Oestrogens in rheumatic diseases: friend or foe?

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Immunological and epidemiological evidences suggest that female sex hormones play an important role in the aetiology and pathophysiology of chronic inflammatory diseases; however, whether (or when) oestrogens are friends or foes in inflammatory/autoimmune-mediated rheumatic diseases is still a matter of debate.

Several significant factors generate confusion and opposite conclusions in evaluating the role of oestrogens in inflammatory/autoimmune diseases. These factors include the relatively superficial translation done from the animal studies to the human condition, the different effects of oestrogens on their different receptors or on different target cells, the different oestrogen concentrations employed and finally, opposite effects (especially on cell proliferation) exerted by different peripheral oestrogen metabolites.

However, as supported by the higher prevalence of autoimmune diseases in women, oestrogens are generally considered as enhancers of cell proliferation and humoral immune response.

Key words: Oestrogens, Autoimmune rheumatic diseases, Rheumatoid arthritis, Systemic lupus erythematous, Aromatase, Sex hormones.

Introduction

Epidemiological and immunological evidences suggest that female gonadal hormones exert an important role in the aetiology and course of chronic inflammatory diseases since the menstrual cycle, pregnancy and menopausal status are recognized as significant influencing factors [1].

However, whether oestrogens are friends or foes in inflammatory/autoimmune-mediated rheumatic diseases is still a matter of debate.

In fact, there is still the unresolved paradox with respect to the immunomodulating role of oestrogens. 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 pro-inflammatory effects in some chronic autoimmune diseases in humans (as an initiating or perpetuating cause).

Therefore, the most significant factors generating confusion and opposite conclusions in evaluating the role of oestrogens in inflammatory/autoimmune diseases, include the relatively superficial translation done from the animal studies to the human condition, the different effects of oestrogens on their different receptors or on different target cells, different oestrogen concentrations studied and finally, opposite effects (especially on cell proliferation) of different peripheral oestrogen metabolites [2].

Animal models vs human disease

Mouse models of RA have inherent immunological shortcomings. Mouse models fall short of the endocrinological conditions that reproduce the female-biased susceptibility to arthritis and other autoimmune rheumatic diseases. Recently, a new mouse model of RA described was reported as the first to share similarities with human RA [3].

In fact, a major difference between RA and CIA models is that, while RA shows a significant sex bias and production of RF and cyclic citrullinated peptide (anti-CCP) autoantibodies, mouse CIA does not. In humans, anti-CCP antibodies precede the onset of RA and are associated with DR4 [4]. The new DR4-transgenic mice described produce both RF and anti-CCP antibodies and large amounts of the pro-inflammatory cytokines, TNF-α and IL-18. Furthermore, the female mice had a much stronger T-cell response to the DR4-restricted peptide than did the male mice, which is consistent with a recent study showing that deprivation of androgen in mice increases the cellularity of primary and peripheral lymphoid organs and increases T-cell proliferation [4].

Clearly, there are major differences in the histories of the human population and inbred laboratory strains of mice as well as no single animal model perfectly mimics a human disease appropriate model but frequently help to clarify the aetiology and pathogenesis of autoimmune diseases such as SLE [5, 6].

The role of oestrogen receptors

The presence of oestrogen receptors (ERs), ER-α and ER-β, is of outstanding importance because a preponderance of one ER subtype over the other might change oestrogen effects [7]. For example, in synovial tissue of patients with RA, macrophage-like and fibroblast-like synoviocytes have been found to be positive for both ER-α [8–10] and ER-β [11].

However, one study demonstrated higher density of ER-β cells than that of ER-α+ cells and other studies confirmed a higher density of ER-β cells in relation to ER-α+ cells in RA synovial tissue compared with controls [11]. ER-β preponderance was observed in all three synovial compartments investigated namely the lining cell layer, in fibroblasts and in inflammatory cells [11]. Similarly, the amount of ER-α was lower in T cells of patients with SLE than controls, but the quantity of ER-β was similar, which indicates a relative increase of ER-β in relation to ER-α in SLE patients [12].

In animals subjected to trauma and haemorrhage, a general inflammatory condition, ER-β mRNA expression was increased, whereas ER-α expression was decreased [8].

These studies all together suggest inflammation-dependent up-regulation of ER-β relative to ER-α. It was further shown that hypoxia, which usually accompanies inflammatory conditions, reduced expression of ER-α, and oxidative stress increased the expression of ER-β [2, 9].

In particular, oxidative stress increased ER-β relative to ER-α in endothelial cells (E304). In activated macrophages, lipopolysaccharide (LPS) plus IFN-γ increased expression of ER-β but not of ER-α in the presence of hypoxia [13]. These studies support a concept of up-regulation of ER-β relative to ER-α under hypoxic conditions, which might lead to a preponderance of signalling through ER-β pathways.

As a consequence, the preponderance of ER-β relative to ER-α under inflammatory and hypoxic conditions might influence...
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Oestrogen effects. One might hypothesize that this depends on the time point of oestrogen application in relation to the state of an inflammatory disease.

Conditions before disease outbreak or at the beginning of a disease with slight tissue inflammation and limited hypoxia might be governed by a balance of ER-α and ER-β whereas in the chronic phase of a disease with much inflammation and a higher degree of hypoxia, ER-β is increased relative to ER-α.

In a recent study, using selective agonist for ER-β (ERB-041) stimulation, the authors identified 49 genes that were activated by TNFα in human osteosarcoma U2OS cells expressing ER-β [10]. 17β-Oestradiol (E2) treatment significantly reduced the activation by TNFα on 18 genes via ER-β and ER-α. Most repressed genes were inflammatory genes, such as TNF-α, IL-6 and CSF2.

The authors suggested that the anti-inflammatory effects of ERB-041 and other ER-β-selective oestrogens in animal models are due to transcriptional repression of pro-inflammatory genes concluding that these compounds might represent a new class of drugs to treat inflammatory disorders.

However, the translation to the human condition, where RA patients were treated with ERB-041, showed negative results [14]. Forty-seven investigator sites enrolled 291 subjects who were administered 5, 25 or 75 mg of ERB-041 or placebo for 12 weeks. For the primary efficacy end-point of ACR20, there were no statistically significant differences between ERB-041 and placebo.

In addition, there were no statistically significant differences for any of the secondary end-points including CRP. Of the 291 subjects who received at least one dose of the test substance, a total of 247 subjects (84.9%) completed the study, and 44 (15.1%) subjects discontinued. Though well tolerated and safe, ERB-041 failed to demonstrate efficacy in any of the dose groups relative to placebo, despite evidence of anti-inflammatory activity in preclinical models of RA [14].

Different roles for different oestrogen concentrations on different cells

Different concentrations of oestrogens exert different and often opposite effects on immune/inflammatory cells (Fig. 1).

In human peripheral blood mononuclear cells (PBMCs) in the presence of LPS (as cell activator/stimulator substance), E2 inhibited TNF at concentrations of 10^-10 to 10^-7 M in male subjects and at 10^-8 to 10^-5 M in female subjects, but E2 had a stimulating effect in the absence of LPS [15].

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 [12].

Furthermore, in PBMCs, E2 inhibited TNF release in post-menopausal women with fractures in a dose-dependent manner between 10^-12 and 10^-7 M but had no consistent effect on PBMCs derived from men or pre-menopausal women [16]. From these data, it seems that E2 at periovulatory to pregnancy levels inhibited pro-inflammatory cytokines from PBMCs, which is not unchallenged [2]. The contrasting results might be due to divergent effects of E2 on different subtypes of immune/inflammatory cells.

By considering the B cells, 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.

It is now evident that autoimmune diseases are not uniform, and often B cells play a major role as recently substantiated by the success of anti-CD20 antibody treatment in humans. 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 reactivity to oestrogens seems to explain why particularly B-cell-dependent diseases such as SLE, MCTD (Sharp syndrome), IgA nephropathy, 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.

On the other hand, by considering the T cells, 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 (oestriol) and E2, respectively, at pregnancy levels inhibit T-cell-dependent delayed-type hypersensitivity.

Since we know that increased IL-4, IL-10 and IFN-γ concentrations in the presence of low TNF support an anti-aggressive immune response, these data suggest that E2 at periovulatory to pregnancy levels might be a favourable hormone leading to down-regulation of T-cell-dependent immunity. The question arises as to what would happen if E2 falls to post-menopausal levels before initiation or during the course of an autoimmune disease. One might assume that the protective effects of E2 are getting lost under these latter conditions.

Interestingly, by considering human and mouse/rat monocyte/macrophage-like cells, secretion of IL-1β is increased at periovulatory/pro-oestrous to early pregnancy levels, whereas IL-1 secretion is inhibited at high pregnancy levels. It is further obvious that pro-oestrous to pregnancy levels of E2 decreased LPS-stimulated TNF secretion in mouse/rat cells.

Therefore, 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 nuclear factor-κB (NF-κB) [2]. 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.

Oestrogen effects on cell apoptosis and proliferation

Finally, by considering the oestrogen effects on cell apoptosis/proliferation, most of the studies demonstrated the anti-apoptotic effects of E2. Given the fact that E2 decreases apoptosis of
immune cells, this particular aspect of oestrogens must be considered a pro-inflammatory effect [2].

The effects of E2 and of testosterone were tested on the cultured human monocytic/macrophage cell line (THP-1) activated with IFN-γ in order to investigate their role in cell proliferation and apoptosis [17]. Activated human THP-1 cells were cultured in the presence of E2 and testosterone (final concentration, 10 nM).

The evaluation of markers of cell proliferation included the NF-κB DNA-binding assay, the NF-κB inhibition complex, the proliferating cell nuclear antigen expression and the methyltriazolium salt test.

Apoptosis was detected by the annexin V propidium assay and by the cleaved poly-ADP ribose polymerase expression. Cell growth inhibition and increased apoptosis were observed in testosterone-treated THP-1 cells. Increased cleaved poly-ADP ribose polymerase expression and decreased proliferating cell nuclear antigen expression, as well as an increase of IκB-a and a decrease of the IκB-a phosphorylated form (serine 32), were found in testosterone-treated THP-1 cells. However, the NF-κB DNA binding was found increased in E2-treated THP-1 cells [17].

The treatment with staurosporine (enhancer of apoptosis) induced decreased NF-κB DNA binding in all conditions, but particularly in testosterone-treated THP-1 cells. Treatment of THP-1 by sex hormones was found to influence cell proliferation and apoptosis.

Thus, androgens were found to increase the apoptosis, and oestrogens tended to protect cells from cell death—both acting as modulators of the NF-κB complex [17].

The forgotten role of active peripheral oestrogen metabolites

Several studies strongly support an accelerated peripheral metabolic conversion of upstream androgen precursors to oestrogens in both male and female RA/SLE patients [18].

High oestrogen concentrations have been found particularly in SFs of RA patients of both sexes. The appropriate explanation might originate from recent studies showing that the inflammatory cytokines (i.e. TNF-α, IL-6, IL-1), particularly increased in RA synovitis, are able to markedly stimulate the aromatase activity in peripheral [19].

As a matter of fact, the aromatase enzyme complex is involved in the peripheral conversion of androgens (testosterone and androstenedione) to oestrogens (oestrone and oestradiol, respectively) (Fig. 2). In tissues rich in macrophages, a significant correlation was found between the aromatase activity and the IL-6 production, and aromatase has also been found in synoviocytes [13].

Therefore, the increased aromatase activity induced by locally produced inflammatory cytokines (i.e. TNF-α, IL-1, IL-6) might explain the altered balance resulting in lower androgens and higher oestrogens in the synovial RA fluids, as well as their effects on synovial cells. The role of local sex hormone concentrations at the level of inflammatory foci is of great value in order to explain the modulatory effects exerted by these hormones on the immune-inflammatory reaction.

Interestingly, in a recent study, dehydroepiandrosterone sulphate (DHEAS) and oestrone concentrations have been found lower and E2 was found higher in male RA patients compared with healthy controls [20].

In this study, oestrone did not correlate with any disease variable, whereas oestradiol correlated strongly and positively with all measured indices of inflammation. Men with RA had aberrations in all sex hormones analysed, although only E2 consistently correlated with inflammation.

In SLE patients, the aromatase activity evaluated in skin and subcutaneous tissue, showed a tendency towards an increase when compared with control subjects.

Among SLE patients, the aromatase activity varied inversely with disease activity and the patients had decreased androgen and increased oestrogen serum levels [21].

Therefore, tissue aromatase activity showed significant direct correlation with oestrogen levels in SLE patients. These data suggest that abnormal regulation of aromatase activity

![Diagram showing the conversion of androgens to oestrogens](https://academic.oup.com/rheumatology/article-abstract/47/suppl_3/iii2/1784504/iii4)

Fig. 2. Pro-inflammatory cytokines increase conversion from androgens to oestrogens. This activation leads to a relative preponderance of oestrogens to androgens of (6:1). In addition, downstream conversion of oestrone and 17β-oestradiol lead to 16α-hydroxylated oestrogens.
(i.e. increased activity) may partially explain the abnormalities of peripheral oestrogen synthesis in SLE, as well as the altered serum sex-hormone levels and ratio (i.e. decreased androgens and DHEAS).

Similarly, a recent study proposed that the urinary excretion of hydroxyoestrogens (namely, 16α-hydroxyoestrone and 2-hydroxyoestrogens) reflects the production in the tissues, because no respective hydroxylase activity is expected in the urine [22].

Interestingly, as recently reviewed, peripheral oestrogen hydroxylation, which is influencing cell proliferation, was found increased in both men and women with SLE and the oestrogenic metabolites have been reported to increase B-cell differentiation and to activate T cells.

Conclusions
Generally, based on epidemiological and immunological evaluation, oestrogens in normal conditions enhance the immune response (friend), but at the same time seem to play a major role in the pathophysiology of autoimmune rheumatic diseases (foe), together with other risk factors. However, the translation of the study results on modulatory effects of oestrogens obtained from animal and in vitro investigations to the human conditions is always difficult and complex. In addition, in most animal studies only E2 was used as the investigated oestrogen. However, in most human studies, a crude mixture of conjugated oestrogens was used, which can have several pro-inflammatory effects as recently reviewed [2].

Different concentrations used in vitro or in vivo testing, might render oestrogen friend or foe in immuno-inflammatory conditions. In addition, different cells involved in the immune system, react in an opposite manner to different oestrogen concentrations. Moreover, the expression of oestrogen receptors (ER-β or ER-α) might be quite different under inflammatory conditions depending on the microenvironment and the type of disease.

Generally, oestrogens enhance cell proliferation and reduce cell apoptosis, favouring cell growth and acting as friend for osteoporosis and longevity, but foe in cancer and autoimmunity.

Finally, the role of local oestrogen concentrations and the type of peripheral oestrogen metabolites at the level of inflammatory foci is of great importance in order to explain the modulatory effects exerted by these hormones on the immune-inflammatory reaction and whether they are friend or foe.

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