Rheumatoid arthritis: a female challenge

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Rheumatoid arthritis (RA) is two- to three-fold more frequent in women than in men and a strong association with sex hormones has been demonstrated. There is strong evidence that autoimmunity is under genetic control, and genes in sexual chromosomes can play a role in supporting the female prevalence. On the other hand, it is widely accepted that sex hormones – estrogens in particular – may regulate the immune response by favoring the survival of forbidden autoreactive clones and ultimately the prevalence of autoimmunity in women. Accordingly, estrogens have been suggested to be associated with the development of RA. Pregnancy in RA women is a common situation and most pregnant patients experience a remission. This has been closely related to a switch from Th1 to Th2 immune responses and to a decreased production of proinflammatory cytokines, at least in part supported by the changes of the hormonal profile in pregnancy. Pregnancy planning is required in RA in order to avoid unwanted complications. In particular, the need to control the disease requires safe use of antirheumatic drugs both during the pregnancy itself and in the breastfeeding period. Hormonal treatment for contraception is contraindicated in the case of positivity for antiphospholipid antibodies owing to the increased thrombophilic risk. Similarly, replacement hormonal treatment in postmenopausal women with RA to control osteoporosis is no longer recommended as a result of its ability to increase the cardiovascular risk closely associated with RA itself.

Rheumatoid arthritis (RA) is more common in women than men. The purpose of this review is to study in detail the aspects of the disease more strictly related to female gender and to analyze thoroughly the problems the physician has to face when taking care of a woman with RA. To better understand these aspects we will give an overview of the relation between sex hormones and RA and we will try to explain why this disease is more common in women. Then we will discuss problems related to pregnancy, with a special eye on the use of drugs in this situation. Finally, we will examine two important conditions related to sex hormones: contraception and menopause.

Background

Rheumatoid arthritis is a chronic and progressive autoimmune disease of still unknown etiology, mainly characterized by joint inflammation and synovial effusion, which can result in destructive changes [1].

Annual incidence of the disease is estimated to be between 0.1 and 0.5 per 1000 inhabitants, while the prevalence ranges from 2 to 10.7 in different populations [2]. Studies from south European countries suggest a relatively lower occurrence of RA when compared with north European and North American countries [2]. The peak of onset is at the fourth and fifth decade of life, with a considerable variation of the disease frequency among different populations [3].

As for other systemic autoimmune diseases, a strong association between RA and sex hormones has been demonstrated and the disease is two- to three-fold more frequent in women than in men [3]. Thus, conditions related to reproductive and endocrine changes, such as pregnancy, contraception and menopause, represent clinical situations to be addressed by physicians in a particular manner.

Autoimmunity & the female gender

Autoimmunity is characterized by a mistake of the immune system that initiates an attack on self, normal tissues. The immune-mediated chronic inflammation leads to tissue damage with a consequent functional loss or abnormalities.

Autoimmune diseases represent a prototypical class of illnesses that display high female:male ratios. Interestingly, the increased prevalence of autoimmune diseases in women does not seem to be associated with a stronger disease severity [4–6].

A difference between male and female immune responses does not apparently account for the high female:male ratios in autoimmune

Keywords: autoimmunity, female gender, rheumatoid arthritis

10.2217/17455057.4.2.195 © 2008 Future Medicine Ltd ISSN 1745-5057

diseases. In fact, no major differences regarding the response to vaccinations or infections have been found between women and men [5]. There is strong evidence that autoimmunity is under genetic control, and genes in sexual chromosomes have been suggested to play a role in such control [7].

Sex hormones, the immune system & autoimmune diseases

Medical case reports and animal models of amelioration of autoimmune diseases after castration, and worsening after hormone treatment, represent the rational for the role of female hormones – estrogens in particular – to explain the prevalence of autoimmunity in women [4–6]. This is a rather simplistic explanation and there is clinical as well as experimental evidence that the situation is more complex. In fact, it is known that estrogen use as replacement or contraceptive therapy does not worsen systemic autoimmune diseases – such as systemic lupus erythematosus (SLE) – in a regular way [8,9]. In addition, there are examples of autoimmune diseases that remit during pregnancy (e.g., RA and multiple sclerosis), while animal models of spontaneously occurring autoimmune conditions are not always associated with female gender [4–6].

On the other hand, sex hormones play an important role in the regulation of the immune response. Estrogen receptors are expressed on the surface of several cells of the immune system, such as $CD4^+$ and $CD8^+$ T cells, B cells, NK cells and macrophages, and can induce intracellular signals involved in proliferation and differentiation [10,11]. Estrogens may interfere with the thymus by reducing T-cell population, with bone marrow by inhibiting B-cell development and with dendritic cell differentiation, and may induce monocyte apoptosis [10,11]. While androgens and progesterone are considered natural immunosuppressors, estrogens can enhance humoral immunity in several systems. Accordingly, females have higher levels of circulating immunoglobulins [5]. In addition, *in vitro* studies have shown that polyclonal activation of human B cells can be induced by estrogens and that lymphocytes and monocytes from females display higher response to polyclonal mitogens and higher antigen-presenting activity [5,10,11]. Moreover, estrogens can increase autoantibody synthesis by peripheral blood mononuclear cells (PBMC) from SLE patients, while testosterone can not [12].

These effects together with the known ability of estrogens to enhance the secretion of pro-inflammatory cytokines and to display anti-apoptotic activity, have been suggested to eventually favor the survival of forbidden autoreactive clones. However, besides the permissive role that estrogens may display on autoimmunity, additional mechanisms – which, in part, are still unknown – may contribute to the higher female susceptibility to develop autoimmune diseases [4,5].

Sex hormones & rheumatoid arthritis

While animal models of SLE, the prototype of systemic autoimmune diseases, strongly suggest an important role of sex hormones and prolactin in the pathogenesis of the disease, data from experimental models of arthritis are controversial [13,14]. Studies on collagen-induced arthritis (CIA) have shown that male mice are more susceptible than females to develop the disease and that estrogen treatment improves disease activity [14]. In addition, KRNxNOD mice, another murine model of RA, develop arthritis independently of gender.

Nevertheless, as stated above, there is an evident gender dimorphism in the prevalence of RA in humans, with a male:female ratio between 1:2 and 1:3. Reduced levels of androgens, such as testosterone, dihydrotestosterone (DHT) and dehydroepiandrosterone (DHEA) have been demonstrated in both male and female RA patients [15]. Moreover, increased levels of estradiol have been found in men affected by RA and the plasma levels correlated with the degree of inflammation; on the other hand, the levels of DHEA and testosterone were low in these patients [16]. A recent study by Masi *et al.* on the potential risk factors for RA has shown that combined low levels of serum cortisol and total testosterone were significantly associated with the risk of developing RA [17]. A negative correlation was demonstrated between levels of testosterone and rheumatoid factor (RF) titers in RA females, while RA males showed a positive correlation between estradiol levels and RF titers [17]. Cutolo *et al.* have found a reduced androgen:estrogen ratio in the synovial fluid of both female and male patients suffering from RA [15,18]. It has been suggested that the reduced androgen:estrogen ratio may depend on the enhanced activation of aromatase. This enzyme is actually involved in the sequential degradation of cholesterol to androgens and estrogens, and can be activated by one of the proinflammatory

cytokines, TNF-α, playing a key pathogenic role in RA synovitis [18]. Finally, the demonstration that human synovial macrophages express androgen and estrogen receptors further supports the hypothesis that an altered balance of sex hormones in the synovial fluid may exert a paracrine action and contribute to RA pathogenesis [10,11,18].

Rheumatoid arthritis & pregnancy

Although the peak of onset of the disease is reported in the fourth and fifth decade of life, the occurrence of pregnancy in RA women is a common clinical situation. In addition, fertility is not affected as for other systemic autoimmune diseases.

More than 75% of women affected by RA experience an improvement of symptoms or even a complete remission during pregnancy, particularly during the second trimester. A disease flare frequently occurs within the first 4–6 months after delivery and some authors also report a higher risk of developing RA in the puerperium than at any other time; during this period an increase of RF titer has also been described in approximately 90% of cases [19]. Initial onset of RA during pregnancy is very rare; however, both breastfeeding and parity have been associated to an increased occurrence and/or severity of the disease.

In order to protect the fetus from the attack of the maternal immune system, several changes in the maternal immune responses occur physiologically during the pregnancy [20,21]. In particular, there is an altered cytokine profile, with a decreased secretion of Th1-associated cytokines, such as IL-1, IL-6 and IFN-γ. These changes lead to an enhanced humoral immunity and to a partial suppression of the cell-mediated responses. At the same time, a decreased production of proinflammatory cytokines (such as TNF-α) does occur, resulting in a noninflammatory milieu [22]. IFN-γ and IL-1β are undetectable and increased titers of soluble TNF receptor (TNFR) and IL-1 receptor antagonist (TNFRa) are present in pregnant compared with nonpregnant women. These findings are independent from the presence of RA. However, in RA patients, levels of IL-1Ra display an increase from the second to the third trimester and correlate to disease activity improvement. A significant decrease of IL-1Ra occurs both in healthy women and in RA patients after delivery [23]. Changes in the hormonal profile during pregnancy may in turn increase Th2 cytokines and further contribute to the modulation of the immune response, favoring clinical improvement [15].

Maternal/fetal HLA disparity was reported to facilitate RA improvement. It has been suggested that placental apoptotic syncytiotrophoblast debris – containing fetal HLA II peptides – are present in the maternal blood. Fetal and self RA-associated antigens are then simultaneously presented by immature maternal dendritic cells to T lymphocytes in the setting of a noninflammatory environment. This process is thought to play a role in inducing fetal tolerance and RA improvement [24,25].

Finally, regulatory T lymphocytes ($CD4^+$, $CD25^+$ and $Foxp3^+$), usually inhibited in RA by elevated levels of TNF-α, can be reactivated during the pregnancy to suppress autoreactive T lymphocytes and to induce a tolerogenic milieu [24,25].

Antirheumatic drugs & pregnancy

Pregnancy planning in RA is not critical as it is in other systemic autoimmune diseases such as SLE, mainly because of the lack of serious complications and of the better prognosis as a whole. Nevertheless, the need to continue the treatment in order to control the disease raises the point of the safe use of specific antirheumatic drugs during pregnancy and breastfeeding.

A recent paper reported a consensus on the use of traditional nonsteroidal anti-inflammatory drugs and immunosuppressants in rheumatic diseases [26]. This aspect has been updated at the 5th International Conference on Sex Hormones, Pregnancy and Rheumatic Diseases held in Florence, Italy, last April [25].

Regarding the most common immunosuppressive agents, azathioprine and cyclosporine are permitted during pregnancy but not during breastfeeding, while methotrexate, mycophenolate-mophetile and leflunomide are all contraindicated. Methotrexate must be withdrawn at least 3 months before conception, while mycophenolate-mophetile must be stopped 6 weeks before conception [25–27]. A wash out from the drug is also strongly recommended before conception for leflunomide [28].

Anti-TNF-α (infliximab, etanercept and adalimumab) and anti-B lymphocyte (rituximab) agents are common treatments for RA. All these molecules may cross the placenta at the fourteenth week of gestation, being potentially able to induce a toxic effect in the fetus. Administration of anti-TNF-α agents should be stopped at conception, while rituximab should be stopped approximately 12 months before pregnancy. However, prospective studies on the safety of

anti-TNF-α agents in pregnancy are ongoing in order to draw definitive conclusions. Moreover, conclusive studies on the presence of anti-TNF-α drugs in maternal milk are still lacking; the well-known fact that all the immunoglobin classes can be present in the milk does contraindicate breast-feeding during treatment [25–27]. The need of a wash out of immunosuppressive drugs (such as methotrexate and leflunomide) before conception raises the possibility of controlling the disease with anti-TNF-α blocking agents during this period. Anti-TNF-α agents should then be stopped at conception as stated before.

Contraception

Pregnancy planning implies safe contraception: rules useful for the majority of the systemic autoimmune diseases can also be applied to RA. Different studies suggested a protective effect of oral contraception (OC) on the pathogenetic course of RA with a decreased risk of developing the disease among OC users, in contrast with postmenopausal estrogen-replacement therapy, which did not affect RA development [29].

The immunomodulatory effect of estrogen could be considered to be dose-related, as supported by the evidence of a stronger effect of OCs in the earlier year of its use, when they typically contained considerably higher doses of estrogen. OC is contraindicated in the case of positivity for antiphospholipid antibodies owing to the increased thrombophilic risk [8]. Intrauterine devices may be the cause of local infectious processes in women receiving an immunosuppressive treatment.

Menopause & osteoporosis

Rheumatoid arthritis is characterized by three different forms of bone damage: generalized osteoporosis, peri-articular osteoporosis and bone erosions.

Generalized osteoporosis is frequent in postmenopausal RA, affecting approximately half of patients, and several studies demonstrated a decreased bone mineral density (BMD) with a high risk for bone fractures [30,31]. The pathogenesis of this phenomenon has been related to estrogen deficiency, caused by both menopause and inflammation. In experimental murine models, both arthritis and ovariectomy are able to induce comparable degrees of trabecular bone loss (26 vs 22%), but arthritic ovariectomized mice display a much higher decrease in BMD (58%). Furthermore, arthritis itself, but not ovariectomy, induces cortical bone loss [32].

Recent studies have also suggested that generalized osteoporosis and local osteoporosis with bone erosions widely share the same pathogenetic mechanisms [33,34]. D'Elia *et al.* have demonstrated a strong association between radiographic joint destruction (expressed as Larsen score) and generalized osteoporosis in postmenopausal women with RA [30].

Bone loss seems to be influenced by macrophage-derived proinflammatory cytokines (TNF-α and IL-1) and other mechanisms able to induce osteoclast activation. Osteoclasts are terminally differentiated cells of the monocyte/macrophage lineage, with the function of resorbing bone matrix. The differentiation of precursor cells into mature osteoclasts is mediated by the activation of nuclear factor-κB (NF-κB) through the binding of its membrane receptor activator (receptor activator NF-κB [RANK]), to its ligand (RANKL) expressed on activated T lymphocytes and osteoblasts [35]. Osteoclast activation in RA joints is a complex phenomenon mediated by different mechanisms: IL-17-producing T helper cells (Th17) secrete large amounts of IL-17 that induces RANKL on synovial fibroblasts. IL-17 also stimulates the secretion of proinflammatory cytokines (TNF-α, IL-1 and -6) that are able, in turn, to activate osteoclast precursors and to induce RANKL expression on synovial fibroblasts. Th17 cells may express RANKL on their membrane further contributing to the enhanced osteoclastogenesis [32–36]. Furthermore, impaired physical activity, low body mass and steroid therapy in RA patients contribute to decreased BMD and increased risk for fractures [31].

Thus, until recent years, several authors have suggested that estrogen-replacement therapy in menopausal women with RA could be useful in osteoporosis and joint erosion prevention [31,39]. Several experimental studies on animal models have demonstrated that administration of physiological dosages of estrogens improves arthritis and also osteoporosis [39]. Studies on the use of hormone-replacement therapy (HRT) in postmenopausal women with RA reported its effectiveness in improving BMD [40,41]. However, the side-effects demonstrated with the long-term use of HRT have recently limited the use of this therapy in women in general. In fact, two large studies in normal postmenopausal women demonstrated an increased risk of breast cancer, coronary heart disease, stroke and pulmonary embolism in patients treated with HRT [42,43]. Owing to the increased cardiovascular risk closely associated to RA itself, the use of HRT to control RA systemic osteoporosis is no longer recommended [44].

Alternative therapies have been proposed for RA osteoporosis in women. Anti-TNF agents have been shown to be effective not only in reducing inflammation, but also in improving bone density. Treatment with TNF blockers displays beneficial effects on bone metabolism reducing serological markers of bone resorption and increasing plasma levels of bone-formation markers [45–47]. Experimental evidence from animal models also supports these findings [48]. Biphosphonates have been demonstrated to display a favorable effect on BMD in RA patients, especially in those treated with steroids. However, the effect was less evident than in normal menopausal women with osteoporosis. It has been suggested that the disease-associated inflammation is likely to be responsible for this impaired response [49,50].

Future perspective

In the last few decades, much progress in the knowledge of the pathogenesis and therapy of RA has been made. This has highly improved the quality of life of RA female patients and has increased the number of RA women that experience a pregnancy.

New therapies, such as anti-TNF-α and anti-B lymphocyte agents, are now available and they have dramatically changed the course of the disease, but their use is not yet allowed during pregnancy because data in humans are limited with regard to safety for a developing fetus.

However, some preliminary data on the possible effects of these drugs on the fetus seem to suggest that anti-TNF-α agents do not exert a teratogenic effect [51,52]. The future of women with RA in the childbearing age group depends for a great part on the definitive results of these studies.

These drugs are also changing long-term prognosis of the disease in older women. Actually, several data suggest that anti-TNF-α agents can have a beneficial effect, not only on RA course but also on osteoporosis, which represents one of the most important long-term complications of RA in women.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- Sex hormones play an important role in the regulation of the immune response: while androgens and progesterone are considered natural immunosuppressors, estrogens can enhance humoral immunity in several systems.
- Rheumatoid arthritis (RA) is two- to three-fold more frequent in women than in men and a strong association between RA and sex hormones has been demonstrated, as for other systemic autoimmune diseases.
- Several data from animal models and from clinical studies on humans support the hypothesis that sex hormones are involved in the pathogenesis of RA.
- Maternal/fetal HLA disparity was reported to facilitate RA improvement, and in fact more than 75% of women experience an improvement of symptoms or even a complete remission during pregnancy, while flares are very common during the puerperium.
- RA is characterized by three different forms of bone affections: generalized osteoporosis, peri-articular osteoporosis and bone erosions, and some authors have proposed an association between radiographic joint destruction and generalized osteoporosis in postmenopausal women with RA.
- Anti-TNF agents have been shown to be effective not only in reducing inflammation, but also in improving bone density.

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