New roles for estrogens in rheumatoid arthritis

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ABSTRACT

Sex hormones appear to play an important role as modulators of autoimmune disease onset/perpetuation. Steroid hormones are implicated in the immune response, with estrogens as enhancers at least of humoral immunity, and androgens and progesterone (and glucocorticoids) as natural immune suppressors. Serum levels of estrogens have been found to be normal in rheumatoid arthritis (RA) patients. Synovial fluid levels (SF) of proinflammatory estrogens relative to androgens are significantly elevated in both male and female RA patients as compared to controls, which is most probably due to an increase in local aromatase activity. Thus, available steroid pre-hormones are rapidly converted to proinflammatory estrogens in the synovial tissue in the presence of inflammatory cytokines (i.e. TNFα, IL-1, IL-6). The increased estrogen concentrations observed in RA SF of both sexes are characterized mainly by the hydroxylated forms, in particular 16α-hydroxyestrone, showing a mitogenic stimulating role. Indeed, recent studies by us indicate that 17-β estradiol (E_2) clearly enhanced the expression of markers of cell growth and proliferation, whereas testosterone (T) induced an increase in markers indicating DNA damage and apoptosis. In particular, our data further shows that the enhancing role of estrogens on the immune/inflammatory response is exerted by activating the NFkB complex. In conclusion, locally increased estrogens may exert activating effects on synovial cell proliferation, including macrophages and fibroblasts, suggesting new roles for estrogens in RA.

Serum estrogens in RA

Striking differences in age- and sex-specific rates are seen in many rheumatic diseases. Epidemiological evidence indicates that during the fertile age women are more often affected by rheumatic diseases than men, particularly autoimmune diseases (1). As a matter of fact, autoimmune disorders such as rheumatoid arthritis (RA) result from a combination of several predisposing factors that include the relationships between epitopes of the trigger agent (i.e., a virus) and histocompatibility epitopes (i.e., HLA), the status of the stress response system including the hypothalamic-pituitary-adrenocortical axis (HPA) and the sympathetic nervous system (SNS), and above all the effects of the gonadal hormones [hypothalamic-pituitary-gonadal axis (HPG)] (2).

The pre-or post-menopausal serum sex hormonal status is a further factor influencing the occurrence of rheumatic diseases. It is therefore important, whenever possible, to study epidemiologic data broken down by age (for example, 10-year age bands) and sex before making inferences (3). Sex hormones seem to play an important role as modulators of the disease onset/perpetuation (4). Steroid hormones are implicated in the immune response, with estrogens as enhancers at least of humoral immunity, and androgens and progesterone (and glucocorticoids) as natural immune suppressors (4,5). Low gonadal and adrenal androgens [testosterone (T)/dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA) and its sulphate (DHEAS), respectively] levels, as well as a reduced androgen/estrogen ratio, have been detected in serum and body fluids [i.e. blood, synovial fluid (SF), smears, salivary fluid] of both male and female RA patients, supporting a possible pathogenic role for decreased levels of the immune suppressive androgens (6). Interestingly, however, they are not changed with respect to serum levels of estrogens, which is in strict contrast to androgen levels in RApatients (4).

Several physiological, pathological and therapeutic conditions may change the serum estrogen milieu and/or peripheral conversion rate; these include the menstrual cycle, pregnancy, postpartum period, menopause, elderly, chronic stress, inflammatory cytokines, use of corticosteroids, oral contraceptives, and steroid hormonal replacements, inducing altered androgen/estrogen ratios and related effects (5-7).

As a matter of fact, sex hormones can exert also local actions (paracrine) in the tissues in which they are formed or enter the circulation and both T and 17-β estradiol (E_2) seem to exert dose and

time-dipendent effects on cell growth and apoptosis (2, 4). These effects, as well as important influences on gene promoter of Th1/Th2 cytokines and the recently discovered increased SF estrogen concentrations, might suggest new roles for estrogens in RA(8-11).

Peripheral estrogen metabolism in rheumatoid arthritis synovial tissue

Wethever serum estrogen concentrations are normal in RA patients, lower androgen concentrations (i.e. T, androstenedione (AD) and DHEAS) are detected in the serum as well as in the SF of both male and female RA patients (11,12). The presence in the RA SF of an altered sex hormone balance resulting in lower immunosuppressive androgens and higher immuno-enhancing estrogens in both male and female patients, might determine a favourable condition for the development of the immuno-mediated RA synovitis (13-15).

How can one explain the recent detection of lower androgen and higher estrogen levels in both female and male RA SF ? The appropriate explanation might originate from recent studies showing that the inflammatory cytokines (i.e. TNFα, IL-6, IL-1), which are particularly increased in RA synovitis, are able to markedly stimulate aromatase activity in peripheral tissues (16-18). As a matter of fact, the aromatase enzyme complex is involved in the peripheral conversion of androgens (testosterone and androstenedione) to estrogens (estrone and estradiol, respectively) (see Fig.1). In tissues rich in macrophages a significant correlation was found between aromatase activity and IL-6 production, and aromatase has also been found in synoviocytes (19,20). Therefore, the increased aromatase activity induced by locally produced inflammatory cytokines (i.e. TNFα, IL-1, IL-6) might explain the altered balance resulting in lower androgen and higher estrogen levels in all the synovial RA fluids, as well as their effects on synovial cells, first described by us (21). The role of local sex hormone concentrations at the level of inflammatory foci is of great importance in explaining the modulating effects exerted by these hormones on the immune-inflammatory reaction, especially in RA synovitis. Recently, in 12 patients with RA and 8 patients with traumatic knee injury (non-inflammatory controls), we measured SF steroid concentrations by HPLC and mass spectrometry (22). In a further 3 patients with RA and 3 patients with OA, conversion of DHEA to downstream hormones was detected by phosphoimaging and quantified by thin layer chromatography.

Overall the SF concentration of free estrogens tended to be higher in RA as compared to controls ($p < 0.06$) (Fig.1). The molar ratio of free SF estrogens/ free SF androgens was elevated in RA compared to controls ($p = 0.017$). The free SF concentration of the precursor AD was higher in RAcompared to controls ($p = 0.011$), and SF estrone – the aromatase conversion product of AD – was also elevated in RAversus controls ($p = 0.035$). The most biologically active estrogens, 16α-hydroxyestrone and 4-hydroxyestradiol, were higher in RAcompared to controls ($p = 0.085$ and $p = 0.044$, respectively). In mixed synoviocytes, DHEA conversion yielded high local levels of estrogens compared to androgens. This study clearly demonstrates that local levels of proinflammatory estrogens relative to androgens are significantly elevated in patients with RA compared to controls, which is most probably due to increased aromatase activity. Thus, available steroid pre-hormones are rapidly converted to proinflammatory estrogens in the synovial tissue.

Prevalence of hydroxylated estrogens in rheumatoid arthritis synovial fluid

The increased estrogen concentrations observed in RA SF of both sexes are mainly characterized by the hydroxylated forms, in particular 16α-hydroxyestrone and 4-hydroxyestradiol, whereas the 2-hydroxyestrone level was found to be similar to the controls (22) (Fig. 1). Data from breast cancer studies underlined that 16α-hydroxyestrone is a mitogenic and proliferative endogenous hormone that covalently binds to the estrogen receptor, leading to nuclear translocation (23,24). 16α-hydroxyestrone is converted from upstream estrone and E2, and because of this covalent linkage to the receptor, shows persistent biological responses consisting of a mitogenic tumor growth stimulating role (25) (Fig. 1).

Other conversion products of estrone and E2 are the 2-hydroxylated estrogens such as 2-hydroxyestrone and 2-hydroxyestradiol. In contrast to 16α-hydroxylated estrogens, the 2-hydroxylated forms inhibit the growth promoting effects of E2 (25). In this respect, 2-hydroxyestrone has anti-carcinogenic properties and thus is most likely a naturally occurring anti-estrogen (26). In a recent study, urinary levels of 2-hydroxylated estrogens were found to be 10 times lower in RA patients than in healthy controls, whereas the 16α-hydroxyestrone/2-hydroxyestrogen ratio was 20 times higher in RApatients then in healthy controls (Straub, in press). The relative loss of 2-hydroxylated estrogens in relation to the mitogenic 16α-hydroxyestrone might thus be an important switch to support the proliferative state of synovial cells in RA. Therefore, dose-related conversion to pro- or anti-inflammatory downstream metabolites of estrogens might also support the dual role of estrogens (pro- or anti-inflammatory), for example during estrogen replacement therapy, depending on the local concentration (i.e., SF in RA) of 16α-hydroxyestrone or 2-hydroxyestrogens. Therefore, SF estrogens (mainly hydroxylated metabolites) in RA, by inducing and potentiatiating the immune/inflammatory response, might exert more important roles than their serum concentrations, the latter being just one of the predisposing risk factors (27).

Synovial fluid estrogens induce cell proliferation and reduce cell apoptosis: Apossible role in synovial tissue hyperplasia in RA

An evident modulation of cell growth and apoptosis of synovial cells support the hypothesis that estrogens and androgens play a key role in the homeostasis between cell replication and death, confirming their opposite roles on the immune and inflammatory re-

sponses (28-31).

The signalling pathways for the physiological balance between pro- and anti-inflammatory mechanisms are different and complex, but based on recent research it seems to involve the NFκB mechanism and steroidal hormone receptor activation. Studies concerning the functional interaction of NFκB with members of the steroid hormone receptor family and its role in synovial inflammation have advanced significantly (32). In particular, estrogen receptors (ER) after binding with E_2, have been shown to interact with NFκB factors via transcriptional co-factors, resulting in mutual or non-mutual antagonism (33). In contrast, the androgen receptor (AR) seems to play a homeostatic role in the immune system and it is closely related to the glucocorticoid receptor (GR) in terms of both structure and sequence specificity (they share 78% sequence identity in their DBDs) after binding with their respective hormones (34).In addition, AR and GR have been shown to interact with and to repress AP-1 via a similar mechanism. Consequently, it would not be surprising if AR might also interact with NFκB in a manner very similar to that observed for GR (35).

Indeed, recent studies by us have shown that E_2 clearly increased the expression of markers of cell growth and proliferation, whereas T induced an increase of cell markers indicating DNA damage and apoptosis (31, 36).

In support of the proliferative role exerted by E_2, the cells pre-treated with estrogen (Cutolo submitted) were found to be resistent to the apoptosis-inducer staurosporine, and showed decreased apoptosis when compared with T-treated and untreated cells (37). Therefore, all of these data further suggest the enhancing role of estrogens on the immune/inflammatory response by activating the NFκB complex. Other studies support these results, showing that E_2 inhibits apoptosis in different cells type (cardiac myocytes and others), whereas androgens have been found to induce apoptosis (38-43).

Increased concentrations of estrogens (and low androgens) recently described in the SF of RA patients of both sexes seem to support their role on synovial tissue hyperplasia and synovial cell chronic activation, taking into consideration their effects on cell proliferation and apoptosis (21, 44) (Fig. 1). Such findings have recently been reported for human breast cancer cells as well (45).

Therefore, 17-bestradiol and testosterone may modulate the activity of NFκB in monocytic/macrophage cells, since the expression of a number of genes relevant to pro-inflammatory responses are regulated by the NFκB complex. In conclusion, locally increased estrogens might exert activating effects on synovial cell proliferation, including macrophages and fibroblasts, suggesting new roles for estrogens in RA. In addition, these observations seem to provide a further strong biological link between sex hormones and the inflammatory process in hyperplastic RAsynovial tissue (46, 47).

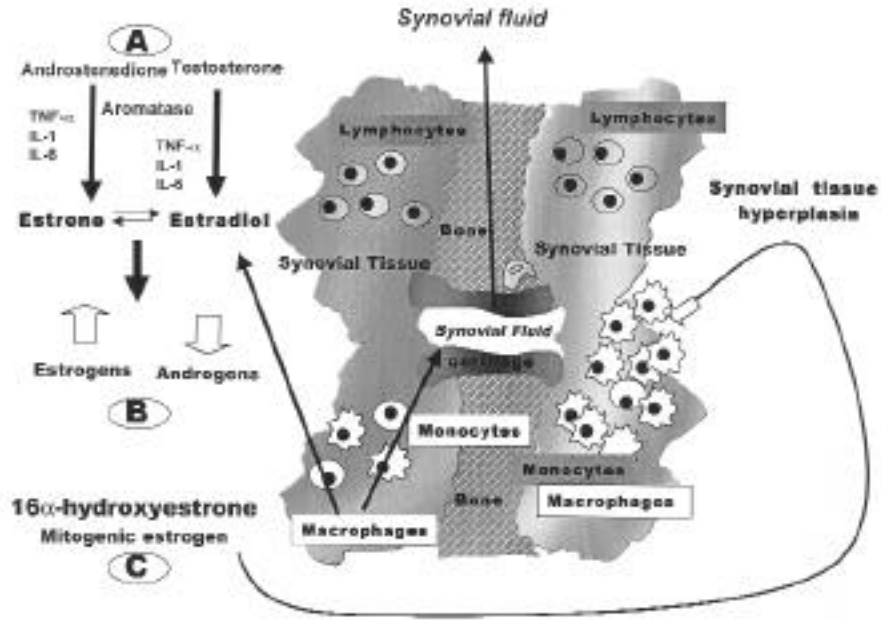

Fig. 1. (**A**) Increased formation in rheumatoid arthritis (RA) synovial fluid (SF) of estrogens from androgens for the increased local aromatase activity induced by proinflammatory cytokines at the level of the synovial tissue. (**B**) Altered ratio in the SF of male and female RApatients with increased estrogens and decreased androgens. (**C**) Increased formation of hydroxylated estrogens in RASF, in particular an increase in the mitogen 16α-hydroxyestrone that might induce synovial tissue hyperplasia and cell proliferation.

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