytokine activity after menopause. Endocr Rev 23:90–119
7. **Hall JM, McDonnell DP** 1999 The estrogen receptor β-isoform (ERβ) of the human estrogen receptor modulates ERα transcriptional activity and is a key regulator of the cellular response to estrogens and antiestrogens. Endocrinology 140:5566–5578
8. **Weihua Z, Saji S, Makinen S, Cheng G, Jensen EV, Warner M, Gustafsson JA** 2000 Estrogen receptor (ER) β, a modulator of ERα in the uterus. Proc Natl Acad Sci USA 97:5936–5941
9. **Cutolo M, Accardo S, Villaggio B, Clerico P, Bagnasco M, Coviello DA, Carruba G, Io Casto M, Castagnetta L** 1993 Presence of estrogen-binding sites on macrophage-like synoviocytes and CD8+, CD29+, CD45RO+ T lymphocytes in normal and rheumatoid synovium. Arthritis Rheum 36:1087–1097
10. **Ushiyama T, Inoue K, Nishioka J** 1995 Expression of estrogen receptor related protein (p29) and estradiol binding in human arthritic synovium. J Rheumatol 22:421–426
11. **Cutolo M, Accardo S, Villaggio B, Barone A, Sulli A, Coviello DA, Carabbio C, Felli L, Miceli D, Farruggio R, Carruba G, Castagnetta L** 1996 Androgen and estrogen receptors are present in primary cultures of human synovial macrophages. J Clin Endocrinol Metab 81:820–827
12. **Ishizuka M, Hatori M, Suzuki T, Miki Y, Darnel AD, Tazawa C, Sawai T, Uzuki M, Tanaka Y, Kokubun S, Sasano H** 2004 Sex steroid receptors in rheumatoid arthritis. Clin Sci (Lond) 106:293–300
13. **Capellino S, Riepl B, Rauch L, Angele P, Cutolo M, Straub RH** 2007 Quantitative determination of steroid hormone receptor positive cells in synovium of RA and OA patients: is there a link to inflammation? Ann Rheum Dis 66:53–58
14. **Rider V, Li X, Peterson G, Dawson J, Kimler BF, Abdou NI** 2006 Differential expression of estrogen receptors in women with systemic lupus erythematosus. J Rheumatol 33:1093–1101
15. **Schneider CP, Nickel EA, Samy TS, Schwacha MG, Cioffi WG, Bland KI, Chaudry IH** 2000 The aromatase inhibitor, 4-hydroxyandrostenedione, restores immune responses following trauma-hemorrhage in males and decreases mortality from subsequent sepsis. Shock 14:347–353
16. **Kurebayashi J, Otsuki T, Moriya T, Sonoo H** 2001 Hypoxia reduces hormone responsiveness of human breast cancer cells. Jpn J Cancer Res 92:1093–1101
17. **Stoner M, Saville B, Wormke M, Dean D, Burghardt R, Safe S** 2002 Hypoxia induces proteasome-dependent degradation of estrogen receptor α in ZR-75 breast cancer cells. Mol Endocrinol 16:2231–2242
18. **Tamir S, Izrael S, Vaya J** 2002 The effect of oxidative stress on ERα and ERβ expression. J Steroid Biochem Mol Biol 81:327–332
19. **Katzenellenbogen BS, Nardulli AM, Read LD** 1990 Estrogen regulation of proliferation and hormonal modulation of estrogen and progesterone receptor biosynthesis and degradation in target cells. Prog Clin Biol Res 322:201–211
20. **Asai K, Hiki N, Mimura Y, Ogawa T, Unou K, Kaminishi M** 2001 Gender differences in cytokine secretion by human peripheral blood mononuclear cells: role of estrogen in modulating LPS-induced cytokine secretion in an ex vivo septic model. Shock 16: 340–343
21. **Rogers A, Eastell R** 2001 The effect of 17β-estradiol on production of cytokines in cultures of peripheral blood. Bone 29:30–34
22. **Ralston SH, Russell RG, 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
23. **Bernard-Poenaru O, Roux C, Blanque R, Gardner C, de Vemejoul MC, Cohen-Solal ME** 2001 Bone-resorbing cytokines from periph-

eral blood mononuclear cells after hormone replacement therapy: a longitudinal study. Osteoporos Int 12:769–776
24. **Paavonen T, Andersson LC, Adlercreutz H** 1981 Sex hormone regulation of in vitro immune response. Estradiol enhances human B cell maturation via inhibition of suppressor T cells in pokeweed mitogen-stimulated cultures. J Exp Med 154:1935–1945
25. **Clerici E, Bergamasco E, Ferrario E, Villa ML** 1991 Influence of sex steroids on the antigen-specific primary antibody response in vitro. J Clin Lab Immunol 34:71–78
26. **Mizoguchi Y, Ikemoto Y, Yamamoto S, Morisawa S** 1985 Studies on the effects of estrogen on antibody responses in asymptomatic HB virus carriers and non-responders to HB vaccine inoculation. Gastroenterol Jpn 20:324–329
27. **Sthoeger ZM, Chiorazzi N, Lahita RG** 1988 Regulation of the immune response by sex hormones. I. In vitro effects of estradiol and testosterone on pokeweed mitogen-induced human B cell differentiation. J Immunol 141:91–98
28. **Kanda N, Tamaki K** 1999 Estrogen enhances immunoglobulin production by human PBMCs. J Allergy Clin Immunol 103:282–288
29. **Kanda N, Tsuchida T, Tamaki K** 1999 Estrogen enhancement of anti-double-stranded DNA antibody and immunoglobulin G production in peripheral blood mononuclear cells from patients with systemic lupus erythematosus. Arthritis Rheum 42:328–337
30. **Lü FX, Abel K, Ma Z, Rourke T, Lu D, Torten J, McChesney M, Miller CJ** 2002 The strength of B cell immunity in female rhesus macaques is controlled by CD8+ T cells under the influence of ovarian steroid hormones. Clin Exp Immunol 128:10–20
31. **Franklin RD, Kutteh WH** 1999 Characterization of immunoglobulins and cytokines in human cervical mucus: influence of exogenous and endogenous hormones. J Reprod Immunol 42:93–106
32. **Ahmed SA, Aufdemorte TB, Chen JR, Montoya AI, Olive D, Talal N** 1989 Estrogen induces the development of autoantibodies and promotes salivary gland lymphoid infiltrates in normal mice. J Autoimmun 2:543–552
33. **Nilsson N, Carlsten H** 1994 Estrogen induces suppression of natural killer cell cytotoxicity and augmentation of polyclonal B cell activation. Cell Immunol 158:131–139
34. **Verthelyi D, Ahmed SA** 1994 17β-Estradiol, but not 5 α-dihydrotestosterone, augments antibodies to double-stranded deoxyribonucleic acid in nonautoimmune C57BL/6J mice. Endocrinology 135:2615–2622
35. **Latham KA, Zamora A, Drought H, Subramanian S, Matejuk A, Offner H, Rosloniec EF** 2003 Estradiol treatment redirects the isotype of the autoantibody response and prevents the development of autoimmune arthritis. J Immunol 171:5820–5827
36. **Peeva E, Venkatesh J, Diamond B** 2005 Tamoxifen blocks estrogen-induced B cell maturation but not survival. J Immunol 175:1415–1423
37. **Kim S, Liva SM, Dalal MA, Verity MA, Voskuhl RR** 1999 Estriol ameliorates autoimmune demyelinating disease: implications for multiple sclerosis. Neurology 52:1230–1238
38. **Medina KL, Kincade PW** 1994 Pregnancy-related steroids are potential negative regulators of B lymphopoiesis. Proc Natl Acad Sci USA 91:5382–5386
39. **Smithson G, Beamer WG, Shultz KL, Christianson SW, Shultz LD, Kincade PW** 1994 Increased B lymphopoiesis in genetically sex steroid-deficient hypogonadal (hpg) mice. J Exp Med 180:717–720
40. **Smithson G, Couse JF, Lubahn DB, Korach KS, Kincade PW** 1998 The role of estrogen receptors and androgen receptors in sex steroid regulation of B lymphopoiesis. J Immunol 161:27–34
41. **Islander U, Erlandsson MC, Hasseus B, Jonsson CA, Ohlsson C, Gustafsson JA, Dahlgren U, Carlsten H** 2003 Influence of oestrogen receptor α and β on the immune system in aged female mice. Immunology 110:149–157
42. **Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL** 1986 Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 136:2348–2357
43. **Steinman L** 2007 A brief history of T(H)17, the first major revision in the T(H)1/T(H)2 hypothesis of T cell-mediated tissue damage. Nat Med 13:139–145
44. **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
45. **Correale J, Arias M, Gilmore W** 1998 Steroid hormone regulation of cytokine secretion by proteolipid protein-specific CD4+ T cell clones isolated from multiple sclerosis patients and normal control subjects. J Immunol 161:3365–3374
46. **Zang YC, Halder JB, Hong J, Rivera VM, Zhang JZ** 2002 Regulatory effects of estriol on T cell migration and cytokine profile: inhibition of transcription factor NF-κB. J Neuroimmunol 124:106–114
47. **Sicotte NL, Liva SM, Klutch R, Pfeiffer P, Bouvier S, Odesa S, Wu TC, Voskuhl RR** 2002 Treatment of multiple sclerosis with the pregnancy hormone estriol. Ann Neurol 52:421–428
48. **Carlsten H, Verdrengh M, Taube M** 1996 Additive effects of suboptimal doses of estrogen and cortisone on the suppression of T lymphocyte dependent inflammatory responses in mice. Inflamm Res 45:26–30
49. **Erlandsson MC, Gomori E, Taube M, Carlsten H** 2000 Effects of raloxifene, a selective estrogen receptor modulator, on thymus, T cell reactivity, and inflammation in mice. Cell Immunol 205:103–109
50. **Gregory MS, Duffner LA, Faunce DE, Kovacs EJ** 2000 Estrogen mediates the sex difference in post-burn immunosuppression. J Endocrinol 164:129–138
51. **Bao M, Yang Y, Jun HS, Yoon JW** 2002 Molecular mechanisms for gender differences in susceptibility to T cell-mediated autoimmune diabetes in nonobese diabetic mice. J Immunol 168:5369–5375
52. **Gourdy P, Araujo LM, Zhu R, Garmy-Susini B, Diem S, Laurell H, Leite-de-Moraes M, Dy M, Arnal JF, Bayard F, Herbelin A** 2005 Relevance of sexual dimorphism to regulatory T cells: estradiol promotes IFN-γ production by invariant natural killer T cells. Blood 105:2415–2420
53. **Karpuzoglu-Sahin E, Zhi-Jun Y, Lengi A, Sriranganathan N, Ansar AS** 2001 Effects of long-term estrogen treatment on IFN-γ, IL-2 and IL-4 gene expression and protein synthesis in spleen and thymus of normal C57BL/6 mice. Cytokine 14:208–217
54. **Maret A, Coudert JD, Garidou L, Foucras G, Gourdy P, Krust A, Dupont S, Chambon P, Druet P, Bayard F, Guery JC** 2003 Estradiol enhances primary antigen-specific CD4 T cell responses and Th1 development in vivo. Essential role of estrogen receptor α expression in hematopoietic cells. Eur J Immunol 33:512–521
55. **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
56. **Roggia C, Tamone C, Cenci S, Pacifici R, Isaia GC** 2004 Role of TNF-α producing T-cells in bone loss induced by estrogen deficiency. Minerva Med 95:125–132
57. **Lambert KC, Curran EM, Judy BM, Milligan GN, Lubahn DB, Estes DM** 2005 Estrogen receptor α (ERα) deficiency in macrophages results in increased stimulation of CD4+ T cells while 17β-estradiol acts through ERα to increase IL-4 and GATA-3 expression in CD4+ T cells independent of antigen presentation. J Immunol 175:5716–5723
58. **Polanczyk MJ, Carson BD, Subramanian S, Afentoulis M, Vandenbark AA, Ziegler SF, Offner H** 2004 Cutting edge: estrogen drives expansion of the CD4+CD25+ regulatory T cell compartment. J Immunol 173:2227–2230
59. **Staples JE, Gasiewicz TA, Fiore NC, Lubahn DB, Korach KS, Silverstone AE** 1999 Estrogen receptor α is necessary in thymic development and estradiol-induced thymic alterations. J Immunol 163:4168–4174
60. **Erlandsson MC, Ohlsson C, Gustafsson JA, Carlsten H** 2001 Role of oestrogen receptors α and β in immune organ development and in oestrogen-mediated effects on thymus. Immunology 103:17–25
61. **Hirahara H, Ogawa M, Kimura M, Iiai T, Tsuchida M, Hanawa H, Watanabe H, Abo T** 1994 Glucocorticoid independence of acute thymic involution induced by lymphotoxin and estrogen. Cell Immunol 153:401–411
62. **Islander U, Erlandsson MC, Chavoshi T, Jochems C, Moverare S, Nilsson S, Ohlsson C, Gustafsson JA, Carlsten H** 2005 Estren-mediated inhibition of T lymphopoiesis is estrogen receptor-independent whereas its suppression of T cell-mediated inflammation is estrogen receptor-dependent. Clin Exp Immunol 139:210–215

63. **Jain SK, Rogier K, Prouty L, Jain SK** 2004 Protective effects of 17β-estradiol and trivalent chromium on interleukin-6 secretion, oxidative stress, and adhesion of monocytes: relevance to heart disease in postmenopausal women. Free Radic Biol Med 37:1730–1735
64. **Kramer PR, Kramer SF, Guan G** 2004 17 β-Estradiol regulates cytokine release through modulation of CD16 expression in monocytes and monocyte-derived macrophages. Arthritis Rheum 50: 1967–1975
65. **Deshpande R, Khalili H, Pergolizzi RG, Michael SD, Chang MD** 1997 Estradiol down-regulates LPS-induced cytokine production and NFκB activation in murine macrophages. Am J Reprod Immunol 38:46–54
66. **Zhang X, Wang L, Zhang H, Guo D, Qiao Z, Qiao J** 2001 Estrogen inhibits lipopolysaccharide-induced tumor necrosis factor-α release from murine macrophages. Methods Find Exp Clin Pharmacol 23:169–173
67. **Kim JY, Jeong HG** 2003 Down-regulation of inducible nitric oxide synthase and tumor necrosis factor-α expression by bisphenol A via nuclear factor-κB inactivation in macrophages. Cancer Lett 196: 69–76
68. **Tomaszewska A, Guevara I, Wilczok T, Dembinska-Kiec A** 2003 17β-Estradiol- and lipopolysaccharide-induced changes in nitric oxide, tumor necrosis factor-α and vascular endothelial growth factor release from RAW 264.7 macrophages. Gynecol Obstet Invest 56:152–159
69. **Liu HY, Buenafe AC, Matejuk A, Ito A, Zamora A, Dwyer J, Vandenbark AA, Offner H** 2002 Estrogen inhibition of EAE involves effects on dendritic cell function. J Neurosci Res 70:238–248
70. **Pettersson A, Ciumas C, Chirsky V, Link H, Huang YM, Xiao BG** 2004 Dendritic cells exposed to estrogen in vitro exhibit therapeutic effects in ongoing experimental allergic encephalomyelitis. J Neuroimmunol 156:58–65
71. **Xiao BG, Liu X, Link H** 2004 Antigen-specific T cell functions are suppressed over the estrogen-dendritic cell-indoleamine 2,3-dioxygenase axis. Steroids 69:653–659
72. **Polan ML, Daniele A, Kuo A** 1988 Gonadal steroids modulate human monocyte interleukin-1 (IL-1) activity. Fertil Steril 49:964–968
73. **Shanker G, Sorci-Thomas M, Register TC, Adams MR** 1994 The inducible expression of THP-1 cell interleukin-1 mRNA: effects of estrogen on differential response to phorbol ester and lipopolysaccharide. Lymphokine Cytokine Res 13:1–7
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. **Hu SK, Mitcho YL, Rath NC** 1988 Effect of estradiol on interleukin 1 synthesis by macrophages. Int J Immunopharmacol 10:247–252
76. **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
77. **Viviani B, Corsini E, Binaglia M, Lucchi L, Galli CL, Marinovich M** 2002 The anti-inflammatory activity of estrogen in glial cells is regulated by the PKC-anchoring protein RACK-1. J Neurochem 83:1180–1187
78. **Dhandapani KM, Wade FM, Mahesh VB, Brann DW** 2005 Astrocyte-derived transforming growth factor-β mediates the neuroprotective effects of 17β-estradiol: involvement of nonclassical genomic signaling pathways. Endocrinology 146:2749–2759
79. **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
80. **Dimayuga FO, Reed JL, Carnero GA, Wang C, Dimayuga ER, Dimayuga VM, Perger A, Wilson ME, Keller JN, Bruce-Keller AJ** 2005 Estrogen and brain inflammation: effects on microglial expression of MHC, costimulatory molecules and cytokines. J Neuroimmunol 161:123–136
81. **Vegeto E, Bonincontro C, Pollio G, Sala A, Viappiani S, Nardi F, Brusadelli A, Viviani B, Ciana P, Maggi A** 2001 Estrogen prevents the lipopolysaccharide-induced inflammatory response in microglia. J Neurosci 21:1809–1818
82. **Baker AE, Brautigam VM, Watters JJ** 2004 Estrogen modulates microglial inflammatory mediator production via interactions with estrogen receptor β. Endocrinology 145:5021–5032
83. **Seaman WE, Merigan TC, Talal N** 1979 Natural killing in estrogen-treated mice responds poorly to poly I.C despite normal stimulation of circulating interferon. J Immunol 123:2903–2905
84. **Warri A, Kangas L** 1990 Effect of toremifene on the activity of NK-cells in NZB/NZW mice. J Steroid Biochem 36:207–209
85. **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
86. **Fujimoto J, Hori M, Ichigo S, Tamaya T** 1997 Ovarian steroids regulate the expression of basic fibroblast growth factor and its mRNA in fibroblasts derived from uterine endometrium. Ann Clin Biochem 34:91–96
87. **Kawasaki T, Ushiyama T, Inoue K, Hukuda S** 2000 Effects of estrogen on interleukin-6 production in rheumatoid fibroblast-like synoviocytes. Clin Exp Rheumatol 18:743–745
88. **Deb S, Amin S, Imir AG, Yilmaz MB, Suzuki T, Sasano H, Bulun SE** 2004 Estrogen regulates expression of tumor necrosis factor receptors in breast adipose fibroblasts. J Clin Endocrinol Metab 89:4018–4024
89. **Kollias G, Kontoyiannis D** 2002 Role of TNF/TNFR in autoimmunity: specific TNF receptor blockade may be advantageous to anti-TNF treatments. Cytokine Growth Factor Rev 13:315–321
90. **Arici A, Senturk LM, Seli E, Bahtiyar MO, Kim G** 1999 Regulation of monocyte chemotactic protein-1 expression in human endometrial stromal cells by estrogen and progesterone. Biol Reprod 61: 85–90
91. **Kovacs EJ, Faunce DE, Ramer-Quinn DS, Mott FJ, Dy PW, Frazier-Jessen MR** 1996 Estrogen regulation of JE/MCP-1 mRNA expression in fibroblasts. J Leukoc Biol 59:562–568
92. **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
93. **Sato T, Ito A, Mori Y, Yamashita K, Hayakawa T, Nagase H** 1991 Hormonal regulation of collagenolysis in uterine cervical fibroblasts. Modulation of synthesis of procollagenase, prostromelysin and tissue inhibitor of metalloproteinases (TIMP) by progesterone and oestradiol-17 β. Biochem J 275:645–650
94. **Kikuchi N, Urabe M, Iwasa K, Okubo T, Tsuchiya H, Hosoda T, Tatsumi H, Honjo H** 2000 Atheroprotective effect of estriol and estrone sulfate on human vascular smooth muscle cells. J Steroid Biochem Mol Biol 72:71–78
95. **Nakamura Y, Miki Y, Suzuki T, Nakata T, Darnel AD, Moriya T, Tazawa C, Saito H, Ishibashi T, Takahashi S, Yamada S, Sasano H** 2003 Steroid sulfatase and estrogen sulfotransferase in the atherosclerotic human aorta. Am J Pathol 163:1329–1339
96. **Hennebold JD, Daynes RA** 1994 Regulation of macrophage dehydroepiandrosterone sulfate metabolism by inflammatory cytokines. Endocrinology 135:67–75
97. **Weidler C, Struharova S, Schmidt M, Ugele B, Schölmerich J, Straub RH** 2005 Tumor necrosis factor inhibits conversion of dehydroepiandrosterone sulfate (DHEAS) to DHEA in rheumatoid arthritis synovial cells: a prerequisite for local androgen deficiency. Arthritis Rheum 52:1721–1729
98. **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
99. **Vegeto E, Pollio G, Ciana P, Maggi A** 2000 Estrogen blocks inducible nitric oxide synthase accumulation in LPS-activated microglia cells. Exp Gerontol 35:1309–1316
100. **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
101. **Sharma RV, Gurjar MV, Bhalla RC** 2001 Selected contribution: estrogen receptor-α gene transfer inhibits proliferation and NF-κB activation in VSM cells from female rats. J Appl Physiol 91:2400–2406
102. **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
103. **Ghisletti S, Meda C, Maggi A, Vegeto E** 2005 17β-Estradiol inhibits inflammatory gene expression by controlling NF-κB intracellular localization. Mol Cell Biol 25:2957–2968
104. **Sun WH, Keller ET, Stebler BS, Ershler WB** 1998 Estrogen inhibits phorbol ester-induced I κB α transcription and protein degradation. Biochem Biophys Res Commun 244:691–695
105. **Chadwick CC, Chippari S, Matelan E, Borges-Marcucci L, Eckert AM, Keith Jr JC, Albert LM, Leathurby Y, Harris HA, Bhat RA, Ashwell M, Trybulski E, Winneker RC, Adelman SJ, Steffan RJ, Harnish DC** 2005 Identification of pathway-selective estrogen receptor ligands that inhibit NF-κB transcriptional activity. Proc Natl Acad Sci USA 102:2543–2548
106. **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
107. **Kurebayashi S, Miyashita Y, Hirose T, Kasayama S, Akira S, Kishimoto T** 1997 Characterization of mechanisms of interleukin-6 gene repression by estrogen receptor. J Steroid Biochem Mol Biol 60:11–17
108. **McCracken SA, Drury CL, Lee HS, Morris JM** 2003 Pregnancy is associated with suppression of the nuclear factor κB/IκB activation pathway in peripheral blood mononuclear cells. J Reprod Immunol 58:27–47
109. **Messingham KA, Heinrich SA, Kovacs EJ** 2001 Estrogen restores cellular immunity in injured male mice via suppression of interleukin-6 production. J Leukoc Biol 70:887–895
110. **Wen Y, Yang S, Liu R, Perez E, Yi KD, Koulen P, Simpkins JW** 2004 Estrogen attenuates nuclear factor-κB activation induced by transient cerebral ischemia. Brain Res 1008:147–154
111. **Ospina JA, Brevig HN, Krause DN, Duckles SP** 2004 Estrogen suppresses IL-1β-mediated induction of COX-2 pathway in rat cerebral blood vessels. Am J Physiol Heart Circ Physiol 286:H2010–H2019
112. **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
113. **Caulin-Glaser T, Watson CA, Pardi R, Bender JR** 1996 Effects of 17β-estradiol on cytokine-induced endothelial cell adhesion molecule expression. J Clin Invest 98:36–42
114. **Mukherjee TK, Nathan L, Dinh H, Reddy ST, Chaudhuri G** 2003 17-Epiestriol, an estrogen metabolite, is more potent than estradiol in inhibiting vascular cell adhesion molecule 1 (VCAM-1) mRNA expression. J Biol Chem 278:11746–11752
115. **Mori M, Tsukahara F, Yoshioka T, Irie K, Ohta H** 2004 Suppression by 17β-estradiol of monocyte adhesion to vascular endothelial cells is mediated by estrogen receptors. Life Sci 75:599–609
116. **Piercy KT, Donnell RL, Kirkpatrick SS, Timaran CH, Stevens SL, Freeman MB, Goldman MH** 2002 Effects of estrogen, progesterone, and combination exposure on interleukin-1 β-induced expression of VCAM-1, ICAM-1, PECAM, and E-selectin by human female iliac artery endothelial cells. J Surg Res 105:215–219
117. **Tyree CM, Zou A, Allegretto EA** 2002 17β-Estradiol inhibits cytokine induction of the human E-selectin promoter. J Steroid Biochem Mol Biol 80:291–297
118. **Galea E, Santizo R, Feinstein DL, Adamsom P, Greenwood J, Koenig HM, Pelligrino DA** 2002 Estrogen inhibits NF κB-dependent inflammation in brain endothelium without interfering with I κB degradation. Neuroreport 13:1469–1472
119. **Mikkola TS, St Clair RW** 2002 Estradiol reduces basal and cytokine induced monocyte adhesion to endothelial cells. Maturitas 41:313–319
120. **Rodriguez E, Lopez R, Paez A, Masso F, Montano LF** 2002 17β-Estradiol inhibits the adhesion of leukocytes in TNF-α stimulated human endothelial cells by blocking IL-8 and MCP-1 secretion, but not its transcription. Life Sci 71:2181–2193
121. **Friedrich EB, Clever YP, Wassmann S, Hess C, Nickenig G** 2006 17β-Estradiol inhibits monocyte adhesion via down-regulation of Rac1 GTPase. J Mol Cell Cardiol 40:87–95
122. **Squadrito F, Altavilla D, Squadrito G, Campo GM, Arlotta M, Arcoraci V, Minutoli L, Serrano M, Saitta A, Caputi AP** 1997 17β-Oestradiol reduces cardiac leukocyte accumulation in myocardial ischaemia reperfusion injury in rat. Eur J Pharmacol 335:185–192
123. **Verdu EF, Deng Y, Bercik P, Collins SM** 2002 Modulatory effects of estrogen in two murine models of experimental colitis. Am J Physiol Gastrointest Liver Physiol 283:G27–G36
124. **Cuzzocrea S, Mazzon E, Sautebin L, Serraino I, Dugo L, Calabro G, Caputi AP, Maggi A** 2001 The protective role of endogenous estrogens in carrageenan-induced lung injury in the rat. Mol Med 7:478–487
125. **Chantakru S, Wang WC, van den HM, Bashar S, Simpson A, Chen Q, Croy BA, Evans SS** 2003 Coordinate regulation of lymphocyte-endothelial interactions by pregnancy-associated hormones. J Immunol 171:4011–4019
126. **Caulin-Glaser T, Farrell WJ, Pfau SE, Zaret B, Bunger K, Setaro JF, Brennan JJ, Bender JR, Cleman MW, Cabin HS, Remetz MS** 1998 Modulation of circulating cellular adhesion molecules in postmenopausal women with coronary artery disease. J Am Coll Cardiol 31:1555–1560
127. **Van Baal WM, Emeis JJ, Kenemans P, Kessel H, Peters-Muller ER, Schalkwijk CG, van der Mooren MJ, Stehouwer CD** 1999 Short-term hormone replacement therapy: reduced plasma levels of soluble adhesion molecules. Eur J Clin Invest 29:913–921
128. **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
129. **Koh KK, Jin DK, Yang SH, Lee SK, Hwang HY, Kang MH, Kim W, Kim DS, Choi IS, Shin EK** 2001 Vascular effects of synthetic or natural progestagen combined with conjugated equine estrogen in healthy postmenopausal women. Circulation 103:1961–1966
130. **Herrington DM, Brosnihan KB, Pusser BE, Seely EW, Ridker PM, Rifai N, MacLean DB** 2001 Differential effects of E and droloxifene on C-reactive protein and other markers of inflammation in healthy postmenopausal women. J Clin Endocrinol Metab 86:4216–4222
131. **Silvestri A, Gebara O, Vitale C, Wajngarten M, Leonardo F, Ramires JA, Fini M, Mercuro G, Rosano GM** 2003 Increased levels of C-reactive protein after oral hormone replacement therapy may not be related to an increased inflammatory response. Circulation 107:3165–3169
132. **Souter I, Janzen C, Martinez-Maza O, Breen EC, Stanczyk F, Chaudhuri G, Nathan L** 2005 Serum levels of soluble vascular cell adhesion molecule-1 are decreased in women receiving oral contraceptives compared with normally menstruating women: implications in atherosclerosis. Fertil Steril 83:1480–1488
133. **Register TC, Cann JA, Kaplan JR, Williams JK, Adams MR, Morgan TM, Anthony MS, Blair RM, Wagner JD, Clarkson TB** 2005 Effects of soy isoflavones and conjugated equine estrogens on inflammatory markers in atherosclerotic, ovariectomized monkeys. J Clin Endocrinol Metab 90:1734–1740
134. **Qu Q, Harkonen PL, Monkkonen J, Vaananen HK** 1999 Conditioned medium of estrogen-treated osteoblasts inhibits osteoclast maturation and function in vitro. Bone 25:211–215
135. **Polan ML, Loukides J, Nelson P, Carding S, Diamond M, Walsh A, Bottomly K** 1989 Progesterone and estradiol modulate interleukin-1 β messenger ribonucleic acid levels in cultured human peripheral monocytes. J Clin Endocrinol Metab 69:1200–1206
136. **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
137. **Stein B, Yang MX** 1995 Repression of the interleukin-6 promoter by estrogen receptor is mediated by NF-κB and C/EBP β. Mol Cell Biol 15:4971–4979
138. **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
139. **Cheung J, Mak YT, Papaioannou S, Evans BA, Fogelman I, Hampson G** 2003 Interleukin-6 (IL-6), IL-1, receptor activator of nuclear factor κB ligand (RANKL) and osteoprotegerin production by hu-

man osteoblastic cells: comparison of the effects of 17-β oestradiol and raloxifene. J Endocrinol 177:423–433
140. **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
141. **Hogasen AK, Nordsletten L, Aasen AO, Falch JA** 1998 17β-Oestradiol and 1 α,25-dihydroxycholecalciferol modulate constitutive and bone matrix-induced interleukin-1 β (IL-1 β) production by peripheral blood mononuclear cells isolated from postmenopausal women. Scand J Clin Lab Invest 58:97–102
142. **Mori H, Nakagawa M, Itoh N, Wada K, Tamaya T** 1990 Danazol suppresses the production of interleukin-1 β and tumor necrosis factor by human monocytes. Am J Reprod Immunol 24:45–50
143. **Sunyer T, Lewis J, Collin-Osdoby P, Osdoby P** 1999 Estrogen's bone-protective effects may involve differential IL-1 receptor regulation in human osteoclast-like cells. J Clin Invest 103:1409–1418
144. **Schaefer TM, Wright JA, Pioli PA, Wira CR** 2005 IL-1β-mediated proinflammatory responses are inhibited by estradiol via downregulation of IL-1 receptor type I in uterine epithelial cells. J Immunol 175:6509–6516
145. **Lin Y, Huang R, Chen L, Li S, Shi Q, Jordan C, Huang RP** 2004 Identification of interleukin-8 as estrogen receptor-regulated factor involved in breast cancer invasion and angiogenesis by protein arrays. Int J Cancer 109:507–515
146. **Kanda N, Watanabe S** 2001 17β-Estradiol, progesterone, and dihydrotestosterone suppress the growth of human melanoma by inhibiting interleukin-8 production. J Invest Dermatol 117:274–283
147. **Rachon D, Mysliwska J, Suchecka-Rachon K, Wieckiewicz J, Mysliwski A** 2002 Effects of oestrogen deprivation on interleukin-6 production by peripheral blood mononuclear cells of postmenopausal women. J Endocrinol 172:387–395
148. **Gordon CM, LeBoff MS, Glowacki J** 2001 Adrenal and gonadal steroids inhibit IL-6 secretion by human marrow cells. Cytokine 16:178–186
149. **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
150. **Kim MS, Chae HJ, Shin TY, Kim HM, Kim HR** 2001 Estrogen regulates cytokine release in human mast cells. Immunopharmacol Immunotoxicol 23:495–504
151. **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
152. **Koka S, Petro TM, Reinhardt RA** 1998 Estrogen inhibits interleukin-1β-induced interleukin-6 production by human osteoblast-like cells. J Interferon Cytokine Res 18:479–483
153. **Chen XW, Garner SC, Anderson JJ** 2002 Isoflavones regulate interleukin-6 and osteoprotegerin synthesis during osteoblast cell differentiation via an estrogen-receptor-dependent pathway. Biochem Biophys Res Commun 295:417–422
154. **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
155. **Schiller C, Gruber R, Redlich K, Ho GM, Katzgraber F, Willheim M, Pietschmann P, Peterlik M** 1997 17β-Estradiol antagonizes effects of 1α,25-dihydroxyvitamin D3 on interleukin-6 production and osteoclast-like cell formation in mouse bone marrow primary cultures. Endocrinology 138:4567–4571
156. **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
157. **Khalkhali-Ellis Z, Handa RJ, Price Jr RH, Adams BD, Callaghan JJ, Hendrix MJ** 2002 Androgen receptors in human synoviocytes and androgen regulation of interleukin 1β (IL-1β) induced IL-6 production: a link between hypoandrogenicity and rheumatoid arthritis? J Rheumatol 29:1843–1846
158. **Grant-Tschudy KS, Wira CR** 2005 Effect of oestradiol on mouse uterine epithelial cell tumour necrosis factor-α release is mediated through uterine stromal cells. Immunology 115:99–107
159. **Crane-Godreau MA, Wira CR** 2005 Effects of estradiol on lipopolysaccharide and Pam3Cys stimulation of CCL20/macrophage inflammatory protein 3 α and tumor necrosis factor α production by uterine epithelial cells in culture. Infect Immun 73:4231–4237
160. **Carruba G, D'Agostino P, Miele M, Calabro M, Barbera C, Bella GD, Milano S, Ferlazzo V, Caruso R, Rosa ML, Cocciadiferro L, Campisi I, Castagnetta L, Cillari E** 2003 Estrogen regulates cytokine production and apoptosis in PMA-differentiated, macrophage-like U937 cells. J Cell Biochem 90:187–196
161. **Zhang QH, Hu YZ, Cao J, Zhong YQ, Zhao YF, Mei QB** 2004 Estrogen influences the differentiation, maturation and function of dendritic cells in rats with experimental autoimmune encephalomyelitis. Acta Pharmacol Sin 25:508–513
162. **Nakaya M, Yamasaki M, Miyazaki Y, Tachibana H, Yamada K** 2003 Estrogenic compounds suppressed interferon-γ production in mouse splenocytes through direct cell-cell interaction. In Vitro Cell Dev Biol Anim 39:383–387
163. **Fox HS, Bond BL, Parslow TG** 1991 Estrogen regulates the IFN-γ promoter. J Immunol 146:4362–4367
164. **Gerli R, Bistoni O, Russano A, Fiorucci S, Borgato L, Cesarotti ME, Lunardi C** 2002 In vivo activated T cells in rheumatoid synovitis. Analysis of Th1- and Th2-type cytokine production at clonal level in different stages of disease. Clin Exp Immunol 129: 549–555
165. **Ruschpler P, Stiehl P** 2002 Shift in Th1 (IL-2 and IFN-γ) and Th2 (IL-10 and IL-4) cytokine mRNA balance within two new histological main-types of rheumatoid-arthritis (RA). Cell Mol Biol (Noisy-le-grand) 48:285–293
166. **Sospedra M, Martin R** 2005 Immunology of multiple sclerosis. Annu Rev Immunol 23:683–747
167. **Lassmann H, Bruck W, Lucchinetti C** 2001 Heterogeneity of multiple sclerosis pathogenesis: implications for diagnosis and therapy. Trends Mol Med 7:115–121
168. **Kamada M, Irahara M, Maegawa M, Ohmoto Y, Murata K, Yasui T, Yamano S, Aono T** 2001 Transient increase in the levels of T-helper 1 cytokines in postmenopausal women and the effects of hormone replacement therapy. Gynecol Obstet Invest 52:82–88
169. **Kumru S, Godekmerdan A, Yilmaz B** 2004 Immune effects of surgical menopause and estrogen replacement therapy in perimenopausal women. J Reprod Immunol 63:31–38
170. **Hiramoto K, Tanaka H, Yanagihara N, Sato EF, Inoue M** 2004 Effect of 17β-estradiol on immunosuppression induced by ultraviolet B irradiation. Arch Dermatol Res 295:307–311
171. **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
172. **Hatthachote P, Gillespie JI** 1999 Complex interactions between sex steroids and cytokines in the human pregnant myometrium: evidence for an autocrine signaling system at term. Endocrinology 140:2533–2540
173. **Soares R, Guo S, Gartner F, Schmitt FC, Russo J** 2003 17 β-Estradiol-mediated vessel assembly and stabilization in tumor angiogenesis requires TGF β and EGFR crosstalk. Angiogenesis 6:271–281
174. **Mercier I, Colombo F, Mader S, Calderone A** 2002 Ovarian hormones induce TGF-β (3) and fibronectin mRNAs but exhibit a disparate action on cardiac fibroblast proliferation. Cardiovasc Res 53:728–739
175. **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
176. **Lu D, Giguere V** 2001 Requirement of Ras-dependent pathways for activation of the transforming growth factor β3 promoter by estradiol. Endocrinology 142:751–759
177. **Matsuda T, Yamamoto T, Muraguchi A, Saatcioglu F** 2001 Crosstalk between transforming growth factor-β and estrogen receptor signaling through Smad3. J Biol Chem 276:42908–42914
178. **Zhang Y, Sugimoto Y, Kulp SK, Farrar WB, Brueggemeier RW, Lin YC** 1998 Estrogen-induced keratinocyte growth factor mRNA

expression in normal and cancerous human breast cells. Oncol Rep 5:577–583
179. **Pedchenko VK, Imagawa W** 2000 Estrogen treatment in vivo increases keratinocyte growth factor expression in the mammary gland. J Endocrinol 165:39–49
180. **Zhou S, Turgeman G, Harris SE, Leitman DC, Komm BS, Bodine PV, Gazit D** 2003 Estrogens activate bone morphogenetic protein-2 gene transcription in mouse mesenchymal stem cells. Mol Endocrinol 17:56–66
181. **Rickard DJ, Hofbauer LC, Bonde SK, Gori F, Spelsberg TC, Riggs BL** 1998 Bone morphogenetic protein-6 production in human osteoblastic cell lines. Selective regulation by estrogen. J Clin Invest 101:413–422
182. **Kanda N, Watanabe S** 2003 17β-Estradiol enhances the production of nerve growth factor in THP-1-derived macrophages or peripheral blood monocyte-derived macrophages. J Invest Dermatol 121: 771–780
183. **Ralston SH** 1997 The Michael Mason Prize Essay 1997. Nitric oxide and bone: what a gas! Br J Rheumatol 36:831–838
184. **Chambliss KL, Shaul PW** 2002 Estrogen modulation of endothelial nitric oxide synthase. Endocr Rev 23:665–686
185. **Weiner CP, Lizasoain I, Baylis SA, Knowles RG, Charles IG, Moncada S** 1994 Induction of calcium-dependent nitric oxide synthases by sex hormones. Proc Natl Acad Sci USA 91:5212–5216
186. **Miller L, Alley EW, Murphy WJ, Russell SW, Hunt JS** 1996 Progesterone inhibits inducible nitric oxide synthase gene expression and nitric oxide production in murine macrophages. J Leukoc Biol 59:442–450
187. **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 Pharmacol 31:292–298
188. **Kauser K, Sonnenberg D, Diel P, Rubanyi GM** 1998 Effect of 17β-oestradiol on cytokine-induced nitric oxide production in rat isolated aorta. Br J Pharmacol 123:1089–1096
189. **Karpuzoglu E, Fenaux JB, Phillips RA, Lengi AJ, Elvinger F, Ansar AS** 2006 Estrogen up-regulates inducible nitric oxide synthase, nitric oxide, and cyclooxygenase-2 in splenocytes activated with T cell stimulants: role of interferon-γ. Endocrinology 147:662–671
190. **Stefano GB, Cadet P, Breton C, Goumon Y, Prevot V, Dessaint JP, Beauvillain JC, Roumier AS, Welters I, Salzet M** 2000 Estradiol-stimulated nitric oxide release in human granulocytes is dependent on intracellular calcium transients: evidence of a cell surface estrogen receptor. Blood 95:3951–3958
191. **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
192. **Beauregard C, Brandt P** 2004 Down regulation of interleukin-1β-induced nitric oxide production in lacrimal gland acinar cells by sex steroids. Curr Eye Res 29:59–66
193. **Drew PD, Chavis JA** 2000 Female sex steroids: effects upon microglial cell activation. J Neuroimmunol 111:77–85
194. **Arnal JF, Clamens S, Pechet C, Negre-Salvayre A, Allera C, Girolami JP, Salvayre R, Bayard F** 1996 Ethinylestradiol does not enhance the expression of nitric oxide synthase in bovine endothelial cells but increases the release of bioactive nitric oxide by inhibiting superoxide anion production. Proc Natl Acad Sci USA 93:4108–4113
195. **Ayres S, Abplanalp W, Liu JH, Subbiah MT** 1998 Mechanisms involved in the protective effect of estradiol-17β on lipid peroxidation and DNA damage. Am J Physiol 274:E1002–E1008
196. **Sawada H, Ibi M, Kihara T, Urushitani M, Akaike A, Shimohama S** 1998 Estradiol protects mesencephalic dopaminergic neurons from oxidative stress-induced neuronal death. J Neurosci Res 54: 707–719
197. **Barth A, Landmann G, Liepold K, Zapf H, Muller D, Karge E, Klinger W** 1999 Influence of oestrogens on formation of reactive oxygen species in liver microsomes of differently aged male Wistar rats. Exp Toxicol Pathol 51:282–288
198. **Si ML, Al Sharafi B, Lai CC, Khardori R, Chang C, Su CY** 2001 Gender difference in cytoprotection induced by estrogen on female and male bovine aortic endothelial cells. Endocrine 15:255–262
199. **Wang X, Dykens JA, Perez E, Liu R, Yang S, Covey DF, Simpkins JW** 2006 Neuroprotective effects of 17β-estradiol and nonfeminizing estrogens against H2O2 toxicity in human neuroblastoma SK-N-SH cells. Mol Pharmacol 70:395–404
200. **Urata Y, Ihara Y, Murata H, Goto S, Koji T, Yodoi J, Inoue S, Kondo T** 2006 17β-Estradiol protects against oxidative stress-induced cell death through the glutathione/glutaredoxin-dependent redox regulation of Akt in myocardiac H9c2 cells. J Biol Chem 281:13092–13102
201. **Wagner AH, Schroeter MR, Hecker M** 2001 17β-Estradiol inhibition of NADPH oxidase expression in human endothelial cells. FASEB J 15:2121–2130
202. **Sumi D, Hayashi T, Jayachandran M, Iguchi A** 2001 Estrogen prevents destabilization of endothelial nitric oxide synthase mRNA induced by tumor necrosis factor α through estrogen receptor mediated system. Life Sci 69:1651–1660
203. **Bekesi G, Kakucs R, Varbiro S, Feher J, Pazmany T, Magyar Z, Sprintz D, Szekacs B** 2001 Induced myeloperoxidase activity and related superoxide inhibition during hormone replacement therapy. BJOG 108:474–481
204. **Chen ZH, Na HK, Hurh YJ, Surh YJ** 2005 4-Hydroxyestradiol induces oxidative stress and apoptosis in human mammary epithelial cells: possible protection by NF-κB and ERK/MAPK. Toxicol Appl Pharmacol 208:46–56
205. **Takao T, Kumagai C, Hisakawa N, Matsumoto R, Hashimoto K** 2005 Effect of 17β-estradiol on tumor necrosis factor-α-induced cytotoxicity in the human peripheral T lymphocytes. J Endocrinol 184:191–197
206. **Vegeto E, Pollio G, Pellicciari C, Maggi A** 1999 Estrogen and progesterone induction of survival of monoblastoid cells undergoing TNF-α-induced apoptosis. FASEB J 13:793–803
207. **Cutolo M, Capellino S, Montagna P, Ghiorzo P, Sulli A, Villaggio B** 2005 Sex hormone modulation of cell growth and apoptosis of the human monocytic/macrophage cell line. Arthritis Res Ther 7:R1124–R1132
208. **Kajta M, Budziszewska B, Marszal M, Lason W** 2001 Effects of 17-β estradiol and estriol on NMDA-induced toxicity and apoptosis in primary cultures of rat cortical neurons. J Physiol Pharmacol 52:437–446
209. **Linford NJ, Dorsa DM** 2002 17β-Estradiol and the phytoestrogen genistein attenuate neuronal apoptosis induced by the endoplasmic reticulum calcium-ATPase inhibitor thapsigargin. Steroids 67: 1029–1040
210. **Sribnick EA, Ray SK, Nowak MW, Li L, Banik NL** 2004 17β-Estradiol attenuates glutamate-induced apoptosis and preserves electrophysiologic function in primary cortical neurons. J Neurosci Res 76:688–696
211. **Billig H, Furuta I, Hsueh AJ** 1993 Estrogens inhibit and androgens enhance ovarian granulosa cell apoptosis. Endocrinology 133:2204–2212
212. **Patten RD, Pourati I, Aronovitz MJ, Baur J, Celestin F, Chen X, Michael A, Haq S, Nuedling S, Grohe C, Force T, Mendelsohn ME, Karas RH** 2004 17β-Estradiol reduces cardiomyocyte apoptosis in vivo and in vitro via activation of phospho-inositide-3 kinase/Akt signaling. Circ Res 95:692–699
213. **Zecchi-Orlandini S, Formigli L, Tani A, Benvenuti S, Fiorelli G, Papucci L, Capaccioli S, Orlandini GE, Brandi ML** 1999 17β-Estradiol induces apoptosis in the preosteoclastic FLG 29.1 cell line. Biochem Biophys Res Commun 255:680–685
214. **Acconcia F, Totta P, Ogawa S, Cardillo I, Inoue S, Leone S, Trentalance A, Muramatsu M, Marino M** 2005 Survival versus apoptotic 17β-estradiol effect: role of ER α and ER β activated non-genomic signaling. J Cell Physiol 203:193–201
215. **Kincade PW, Medina KL, Smithson G** 1994 Sex hormones as negative regulators of lymphopoiesis. Immunol Rev 137:119–134
216. **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
217. **Blobel GA, Orkin SH** 1996 Estrogen-induced apoptosis by inhibition of the erythroid transcription factor GATA-1. Mol Cell Biol 16:1687–1694

218. **Aronica SM, Dozier A, Fanti P, Nazareth M** 2000 Altered bone marrow cell sensitivity in the lupus-prone NZB/W mouse: regulation of CFU-GM colony formation by estrogen, tamoxifen and thrombopoietin. Lupus 9:271–277
219. **Hoffman-Goetz L, Fietsch CL, McCutcheon D, Duerrstein L** 2001 Effect of 17β-estradiol and voluntary exercise on lymphocyte apoptosis in mice. Physiol Behav 74:653–658
220. **Jochems C, Islander U, Erlandsson M, Verdrengh M, Ohlsson C, Carlsten H** 2005 Osteoporosis in experimental postmenopausal polyarthritis: the relative contributions of estrogen deficiency and inflammation. Arthritis Res Ther 7:R837–R843
221. **Kanda N, Watanabe S** 2003 17β-Estradiol inhibits the production of RANTES in human keratinocytes. J Invest Dermatol 120:420–427
222. **Kanda N, Watanabe S** 2003 17β-Estradiol inhibits MCP-1 production in human keratinocytes. J Invest Dermatol 120:1058–1066
223. **Yamada K, Hayashi T, Kuzuya M, Naito M, Asai K, Iguchi A** 1996 Physiological concentration of 17 β-estradiol inhibits chemotaxis of human monocytes in response to monocyte chemotactic protein 1. Artery 22:24–35
224. **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
225. **Seli E, Pehlivan T, Selam B, Garcia-Velasco JA, Arici A** 2002 Estradiol down-regulates MCP-1 expression in human coronary artery endothelial cells. Fertil Steril 77:542–547
226. **Frazier-Jessen MR, Kovacs EJ** 1995 Estrogen modulation of JE/monocyte chemoattractant protein-1 mRNA expression in murine macrophages. J Immunol 154:1838–1845
227. **Störk S, Baumann K, von Schacky C, Angerer P** 2002 The effect of 17 β-estradiol on MCP-1 serum levels in postmenopausal women. Cardiovasc Res 53:642–649
228. **Lundgren D** 1973 Influence of estrogen and progesterone on exudation, inflammatory cell migration and granulation tissue formation in preformed cavities. Scand J Plast Reconstr Surg 7:10–14
229. **Josefsson E, Tarkowski A, Carlsten H** 1992 Anti-inflammatory properties of estrogen. I. In vivo suppression of leukocyte production in bone marrow and redistribution of peripheral blood neutrophils. Cell Immunol 142:67–78
230. **Xing D, Miller A, Novak L, Rocha R, Chen YF, Oparil S** 2004 Estradiol and progestins differentially modulate leukocyte infiltration after vascular injury. Circulation 109:234–241
231. **Miller AP, Feng W, Xing D, Weathington NM, Blalock JE, Chen YF, Oparil S** 2004 Estrogen modulates inflammatory mediator expression and neutrophil chemotaxis in injured arteries. Circulation 110:1664–1669
232. **Cuzzocrea S, Santagati S, Sautebin L, Mazzon E, Calabro G, Serraino I, Caputi AP, Maggi A** 2000 17β-Estradiol antiinflammatory activity in carrageenan-induced pleurisy. Endocrinology 141:1455–1463
233. **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
234. **Subramanian S, Matejuk A, Zamora A, Vandenbark AA, Offner H** 2003 Oral feeding with ethinyl estradiol suppresses and treats experimental autoimmune encephalomyelitis in SJL mice and inhibits the recruitment of inflammatory cells into the central nervous system. J Immunol 170:1548–1555
235. **Janis K, Hoeltke J, Nazareth M, Fanti P, Poppenberg K, Aronica SM** 2004 Estrogen decreases expression of chemokine receptors, and suppresses chemokine bioactivity in murine monocytes. Am J Reprod Immunol 51:22–31
236. **Hunt JS, Miller L, Platt JS** 1998 Hormonal regulation of uterine macrophages. Dev Immunol 6:105–110
237. **Tibbetts TA, Conneely OM, O'Malley BW** 1999 Progesterone via its receptor antagonizes the pro-inflammatory activity of estrogen in the mouse uterus. Biol Reprod 60:1158–1165
238. **Akoum A, Jolicoeur C, Boucher A** 2000 Estradiol amplifies interleukin-1-induced monocyte chemotactic protein-1 expression by ectopic endometrial cells of women with endometriosis. J Clin Endocrinol Metab 85:896–904
239. **Akoum A, Lawson C, McColl S, Villeneuve M** 2001 Ectopic endometrial cells express high concentrations of interleukin (IL)-8 in vivo regardless of the menstrual cycle phase and respond to oestradiol by up-regulating IL-1-induced IL-8 expression in vitro. Mol Hum Reprod 7:859–866
240. **DeLoia JA, Stewart-Akers AM, Brekosky J, Kubik CJ** 2002 Effects of exogenous estrogen on uterine leukocyte recruitment. Fertil Steril 77:548–554
241. **Sentman CL, Meadows SK, Wira CR, Eriksson M** 2004 Recruitment of uterine NK cells: induction of CXC chemokine ligands 10 and 11 in human endometrium by estradiol and progesterone. J Immunol 173:6760–6766
242. **Groten T, Pierce AA, Huen AC, Schnaper HW** 2005 17β-Estradiol transiently disrupts adherens junctions in endothelial cells. FASEB J 19:1368–1370
243. **Schnaper HW, McGowan KA, Kim-Schulze S, Cid MC** 1996 Oestrogen and endothelial cell angiogenic activity. Clin Exp Pharmacol Physiol 23:247–250
244. **Losordo DW, Isner JM** 2001 Estrogen and angiogenesis: a review. Arterioscler Thromb Vasc Biol 21:6–12
245. **Rubanyi GM, Johns A, Kauser K** 2002 Effect of estrogen on endothelial function and angiogenesis. Vascul Pharmacol 38:89–98
246. **Miyamoto N, Mandai M, Takagi H, Suzuma I, Suzuma K, Koyama S, Otani A, Oh H, Honda Y** 2002 Contrasting effect of estrogen on VEGF induction under different oxygen status and its role in murine ROP. Invest Ophthalmol Vis Sci 43:2007–2014
247. **Newman SP, Leese MP, Purohit A, James DR, Rennie CE, Potter BV, Reed MJ** 2004 Inhibition of in vitro angiogenesis by 2-methoxy- and 2-ethyl-estrogen sulfamates. Int J Cancer 109:533–540
248. **Fotsis T, Zhang Y, Pepper MS, Adlercreutz H, Montesano R, Nawroth PP, Schweigerer L** 1994 The endogenous oestrogen metabolite 2-methoxyoestradiol inhibits angiogenesis and suppresses tumour growth. Nature 368:237–239
249. **Lippert C, Seeger H, Mueck AO, Lippert TH** 2000 The effects of A-ring and D-ring metabolites of estradiol on the proliferation of vascular endothelial cells. Life Sci 67:1653–1658
250. **Dubey RK, Gillespie DG, Zacharia LC, Rosselli M, Korzekwa KR, Fingerle J, Jackson EK** 2000 Methoxyestradiols mediate the antimitogenic effects of estradiol on vascular smooth muscle cells via estrogen receptor-independent mechanisms. Biochem Biophys Res Commun 278:27–33
251. **Holmdahl R, Carlsten H, Jansson L, Larsson P** 1989 Oestrogen is a potent immunomodulator of murine experimental rheumatoid disease. Br J Rheumatol 28 (Suppl 1):54–58
252. **Masi AT** 1995 Sex hormones and rheumatoid arthritis: cause or effect relationships in a complex pathophysiology? Clin Exp Rheumatol 13:227–240
253. **Julkunen H** 2000 Hormone replacement therapy in women with rheumatic diseases. Scand J Rheumatol 29:146–153
254. **Kanik KS, Wilder RL** 2000 Hormonal alterations in rheumatoid arthritis, including the effects of pregnancy. Rheum Dis Clin North Am 26:805–823
255. **Cutolo M, Lahita RG** 2005 Estrogens and arthritis. Rheum Dis Clin North Am 31:19–27, vii
256. **Dubey RK, Imthurn B, Barton M, Jackson EK** 2005 Vascular consequences of menopause and hormone therapy: importance of timing of treatment and type of estrogen. Cardiovasc Res 66:295–306
257. **Holmdahl R, Jansson L, Meyerson B, Klareskog L** 1987 Oestrogen induced suppression of collagen arthritis. I. Long term oestradiol treatment of DBA/1 mice reduces severity and incidence of arthritis and decreases the anti type II collagen immune response. Clin Exp Immunol 70:372–378
258. **Holmdahl R, Jansson L** 1988 Estrogen-induced suppression of collagen arthritis. III. Adult thymectomy does not affect the course of arthritis or the estrogen-mediated suppression of T-cell immunity. Brain Behav Immun 2:123–132
259. **Larsson P, Holmdahl R** 1987 Oestrogen-induced suppression of collagen arthritis. II. Treatment of rats suppresses development of arthritis but does not affect the anti-type II collagen humoral response. Scand J Immunol 26:579–583
260. **Larsson P, Goldschmidt TJ, Klareskog L, Holmdahl R** 1989 Oestrogen-mediated suppression of collagen-induced arthritis in rats.

Studies on the role of the thymus and of peripheral CD8+ T lymphocytes. Scand J Immunol 30:741–747
261. **Schlaghecke R, Lenhardt E, Fortkamp E, Juli E, Zeidler H** 1989 Preventive and therapeutic effect of estrogen administration in adjuvant arthritis. Z Rheumatol 48:313–316
262. **Badger AM, Blake SM, Dodds RA, Griswold DE, Swift BA, Rieman DJ, Stroup GB, Hoffman SJ, Gowen M** 1999 Idoxifene, a novel selective estrogen receptor modulator, is effective in a rat model of adjuvant-induced arthritis. J Pharmacol Exp Ther 291: 1380–1386
263. **Yoneda T, Ishimaru N, Arakaki R, Kobayashi M, Izawa T, Moriyama K, Hayashi Y** 2004 Estrogen deficiency accelerates murine autoimmune arthritis associated with receptor activator of nuclear factor-κB ligand-mediated osteoclastogenesis. Endocrinology 145:2384–2391
264. **Subramanian S, Tovey M, Afentoulis M, Krogstad A, Vandenbark AA, Offner H** 2005 Ethinyl estradiol treats collagen-induced arthritis in DBA/1LacJ mice by inhibiting the production of TNF-α and IL-1β. Clin Immunol 115:162–172
265. **Jansson L, Holmdahl R** 1992 Oestrogen-induced suppression of collagen arthritis; 17 β-oestradiol is therapeutically active in normal and castrated F1 hybrid mice of both sexes. Clin Exp Immunol 89:446–451
266. **Nandakumar KS, Svensson L, Holmdahl R** 2003 Collagen type II-specific monoclonal antibody-induced arthritis in mice: description of the disease and the influence of age, sex, and genes. Am J Pathol 163:1827–1837
267. **Jansson L, Olsson T, Holmdahl R** 1994 Estrogen induces a potent suppression of experimental autoimmune encephalomyelitis and collagen-induced arthritis in mice. J Neuroimmunol 53:203–207
268. **Jansson L, Holmdahl R** 2001 Enhancement of collagen-induced arthritis in female mice by estrogen receptor blockage. Arthritis Rheum 44:2168–2175
269. **Harris HA, Albert LM, Leathurby Y, Malamas MS, Mewshaw RE, Miller CP, Kharode YP, Marzolf J, Komm BS, Winneker RC, Frail DE, Henderson RA, Zhu Y, Keith Jr JC** 2003 Evaluation of an estrogen receptor-β agonist in animal models of human disease. Endocrinology 144:4241–4249
270. **Josefsson E, Tarkowski A** 1997 Suppression of type II collagen-induced arthritis by the endogenous estrogen metabolite 2-methoxyestradiol. Arthritis Rheum 40:154–163
271. **Mattsson R, Mattsson A, Holmdahl R, Whyte A, Rook GA** 1991 Maintained pregnancy levels of oestrogen afford complete protection from post-partum exacerbation of collagen-induced arthritis. Clin Exp Immunol 85:41–47
272. **Ratkay LG, Zhang D, Tonzetich J, Levy JG, Waterfield JD** 1994 Evaluation of a model for post-partum arthritis and the role of oestrogen in prevention of MRL-lpr associated rheumatic conditions. Clin Exp Immunol 98:52–59
273. **da Silva JA, Larbre JP, Seed MP, Cutolo M, Villaggio B, Scott DL, Willoughby DA** 1994 Sex differences in inflammation induced cartilage damage in rodents. The influence of sex steroids. J Rheumatol 21:330–337
274. **Green PG, Dahlqvist SR, Isenberg WM, Strausbaugh HJ, Miao FJ, Levine JD** 1999 Sex steroid regulation of the inflammatory response: sympathoadrenal dependence in the female rat. J Neurosci 19:4082–4089
275. **Kuiper S, van Gestel AM, Swinkels HL, de Boo TM, da Silva JA, van Riel PL** 2001 Influence of sex, age, and menopausal state on the course of early rheumatoid arthritis. J Rheumatol 28:1809–1816
276. **Tengstrand B, Ahlmen M, Hafstrom I** 2004 The influence of sex on rheumatoid arthritis: a prospective study of onset and outcome after 2 years. J Rheumatol 31:214–222
277. **Jorgensen C, Picot MC, Bologna C, Sany J** 1996 Oral contraception, parity, breast feeding, and severity of rheumatoid arthritis. Ann Rheum Dis 55:94–98
278. **Nelson JL** 1996 Maternal-fetal immunology and autoimmune disease: is some autoimmune disease auto-alloimmune or allo-autoimmune? Arthritis Rheum 39:191–194
279. **Brennan MT, Sankar V, Leakan RA, Grisius MM, Collins MT, Fox PC, Baum BJ, Pillemer SR** 2003 Sex steroid hormones in primary Sjogren's syndrome. J Rheumatol 30:1267–1271
280. **Straub RH, Härle P, Atzeni F, Weidler C, Cutolo M, Sarzi-Puttini P** 2005 Sex hormone concentrations in patients with rheumatoid arthritis are not normalized during 12 weeks of anti-tumor necrosis factor therapy. J Rheumatol 32:1253–1258
281. **Seriolo B, Cutolo M, Garnero A, Accardo S** 1999 Relationships between serum 17β-oestradiol and anticardiolipin antibody concentrations in female patients with rheumatoid arthritis. Rheumatology (Oxford) 38:1159–1161
282. **Tengstrand B, Carlstrom K, Fellander-Tsai L, Hafstrom I** 2003 Abnormal levels of serum dehydroepiandrosterone, estrone, and estradiol in men with rheumatoid arthritis: high correlation between serum estradiol and current degree of inflammation. J Rheumatol 30:2338–2343
283. **Enzinger J** 1954 Urinary estrogen excretion in polyarthritis. Wien Z Inn Med 35:43–45
284. **Weidler C, Härle P, Schedel J, Schmidt M, Schölmerich J, Straub RH** 2004 Patients with rheumatoid arthritis and systemic lupus erythematosus have increased renal excretion of mitogenic estrogens in relation to endogenous antiestrogens. J Rheumatol 31:489–494
285. **Castagnetta LA, Carruba G, Granata OM, Stefano R, Miele M, Schmidt M, Cutolo M, Straub RH** 2003 Increased estrogen formation and estrogen to androgen ratio in the synovial fluid of patients with rheumatoid arthritis. J Rheumatol 30:2597–2605
286. **Cutolo M, Balleari E, Giusti M, Monachesi M, Accardo S** 1986 Sex hormone status in women suffering from rheumatoid arthritis. J Rheumatol 13:1019–1023
287. **Flaisler F, Hedon B, Sany J, Combe B** 1995 A study of ovarian function in rheumatoid arthritis. Rev Rhum Engl Ed 62:549–554
288. **Deighton CM, Watson MJ, Walker DJ** 1992 Sex hormones in postmenopausal HLA-identical rheumatoid arthritis discordant sibling pairs. J Rheumatol 19:1663–1667
289. **Cutolo M, Balleari E, Giusti M, Monachesi M, Accardo S** 1988 Sex hormone status of male patients with rheumatoid arthritis: evidence of low serum concentrations of testosterone at baseline and after human chorionic gonadotropin stimulation. Arthritis Rheum 31:1314–1317
290. **Mackworth-Young CG, Parke AL, Morley KD, Fotherby K, Hughes GR** 1983 Sex hormones in male patients with systemic lupus erythematosus: a comparison with other disease groups. Eur J Rheumatol Inflamm 6:228–232
291. **Sambrook PN, Eisman JA, Champion GD, Pocock NA** 1988 Sex hormone status and osteoporosis in postmenopausal women with rheumatoid arthritis. Arthritis Rheum 31:973–978
292. **Schmidt M, Weidler C, Naumann H, Anders S, Schölmerich J, Straub RH** 2005 Androgen conversion in osteoarthritis and rheumatoid arthritis synoviocytes—androstenedione and testosterone inhibit estrogen formation and favor production of more potent 5α-reduced androgens. Arthritis Res Ther 7:R938–R948
293. **Ushiyama T, Mori K, Inoue K, Huang J, Nishioka J, Hukuda S** 1999 Association of oestrogen receptor gene polymorphisms with age at onset of rheumatoid arthritis. Ann Rheum Dis 58:7–10
294. **Takagi H, Ishiguro N, Iwata H, Kanamono T** 2000 Genetic association between rheumatoid arthritis and estrogen receptor microsatellite polymorphism. J Rheumatol 27:1638–1642
295. **Hall GM, Daniels M, Huskisson EC, Spector TD** 1994 A randomised controlled trial of the effect of hormone replacement therapy on disease activity in postmenopausal rheumatoid arthritis. Ann Rheum Dis 53:112–116
296. **Wilder RL** 1996 Hormones and autoimmunity: animal models of arthritis. Baillieres Clin Rheumatol 10:259–271
297. **Lahita RG** 1999 The role of sex hormones in systemic lupus erythematosus. Curr Opin Rheumatol 11:352–356
298. **McMurray RW** 2001 Sex hormones in the pathogenesis of systemic lupus erythematosus. Front Biosci 6:E193–E206
299. **Peeva E, Zouali M** 2005 Spotlight on the role of hormonal factors in the emergence of autoreactive B-lymphocytes. Immunol Lett 101:123–143
300. **Doria A, Iaccarino L, Arienti S, Ghirardello A, Zampieri S, Rampudda ME, Cutolo M, Tincani A, Todesco S** 2006 Th2 immune deviation induced by pregnancy: the two faces of autoimmune rheumatic diseases. Reprod Toxicol 22:234–241
301. **Cohen-Solal JF, Jeganathan V, Grimaldi CM, Peeva E, Diamond**

B 2006 Sex hormones and SLE: influencing the fate of autoreactive B cells. Curr Top Microbiol Immunol 305:67–88
302. **Steinberg AD, Melez KA, Raveche ES, Reeves JP, Boegel WA, Smathers PA, Taurog JD, Weinlein L, Duvic M** 1979 Approach to the study of the role of sex hormones in autoimmunity. Arthritis Rheum 22:1170–1176
303. **Blank M, Mendlovic S, Fricke H, Mozes E, Talal N, Shoenfeld Y** 1990 Sex hormone involvement in the induction of experimental systemic lupus erythematosus by a pathogenic anti-DNA idiotype in naive mice. J Rheumatol 17:311–317
304. **Carlsten H, Nilsson N, Jonsson R, Backman K, Holmdahl R, Tarkowski A** 1992 Estrogen accelerates immune complex glomerulonephritis but ameliorates T cell-mediated vasculitis and sialadenitis in autoimmune MRL lpr/lpr mice. Cell Immunol 144:190–202
305. **Rider V, Foster RT, Evans M, Suenaga R, Abdou NI** 1998 Gender differences in autoimmune diseases: estrogen increases calcineurin expression in systemic lupus erythematosus. Clin Immunol Immunopathol 89:171–180
306. **Rider V, Jones S, Evans M, Bassiri H, Afsar Z, Abdou NI** 2001 Estrogen increases CD40 ligand expression in T cells from women with systemic lupus erythematosus. J Rheumatol 28:2644–2649
307. **Lahita RG, Bradlow HL, Kunkel HG, Fishman J** 1981 Increased 16 α-hydroxylation of estradiol in systemic lupus erythematosus. J Clin Endocrinol Metab 53:174–178
308. **Lahita RG, Bradlow HL, Kunkel HG, Fishman J** 1979 Alterations of estrogen metabolism in systemic lupus erythematosus. Arthritis Rheum 22:1195–1198
309. **Athreya BH, Rafferty JH, Sehgal GS, Lahita RG** 1993 Adenohypophyseal and sex hormones in pediatric rheumatic diseases. J Rheumatol 20:725–730
310. **Aguirre MA, Jimena P, Gomez-Vidal MM, Gracia-Courtoy C, Salmoral A, Cuadrado MJ** 1997 Sex hormone levels in patients with systemic lupus erythematosus: a case control study. Arthritis Rheum 40 (Suppl):S308
311. **Offner H** 2004 Neuroimmunoprotective effects of estrogen and derivatives in experimental autoimmune encephalomyelitis: therapeutic implications for multiple sclerosis. J Neurosci Res 78:603–624
312. **Bebo Jr BF, Fyfe-Johnson A, Adlard K, Beam AG, Vandenbark AA, Offner H** 2001 Low-dose estrogen therapy ameliorates experimental autoimmune encephalomyelitis in two different inbred mouse strains. J Immunol 166:2080–2089
313. **Hoffman GE, Le WW, Murphy AZ, Koski CL** 2001 Divergent effects of ovarian steroids on neuronal survival during experimental allergic encephalitis in Lewis rats. Exp Neurol 171:272–284
314. **Ito A, Matejuk A, Hopke C, Drought H, Dwyer J, Zamora A, Subramanian S, Vandenbark AA, Offner H** 2003 Transfer of severe experimental autoimmune encephalomyelitis by IL-12- and IL-18-potentiated T cells is estrogen sensitive. J Immunol 170:4802–4809
315. **Palaszynski KM, Liu H, Loo KK, Voskuhl RR** 2004 Estriol treatment ameliorates disease in males with experimental autoimmune encephalomyelitis: implications for multiple sclerosis. J Neuroimmunol 149:84–89
316. **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
317. **Ito A, Buenafe AC, Matejuk A, Zamora A, Silverman M, Dwyer J, Vandenbark AA, Offner H** 2002 Estrogen inhibits systemic T cell expression of TNF-α and recruitment of TNF-α (+) T cells and macrophages into the CNS of mice developing experimental encephalomyelitis. Clin Immunol 102:275–282
318. **Polanczyk M, Zamora A, Subramanian S, Matejuk A, Hess DL, Blankenhorn EP, Teuscher C, Vandenbark AA, Offner H** 2003 The protective effect of 17β-estradiol on experimental autoimmune encephalomyelitis is mediated through estrogen receptor-α. Am J Pathol 163:1599–1605
319. **Liu HB, Loo KK, Palaszynski K, Ashouri J, Lubahn DB, Voskuhl RR** 2003 Estrogen receptor α mediates estrogen's immune protection in autoimmune disease. J Immunol 171:6936–6940
320. **Dubal DB, Zhu H, Yu J, Rau SW, Shughrue PJ, Merchenthaler I, Kindy MS, Wise PM** 2001 Estrogen receptor α, not β, is a critical link in estradiol-mediated protection against brain injury. Proc Natl Acad Sci USA 98:1952–1957
321. **Elloso MM, Phiel K, Henderson RA, Harris HA, Adelman SJ** 2005 Suppression of experimental autoimmune encephalomyelitis using estrogen receptor-selective ligands. J Endocrinol 185:243–252
322. **Garidou L, Laffont S, Douin-Echinard V, Coureau C, Krust A, Chambon P, Guery JC** 2004 Estrogen receptor α signaling in inflammatory leukocytes is dispensable for 17β-estradiol-mediated inhibition of experimental autoimmune encephalomyelitis. J Immunol 173:2435–2442
323. **Soldan SS, Alvarez Retuerto AI, Sicotte NL, Voskuhl RR** 2003 Immune modulation in multiple sclerosis patients treated with the pregnancy hormone estriol. J Immunol 171:6267–6274
324. **Nordell VL, Scarborough MM, Buchanan AK, Sohrabji F** 2003 Differential effects of estrogen in the injured forebrain of young adult and reproductive senescent animals. Neurobiol Aging 24: 733–743
325. **Vegeto E, Belcredito S, Etteri S, Ghisletti S, Brusadelli A, Meda C, Krust A, Dupont S, Ciana P, Chambon P, Maggi A** 2003 Estrogen receptor-α mediates the brain antiinflammatory activity of estradiol. Proc Natl Acad Sci USA 100:9614–9619
326. **Vegeto E, Belcredito S, Ghisletti S, Meda C, Etteri S, Maggi A** 2006 The endogenous estrogen status regulates microglia reactivity in animal models of neuroinflammation. Endocrinology 147:2263–2272
327. **Liao SL, Chen WY, Chen CJ** 2002 Estrogen attenuates tumor necrosis factor-α expression to provide ischemic neuroprotection in female rats. Neurosci Lett 330:159–162
328. **Zemlyak I, Brooke S, Sapolsky R** 2005 Estrogenic protection against gp120 neurotoxicity: role of microglia. Brain Res 1046:130–136
329. **Barbesino G, Tomer Y, Concepcion ES, Davies TF, Greenberg DA** 1998 Linkage analysis of candidate genes in autoimmune thyroid disease. II. Selected gender-related genes and the X-chromosome. International Consortium for the Genetics of Autoimmune Thyroid Disease. J Clin Endocrinol Metab 83:3290–3295
330. **Strieder TG, Prummel MF, Tijssen JG, Endert E, Wiersinga WM** 2003 Risk factors for and prevalence of thyroid disorders in a cross-sectional study among healthy female relatives of patients with autoimmune thyroid disease. Clin Endocrinol (Oxf) 59:396–401
331. **De M, Jaap A, Dempster J** 2002 Tamoxifen therapy in steroid-resistant Riedels disease. Scott Med J 47:12–13
332. **Okayasu I, Kong YM, Rose NR** 1981 Effect of castration and sex hormones on experimental autoimmune thyroiditis. Clin Immunol Immunopathol 20:240–245
333. **Ahmed SA, Penhale WJ** 1982 The influence of testosterone on the development of autoimmune thyroiditis in thymectomized and irradiated rats. Clin Exp Immunol 48:367–374
334. **Ahmed SA, Young PR, Penhale WJ** 1983 The effects of female sex steroids on the development of autoimmune thyroiditis in thymectomized and irradiated rats. Clin Exp Immunol 54:351–358
335. **Wang SH, Myc A, Koenig RJ, Bretz JD, Arscott PL, Baker JR** 2000 2-Methoxyestradiol, an endogenous estrogen metabolite, induces thyroid cell apoptosis. Mol Cell Endocrinol 165:163–172
336. **Chang L, Heitkemper MM** 2002 Gender differences in irritable bowel syndrome. Gastroenterology 123:1686–1701
337. **Bradesi S, Eutamene H, Garcia-Villar R, Fioramonti J, Bueno L** 2003 Stress-induced visceral hypersensitivity in female rats is estrogen-dependent and involves tachykinin NK1 receptors. Pain 102:227–234
338. **Harnish DC, Albert LM, Leathurby Y, Eckert AM, Ciarletta A, Kasaian M, Keith Jr JC** 2004 Beneficial effects of estrogen treatment in the HLA-B27 transgenic rat model of inflammatory bowel disease. Am J Physiol Gastrointest Liver Physiol 286:G118–G125
339. **Yasuda M, Shimizu I, Shiba M, Ito S** 1999 Suppressive effects of estradiol on dimethylnitrosamine-induced fibrosis of the liver in rats. Hepatology 29:719–727
340. **Xu JW, Gong J, Chang XM, Luo JY, Dong L, Hao ZM, Jia A, Xu GP** 2002 Estrogen reduces CCL4- induced liver fibrosis in rats. World J Gastroenterol 8:883–887

341. **Eckhoff DE, Bilbao G, Frenette L, Thompson JA, Contreras JL** 2002 17-β-Estradiol protects the liver against warm ischemia/reperfusion injury and is associated with increased serum nitric oxide and decreased tumor necrosis factor-α. Surgery 132:302–309
342. **Vilatoba M, Eckstein C, Bilbao G, Frennete L, Eckhoff DE, Contreras JL** 2005 17β-Estradiol differentially activates mitogen-activated protein-kinases and improves survival following reperfusion injury of reduced-size liver in mice. Transplant Proc 37:399–403
343. **Evans MJ, Eckert A, Lai K, Adelman SJ, Harnish DC** 2001 Reciprocal antagonism between estrogen receptor and NF-κB activity in vivo. Circ Res 89:823–830
344. **Omoya T, Shimizu I, Zhou Y, Okamura Y, Inoue H, Lu G, Itonaga M, Honda H, Nomura M, Ito S** 2001 Effects of idoxifene and estradiol on NF-κB activation in cultured rat hepatocytes undergoing oxidative stress. Liver 21:183–191
345. **Evans MJ, Lai K, Shaw LJ, Harnish DC, Chadwick CC** 2002 Estrogen receptor α inhibits IL-1β induction of gene expression in the mouse liver. Endocrinology 143:2559–2570
346. **Yin M, Ikejima K, Wheeler MD, Bradford BU, Seabra V, Forman DT, Sato N, Thurman RG** 2000 Estrogen is involved in early alcohol-induced liver injury in a rat enteral feeding model. Hepatology 31:117–123
347. **Enomoto N, Yamashina S, Schemmer P, Rivera CA, Bradford BU, Enomoto A, Brenner DA, Thurman RG** 1999 Estriol sensitizes rat Kupffer cells via gut-derived endotoxin. Am J Physiol 277:G671–G677
348. **Jarvelainen HA, Lukkari TA, Heinaro S, Sippel H, Lindros KO** 2001 The antiestrogen toremifene protects against alcoholic liver injury in female rats. J Hepatol 35:46–52
349. **Blush J, Lei J, Ju W, Silbiger S, Pullman J, Neugarten J** 2004 Estradiol reverses renal injury in Alb/TGF-β1 transgenic mice. Kidney Int 66:2148–2154
350. **Maric C, Sandberg K, Hinojosa-Laborde C** 2004 Glomerulosclerosis and tubulointerstitial fibrosis are attenuated with 17β-estradiol in the aging Dahl salt sensitive rat. J Am Soc Nephrol 15: 1546–1556
351. **Antus B, Liu S, Yao Y, Zou H, Song E, Lutz J, Heemann U** 2005 Effects of progesterone and selective oestrogen receptor modulators on chronic allograft nephropathy in rats. Nephrol Dial Transplant 20:329–335
352. **Neugarten J, Acharya A, Lei J, Silbiger S** 2000 Selective estrogen receptor modulators suppress mesangial cell collagen synthesis. Am J Physiol Renal Physiol 279:F309–F318
353. **Birch Nielsen C, Krag S, Osterby R, Flyvbjerg A, Nyengaard J, Forman A, Wogensen L** 2004 Transforming growth factor β1-induced glomerulopathy is prevented by 17β-estradiol supplementation. Virchows Arch 444:561–566
354. **Esposito E, Iacono A, Raso GM, Pacilio M, Coppola A, Di Carlo R, Meli R** 2005 Raloxifene, a selective estrogen receptor modulator, reduces carrageenan-induced acute inflammation in normal and ovariectomized rats. Endocrinology 146:3301–3308
355. **Speyer CL, Rancilio NJ, McClintock SD, Crawford JD, Gao H, Sarma JV, Ward PA** 2005 Regulatory effects of estrogen on acute lung inflammation in mice. Am J Physiol Cell Physiol 288:C881–C890
356. **Sullivan DA** 1997 Sex hormones and Sjogren's syndrome. J Rheumatol Suppl 50:17–32
357. **Buggage RR, Matteson DM, Shen dF, Sun B, Tuaillon N, Chan CC** 2003 Effect of sex hormones on experimental autoimmune uveoretinitis (EAU). Immunol Invest 32:259–273
358. **de Kozak Y, Andrieux K, Villarroya H, Klein C, Thillaye-Goldenberg B, Naud MC, Garcia E, Couvreur P** 2004 Intraocular injection of tamoxifen-loaded nanoparticles: a new treatment of experimental autoimmune uveoretinitis. Eur J Immunol 34:3702–3712
359. **Sanghvi C, Aziz K, Jones NP** 2004 Uveitis and the menstrual cycle. Eye 18:451–454
360. **Chan CC, Reed GF, Kim Y, Agron E, Buggage RR** 2004 A correlation of pregnancy term, disease activity, serum female hormones, and cytokines in uveitis. Br J Ophthalmol 88:1506–1509
361. **Geisler JG, Zawalich W, Zawalich K, Lakey JR, Stukenbrok H, Milici AJ, Soeller WC** 2002 Estrogen can prevent or reverse obesity and diabetes in mice expressing human islet amyloid polypeptide. Diabetes 51:2158–2169
362. **Samaras K, Hayward CS, Sullivan D, Kelly RP, Campbell LV** 1999 Effects of postmenopausal hormone replacement therapy on central abdominal fat, glycemic control, lipid metabolism, and vascular factors in type 2 diabetes: a prospective study. Diabetes Care 22:1401–1407
363. **Cornu C, Mercier C, Ffrench P, Bully C, Pugeat M, Cousin P, Riou JP, Bajart L, Orgiazzi J, Pommet-Nicot C, Darsy P, Boissel JP, Berthezene F** 2000 Postmenopause hormone treatment in women with NIDDM or impaired glucose tolerance: the MEDIA randomized clinical trial. Maturitas 37:95–104
364. **Perera M, Sattar N, Petrie JR, Hillier C, Small M, Connell JM, Lowe GD, Lumsden MA** 2001 The effects of transdermal estradiol in combination with oral norethisterone on lipoproteins, coagulation, and endothelial markers in postmenopausal women with type 2 diabetes: a randomized, placebo-controlled study. J Clin Endocrinol Metab 86:1140–1143
365. **Contreras JL, Smyth CA, Bilbao G, Young CJ, Thompson JA, Eckhoff DE** 2002 17β-Estradiol protects isolated human pancreatic islets against proinflammatory cytokine-induced cell death: molecular mechanisms and islet functionality. Transplantation 74:1252–1259
366. **Robinette CL** 1988 Sex-hormone-induced inflammation and fibromuscular proliferation in the rat lateral prostate. Prostate 12:271–286
367. **Harris MT, Feldberg RS, Lau KM, Lazarus NH, Cochrane DE** 2000 Expression of proinflammatory genes during estrogen-induced inflammation of the rat prostate. Prostate 44:19–25
368. **Wilson MJ, Woodson M, Wiehr C, Reddy A, Sinha AA** 2004 Matrix metalloproteinases in the pathogenesis of estradiol-induced nonbacterial prostatitis in the lateral prostate lobe of the Wistar rat. Exp Mol Pathol 77:7–17
369. **Nothnick WB** 2001 Treating endometriosis as an autoimmune disease. Fertil Steril 76:223–231
370. **Gleicher N, Pratt D** 1993 Abnormal (auto)immunity and endometriosis. Int J Gynaecol Obstet 40 Suppl:S21—S27
371. **Giudice LC, Kao LC** 2004 Endometriosis. Lancet 364:1789–1799
372. **Burney RO, Giudice LC** 2007 Pathogenesis of endometriosis. In: Nezhat C, ed. Comprehensive gynecologic endoscopic surgery: principles and techniques. Cambridge, UK: Cambridge University Press; in press
373. **Burney RO, Talbi S, Hamilton AE, Vo KC, Nyegaard M, Nezhat CR, Lessey BA, Giudice LC** 2007 Gene expression analysis of endometrium reveals progesterone resistance and candidate susceptibility genes in women with endometriosis. Endocrinology 148:3814–3826
374. **Kovacs EJ, Messingham KA, Gregory MS** 2002 Estrogen regulation of immune responses after injury. Mol Cell Endocrinol 193: 129–135
375. **Kovacs EJ, Plackett TP, Witte PL** 2004 Estrogen replacement, aging, and cell-mediated immunity after injury. J Leukoc Biol 76: 36–41
376. **Kanda N, Watanabe S** 2005 Regulatory roles of sex hormones in cutaneous biology and immunology. J Dermatol Sci 38:1–7
377. **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
378. **Ashcroft GS, Mills SJ, Lei K, Gibbons L, Jeong MJ, Taniguchi M, Burow M, Horan MA, Wahl SM, Nakayama T** 2003 Estrogen modulates cutaneous wound healing by downregulating macrophage migration inhibitory factor. J Clin Invest 111:1309–1318
379. **Flores JM, Jimenez PI, Rincon MD, Marquez JA, Navarro H, Arteta D, Murillo F** 2001 Early risk factors for sepsis in patients with severe blunt trauma. Injury 32:5–12
380. **Yokoyama Y, Schwacha MG, Samy TS, Bland KI, Chaudry IH** 2002 Gender dimorphism in immune responses following trauma and hemorrhage. Immunol Res 26:63–76
381. **Angele MK, Knoferl MW, Schwacha MG, Ayala A, Cioffi WG, Bland KI, Chaudry IH** 1999 Sex steroids regulate pro- and anti-inflammatory cytokine release by macrophages after trauma-hemorrhage. Am J Physiol 277:C35–C42
382. **Knoferl MW, Jarrar D, Angele MK, Ayala A, Schwacha MG, Bland KI, Chaudry IH** 2001 17 β-Estradiol normalizes immune

responses in ovariectomized females after trauma-hemorrhage. Am J Physiol Cell Physiol 281:C1131–C1138
383. **Knoferl MW, Angele MK, Schwacha MG, Anantha Samy TS, Bland KI, Chaudry IH** 2003 Immunoprotection in proestrus females following trauma-hemorrhage: the pivotal role of estrogen receptors. Cell Immunol 222:27–34
384. **Knoferl MW, Schwacha MG, Jarrar D, Angele MK, Fragoza K, Bland KI, Chaudry IH** 2002 Estrogen pretreatment protects males against hypoxia-induced immune depression. Am J Physiol Cell Physiol 282:C1087–C1092
385. **Özveri ES, Bozkurt A, Haklar G, Cetinel S, Arbak S, Yegen C, Yegen BC** 2001 Estrogens ameliorate remote organ inflammation induced by burn injury in rats. Inflamm Res 50:585–591
386. **Kovacs EJ, Duffner LA, Plackett TP** 2004 Immunosuppression after injury in aged mice is associated with a TH1-TH2 shift, which can be restored by estrogen treatment. Mech Ageing Dev 125:121–123
387. **Ikejima K, Enomoto N, Iimuro Y, Ikejima A, Fang D, Xu J, Forman DT, Brenner DA, Thurman RG** 1998 Estrogen increases sensitivity of hepatic Kupffer cells to endotoxin. Am J Physiol 274:G669–G676
388. **Zuckerman SH, Ahmari SE, Bryan-Poole N, Evans GF, Short L, Glasebrook AL** 1996 Estriol: a potent regulator of TNF and IL-6 expression in a murine model of endotoxemia. Inflammation 20: 581–597
389. **Manolagas SC, Jilka RL, Girasole G, Passeri G, Bellido T** 1993 Estrogen, cytokines, and the control of osteoclast formation and bone resorption in vitro and in vivo. Osteoporos Int 3(Suppl 1): 114–116
390. **Ershler WB, Harman SM, Keller ET** 1997 Immunologic aspects of osteoporosis. Dev Comp Immunol 21:487–499
391. **Oursler MJ** 1998 Mechanisms of steroid action on osteoclasts and osteoblasts. Biochem Soc Trans 26:33–38
392. **Windahl SH, Andersson G, Gustafsson JA** 2002 Elucidation of estrogen receptor function in bone with the use of mouse models. Trends Endocrinol Metab 13:195–200
393. **Carlsten H** 2005 Immune responses and bone loss: the estrogen connection. Immunol Rev 208:194–206
394. **Weitzmann MN, Pacifici R** 2006 Estrogen deficiency and bone loss: an inflammatory tale. J Clin Invest 116:1186–1194
395. **Eriksen EF, Langdahl B, Vesterby A, Rungby J, Kassem M** 1999 Hormone replacement therapy prevents osteoclastic hyperactivity: a histomorphometric study in early postmenopausal women. J Bone Miner Res 14:1217–1221
396. **Jilka RL, Hangoc G, Girasole G, Passeri 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
397. **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-β 1 and increases the mRNA levels of osteocalcin in rat bone in vivo. Biochem Biophys Res Commun 194:1228–1233
398. **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
399. **Cenci S, Toraldo G, Weitzmann MN, Roggia C, Gao Y, Qian WP, Sierra O, Pacifici R** 2003 Estrogen deficiency induces bone loss by increasing T cell proliferation and lifespan through IFN-γ-induced class II transactivator. Proc Natl Acad Sci USA 100:10405–10410
400. **Miyaura C, Onoe Y, Inada M, Maki K, Ikuta K, Ito M, Suda T** 1997 Increased B-lymphopoiesis by interleukin 7 induces bone loss in mice with intact ovarian function: similarity to estrogen deficiency. Proc Natl Acad Sci USA 94:9360–9365
401. **Valenzona HO, Pointer R, Ceredig R, Osmond DG** 1996 Prelymphomatous B cell hyperplasia in the bone marrow of interleukin-7 transgenic mice: precursor B cell dynamics, microenvironmental organization and osteolysis. Exp Hematol 24:1521–1529
402. **Ross FP** 2003 Interleukin 7 and estrogen-induced bone loss. Trends Endocrinol Metab 14:147–149
403. **Steinbeck MJ, Appel Jr WH, Verhoeven AJ, Karnovsky MJ** 1994 NADPH-oxidase expression and in situ production of superoxide by osteoclasts actively resorbing bone. J Cell Biol 126:765–772
404. **Lean JM, Jagger CJ, Kirstein B, Fuller K, Chambers TJ** 2005 Hydrogen peroxide is essential for estrogen-deficiency bone loss and osteoclast formation. Endocrinology 146:728–735
405. **Jagger CJ, Lean JM, Davies JT, Chambers TJ** 2005 Tumor necrosis factor-α mediates osteopenia caused by depletion of antioxidants. Endocrinology 146:113–118
406. **Eielson C, Kaplan D, Mitnick MA, Paliwal I, Insogna K** 1994 Estrogen modulates parathyroid hormone-induced fibronectin production in human and rat osteoblast-like cells. Endocrinology 135:1639–1644
407. **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
408. **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
409. **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
410. **Hall GM, Spector TD, Delmas PD** 1995 Markers of bone metabolism in postmenopausal women with rheumatoid arthritis. Effects of corticosteroids and hormone replacement therapy. Arthritis Rheum 38:902–906
411. **Forsblad D'Elia H, Christgau S, Mattsson LA, Saxne T, Ohlsson C, Nordborg E, Carlsten H** 2004 Hormone replacement therapy, calcium and vitamin D3 versus calcium and vitamin D3 alone decreases markers of cartilage and bone metabolism in rheumatoid arthritis: a randomized controlled trial [ISRCTN46523456]. Arthritis Res Ther 6:R457–R468
412. **Brühl H, Cihak J, Schneider MA, Plachy J, Rupp T, Wenzel I, Shakarami M, Milz S, Ellwart JW, Stangassinger M, Schlöndorff D, Mack M** 2004 Dual role of CCR2 during initiation and progression of collagen-induced arthritis: evidence for regulatory activity of CCR2+ T cells. J Immunol 172:890–898
413. **Härle P, Möbius D, Carr DJ, Schölmerich J, Straub RH** 2005 An opposing time-dependent immune-modulating effect of the sympathetic nervous system conferred by altering the cytokine profile in the local lymph nodes and spleen of mice with type II collagen-induced arthritis. Arthritis Rheum 52:1305–1313
414. **Honjo H, Ogino Y, Naitoh K, Urabe M, Kitawaki J, Yasuda J, Yamamoto T, Ishihara S, Okada H, Yonezawa T** 1989 In vivo effects by estrone sulfate on the central nervous system-senile dementia (Alzheimer's type). J Steroid Biochem 34:521–525
415. **Tang MX, Jacobs D, Stern Y, Marder K, Schofield P, Gurland B, Andrews H, Mayeux R** 1996 Effect of oestrogen during menopause on risk and age at onset of Alzheimer's disease. Lancet 348:429–432
416. **Kawas C, Resnick S, Morrison A, Brookmeyer R, Corrada M, Zonderman A, Bacal C, Lingle DD, Metter E** 1997 A prospective study of estrogen replacement therapy and the risk of developing Alzheimer's disease: the Baltimore Longitudinal Study of Aging. Neurology 48:1517–1521
417. **Mulnard RA, Cotman CW, Kawas C, van Dyck CH, Sano M, Doody R, Koss E, Pfeiffer E, Jin S, Gamst A, Grundman M, Thomas R, Thal LJ** 2000 Estrogen replacement therapy for treatment of mild to moderate Alzheimer disease: a randomized controlled trial. Alzheimer's Disease Cooperative Study. JAMA 283: 1007–1015
418. **Mikkola TS, Clarkson TB** 2002 Estrogen replacement therapy, atherosclerosis, and vascular function. Cardiovasc Res 53:605–619
419. **Mori T, Durand J, Chen Y, Thompson JA, Bakir S, Oparil S** 2000 Effects of short-term estrogen treatment on the neointimal response to balloon injury of rat carotid artery. Am J Cardiol 85:1276–1279
420. **Jansson L, Mattsson A, Mattsson R, Holmdahl R** 1990 Estrogen induced suppression of collagen arthritis. V: Physiological level of estrogen in DBA/1 mice is therapeutic on established arthritis, suppresses anti-type II collagen T-cell dependent immunity and stimulates polyclonal B-cell activity. J Autoimmun 3:257–270
421. **Delpy L, Douin-Echinard V, Garidou L, Bruand C, Saoudi A, Guery JC** 2005 Estrogen enhances susceptibility to experimental autoimmune myasthenia gravis by promoting type 1-polarized immune responses. J Immunol 175:5050–5057

422. **Simpson ER** 2003 Sources of estrogen and their importance. J Steroid Biochem Mol Biol 86:225–230
423. **Schmidt M, Kreutz M, Löffler G, Schölmerich J, Straub RH** 2000 Conversion of dehydroepiandrosterone to downstream steroid hormones in macrophages. J Endocrinol 164:161–169
424. **Shozu M, Zhao Y, Simpson ER** 1997 Estrogen biosynthesis in THP1 cells is regulated by promoter switching of the aromatase (CYP19) gene. Endocrinology 138:5125–5135
425. **Reed MJ, Purohit A** 1997 Breast cancer and the role of cytokines in regulating estrogen synthesis: an emerging hypothesis. Endocr Rev 18:701–715
426. **Jakob F, Siggelkow H, Homann D, Kohrle J, Adamski J, Schutze N** 1997 Local estradiol metabolism in osteoblast- and osteoclast-like cells. J Steroid Biochem Mol Biol 61:167–174
427. **Simard J, Gingras S** 2001 Crucial role of cytokines in sex steroid formation in normal and tumoral tissues. Mol Cell Endocrinol 171:25–40
428. **Purohit A, Reed MJ** 2002 Regulation of estrogen synthesis in postmenopausal women. Steroids 67:979–983
429. **Schumacher G, Neuhaus P** 2001 The physiological estrogen metabolite 2-methoxyestradiol reduces tumor growth and induces apoptosis in human solid tumors. J Cancer Res Clin Oncol 127: 405–410
430. **Dubey RK, Jackson EK** 2001 Cardiovascular protective effects of 17β-estradiol metabolites. J Appl Physiol 91:1868–1883
431. **Yager JD, Davidson NE** 2006 Estrogen carcinogenesis in breast cancer. N Engl J Med 354:270–282
432. **Lattanzi ML, Santos CB, Mudry MD, Baranao JL** 2003 Exposure of bovine oocytes to the endogenous metabolite 2-methoxyestradiol during in vitro maturation inhibits early embryonic development. Biol Reprod 69:1793–1800
433. **Lottering ML, Haag M, Seegers JC** 1992 Effects of 17 β-estradiol metabolites on cell cycle events in MCF-7 cells. Cancer Res 52: 5926–5932
434. **Lewis JS, Thomas TJ, Pestell RG, Albanese C, Gallo MA, Thomas T** 2005 Differential effects of 16α-hydroxyestrone and 2-methoxyestradiol on cyclin D1 involving the transcription factor ATF-2 in MCF-7 breast cancer cells. J Mol Endocrinol 34:91–105
435. **Zacharia LC, Jackson EK, Gillespie DG, Dubey RK** 2001 Catecholamines abrogate antimitogenic effects of 2-hydroxyestradiol on human aortic vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 21:1745–1750
436. **Ho YT, Newman SP, Purohit A, Leese MP, Potter BV, Reed MJ** 2003 The effects of 2-methoxy oestrogens and their sulphamoylated derivatives in conjunction with TNF-α on endothelial and fibroblast cell growth, morphology and apoptosis. J Steroid Biochem Mol Biol 86:189–196
437. **Maran A, Gorny G, Oursler MJ, Zhang M, Shogren KL, Yaszemski MJ, Turner RT** 2006 2-Methoxyestradiol inhibits differentiation and is cytotoxic to osteoclasts. J Cell Biochem 99:425–434
438. **Chauhan D, Li G, Auclair D, Hideshima T, Richardson P, Podar K, Mitsiades N, Mitsiades C, Li C, Kim RS, Munshi N, Chen LB, Wong W, Anderson KC** 2003 Identification of genes regulated by 2-methoxyestradiol (2ME2) in multiple myeloma cells using oligonucleotide arrays. Blood 101:3606–3614
439. **Lambert C, Apel K, Biesalski HK, Frank J** 2004 2-Methoxyestradiol induces caspase-independent, mitochondria-centered apoptosis in DS-sarcoma cells. Int J Cancer 108:493–501
440. **Banerjeei SK, Zoubine MN, Sarkar DK, Weston AP, Shah JH, Campbell DR** 2000 2-Methoxyestradiol blocks estrogen-induced rat pituitary tumor growth and tumor angiogenesis: possible role of vascular endothelial growth factor. Anticancer Res 20:2641–2645
441. **Tofovic SP, Dubey R, Salah EM, Jackson EK** 2002 2-Hydroxyestradiol attenuates renal disease in chronic puromycin aminonucleoside nephropathy. J Am Soc Nephrol 13:2737–2747
442. **Fowke JH, Qi D, Bradlow HL, Shu XO, Gao YT, Cheng JR, Jin F, Zheng W** 2003 Urinary estrogen metabolites and breast cancer: differential pattern of risk found with pre- versus post-treatment collection. Steroids 68:65–72
443. **Bradlow HL, Telang NT, Sepkovic DW, Osborne MP** 1996 2-Hydroxyestrone: the 'good' estrogen. J Endocrinol 150 Suppl:S259–S265
444. **Sowers MR, McConnell D, Jannausch M, Buyuktur AG, Hochberg M, Jamadar DA** 2006 Estradiol and its metabolites and their association with knee osteoarthritis. Arthritis Rheum 54:2481–2487
445. **Chen J, Li Y, Lavigne JA, Trush MA, Yager JD** 1999 Increased mitochondrial superoxide production in rat liver mitochondria, rat hepatocytes, and HepG2 cells following ethinyl estradiol treatment. Toxicol Sci 51:224–235
446. **Dubey RK, Tyurina YY, Tyurin VA, Gillespie DG, Branch RA, Jackson EK, Kagan VE** 1999 Estrogen and tamoxifen metabolites protect smooth muscle cell membrane phospholipids against peroxidation and inhibit cell growth. Circ Res 84:229–239
447. **Spencer DL, Masten SA, Lanier KM, Yang X, Grassman JA, Miller CR, Sutter TR, Lucier GW, Walker NJ** 1999 Quantitative analysis of constitutive and 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced cytochrome P450 1B1 expression in human lymphocytes. Cancer Epidemiol Biomarkers Prev 8:139–146
448. **Chao TC, Phuangsab A, Van Alten PJ, Walter RJ** 1996 Steroid sex hormones and macrophage function: regulation of chemiluminescence and phagocytosis. Am J Reprod Immunol 35:106–113
449. **Piccinni MP, Giudizi MG, Biagiotti R, Beloni L, Giannarini L, Sampognaro S, Parronchi P, Manetti R, Annunziato F, Livi C** 1995 Progesterone favors the development of human T helper cells producing Th2-type cytokines and promotes both IL-4 production and membrane CD30 expression in established Th1 cell clones. J Immunol 155:128–133
450. **Montes MJ, Tortosa CG, Borja C, Abadia AC, Gonzalez-Gomez F, Ruiz C, Olivares EG** 1995 Constitutive secretion of interleukin-6 by human decidual stromal cells in culture. Regulatory effect of progesterone 577. Am J Reprod Immunol 34:188–194
451. **Szekeres-Bartho J, Wegmann TG** 1996 A progesterone-dependent immunomodulatory protein alters the Th1/Th2 balance 579. J Reprod Immunol 31:81–95
452. **Le N, Yousefi S, Vaziri N, Carandang G, Ocariz J, Cesario T** 1988 The effect of β-estradiol, progesterone and testosterone on the production of human leukocyte derived interferons 571. J Biol Regul Homeost Agents 2:199–204
453. **Szekeres-Bartho J, Kinsky R, Chaouat G** 1990 The effect of a progesterone-induced immunologic blocking factor on NK-mediated resorption 1391. Am J Reprod Immunol 24:105–107
454. **Mantovani G, Maccio A, Esu S, Lai P, Santona MC, Massa E, Dessi D, Melis GB, Del Giacco GS** 1997 Medroxyprogesterone acetate reduces the in vitro production of cytokines and serotonin involved in anorexia/cachexia and emesis by peripheral blood mononuclear cells of cancer patients 574. Eur J Cancer 33:602–607
455. **Khamashta MA, Ruiz-Irastorza G, Hughes GR** 1997 Systemic lupus erythematosus flares during pregnancy. Rheum Dis Clin North Am 23:15–30
456. **Lockshin MD, Sammaritano LR** 2003 Lupus pregnancy. Autoimmunity 36:33–40
457. **Petri M** 1997 Hopkins Lupus Pregnancy Center: 1987 to 1996. Rheum Dis Clin North Am 23:1–13
458. **Rahman P, Gladman DD, Urowitz MB** 1998 Clinical predictors of fetal outcome in systemic lupus erythematosus. J Rheumatol 25: 1526–1530
459. **Doria A, Cutolo M, Ghirardello A, Zampieri S, Vescovi F, Sulli A, Giusti M, Piccoli A, Grella P, Gambari PF** 2002 Steroid hormones and disease activity during pregnancy in systemic lupus erythematosus. Arthritis Rheum 47:202–209
460. **Hench PS** 1938 The ameliorating effect of pregnancy on chronic atrophic (infectious, rheumatoid) arthritis, fibrositis, and intermittent hydrarthrosis. Proc Staff Meetings Mayo Clinic 13:161–167
461. **Ostensen M, Aune B, Husby G** 1983 Effect of pregnancy and hormonal changes on the activity of rheumatoid arthritis. Scand J Rheumatol 12:69–72
462. **Russell AS, Johnston C, Chew C, Maksymowych WP** 1997 Evidence for reduced Th1 function in normal pregnancy: a hypothesis for the remission of rheumatoid arthritis. J Rheumatol 24:1045–1050
463. **Tchorzewski H, Krasomski G, Biesiada L, Glowacka E, Banasik M, Lewkowicz P** 2000 IL-12, IL-6 and IFN-γ production by lymphocytes of pregnant women with rheumatoid arthritis remission during pregnancy. Mediators Inflamm 9:289–293
464. **Elenkov IJ, Wilder RL, Bakalov VK, Link AA, Dimitrov MA, Fisher S, Crane M, Kanik KS, Chrousos GP** 2001 IL-12, TNF-α, and hormonal changes during late pregnancy and early postpartum:

implications for autoimmune disease activity during these times. J Clin Endocrinol Metab 86:4933–4938
465. **Munoz-Valle JF, Vazquez-Del Mercado M, Garcia-Iglesias T, Orozco-Barocio G, Bernard-Medina G, Martinez-Bonilla G, Bastidas-Ramirez BE, Navarro AD, Bueno M, Martinez-Lopez E, Best-Aguilera CR, Kamachi M, Armendariz-Borunda J** 2003 T(H)1/T(H)2 cytokine profile, metalloprotease-9 activity and hormonal status in pregnant rheumatoid arthritis and systemic lupus erythematosus patients. Clin Exp Immunol 131:377–384
466. **Ostensen M, Sicher P, Forger F, Villiger PM** 2005 Activation markers of peripheral blood mononuclear cells in late pregnancy and after delivery: a pilot study. Ann Rheum Dis 64:318–320
467. **Confavreux C, Hutchinson M, Hours MM, Cortinovis-Tourniaire P, Moreau T** 1998 Rate of pregnancy-related relapse in multiple sclerosis. Pregnancy in Multiple Sclerosis Group. N Engl J Med 339:285–291
468. **Linos A, Worthington JW, O'Fallon WM, Kurland LT** 1983 Case-control study of rheumatoid arthritis and prior use of oral contraceptives. Lancet 1:1299–1300
469. **Allebeck P, Ahlbom A, Ljungstrom K, Allander E** 1984 Do oral contraceptives reduce the incidence of rheumatoid arthritis? A pilot study using the Stockholm County medical information system. Scand J Rheumatol 13:140–146
470. **del Junco DJ, Annegers JF, Luthra HS, Coulam CB, Kurland LT** 1985 Do oral contraceptives prevent rheumatoid arthritis? JAMA 254:1938–1941
471. **Vessey MP, Villard-Mackintosh L, Yeates D** 1987 Oral contraceptives, cigarette smoking and other factors in relation to arthritis. Contraception 35:457–464
472. **Romieu I, Hernandez-Avila M, Liang MH** 1989 Oral contraceptives and the risk of rheumatoid arthritis: a meta-analysis of a conflicting literature. Br J Rheumatol 28(Suppl 1):13–17; discussion 18–23
473. **Hernandez-Avila M, Liang MH, Willett WC, Stampfer MJ, Colditz GA, Rosner B, Chang RW, Hennekens CH, Speizer FE** 1990 Exogenous sex hormones and the risk of rheumatoid arthritis. Arthritis Rheum 33:947–953
474. **Moskowitz MA, Jick SS, Burnside S, Wallis WJ, Dickson JF, Hunter JR, Jick H** 1990 The relationship of oral contraceptive use to rheumatoid arthritis. Epidemiology 1:153–156
475. **Hannaford PC, Kay CR, Hirsch S** 1990 Oral contraceptives and rheumatoid arthritis: new data from the Royal College of General Practitioners' oral contraception study. Ann Rheum Dis 49:744–746
476. **Brennan P, Bankhead C, Silman A, Symmons D** 1997 Oral contraceptives and rheumatoid arthritis: results from a primary care-based incident case-control study. Semin Arthritis Rheum 26:817–823
477. **Vandenbroucke JP, Witteman JC, Valkenburg HA, Boersma JW, Cats A, Festen JJ, Hartman AP, Huber-Bruning O, Rasker JJ, Weber J** 1986 Noncontraceptive hormones and rheumatoid arthritis in perimenopausal and postmenopausal women. JAMA 255: 1299–1303
478. **van Zeben D, Hazes JM, Vandenbroucke JP, Dijkmans BA, Cats A** 1990 Diminished incidence of severe rheumatoid arthritis associated with oral contraceptive use. Arthritis Rheum 33:1462–1465
479. **Doran MF, Crowson CS, O'Fallon WM, Gabriel SE** 2004 The effect of oral contraceptives and estrogen replacement therapy on the risk of rheumatoid arthritis: a population based study. J Rheumatol 31:207–213
480. **Tarzy BJ, Garcia CR, Wallach EE, Zweiman B, Myers AR** 1972 Rheumatic disease, abnormal serology, and oral contraceptives. Lancet 2:501–503
481. **Hazes JM, Dijkmans BA, Vandenbroucke JP, Cats A** 1989 Oral contraceptive treatment for rheumatoid arthritis: an open study in 10 female patients. Br J Rheumatol 28(Suppl 1):28–30
482. **Drossaers-Bakker KW, Zwinderman AH, van Zeben D, Breedveld FC, Hazes JM** 2002 Pregnancy and oral contraceptive use do not significantly influence outcome in long term rheumatoid arthritis. Ann Rheum Dis 61:405–408
483. **Grimes DA, LeBolt SA, Grimes KR, Wingo PA** 1985 Systemic lupus erythematosus and reproductive function: a case-control study. Am J Obstet Gynecol 153:179–186
484. **Strom BL, Reidenberg MM, West S, Snyder ES, Freundlich B, Stolley PD** 1994 Shingles, allergies, family medical history, oral contraceptives, and other potential risk factors for systemic lupus erythematosus. Am J Epidemiol 140:632–642
485. **Sanchez-Guerrero J, Karlson EW, Liang MH, Hunter DJ, Speizer FE, Colditz GA** 1997 Past use of oral contraceptives and the risk of developing systemic lupus erythematosus. Arthritis Rheum 40: 804–808
486. **Sanchez-Guerrero J, Uribe AG, Jimenez-Santana L, Mestanza-Peralta M, Lara-Reyes P, Seuc AH, Cravioto MD** 2005 A trial of contraceptive methods in women with systemic lupus erythematosus. N Engl J Med 353:2539–2549
487. **Petri M, Kim MY, Kalunian KC, Grossman J, Hahn BH, Sammaritano LR, Lockshin M, Merrill JT, Belmont HM, Askanase AD, McCune WJ, Hearth-Holmes M, Dooley MA, Von Feldt J, Friedman A, Tan M, Davis J, Cronin M, Diamond B, Mackay M, Sigler L, Fillius M, Rupel A, Licciardi F, Buyon JP** 2005 Combined oral contraceptives in women with systemic lupus erythematosus. N Engl J Med 353:2550–2558
488. **Thorogood M, Hannaford PC** 1998 The influence of oral contraceptives on the risk of multiple sclerosis. Br J Obstet Gynaecol 105:1296–1299
489. **Hernan MA, Hohol MJ, Olek MJ, Spiegelman D, Ascherio A** 2000 Oral contraceptives and the incidence of multiple sclerosis. Neurology 55:848–854
490. **Alonso A, Jick SS, Olek MJ, Ascherio A, Jick H, Hernan MA** 2005 Recent use of oral contraceptives and the risk of multiple sclerosis. Arch Neurol 62:1362–1365
491. **Aune B, Oian P, Omsjo I, Osterud B** 1995 Hormone replacement therapy reduces the reactivity of monocytes and platelets in whole blood–a beneficial effect on atherogenesis and thrombus formation? Am J Obstet Gynecol 173:1816–1820
492. **Uemura H, Kamada M, Maegawa M, Ohmoto Y, Murata K, Kuwahara A, Matsuzaki T, Yasui T, Takeji T, Irahara M** 2005 Effect of hormone replacement therapy on the production of bone-resorbing cytokines by peripheral blood cells in postmenopausal women. Horm Metab Res 37:226–230
493. **Berg G, Ekerfelt C, Hammar M, Lindgren R, Matthiesen L, Ernerudh J** 2002 Cytokine changes in postmenopausal women treated with estrogens: a placebo-controlled study. Am J Reprod Immunol 48:63–69
494. **Lacut K, Oger E, Le Gal G, Blouch MT, Abgrall JF, Kerlan V, Scarabin PY, Mottier D** 2003 Differential effects of oral and transdermal postmenopausal estrogen replacement therapies on C-reactive protein. Thromb Haemost 90:124–131
495. **Prestwood KM, Unson C, Kulldorff M, Cushman M** 2004 The effect of different doses of micronized 17β-estradiol on C-reactive protein, interleukin-6, and lipids in older women. J Gerontol A Biol Sci Med Sci 59:827–832
496. **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
497. **Straub RH, Hense HW, Andus T, Schölmerich 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
498. **Porter VR, Greendale GA, Schocken M, Zhu X, Effros RB** 2001 Immune effects of hormone replacement therapy in post-menopausal women. Exp Gerontol 36:311–326
499. **Brooks-Asplund EM, Tupper CE, Daun JM, Kenney WL, Cannon JG** 2002 Hormonal modulation of interleukin-6, tumor necrosis factor and associated receptor secretion in postmenopausal women. Cytokine 19:193–200
500. **Brunelli R, Frasca D, Perrone G, Pioli C, Fattorossi A, Zichella L, Doria G** 1996 Hormone replacement therapy affects various immune cell subsets and natural cytotoxicity. Gynecol Obstet Invest 41:128–131
501. **Attanasio R, Gust DA, Wilson ME, Meeker T, Gordon TP** 2002 Immunomodulatory effects of estrogen and progesterone replacement in a nonhuman primate model. J Clin Immunol 22: 263–269
502. **Carette S, Marcoux S, Gingras S** 1989 Postmenopausal hor-

mones and the incidence of rheumatoid arthritis. J Rheumatol 16:911–913
503. **Spector TD, Brennan P, Harris P, Studd JW, Silman AJ** 1991 Does estrogen replacement therapy protect against rheumatoid arthritis? J Rheumatol 18:1473–1476
504. **Koepsell TD, Dugowson CE, Nelson JL, Voigt LF, Daling JR** 1994 Non-contraceptive hormones and the risk of rheumatoid arthritis in menopausal women. Int J Epidemiol 23:1248–1255
505. **Merlino LA, Cerhan JR, Criswell LA, Mikuls TR, Saag KG** 2003 Estrogen and other female reproductive risk factors are not strongly associated with the development of rheumatoid arthritis in elderly women. Semin Arthritis Rheum 33:72–82
506. **Gilbert M, Rotstein J, Cunningham C, Estrin I, Davidson A, Pincus G** 1964 Norethynodrel with mestranol in treatment of rheumatoid arthritis. JAMA 190:235
507. **Demers R, Blais JA, Pretty H** 1966 Rheumatoid arthritis treated by norethynodrel associated with mestranol: clinical aspects and laboratory tests. Can Med Assoc J 95:350–354
508. **Bijlsma JW, Huber-Bruning O, Thijssen JH** 1987 Effect of oestrogen treatment on clinical and laboratory manifestations of rheumatoid arthritis. Ann Rheum Dis 46:777–779
509. **van den Brink HR, Lems WF, van Everdingen AA, Bijlsma JW** 1993 Adjuvant oestrogen treatment increases bone mineral density in postmenopausal women with rheumatoid arthritis. Ann Rheum Dis 52:302–305
510. **van den Brink HR, van Everdingen AA, van Wijk MJ, Jacobs JW, Bijlsma JW** 1993 Adjuvant oestrogen therapy does not improve disease activity in postmenopausal patients with rheumatoid arthritis. Ann Rheum Dis 52:862–865
511. **MacDonald AG, Murphy EA, Capell HA, Bankowska UZ, Ralston SH** 1994 Effects of hormone replacement therapy in rheumatoid arthritis: a double blind placebo-controlled study. Ann Rheum Dis 53:54–57
512. **Forsblad D'Elia H, Mattsson LA, Ohlsson C, Nordborg E, Carlsten H** 2003 Hormone replacement therapy in rheumatoid arthritis is associated with lower serum levels of soluble IL-6 receptor and higher insulin-like growth factor 1. Arthritis Res Ther 5:R202–R209
513. **Forsblad D'Elia H, Larsen A, Mattsson LA, Waltbrand E, Kvist G, Mellstrom D, Saxne T, Ohlsson C, Nordborg E, Carlsten H** 2003 Influence of hormone replacement therapy on disease progression and bone mineral density in rheumatoid arthritis. J Rheumatol 30:1456–1463
514. **Sanchez-Guerrero J, Liang MH, Karlson EW, Hunter DJ, Colditz GA** 1995 Postmenopausal estrogen therapy and the risk for developing systemic lupus erythematosus. Ann Intern Med 122: 430–433
515. **Meier CR, Sturkenboom MC, Cohen AS, Jick H** 1998 Postmenopausal estrogen replacement therapy and the risk of developing systemic lupus erythematosus or discoid lupus. J Rheumatol 25:1515–1519
516. **Kreidstein S, Urowitz MB, Gladman DD, Gough J** 1997 Hormone replacement therapy in systemic lupus erythematosus. J Rheumatol 24:2149–2152
517. **Buyon JP, Petri MA, Kim MY, Kalunian KC, Grossman J, Hahn BH, Merrill JT, Sammaritano L, Lockshin M, Alarcon GS, Manzi S, Belmont HM, Askanase AD, Sigler L, Dooley MA, Von Feldt J, McCune WJ, Friedman A, Wachs J, Cronin M, Hearth-Holmes M, Tan M, Licciardi F** 2005 The effect of combined estrogen and progesterone hormone replacement therapy on disease activity in systemic lupus erythematosus: a randomized trial. Ann Intern Med 142:953–962
518. **Holmqvist P, Wallberg M, Hammar M, Landtblom AM, Brynhildsen J** 2006 Symptoms of multiple sclerosis in women in relation to sex steroid exposure. Maturitas 54:149–153
519. **Smith R, Studd JW** 1992 A pilot study of the effect upon multiple sclerosis of the menopause, hormone replacement therapy and the menstrual cycle. J R Soc Med 85:612–613
520. **Straub RH, Cutolo M** 2001 Involvement of the hypothalamic–pituitary–adrenal/gonadal axis and the peripheral nervous system in rheumatoid arthritis: viewpoint based on a systemic pathogenetic role. Arthritis Rheum 44:493–507
521. **Chrousos GP** 1995 The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. N Engl J Med 332:1351–1362
522. **Besedovsky HO, del Rey A** 1996 Immune-neuro-endocrine interactions: facts and hypotheses. Endocr Rev 17:64–102
523. **Turnbull AV, Rivier CL** 1999 Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. Physiol Rev 79:1–71
524. **Webster JI, Sternberg EM** 2004 Role of the hypothalamic-pituitary-adrenal axis, glucocorticoids and glucocorticoid receptors in toxic sequelae of exposure to bacterial and viral products. J Endocrinol 181:207–221
525. **Ader R** 2006 Psychoneuroimmunology. 4th Ed. San Diego: Academic Press
526. **Hvidberg E, Schou J, Jansen JA, Clausen JE** 1968 Tissue and plasma cortisol in man under various conditions. Acta Med Scand 184:215–223
527. **Giltay EJ, Fonk JC, von Blomberg BM, Drexhage HA, Schalkwijk C, Gooren LJ** 2000 *In vivo* effects of sex steroids on lymphocyte responsiveness and immunoglobulin levels in humans. J Clin Endocrinol Metab 85:1648–1657
528. **Kirschbaum C, Schommer N, Federenko I, Gaab J, Neumann O, Oellers M, Rohleder N, Untiedt A, Hanker J, Pirke KM, Hellhammer DH** 1996 Short-term estradiol treatment enhances pituitary-adrenal axis and sympathetic responses to psychosocial stress in healthy young men. J Clin Endocrinol Metab 81: 3639–3643
529. **Vamvakopoulos NC, Chrousos GP** 1993 Evidence of direct estrogenic regulation of human corticotropin-releasing hormone gene expression. Potential implications for the sexual dimorphism of the stress response and immune/inflammatory reaction. J Clin Invest 92:1896–1902
530. **Paulmyer-Lacroix O, Hery M, Pugeat M, Grino M** 1996 The modulatory role of estrogens on corticotropin-releasing factor gene expression in the hypothalamic paraventricular nucleus of ovariectomized rats: role of the adrenal gland. J Neuroendocrinol 8:515–519
531. **Patchev VK, Almeida OF** 1996 Gonadal steroids exert facilitating and "buffering" effects on glucocorticoid-mediated transcriptional regulation of corticotropin-releasing hormone and corticosteroid receptor genes in rat brain. J Neurosci 16:7077–7084
532. **Xia-Zhang L, Xiao E, Ferin M** 1995 A 5-day estradiol therapy, in amounts reproducing concentrations of the early-midfollicular phase, prevents the activation of the hypothalamo-pituitary-adrenal axis by interleukin-1 α in the ovariectomized rhesus monkey. J Neuroendocrinol 7:387–392
533. **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
534. **O'Connor TM, O'Connell J, O'Brien DI, Goode T, Bredin CP, Shanahan F** 2004 The role of substance P in inflammatory disease. J Cell Physiol 201:167–180
535. **Helme RD, Eglezos A, Hosking CS** 1987 Substance P induces chemotaxis of neutrophils in normal and capsaicin-treated rats. Immunol Cell Biol 65:267–269
536. **Ruff MR, Wahl SM, Pert CB** 1985 Substance P receptor-mediated chemotaxis of human monocytes. Peptides 6(Suppl 2):107–111
537. **Papka RE, Storey-Workley M, Shughrue PJ, Merchenthaler I, Collins JJ, Usip S, Saunders PT, Shupnik M** 2001 Estrogen receptor-α and β- immunoreactivity and mRNA in neurons of sensory and autonomic ganglia and spinal cord. Cell Tissue Res 304:193–214
538. **Dufourny L, Warembourg M** 1999 Estrogen modulation of neuropeptides: somatostatin, neurotensin and substance P, in the ventrolateral and arcuate nuclei of the female guinea pig. Neurosci Res 33:223–228
539. **Priest CA, Vink KL, Micevych PE** 1995 Temporal regulation by estrogen of β-preprotachykinin mRNA expression in the rat ventromedial nucleus of the hypothalamus. Brain Res Mol Brain Res 28:61–71
540. **Pinto FM, Armesto CP, Magraner J, Trujillo M, Martin JD,**

Candenas ML 1999 Tachykinin receptor and neutral endopeptidase gene expression in the rat uterus: characterization and regulation in response to ovarian steroid treatment. Endocrinology 140:2526–2532
541. **Villablanca AC, Hanley MR** 1997 17β-Estradiol stimulates substance P receptor gene expression. Mol Cell Endocrinol 135: 109–117
542. **Komisaruk BR, Adler NT, Hutchison J** 1972 Genital sensory field: enlargement by estrogen treatment in female rats. Science 178:1295–1298
543. **Kow LM, Pfaff DW** 1973 Effects of estrogen treatment on the size of receptive field and response threshold of pudendal nerve in the female rat. Neuroendocrinology 13:299–313
544. **Mowa CN, Papka RE** 2004 The role of sensory neurons in cervical ripening: effects of estrogen and neuropeptides. J Histochem Cytochem 52:1249–1258
545. **Blacklock AD, Cauveren JA, Smith PG** 2004 Estrogen selectively increases sensory nociceptor innervation of arterioles in the female rat. Brain Res 1018:55–65
546. **Blacklock AD, Johnson MS, Krizsan-Agbas D, Smith PG** 2005 Estrogen increases sensory nociceptor neuritogenesis in vitro by a direct, nerve growth factor-independent mechanism. Eur J Neurosci 21:2320–2328
547. **Bodnar RJ, Romero MT, Kramer E** 1988 Organismic variables and pain inhibition: roles of gender and aging. Brain Res Bull 21:947–953
548. **Gear RW, Miaskowski C, Gordon NC, Paul SM, Heller PH, Levine JD** 1996 κ-Opioids produce significantly greater analgesia in women than in men. Nat Med 2:1248–1250
549. **Bjorling DE, Wang ZY** 2001 Estrogen and neuroinflammation. Urology 57:40–46
550. **Levine JD, Taiwo YO** 1989 β-Estradiol induced catecholamine-sensitive hyperalgesia: a contribution to pain in Raynaud's phenomenon. Brain Res 487:143–147
551. **Flake NM, Bonebreak DB, Gold MS** 2005 Estrogen and inflammation increase the excitability of rat temporomandibular joint afferent neurons. J Neurophysiol 93:1585–1597
552. **Ceccarelli I, Fiorenzani P, Grasso G, Lariviere WR, Massafra C, Massai L, Muscettola M, Aloisi AM** 2004 Estrogen and μ-opioid receptor antagonists counteract the 17 β-estradiol-induced licking increase and interferon-γ reduction occurring during the formalin test in male rats. Pain 111:181–190
553. **Mogil JS, Chesler EJ, Wilson SG, Juraska JM, Sternberg WF** 2000 Sex differences in thermal nociception and morphine antinociception in rodents depend on genotype. Neurosci Biobehav Rev 24:375–389
554. **Craft RM, Mogil JS, Aloisi AM** 2004 Sex differences in pain and analgesia: the role of gonadal hormones. Eur J Pain 8:397–411
555. **Balthazart J, Ball GF** 2006 Is brain estradiol a hormone or a neurotransmitter? Trends Neurosci 29:241–249
556. **Ryan SM, Maier SF** 1988 The estrous cycle and estrogen modulate stress-induced analgesia. Behav Neurosci 102:371–380
557. **Nomikos G, Spyraki C, Kazandjian A, Sfikakis A** 1987 Estrogen treatment to ovariectomized rats modifies morphine-induced behavior. Pharmacol Biochem Behav 27:611–617
558. **Dawson-Basoa M, Gintzler AR** 1998 Gestational and ovarian sex steroid antinociception: synergy between spinal κ and δ opioid systems. Brain Res 794:61–67
559. **Straub RH, Härle P** 2005 Sympathetic neurotransmitters in joint inflammation. Rheum Dis Clin North Am 31:43–59, viii
560. **Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES** 2000 The sympathetic nerve—an integrative interface between two supersystems: the brain and the immune system. Pharmacol Rev 52:595–638
561. **Zoubina EV, Smith PG** 2002 Distributions of estrogen receptors α and β in sympathetic neurons of female rats: enriched expression by uterine innervation. J Neurobiol 52:14–23
562. **Del Rio G, Verlardo A, Zizzo G, Marrama P, Della CL** 1993 Sex differences in catecholamine response to clonidine. Int J Obes Relat Metab Disord 17:465–469
563. **Morita T, Latifpour J, O'Hollaren B, Wheeler MA, Weiss RM** 1987 Sex differences in function and distribution of α 1- and α 2-adrenoceptors in rabbit urethra. Am J Physiol 252:F1124–F1128
564. **Jones SB, Bylund DB, Rieser CA, Shekim WO, Byer JA, Carr GW** 1983 α2-Adrenergic receptor binding in human platelets: alterations during the menstrual cycle. Clin Pharmacol Ther 34:90–96
565. **Ferrer M, Osol G** 1998 Estrogen replacement modulates resistance artery smooth muscle and endothelial α2-adrenoceptor reactivity. Endothelium 6:133–141
566. **Vass-Lopez A, Garcia-Villar R, Lafontan M, Toutain PL** 1990 [3H]Idazoxan binding to the ovine myometrium. Binding characteristics and changes due to steroid hormones. J Pharmacol Exp Ther 253:819–824
567. **Riemer RK, Goldfien A, Roberts JM** 1987 Rabbit myometrial adrenergic sensitivity is increased by estrogen but is independent of changes in α adrenoceptor concentration. J Pharmacol Exp Ther 240:44–50
568. **Bento AC, de Moraes S** 1992 Effects of estrogen pretreatment of the spare α 1-adrenoceptors and the slow and fast components of the contractile response of the isolated female rat aorta. Gen Pharmacol 23:565–570
569. **Shackelford Jr DP, McConnaughey MM, Iams SG** 1988 The effects of estradiol and mestranol on α-adrenoceptors in select regions of the rat brain. Brain Res Bull 21:329–333
570. **Kam KW, Qi JS, Chen M, Wong TM** 2004 Estrogen reduces cardiac injury and expression of β1-adrenoceptor upon ischemic insult in the rat heart. J Pharmacol Exp Ther 309:8–15
571. **Petitti N, Etgen AM** 1990 α1-Adrenoceptor augmentation of β-stimulated cAMP formation is enhanced by estrogen and reduced by progesterone in rat hypothalamic slices. J Neurosci 10:2842–2849
572. **Riemer RK, Wu YY, Bottari SP, Jacobs MM, Goldfien A, Roberts JM** 1988 Estrogen reduces β-adrenoceptor-mediated cAMP production and the concentration of the guanyl nucleotide-regulatory protein, Gs, in rabbit myometrium. Mol Pharmacol 33: 389–395
573. **Nimmo AJ, Whitaker EM, Carstairs JR, Morrison JF** 1989 The presence of β-adrenoceptors in rat endometrium is dependent on circulating oestrogen. J Endocrinol 122:R1–R4
574. **Sporrong B, Alm P, Owman C, Sjoberg NO, Thorbert G** 1978 Ultrastructural evidence for adrenergic nerve degeneration in the guinea pig uterus during pregnancy. Cell Tissue Res 195: 189–193
575. **Brauer MM, Corbacho AM, Burnstock G** 1995 Effects of chronic and acute oestrogen treatment on the developing noradrenaline-containing nerves of the rat uterus. Int J Dev Neurosci 13:791–798
576. **Zoubina EV, Smith PG** 2001 Sympathetic hyperinnervation of the uterus in the estrogen receptor α knock-out mouse. Neuroscience 103:237–244
577. **Ting AY, Blacklock AD, Smith PG** 2004 Estrogen regulates vaginal sensory and autonomic nerve density in the rat. Biol Reprod 71:1397–1404
578. **Calabrese EJ** 2001 Estrogen and related compounds: biphasic dose responses. Crit Rev Toxicol 31:503–515
579. **An J, Ribeiro RC, Webb P, Gustafsson JA, 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
580. **Garcia Palacios V, Robinson LJ, Borysenko CW, Lehmann T, Kalla SE, Blair HC** 2005 Negative regulation of RANKL-induced osteoclastic differentiation in RAW 264.7 Cells by estrogen and phytoestrogens. J Biol Chem 280:13720–13727
581. **Banerjee SK, Campbell DR, Weston AP, Banerjee DK** 1997 Biphasic estrogen response on bovine adrenal medulla capillary endothelial cell adhesion, proliferation and tube formation. Mol Cell Biochem 177:97–105
582. **Sobel MI, Winkel CA, Macy LB, Liao P, Bjornsson TD** 1995 The regulation of plasminogen activators and plasminogen activator inhibitor type 1 in endothelial cells by sex hormones. Am J Obstet Gynecol 173:801–808

583. **Concina P, Sordello S, Barbacanne MA, Elhage R, Pieraggi MT, Fournial G, Plouet J, Bayard F, Arnal JF** 2000 The mitogenic effect of 17β-estradiol on in vitro endothelial cell proliferation and on in vivo reendothelialization are both dependent on vascular endothelial growth factor. J Vasc Res 37:202–208
584. **Canellada A, Blois S, Gentile T, Margni Idehu RA** 2002 In vitro modulation of protective antibody responses by estrogen, progesterone and interleukin-6. Am J Reprod Immunol 48:334–343
585. **Richette P, Dumontier MF, Francois M, Tsagris L, Korwin-Zmijowska C, Rannou F, Corvol MT** 2004 Dual effects of 17β-oestradiol on interleukin 1β-induced proteoglycan degradation in chondrocytes. Ann Rheum Dis 63:191–199
586. **Rantapaa-Dahlqvist S, de Jong BA, Berglin E, Hallmans G, Wadell G, Stenlund H, Sundin U, van Venrooij WJ** 2003 Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum 48:2741–2749
587. **Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH, Habibuw MR, Vandenbroucke JP, Dijkmans BA** 2004 Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum 50:380–386
588. **Lockshin MD** 2002 Sex ratio and rheumatic disease. Autoimmun Rev 1:162–167

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