Aromatase and regulation of the estrogen-to-androgen ratio in synovial tissue inflammation: common pathway in both sexes

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Sex hormones play an active role in inflammatory responses, with androgens being anti-inflammatory, whereas estrogens have both pro- and anti-inflammatory effects. In rheumatoid arthritis (RA) patients, low levels of androgens and high levels of estrone are found in the synovial fluid. Aromatase is the key enzyme for the conversion of androgens into estrogens. Proinflammatory cytokines stimulate aromatase activity so that the inflammatory milieu can induce conversion of androgens to estrogens. Moreover, testosterone inhibits aromatase activity. As local androgen levels are low in RA, this can contribute to high aromatase activity in the synovium. Importantly, aromatase-converted estrogens are converted into proproliferative and proinflammatory 16-hydroxylated estrogens. A hormone involved in aromatase activity is vitamin D, which downregulates aromatase in human RA macrophages. Collectively, evidence suggests a key role of aromatase in sex hormone balance during chronic inflammation and points to the importance of vitamin D as a possible new tool for aromatase modulation.

Keywords: aromatase; estrogens; androgens; arthritis; synovial inflammation

Introduction

Epidemiological evidence has strongly indicated the involvement of hormones in immune responses. In recent decades, the role of estrogens and androgens in rheumatoid arthritis (RA) has been studied. Because of the different effects of androgens and estrogens in the immune response, the androgen-to-estrogen ratio plays a key role during inflammation.

Estrogens and the immune response in arthritis

Substantial clinical evidence suggests a role of estrogens in RA. A preponderance of female patients with RA occurs, for example, at reproductive ages;1 the incidence of RA in female patients in the United Kingdom is double that of male patients,2 and according to the Arthritis Foundation, the RA rate is rising among women in the United States. Moreover, female RA patients have a higher disease activity score and health assessment questionnaire scores than male RA patients,3 suggesting a correlation between estrogens and disease severity. Even male patients affected by RA have higher estradiol serum levels compared to healthy men, and estradiol levels correlate with disease activity.4 Despite this and other clinical evidence, estrogens do not always function in a proinflammatory manner in arthritis as previously claimed, but can be either pro- or anti-inflammatory (reviewed in Ref. 5). In animal models, estradiol inhibits T cell autoimmunity but stimulates autoantibody production by B cells;6 it is plausible that the same effects also occur in humans. This opposite effect on T and B cells is crucial, as this could influence disease onset and course. If, for example, an autoimmune disease is mostly dependent on B cell activation, then high estrogen levels are
inflammation; in contrast, the estrogen would be anti-inflammatory in T cell–driven autoimmune diseases. It is hypothesized that different types of RA can exist, where either B cells or T cells play the major role. The more classical RA with late onset, at perimenopausal age or later, is presumably driven by T cells; thus, a decrease in estrogens is responsible for T cell activation and disease onset. In contrast, RA with early onset, within the reproductive age, is B cell–driven and high estrogen levels activate B cells.7

Estrogen effects are also related to the disease course (as reviewed in Ref. 5). Estrogens can, for example, delay the onset of atherosclerosis; however, estrogen replacement therapy has been shown to increase the frequency of atherosclerotic events in elderly women.8 In arthritis, the effects of estrogens before disease outbreak have not been tested, but it is well described that estrogens play different roles during the chronic phase, depending on the cell type bound, the number and kind of estrogen receptors expressed on the cells, and estrogen metabolism. However, some evidence suggests a proinflammatory role of estrogens in arthritis patients. Both estrogen receptors, ERα and ERβ, are present in RA synovial cells,9 and the ERβ/ERα ratio is upregulated during synovial inflammation.10,11 Moreover, estrogens induce the production of proinflammatory cytokines;12 and secretion of interleukin (IL)-6 and IL-8 in RA positively correlates with ER+ cells.11

The multifaceted role of estrogens in the inflammatory process is also due to the fact that downstream estrogen metabolites are active hormones and influence the immune response with opposite effects (Table 1). Many studies on breast cancer extensively showed antiproliferative effects of 2-hydroxy- and 2-methoxy-estrogens. One recent study on RA synoviocytes demonstrated a role of estrogen metabolites in synovial inflammation. In particular, 4-hydroxylated (OH) and 2-OH estrogens inhibit tumor necrosis factor α (TNF-α) secretion, while 16-OH estrogens did not influence TNF-α. Levels of 16α-OH estrone were higher than all other estrogen metabolites in synovial cells in RA, leading to an unfavorable shift of estrogen metabolism during inflammation.13 Therefore, high estrogen levels in the chronic phase of arthritis could be unfavorable because of the high conversion to the proinflammatory 16-OH estrone rather than a direct proinflammatory role of estrogen itself.

Taken together, estrogens have a dual role in inflammatory diseases, acting in either a pro- or anti-inflammatory manner. One possible explanation for the different roles of estrogens could be the diverse effects of estrogen metabolites on inflammation: the prevalence of one or the other estrogen metabolite can influence the immune response, and one synthetic estrogen compound might be more favorable than another because it can be easily converted to an anti-inflammatory downstream metabolite. On the basis of this knowledge, it remains difficult to determine the efficacy of a hormonal therapy in arthritis and other inflammatory diseases.

Androgens and the immune response in arthritis

Androgens have mainly an anti-inflammatory activity in arthritis. It is indeed well demonstrated that testosterone inhibits both IL-1β secretion by peripheral blood mononuclear cells (PBMCs) from RA patients14 and IL-1 synthesis in primary human synovial macrophages;15 testosterone also suppresses both IgG anti-dsDNA antibody and total IgG production by PBMCs.16 Moreover, the biologically active androgen dihydrotestosterone represses expression and activity of IL-6 in human fibroblasts.17

In addition to the above, some clinical evidence confirms the anti-inflammatory activity of androgens. For example, low serum concentrations of the adrenal hormone dehydroepiandrosterone (DHEA) is associated with RA,18,19 and androgen treatment of patients with RA leads to significant improvement of joint disease,20 even if the overall effects on disease activity are limited.21–23 This could be due to the rapid conversion of androgens into estrogens and downstream proinflammatory 16-OH estrogens, as previously described. Synthetic steroid compounds could be a valid alternative (see Ref. 24); however, further studies are needed to prove this hypothesis. Given the beneficial effects of androgens during inflammation, the androgen-to-estrogen conversion and the androgen-to-estrogen ratio are critical determinants of the inflammatory process in arthritis.

The systemic estrogen-to-androgen ratio in RA patients

In RA patients, sex hormone levels are altered both systemically and in inflamed joints, but some differences are observed. Serum levels of anti-inflammatory androgens are mainly low in active
Table 1. Effects of downstream estrogens on arthritis

<table>
<thead>
<tr>
<th>Estrogen metabolite</th>
<th>Effect on arthritis</th>
<th>Disease model/samples</th>
<th>Reference</th>
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| 2-MeOH estradiol    | Suppression of collagen type II–induced arthritis  
Inhibits TNF-α secretion | Collagen type II–induced arthritis  
Human synovial cells from RA and OA patients | 44  
13 |
| 2-OH estrogens      | Concentration 10× downregulated in RA compared to control  
Inhibits TNF-α secretion (especially 2-OH estrone) | Human synovial cells from RA and OA patients | 13 |
|                     | 2-OH estrone low, and 2-OH estradiol high in premenopausal OA compared to control | Human serum of Chinese OA patients and controls, pre- and postmenopausal | 45 |
| 16-OH estrogens     | No effects on TNF-α secretion | Human synovial cells from RA and OA patients | 13 |
| 4-OH estrogens      | Inhibits TNF-α secretion (especially 4-OH estradiol) | Human synovial cells from RA and OA patients | 13 |

In summary, there is a higher systemic estrogen-to-androgen ratio in RA patients, especially in males, and estrogen metabolism leads to increased synthesis of proinflammatory 16-OH estrogens compared to the anti-inflammatory 2-OH-estrogens.

Estrogen-to-androgen ratio in RA synovium

Compared to systemic sex hormone levels, the preponderance of estrogens relative to androgens is much stronger in the inflamed joints in RA. In fact, a strong conversion of 19-hydroxyandrostenedione to estrone via the aromatase complex was found in RA synovium but not in control subjects, and there is a preponderance of estrogens relative to androgens in the synovial fluid of RA patients compared to controls. The results in female and male patients were very similar, demonstrating that sex hormone alterations in inflamed joints do not depend on gender.

It is of interest that estrogen levels are similar in the synovium of RA patients compared to those with osteoarthritis (OA), even if the measured serum levels were higher in RA. Therefore, there have to be sources of estrogens and aromatase upregulation in other organs in RA (e.g., fat tissue or the liver).
Also found in the synovial fluid of RA patients, as in the urine samples, were higher levels of 16-OH-estrogens; however, no alterations of 2-OH-estrogens were observed. On the contrary, the synovial fluid amount of 4-OH-estrogens was significantly increased in RA patients compared to controls. Collectively, these results suggest a different but still altered estrogen metabolism in inflamed joints and a preponderance of proinflammatory 16-OH-estrogens.

Recently, an RA-associated polymorphism in the gene CYB5A on chromosome 18 was discovered. Endogenous de novo synthesis of androgens depends mainly on two enzyme activities of cytochrome P450c17: 17α-hydroxylase and 17,20-lyase. A cofactor, particularly for 17,20-lyase activity, is cytochrome b5. Therefore, the newly described polymorphism is associated with the ability of converting androgen from androgenic precursors. These recent results could explain the altered local and systemic sex hormone metabolism found in a group of RA patients.

**Aromatase activity during inflammation**

Aromatase is the enzyme responsible for the conversion of androgens to estrogens. In particular, it converts androstenedione to estrone and testosterone to estradiol. The aromatase enzyme complex is composed of two polypeptides: the first is a specific cytochrome P450, aromatase cytochrome P450 (the product of the CYP19 gene). The second is a flavoprotein, NADPH-cytochrome P450 reductase, ubiquitously distributed in most cells. Therefore, cell-specific expression of aromatase cytochrome P450 (referred to here for practical reasons as “aromatase”) determines the presence or absence of aromatase activity.

Different pathways are involved in aromatase activation. The classical, proximal promoter of the aromatase gene (promoter II) is activated by follicle-stimulating hormone (FSH) via a cAMP-dependent pathway in granulosa cells in the ovary (reviewed in Ref. 27) (Fig. 1). In activated macrophages, fibroblasts, and adipocytes, aromatase is expressed through activation of STAT3 and glucocorticoid receptor binding sites upstream of the aromatase distal promoter (Fig. 1). Therefore, cytokines and other factors involved in inflammatory reactions can activate aromatase and alter the ratio of estrogens to androgens. It has been demonstrated, for example, that IL-1, IL-6, and TNF-α activate aromatase in different cell types. Environmental factors can also alter aromatase expression. It has been shown that after *Chlamydia trachomatis* infection, aromatase was upregulated in human trophoblast, which was associated with a depletion of estrogen and estrogen precursors. Furthermore, in pregnant women affected by syphilis, a reduction of estrogens has been described, and aromatase downregulation may be responsible for hepatic steatosis in hepatitis C virus–infected patients.
Inflammation-related aromatase activation in the synovial tissue is not disease-specific but related to the inflammatory reaction; in fact, no differences in aromatase-positive synovial cells were observed between OA and RA patients. It is important to note that androgens, such as androstenedione and testosterone, decrease aromatase activity in synovial cells from RA patients. Therefore, low levels of androgens demonstrated in RA patients could lead to high aromatase activity and less androgen content.

In summary, the inflammatory reaction is responsible for aromatase activation in the synovial tissue and, consequently, low androgen levels; and a decrease in androgen leads to even stronger aromatase activity and estrogen preponderance (Fig. 2).

**Aromatase inhibitor therapy and RA**

Considering aromatase activation and estrogen preponderance in arthritis, researchers might conclude that aromatase blockade is a good therapeutic tool in RA. However, some clinical evidence from breast cancer studies attributes a negative effect of aromatase inhibitors (AIs) on joints and joint pain.

Aromatase inhibitors are widely prescribed for postmenopausal hormone receptor–positive breast cancer; however, musculoskeletal symptoms often occur and limit tolerability of treatment. Joint pain and morning stiffness are often described during AI therapy, and a possible role of AIs in RA development has been considered. Nevertheless, even if joint pain is common in AI-treated women, RA onset seems to be rare. It is therefore not known whether RA occurrence during AI therapy is due to chance or a causal effect. As described above, estrogens play different roles in inflammation depending on the time of estrogen treatment. It is possible that estrogens play an anti-inflammatory role before arthritis outbreak, which would explain AI treatment side effects on joints, while aromatase blockade after disease onset could reduce estrogen availability and thus further conversion to the pro-inflammatory 16-OH estrogens, exerting a positive effect.

A role for melatonin in joint pain was suggested in a recently published paper. It was hypothesized that if estrogen levels are low, for example, because of AI therapy, light-induced melatonin suppression loses efficacy. This would then lead to high melatonin levels, also observed during RA, that are supposed to be responsible for morning stiffness. This would explain why systemic estrogen blockade is related to musculoskeletal symptoms similar to RA.

Furthermore, it is important to point out that during RA, estrogen levels in women are altered only in the inflamed joints, while AIs block estrogens in the whole body, altering estrogen pathways in many organs; therefore, the effects could be very different. Local aromatase blockade (e.g., by using nanoparticles for drug delivery) would be more effective and preferable for RA treatment. Additional *in vivo* and *in vitro* studies are required to support and further extend these ideas.

**New tool for aromatase inhibition: role of vitamin D**

Vitamin D (VitD) has recently received increased attention for its involvement in reducing risk for several chronic diseases, including autoimmune rheumatic diseases. The active metabolite of VitD (1,25(OH)2D3) is derived from cholesterol and is considered a steroid hormone. Like glucocorticoids, VitD also exerts immunomodulatory activities; and low serum levels of VitD impair immune responses.

Recently, it was shown that VitD controls aromatase expression in human macrophages. In fact, VitD treatment reduces aromatase in activated human macrophages and also inhibits proinflammatory cytokines, such as IL-1, IL-6, and TNF-α. Moreover, when activated human macrophages were pretreated with estrogens (reproducing the hormonal milieu in the inflamed RA joints), both aromatase and proinflammatory cytokines were activated, while co-treatment with VitD completely blocked estrogen-related aromatase activation. These results present a promising way to block aromatase and the estrogen-related synovial inflammatory reaction, especially if it is considered that no side effects of VitD treatment are known. Systemic aromatase blockade is also possible during VitD treatment of RA patients. This would be favorable in order to reduce the estrogen pool and conversion to the proinflammatory 16-OH estrogens. However, *in vivo* studies and clinical tests are still required to determine the VitD amount requested for treatment and the possible efficacy reached.

**Conclusions**

In recent decades, a strong endocrine–immune interaction has been demonstrated. With regard to sex hormones, important and different roles of...
Figure 2. Summary of aromatase regulation during arthritis. Androgen levels are low in arthritis patients, with an estrogen-to-androgen ratio of 10× higher than in controls. This hormonal imbalance might be at least partially related to a newly discovered polymorphism of CYB5A, a cofactor involved in androgen synthesis from androgenic precursors. Downstream estrogen conversion is also altered in arthritis patients, with a preponderance of 16-OH estrogens, which are proinflammatory and proliferative. Therefore, high estrogen levels cause an even greater amount of 16-OH estrogens and are proinflammatory. Synovial androgens inhibit aromatase, while proinflammatory cytokines stimulate aromatase activity. Therefore, low androgen levels and inflammation are responsible for high aromatase activity in RA synovium and estrogen conversion. Aromatase inhibitors (AIs) and vitamin D are able to block aromatase and might be helpful for restoring the physiological estrogen-to-androgen ratio and reduce inflammation.

Estrogens and androgens have been described in RA and many other diseases. Because of the different effects these hormones may exert, the androgen/estrogen balance is important. The enzyme responsible for the androgen-to-estrogen conversion, the aromatase complex, has thus been the focus of many studies, to better understand how this enzyme is regulated. It has been shown that inflammation triggers aromatase in the inflamed synovial tissue in RA, thus leading to synovial estrogen preponderance, which leads to a specific increase in proinflammatory 16-OH estrogens. As androgens normally inhibit aromatase, the low androgen levels during inflammation can be responsible for further aromatase activation and, increasingly, low androgen levels. Furthermore, a study in RA patients describes a polymorphism in CYB5A, a cofactor involved in de novo androgen synthesis. Because of the proinflammatory role of 16-OH estrogens, estrogen preponderance is an unfavorable condition in RA after disease outbreak, and a blockade of aromatase is a promising therapeutic target. It has been shown that VitD strongly inhibits aromatase and proinflammatory cytokines in human macrophages, thus suggesting a possible therapeutic tool to control aromatase and sex hormone balance in RA.

In conclusion, altered aromatase activity and a subsequent strong imbalance of androgens and estrogens are described in RA patients. As sex hormones are key players in chronic inflammatory responses in arthritis, this imbalance strongly influences the inflammatory process; therefore, control of aromatase activity might be a promising new therapeutic approach in arthritis.

Conflicts of interest
The authors declare no conflicts of interest.

References


