

THE ROLE OF SEX HORMONES IN RHEUMATOID ARTHRITIS

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ABSTRACT

Rheumatoid arthritis is a disease characterised primarily by chronic inflammatory synovitis and is well know to be associated with significant sex difference in its prevalence and clinical feature. Sex hormones have been proposed to be involved in the pathogenesis of rheumatoid arthritis but details pertaining to their role and mechanism of action in rheumatoid arthritis (autoimmune disorder) have yet to be fully characterised. In the present review an effort is made to describe both the role and mechanism of action of testosterone, estrogens, dehydroepiandrosterone and its sulphate conjugate (DHEAS) on immune cells and inflammatory factors all together under one shelter along with the available treatment for rheumatoid arthritis.

Keywords: Rheumatoid arthritis, Androgens, Estrogens, Tumour necrosis factor.

INTRODUCTION

Arthritis is an auto immune disorder characterized by pain, swelling and stiffness. Its prevalence depends upon age. It is an inflammation of synovial joint due to immune mediated response¹. Rheumatoid arthritis is characterised by persistent synovitis, systemic inflammation and auto antibodies in blood or serum (particularly to rheumatoid factor and citrullinated peptide)².

Rheumatoid arthritis (RA) is a chronic inflammatory, systemic autoimmune disease that affects about 1% of the general population in Western countries and it is two to three times more common in women than in men³. It is characterized by both local and systemic inflammation with elevated plasma concentration of pro-inflammatory cytokines such as interleukins-6 (IL-6), interleukin 1 β (IL-1 β), tumour necrosis factor- α (TNF- α) and acute phase proteins.

Typical symptoms are sudden inflammation in the affected area and mild stiffness in joints. The symptoms can lead in others to deformations in the feet and hands as a result of the worsening of persisting symptoms over a long period of time. The organs such as

the heart, lungs, muscles and even the skin can be affected in severe cases over a long period of time due to immune response.

Etiology

Although many theories abound concerning possible causative factors for RA, there is no definitive answer has yet been found. The inflammatory events characteristic to RA suggest that an immune response may be the initiating signal but search for a single human pathogen or a unique antigen associated with this disease have come up empty. However sex hormones are believed to contribute to the risk of rheumatoid arthritis (RA) because of the disease's female preponderance, especially during the child-bearing years and dramatic improvements seen during pregnancy. Available controlled data on serum dehydroepiandrosterone sulfate (DHEAS), testosterone (T) and estradiol (E2) in RA patients not treated with glucocorticoids are summarized. These data support hypoandrogenicity in RA patients especially among premenopausal females and males. Limited prospective studies in women and therapeutic trials of testosterone therapy in men further support a role of sex hormones in RA⁴.

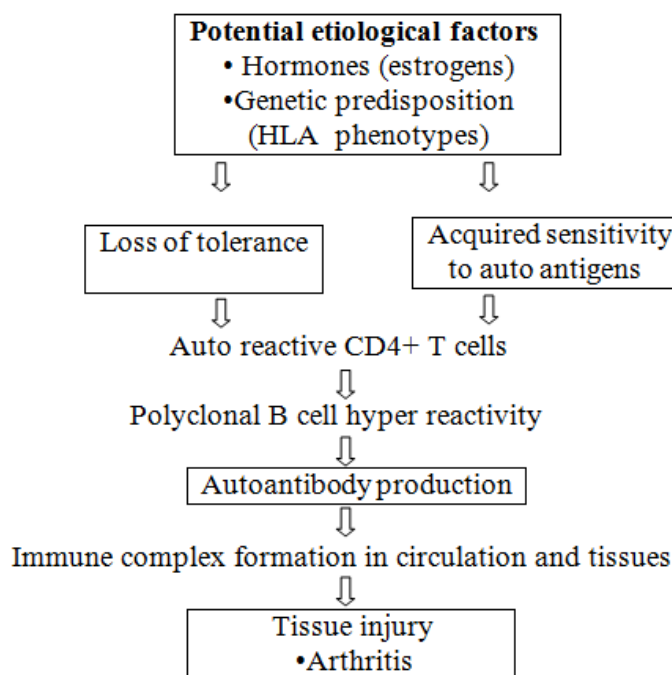


Fig. 1: Potential etiological factors in Rheumatoid arthritis⁵⁹**Pathogenesis**

Despite the inability to identify a causative factor for RA, advances have been made in elucidating subsequent steps in the pathogenesis of this disease. A critical role for T cells in the pathogenesis of RA is suggested by the strong association between RA and certain human leukocyte antigen (HLA) haplotypes and by the observation that RA can be transferred by T cell lines in some animal models of RA. Recent data suggest that the destruction of rheumatoid joints is initiated by complex cell-cell interactions between antigen-presenting cells and CD4+ T cells. The specific roles of antigen presenting cells and T cells in initiating RA and hypothesis regarding the relative importance of the many cells in the synovium in fulfilling these functions remain controversial. However it is thought that these cell-cell interactions result in the activation of macrophages and induction of the inflammatory process, culminating in degradation and resorption of cartilage and bone. Pro-inflammatory cytokines particularly TNF and interleukin 1 (IL-1) are critical components of this process.

Cytokines are believed to play multiple roles in the pathogenesis of RA beginning with activation of antigen presenting cells and T cells. Cytokines also maintain and perpetuate the immune response by interacting with nearby cells that bear the appropriate cell surface receptors. Once activated these cells produce their own cytokines and effector molecules, this sequential expanded production of cytokines constitutes the "cytokine cascade." Although many cells can respond to cytokines but cytokine-activated fibroblasts and macrophages are believed to be particularly important in the pathogenesis of RA⁵.

Among the many cytokines present in the rheumatoid synovium, pro-inflammatory cytokines appears to be most directly linked to the disease process. These cytokines not only promote inflammation but also play a critical role in joint destruction. Evidence indicates that TNF acts as a dominant cytokine in the cytokine cascade, regulating the production of several other pro-inflammatory molecules including IL-1, IL-6 and IL-9 and granulocyte-macrophage colony stimulating factor⁶.

Since sex hormonal levels (testosterone, estradiol, dehydroepiandrosterone and its sulphate conjugates) are correlated

with the regulation of inflammatory factors and immune cells activity they can be considered to participate indirectly in the pathogenesis of RA.

Prevalence

Rheumatoid arthritis has a world wide distribution with an estimated prevalence of 1 to 2%. 46 million U.S. adults reported doctor diagnosed arthritis, among this prevalence rate a quarter million are childrens and 60% are womens. One in five (21%) adults in the U.S. reported having doctor diagnosed arthritis. The number of adults with doctor diagnosed arthritis is projected to increase to 67 million by 2030. Each year arthritis results in 750,000 hospitalizations and 3 million outpatients visits hospital and 9,500 deaths. In India the prevalence of 0.75% is projected to whole population which would give a total of about seven million patients in India. This prevalence is higher than that reported from China, Indonesia, Philippines and rural Africa⁷.

Prevalence in women

The current study led by Sherine Gabriel expanded on prior research (1955-1994) from the Mayo Clinic team by determining RA incidence and prevalence between 1995 and 2007. After thorough review of all medical records, a diagnosis of RA was made in 466 patients whose mean age at RA incidence was 55.6 years with 321 females (69%) in the study cohort. It was observed that a modest increase of RA incidence in women during the study period, which followed a sharp decline in incidence during the previous 4 decades. Results show that RA incidence in women increased by 2.5% per year from 1995 to 2007, while a decrease of 0.5% was noted for men.

Researchers also note that lower doses of estrogens found in modern oral contraceptives offer less protection against RA development than at the previously higher doses found in older medications, which they suspect may contribute to the increased RA incidence among women⁸. The centers for disease control and prevention (CDC) recently analysed data to better understand the overall impact of arthritis as a public health problem with respect to gender and age as given below ^{9,10}.

Table 1: Arthritis prevalence in US with respect to gender

Year	Estimated number of adults with doctor-diagnosed arthritis (in 1,000s)		
	Men	Women	Total
2005	18,480	29,358	47,838
2010	20,178	31,701	51,879
2015	21,732	33,993	55,725
2020	23,164	36,244	59,409
2025	24,622	38,587	63,209
2030	26,053	40,915	66,969

Table 2: Arthritis prevalence in US women and men with respect to age

Age group	Women	Men
18-24	2.9751	1.8388
25-34	6.7362	4.7585
35-44	15.0348	10.721
45-54	26.6342	21.4491
55-64	43.1365	30.7517
65-74	52.13	40.4893
75-84	58.1201	45.7775
85+	60.9193	47.2483

Adrenal and gonadal androgen relationships

Activation of the hypothalamus-pituitary-adrenal axis by pro-inflammatory stimuli and chronic stress leads to parallel decrease in hypothalamus-pituitary-gonadal axis activity¹³. This can be

substantiated by decrease levels of follicle stimulating hormone and luteinizing hormone and it is even more evident by looking at the levels of serum testosterone and the serum androgen DHEAS.

During a chronic inflammatory process like active RA, levels of both serum testosterone and in particular serum DHEAS becomes lower. Since testosterone and its precursors DHEAS and DHEA have anti-inflammatory properties, the decline in levels of these hormones further supports the pro-inflammatory process.

In the adrenal and gonadal glands the loss of DHEA and DHEAS is attributed to a synthetic blockade of the second step of the enzyme P450c17, again induced by inflammatory cytokines such as IL 1 β and TNF. Increased DHEAS levels during treatment with TNF antagonist in active RA patients suggest an improved adrenal function¹⁴.

Androgens in active rheumatoid arthritis

Clinical and epidemiological evidence supports that androgens protect more male than female subjects from the development of immune inflammatory diseases¹¹. Androgens exert anti-inflammatory activities at least at the level of the RA synovial tissue, which contrast with the immune- enhancer activities locally exerted by estrogens and their metabolites. It is well known that serum testosterone levels are inversely correlated with RA disease activity and dehydroepiandrosterone sulfate (DHEAS) levels are inversely correlated with both disease duration and clinical severity¹²

Mechanisms of action of androgens in immune cells

Effects of androgens on B lymphocytes

The effects of testosterone have been studied on human peripheral blood mononuclear cells and a dose dependent inhibition of IgG and IgM production by cells from normal male and female's observed¹⁵. Antibody production in B lymphocytes was suppressed by testosterone, although the magnitude of its effect on B cells was lower than on peripheral blood mononuclear cells (PBMcs). In addition testosterone treatment reduced monocyte IL-6 production compared with controls but did not appear to directly affect isolated B or T cells. Moreover exogenous IL-6 partially restored the testosterone induced decrease in antibody production by PBMcs. Thus these data indicate that testosterone may modulate susceptibility to human autoimmune disease indirectly through action on monocytes. The end results in decreased B cell activity

Effects of androgens on T lymphocytes

The effects of androgens on T cells are also complex and inadequately studied in both humans and animals. Direct exposure of murine T cells to DHT reduces the amount of IL-4, IL-5 and interferon gamma production after activation with anti-CD3 without affecting the production of IL-2¹⁶. The authors observed difference in the production of IL-2, IL-4 and IFN- γ between males and females at a given age. Both IL-4 and IFN- γ production is elevated in females.

Moreover another experimental study revealed that testosterone exert a protective effect on autoimmune encephalomyelitis. The data suggested that this protective effect resulted from the induction of a Th2 bias in auto antigen specific T lymphocytes¹⁷. Enhanced IL-10 production by the auto antigen specific T lymphocytes may explain these observations. This study was first to demonstrate the ability of testosterone to shift an auto antigen specific T lymphocyte response towards the Th2 phenotype *in vivo*, coupled with an observed effect on a clinical autoimmune disease.

Effects of androgens on monocytes/ macrophages

Human macrophages appear to contain the key enzymes of steroidogenesis¹⁸. In particular, macrophages are endowed with 5 α -reductase enzymes that catalyze the formation of DHT from testosterone, the more biologically active metabolite. Recent studies have shown that both physiologic (10⁻⁸ M) and pharmacologic (10⁻⁶ M) concentrations of testosterone inhibit IL-1 β secretion by PBMcs

obtained from RA patients¹⁹. In addition, physiologic concentrations of testosterone inhibit IL-1 synthesis in primary cultured human synovial macrophages²⁰. In related studies DHT was found to repress the expression and activity of the human IL-6 gene promoter in human fibroblasts, thus supporting the concept of anti-inflammatory or immunosuppressive effects of androgens.

Biological aspects and genetic background of androgens

Sex hormone concentrations evaluated particularly in patients with rheumatoid arthritis before glucocorticoid therapy was frequently found to be altered especially in men and premenopausal women²¹. In particular, low gonadal testosterone /dihydrotestosterone and adrenal androgens dehydroepiandrosterone and its sulphate levels as well as reduced androgen/ estrogen ratio were detected in the body fluids (blood, synovial fluid, smears, saliva) of male and female RA patients, supporting the possible pathogenic role for the decreased levels of the immunosuppressive androgens²².

The estrogen synthase (CYP19) locus is the cytochrome p450 that catalyzes the conversion of C19 androgens to C18 estrogens. In RA a linkage to this locus has been described in sibling-pair families having disease at older age (> 50 years)²³. CYP19 polymorphisms that lead to higher levels of CYP19 or a higher enzyme activity lead to reduced levels of androgens and presumably would make such older subjects hypo androgenic and more susceptible to RA.

Huang et al.²⁴ indicated a relationship between CYP17 genotypes and the age at onset of rheumatoid arthritis in female patients. The CYP17 gene coding for the cytochrome P450c 17 α mediates both steroid 17 α -hydroxylase and 17, 20-lyase activity which represent the key point in human steroidogenesis. A single base change in the 5' promoter region of the CYP17 creates an addition Sp-1-type promoter site that might cause increased expression. These authors found a new recognition site presented as two alleles (A1 and A2). Interestingly, they observed that female RA patients with the A2 allele tended to develop the disease at a younger age than those without and having the A2 allele which is a protective factor against older age onset of female RA. The results of study suggest that the A2 allele is related to early onset and A1 allele to late onset. In fact the A2 allele being an expression of increased CYP17 activities is thought to be linked to elevated production of both estrogens and androgens through increased transcription²⁴. Since androgens function as immune suppressors, estrogens as immune stimulants and that having the A2 allele could modify the onset of RA; Huang et al.²⁴ suggests that the effects of the androgen increase induced by the A2 presence might not be biologically influential in younger RA female patients, which is characterised by high estrogens (immune stimulant). However, the same induction of increased production of androgens (immune suppressive) might become an influential resisting factor in older women, who are characterized by physiologically reduced estrogens²⁵. Finally, an association between HLA phenotype and the serum testosterone levels was identified²⁶. In particular the low testosterone levels in men with HLAB15, DR2 and DR5 haplotypes has been demonstrated.

Estrogens in active rheumatoid arthritis

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 noted²⁷. This heightened inflammatory response is advantageous in response to infection and sepsis but is unfavourable in immune responses against self leading to an overall increased rate of autoimmune diseases in women compared to men²⁸. 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. Since rheumatoid arthritis significantly differs from osteoarthritis with respect to inflammatory

condition these sex hormones are considered to be the most biologically active participants in etiology of rheumatoid arthritis.

Estrogens and Inflammatory Factors

A. Nuclear factor κ B

NF- κ B is an important factor in pro-inflammatory signalling that can interact with (estrogen receptor) ER pathways²⁹. With the overview of McKay and Cidlowski²⁹ the following data has been generated in cells relevant for inflammation. In rat vascular smooth muscle cells, E2 (pregnancy levels) inhibited both constitutive and IL-1 β -stimulated NF- κ B activation³⁰. NF- κ B activity is suppressed in PBMCs from pregnant women compared with age-matched non pregnant women³¹. In cerebral arteries of ovariectomized rats, E2 treatment at pregnancy levels reduced cerebrovascular NF- κ B activity³². In conclusion, E2 at periovulatory to pregnancy levels inhibits NF- κ B activation, which must be viewed as an anti-inflammatory signal. It was shown that E2 concentrations equal to or above 10^{-10} M are necessary to inhibit NF- κ B activation. At 10^{-11} M or below (postmenopausal level), no effects were observed. Necessarily, only at these higher E2 concentrations is the anti-inflammatory effect to be expected.

B. 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. E2 effects on IL-1 secretion are heterogeneous. 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^{33, 34} or even exert stimulatory effects on IL-1³⁵. Particularly, the later two studies indicate that E2 at low concentrations might stimulate IL-1 β secretion, whereas it inhibits its secretion at pregnancy levels. With respect to IL-8, E2 at periovulatory to pregnancy levels inhibited secretion of this chemotactic IL as demonstrated in five studies 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. Interestingly, E2 had stimulatory effects on IL-6 secretion in synoviocytes (normal or RA), which happens at pregnancy concentrations. Only two of 20 studies demonstrated no effects of E2 on IL-6 secretion at all.

Similar to IL-6, most studies demonstrated inhibitory effects of estrogens on TNF secretion at mid-follicular to pregnancy levels. 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 secretion³⁶. The latter two studies have often been cited to explain the dual role of estrogens at low vs high concentrations, 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.

In conclusion, important pro-inflammatory 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 pro-inflammatory situation, which might well contribute to the manifestation of chronic inflammatory diseases after the menopause.

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

The interleukins IL-4, IL-10 and TGF- β are assumed to exert anti-inflammatory effects as long as tissue specific autoimmune diseases are considered. However, these cytokines might have a pro-inflammatory role in B cell-oriented autoantibody

driven autoimmunity, allergic diseases or diseases with an overshooting fibrotic repair process (TGF- β).

With respect to IL-4, all available studies demonstrated that E2 stimulated IL-4 secretion in postmenopausal women³⁷. Of the seven studies on IL-10, five demonstrated a stimulating effect of E2 on human monocytes at midfollicular to pregnancy levels³⁸. The two studies that report an inhibitory effect on IL-10 (at pregnancy levels) also delineated that E2 at periovulatory doses had no effect³⁹. All studies devoted to TGF- β demonstrated that E2 at pregnancy levels stimulated secretion of this cytokine⁴⁰.

In conclusion, most of the *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.

Mechanisms of action of Estrogens on immune cells

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder affecting many tissues and organs where autoimmunity plays a pivotal role in both its chronicity and progression, the sex hormone estrogen is observed to influence the immune cells by regulating secretion of various inflammatory factors.

A. 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 cells⁴¹. In women on oral contraceptives (OC), serum levels of IgA and IgG were increased⁴² and in cycling women high levels of Ig were detected before ovulation⁴².

Preclinical study conducted in mice revealed that administration of E2 (pregnancy levels) markedly augments the ability of CD5+ B cells to express their autoimmune potential⁴³. In Swiss male mice, administration of E2 (pregnancy levels) for 4 weeks increased serum levels of IgG1 and in collagen-induced arthritis model, E2-treated mice (pregnancy levels) demonstrated an increase in the levels of IgG1 anti-collagen type II antibodies⁴⁴.

From above mentioned clinical and preclinical 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 precursors⁴⁵. Estrogen replacement in these mice resulted in a dose-dependent reduction in B cell precursors⁴⁶. 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 mice⁴⁷.

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. In chronic inflammatory disorders where B cells play a decisive role, E2 would promote the disease when auto aggressive B cells are already present while chronically elevated E2 would inhibit initiation of an autoimmune disease when no such B cells are available.

B. Estrogens and the T cell

For more than 20 years so-called T helper type 1 (Th1) immune response by CD4-positive T cells were thought to drive cell-mediated immunity leading to tissue damage as in chronic inflammatory autoimmune diseases⁴⁸. In contrast, T helper type 2 (Th2) CD4-positive cells drive certain antibody mediated responses, particularly those that are involved in allergy⁴⁸. 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 view point has changed due to the appearance of anti-inflammatory T regulatory cells producing TGF- β and pro-inflammatory T helper type 17 cells (Th17) producing IL-17⁴⁹. Today, Th17 cells are thought to be the main responsible cells for chronic inflammatory tissue destruction in autoimmune diseases⁴⁹.

Some important aspects concerning the role of estrogens on T cells have been generated in multiple sclerosis (MS) research. In the presence of E2 at pregnancy levels, the majority of the tested neuro antigen-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 secretion⁵⁰. 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 3.6×10^{-8} to 3.6×10^{-7} M⁵⁰. Other important indications for the estrogens mediated inhibition of the pro-inflammatory 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)⁵¹.

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. Because we know that increased IL-4, IL-10 and IFN- γ in the presence of low TNF support an anti-aggressive immune response, collectively these data suggest that E2 at periovulatory to pregnancy levels might be a favourable hormone leading to down-regulation of T cell-dependent immunity. From this we can assume that if E2 falls to postmenopausal levels (metestrus) before initiation or during the course of an autoimmune disease than the protecting effects of E2 are getting lost under these conditions.

C. Estrogens and the monocyte/macrophage/dendritic cell

In human promonocytic cells (U937), E2 (late pregnancy levels) inhibited (lipopolysaccharide) LPS-stimulated IL-6 secretion⁵² and in 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-1 β and IL-6⁵³. This indicates that E2 normally suppresses these cytokines stimulated via cross-linking of CD16. While in mouse splenic macrophages, pre-treatment for 16 h with E2 between 3.6×10^{-11} and 1.8×10^{-9} M decreased LPS-induced TNF production, which was associated with a decreased NF- κ B-binding activity⁵⁴ and also in murine bone marrow-derived macrophages, pre incubation with E2 at pregnancy levels (maximum at 10^{-8} M) inhibited LPS-induced TNF release⁵⁵.

However E2 treatment (pregnancy levels) decreased TNF and IL-12 production in mature mouse dendritic cells⁵⁶. 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 day 12 after immunization with myelin basic protein prevented the expansion 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 , with reduction in TNF production⁵⁷. 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 observed⁵⁸. These studies demonstrate that lower levels of E2 particularly increase IL-1 β secretion.

In conclusion, in human and mouse/rat monocyte/macrophage-like cells, secretion of IL-1 β 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- κ B. The dichotomous effect of E2 on IL-1 β and TNF at high and low concentrations is most probably due to inhibition of NF- κ B at high concentrations.

How is rheumatoid arthritis treated?

There is no known cure for rheumatoid arthritis. To date, the goal of treatment in rheumatoid arthritis is to reduce joint inflammation and pain, maximize joint function, prevent joint destruction and deformity. Two classes of medications are used in treating rheumatoid arthritis: fast-acting "first-line drugs" and slow-acting "second-line drugs" (also referred to as disease-modifying anti rheumatic drugs or DMARDs).

"First-line" rheumatoid arthritis medications

Acetylsalicylate (aspirin), naproxen (Naprosyn), ibuprofen (Advil, Medipren, Motrin), and etodolac (Lodine) are examples of nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs are medications that can reduce tissue inflammation, pain and swelling.

The most common side effects of aspirin and other NSAIDs include stomach upset, abdominal pain, ulcers and even gastrointestinal bleeding. In order to reduce gastrointestinal side effects NSAIDs are usually taken with food. Newer NSAIDs include selective Cox-2 inhibitors, such as celecoxib (Celebrex), which offer anti-inflammatory effects with less risk of stomach irritation and bleeding risk.

Corticosteroid medications can be given orally or injected directly into tissues and joints. They are more potent than NSAIDs in reducing inflammation and in restoring joint mobility and function. However, corticosteroids can have serious side effects especially when given in high doses for long periods of time. These side effects include weight gain, facial puffiness, thinning of skin and bone, easy bruising, cataracts, risk of infection, muscle wasting and destruction of large joints such as the hips⁷.

"Second-line" or "slow-acting" rheumatoid arthritis drugs

(Disease-modifying anti-rheumatic drugs or DMARDs)

While "first-line" medications (NSAIDs and corticosteroids) can relieve joint inflammation and pain, they do not necessarily prevent joint destruction or deformity. Rheumatoid arthritis requires medications other than NSAIDs and corticosteroids to stop progressive damage to cartilage, bone and adjacent soft tissues. The medications needed for ideal management of the disease are also referred to as disease-modifying anti rheumatic drugs or DMARDs.

Recent research suggests that patients who respond to a DMARD with control of the rheumatoid disease may actually decrease the known risk of lymphoma (cancer of lymph nodes) that exists from simply having rheumatoid arthritis. The various available DMARDs are Hydroxychloroquine (Plaquenil) related to quinine and is also used in the treatment of malaria. It is used over long periods for the treatment of rheumatoid arthritis. Possible side effects include upset stomach, skin rashes, muscle weakness and vision changes.

Sulfasalazine (Azulfidine) is an oral medication traditionally used in the treatment of mild to moderately severe inflammatory bowel diseases such as ulcerative colitis and Crohn's colitis. Azulfidine used to treat rheumatoid arthritis in combination with anti-inflammatory medications, Common side effects include rash and stomach upset. Methotrexate is an immunosuppressive drug. It can affect the bone marrow and liver, even rarely causing cirrhosis. All people taking methotrexate require regular blood tests to monitor blood counts and liver function.

Gold salts have been used to treat rheumatoid arthritis throughout most of the past century. Gold thioglucose (Solganal) and gold thiomalate (Myochrysine) are given by injection, initially on a weekly basis, for months to years. Side effects of gold (oral and injectable) include skin rash, mouth sores, kidney damage with leakage of protein in the urine and bone marrow damage

with anemia and low WBC count. D-penicillamine (Depen, Cuprimine) can be helpful in selected cases of progressive forms of rheumatoid arthritis, side effects are similar to those of gold.

Immunosuppressive medicines are powerful medications that suppress the body's immune system. A number of immunosuppressive drugs are used to treat rheumatoid arthritis like, methotrexate, azathioprine (Imuran), cyclophosphamide (Cytoxan), chlorambucil (Leukeran) and cyclosporine (Sandimmune). Immunosuppressive medications can depress bone-marrow function and cause anemia, a low white cell count, and low platelet counts. A low white count can increase the risk of infections, while a low platelet count can increase the risk of bleeding. Methotrexate rarely can lead to liver cirrhosis as described above and allergic reactions in the lung. Cyclosporine can cause kidney damage and high blood pressure⁷.

Herbal therapy

Despite considerable progress in the treatment of arthritis by NSAIDs and other drugs, search for newer drugs continues because the existing synthetic drugs have several limitations. According to reports approximately 60-90% of dissatisfied arthritis patients are likely to seek the option of complementary and alternative medicine. The efficacy of some of valuable herbs like guggul, bhallataka, ginger and ashwagandha that have a history of human use and their anti-inflammatory or anti-arthritis properties have been evaluated preclinically and clinically.

- Guggule extract showed anti-inflammatory activity equal to phenylbutazone and ibuprofen and one-fifth as compared to hydrocortisone, phenylbutazone and ibuprofen in reducing the severity of secondary lesions.
- Ethanol extract of bhallataka nuts inhibited the spontaneous and LPS induced production of pro-inflammatory cytokines IL-1B and IL12p40 but had no effect on TNF- α and IL-6 production both at protein and mRNA level.
- Ashwagandha treated group completely reduced the inflammatory proteins, whereas animals treated with phenylbutazone as well as control groups had increased inflammatory proteins. Hindawi et al found that Withania inhibited the granuloma formation in cotton-pellet implantation in rats and the effect was comparable to hydrocortisone sodium succinate (5mg/kg) treatment.
- In an animal model study boswellic acids significantly reduced the infiltration of leukocytes in to the knee joint, in turn significantly reducing inflammation causing immune WBC response.

Available data suggests that the extracts of most of herbs or compounds derived from them may provide a safe and effective adjunctive therapeutic approach for treatment of arthritis⁶⁰.

CONCLUSION

All preclinical and clinical data discussed here seem to indicate that gonadal androgens (testosterone and DHT) exert their modulatory effects via both direct influence on cytokine production by activated monocytes/macrophages (inhibition of IL-1, IL-6 and tumor necrosis factor- α production) and an indirect influence on cytokine production by activated T cells (inhibition of IL-4, IL-5 and IFN- γ production). On the other hand adrenal androgens (DHEAS and DHEA) may exert a direct effect on cytokine production by T cells (increase of IL-2 and IFN- γ synthesis).

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. In humans E3 and E2 respectively, at pregnancy levels inhibit T cell-dependent delayed type hypersensitivity. Because it is known that increased IL-4, IL-10 and IFN- γ in the presence of low TNF support an anti-aggressive immune response, collectively these data suggest that E2 at

periovulatory to pregnancy levels might be a favourable hormone leading to down-regulation of T cell-dependent immunity. From this we can assume that if E2 falls to postmenopausal levels (metestrus) before initiation or during the course of an autoimmune disease than the protecting effects of E2 are getting lost under these conditions. In conclusion low gonadal testosterone /dihydrotestosterone and androgens dehydroepiandrosterone and its sulphate levels, as well as reduced androgen/estrogen ratio in the body fluids (blood, synovial fluid, smears, saliva) of male and female RA patients and fall in E2 level at postmenopausal stage supports the possible pathogenic role of these sex hormones in chronic and progressive inflammatory disorder (autoimmune disorder) which is responsible for development of rheumatoid arthritis.

The newer NSAIDs though offer anti-inflammatory effect but are associated with stomach irritation and bleeding risk. Where as corticosteroids are potent then NSAIDs in restoring joint mobility and function, however can have serious side effects in high dose for long period of time. But in order to stop progressive damage to cartilage, bone and adjacent soft tissues DMARDs are preferred, which are also considered as medications needed for ideal management of rheumatoid arthritis. Apart from this herbal remedies have been found to make a remarkable contribution in the management of rheumatoid arthritis.

REFERENCE

1. Shivanand P, Arthritis an autoimmune disorder: Demonstration of In-vivo anti-arthritis Activity. International journal of pharmacy and life sciences 2010; 1(1):38-43.
2. Scott DL, Wolfe F, Huizinga TW. J. Rheumatoid arthritis Lancet 2010; 376:1094-108.
3. Hegen M, Keith JC, Collins M, Nickerson-Nutter CL. Utility of animal models for identification of potential therapeutics for rheumatoid arthritis. Ann Rheum Dis 2008; 67: 1505-15.
4. Albani S, Carson DA Etiology and pathogenesis of rheumatoid arthritis. In: Koopman WJ (ed). Baltimore, Williams, Wilkins. Arthritis and Allied Conditions. A Textbook of Rheumatology. 13th ed. 1997. P. 979-92.
5. Feldmann M: The cytokine network in rheumatoid arthritis: definition of TNF α as a therapeutic target. J R Coll Physicians Lond 1996; 30:560-70.
6. Brennan FM. Inhibitory effect of TNF α antibodies on synovial cell interleukin-1 production in rheumatoid arthritis. Lancet 1989; 2:244-7.
7. Irfan AK, Atiya K, editors. Herbal therapy for arthritis. India: Ukaaz publications; 2008; p.71-194.
8. Elena Myasoedova. Is the Incidence of Rheumatoid Arthritis Rising? Arthritis & Rheumatism 2010; 62(6): 1576-82.
9. Theis KA, Helmick CG, Hootman JM. Arthritis burden and impact are greater among U.S. women than men: intervention opportunities. J Women's Health 2007; 16(4):441-53.
10. Hootman JM, Helmick CG. Projections of U.S. prevalence of arthritis and associated activity limitations. Arthritis Rheum 2006; 54(1):226-9.
11. Cutolo M, Straub RH, Bijlsma JW. Neuroendocrine-immune interactions in synovitis. Nat Clin Pract Rheumatol 2007; 3:627-34.
12. Gordon D, Beasall GH, Thomson JA, Sturrock RD. Prolonged hypogonadism in male patients with rheumatoid arthritis during flares in disease activity. Br J Rheumatol 1988; 27: 440-44.
13. Cutolo M, Straub RH. Stress as a risk factor in the pathogenesis of rheumatoid arthritis. Neuroimmunomodulation 2006; 13:277-82.
14. Ernestam S, Hafström I, Werner S, Carlström K, Tengstrand B. Increased DHEAS levels in patients with rheumatoid arthritis after treatment with tumor necrosis factor antagonists: evidence for improved adrenal function. J Rheumatol 2007; 34:1451-58.

15. Kanda N, Tsuchida T, Tamaki K. Testosterone inhibits immunoglobulin production by human peripheral blood mononuclear cells. *Clin Exp Immunol* 1996; 106:410-18.
16. Araneo BA, Dowell T, Diegel M, Daynes RA. Dihydrotestosterone exerts a depressive influence on the production of interleukin-4 (IL-4), IL-5, and IFN-gamma, but not IL-2 by activated murine T cells. *Blood* 1991; 78:688-94.
17. Dalal M, Kim S, Voskuhl R. Testosterone ameliorates experimental autoimmune encephalomyelitis and induces a T helper 2 bias in auto response. *J Immunol* 1997; 159:3-12.
18. Cutolo M, Villaggio B, Barone A, Sulli A, Granata OM, Castagnetta L. Primary cultures of human synovial macrophages metabolize androgens. *Ann N Y. Acad Sci* 1996; 784:237-43.
19. Li ZG, Danis VA, Brooks PM. Effect of gonadal steroids on the production of IL-1 and IL-6 by blood mononuclear cells in vitro. *Clin Exp Rheumatol* 1993; 11:157-64.
20. Cutolo M, Accardo S, Villaggio B, Sulli A, Ballearis E, Bason C et al. Androgen metabolism and inhibition of interleukin-1 synthesis in primary cultured human synovial macrophages. *Mediat Inflamm* 1995; 4:138-47.
21. Masi AT, Feigenbaum SL, Chatterton RT, Cutolo M. Integrated hormonal immunological-vascular (H-I-V) systems interactions in the rheumatic diseases. *Clin Exp Rheumatol* 1995; 13:203-4.
23. Cutolo M., Castagnetta L. Immunomodulatory mechanisms mediated by sex hormones in rheumatoid arthritis. *Ann N Y Acad Sci* 1996; 784:534-39.
24. John S, Eyre S, Myerscough A, Barrett J, Silman A, Ollier W et al. Linkage of a marker in intron D of the estrogen synthase locus to rheumatoid arthritis. *Arthritis Rheum* 1999; 42:1617-22.
25. Huang J, Ushiyama K, Mori K, Hukuda S. Possible association of CYP17 gene polymorphism with the onset of rheumatoid arthritis. *Clin Exp Rheumatol* 1999; 17: 721-24.
26. Cutolo M. The role of the hypothalamus-pituitary-adrenocortical and gonadal axis in rheumatoid arthritis. *Clin Exp Rheumatol* 1998; 16:3-6.
27. Cutolo M, Accardo S. Sex hormones, HLA and rheumatoid arthritis. *Clin Exp Rheumatol* 1991; 9:641-46.
28. Olsen NJ, KovacsWJ. Gonadal steroids and immunity. *Endocr Rev* 1996; 17:369-84.
29. Talal N. Sex steroid hormones and systemic lupus erythematosus. *Arthritis Rheum* 1981; 24:1054-56.
30. McKay LI, Cidlowski JA. Molecular control of immune/ Straub, Estrogens and Inflammation *Endocrine Reviews* 2007; 28(5):521-74.
31. Sharma RV, Gurjar MV, Bhalla RC. Selected contribution: estrogen receptor- gene transfer inhibits proliferation and NF-B activation in VSM cells from female rats. *J Appl Physiol* 2001; 91:2400-06.
32. McCracken SA, Drury CL, Lee HS, Morris JM. Pregnancy is associated with suppression of the nuclear factor B/IB activation pathway in peripheral blood mononuclear cells. *J Reprod Immunol* 2003; 58:27-47.
33. Ospina JA, Brevig HN, Krause DN, Duckles SP. Estrogen suppresses IL-1-mediated induction of COX-2 pathway in rat cerebral blood vessels. *Am J Physiol Heart Circ Physiol* 286:2010-9.
34. Ruh MF, Bi Y, D'Alonzo R, Bellone CJ. Effect of estrogens on IL-1 promoter activity. *J Steroid Biochem Mol Biol* 1998 ; 66:203-10.
35. Hu SK, Mitcho YL, Rath NC. Effect of estradiol on interleukin-1 synthesis by macrophages. *Int J Immunopharmacol* 1988; 10:247-52.
36. Polan ML, Loukides J, Nelson P, Carding S, Diamond M, Walsh A et al. Progesterone and estradiol modulate interleukin-1 messenger ribonucleic acid levels in cultured human peripheral monocytes. *J Clin Endocrinol Metab* 1989; 69:1200-6.
37. Gilmore W, Weiner LP, Correale J. Effect of estradiol on cytokine secretion by proteolipid protein-specific T cell clones isolated from multiple sclerosis patients and normal control subjects. *J Immunol* 1997; 158:446-51.
38. Kamada M, Irahara M, Maegawa M, Ohmoto Y, Murata K, Yasui T et al. Transient increase in the levels of T-helper 1 cytokines in postmenopausal women and the effects of hormone replacement therapy. *Gynecol Obstet Invest* 2001; 52:82-8.
39. Kanda N, Tamaki K. Estrogen enhances immunoglobulin production by human PBMCs. *J Allergy Clin Immunol* 1999; 103:282-8.
40. Carruba G, D' Agostino P, Miele M, Calabro M, Barbera C, Bella GD et al. Estrogen regulates cytokine production and apoptosis in PMA-differentiated, macrophage- like U937 cells. *J Cell Biochem* 2003; 90:187-96.
41. Oursler MJ, Cortese C, Keeting P, Anderson MA, Bonde SK, Riggs BL et al. Modulation of transforming growth factor- production in normal human osteoblast-like cells by 17-estradiol and parathyroid hormone. *Endocrinology* 1991; 129:3313-20.
42. Paavonen T, Andersson LC, Adlercreutz H. 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* 1981; 154:1935-45.
43. Franklin RD, KuttehWH. Characterization of immunoglobulins and cytokines in human cervical mucus: influence of exogenous and endogenous hormones. *J Reprod Immunol* 1999; 42:93-106.
44. Ahmed SA, Aufdemorte TB, Chen JR, Montoya AI, Olive D, Talal N et al. Estrogen induces the development of autoantibodies and promotes salivary gland lymphoid infiltrates in normal mice. *J Autoimmun* 1989; 2:543-52.
45. Latham KA, Zamora A, Drought H, Subramanian S, Matejuk A, Offner H et al. Estradiol treatment redirects the isotype of the autoantibody response and prevents the development of autoimmune arthritis. *J Immunol* 2003; 171:5820-27.
46. Medina KL, Kincade PW. Pregnancy-related steroids are potential negative regulators of B lymphopoiesis. *Proc Natl Acad Sci* 1994; 91:5382-6.
47. Smithson G et al. Increased B lymphopoiesis in genetically sex steroid-deficient hypogonadal (hpg) mice. *J Exp Med* 1994; 180:717-20.
48. Smithson G, Couse JF, Lubahn DB, Korach KS, Kincade PW. The role of estrogen receptors and androgen receptors in sex steroid regulation of B lymphopoiesis. *J Immunol* 1998; 161:27-34.
49. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986; 136:2348-57.
50. Steinman L. 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* 2007; 13:139-45.
51. Gilmore W, Weiner LP, Correale J. Effect of estradiol on cytokine secretion by proteolipid protein-specific T cell clones isolated from multiple sclerosis patients and normal control subjects. *J Immunol* 1997; 158:446-51.
52. Cenci S, Weitzmann MN, Roggia C, Namba N, Novack D, Woodring J et al. Estrogen deficiency induces bone loss by enhancing T-cell production of TNF. *J Clin Invest* 2000; 106:1229-37.
53. Jain SK, Rogier K, Prouty L, Jain SK. Protective effects of 17-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* 2004; 37:1730-5.
54. Kramer PR, Kramer SF, Guan G. 17-Estradiol regulates cytokine release through modulation of CD16 expression in monocytes and monocyte-derived macrophages. *Arthritis Rheum* 2004; 50:1967-75.
55. Deshpande R, Khalili H, Pergolizzi RG, Michael SD, Chang MD. Estradiol down-regulates LPS-induced cytokine production and

- NF- κ B activation in murine macrophages. *Am J Reprod Immunol* 1997; 38:46-54.
56. Zhang X, Wang L, Zhang H, Guo D, Qiao Z, Qiao J. Estrogen inhibits lipopolysaccharide-induced tumor necrosis factor- α release from murine macrophages. *Methods Find Exp Clin Pharmacol* 2001; 23:169-73.
57. Liu HY, Buenafe AC, Matejuk J, Ito A, Zamora A, Dwyer J et al. Estrogen inhibition of EAE involves effects on dendritic cell function. *J Neurosci Res* 2002; 70:238-48.
58. Pettersson A, Ciumas C, Chirsky V, Link H, Huang YM, Xiao BG. Dendritic cells exposed to estrogen in vitro exhibit therapeutic effects in ongoing experimental allergic encephalomyelitis. *J Neuroimmunol* 2004; 156:58-65.
59. Polan ML, Daniele A, Kuo A. Gonadal steroids modulate human monocyte interleukin-1 (IL-1) activity. *Fertil Steril* 1988; 49:964-8.
60. Emanuel Rubin, Howard M Reisner. *Essentials of Rubin's Pathology*. 5th ed. Lippincott Williams & Wilkins publisher; 2008. p. 67.
61. Sunetra K Patwardhan, Kaumudee S Bodas, Sameer Gundewar. Coping with arthritis using safer herbal options. *Int J Pharm Pharm Sci* 2010; 2(1):1-10.