Study of the Possible Potential Role of Testosterone in Modulating Collagen-Induced Arthritis in Castrated Male Albino Rats

MAGDA ELHAMZAWY, M.D.; ZAINAB ABD ELWAHAB, M.D.; MOHAMMED ELSAYED SALEH, M.D. and MOHAMMED MAHER, M.B.B.Ch.

The Department of Physiology, Faculty of Medicine, Cairo University

Abstract

Rheumatoid arthritis (RA) is a chronic, inflammatory, systemic autoimmune disease that affects about 1% of the general population. Experimental and clinical evidence indicates that immune reactivity is greater in females than in males and suggests that gonadal steroids may play an important role in the regulation of the immune response. Collagen-induced arthritis (CIA) is an experimental autoimmune mediated poly-arthritis that is well accepted in different types of rodents. The aim of the present work is to study the effects of testosterone deprivation, induced by castration of male albino rats, and inhibition of aromatase- induced conversion of androgen into estrogen on the severity of collagen induced arthritis evaluated by peripheral total lymphocytic count, T- and B - lymphocytic count, monocyte counts, plasma levels of tumor necrosis factor-α (TNF-α), prostaglandin E2 (PGE2), immunoglobulin G (IgG), rheumatoid factor (RF) and the level of TNF-α in the synovial fluid. The severity of collagen-induced arthritis was also histopathologically assessed.

Materials and Methods: 48 adult male albino rats were randomly divided into 3 groups. Group I (control group, n=8 rats) which represented the placebo group, Group II (sham-operated, CIA, n=8 rats) in which rats were injected with collagen type II to induce CIA, and Group III (castrated group, n=32 rats) in which bilateral orchiectomy was performed and rats were subdivided into four subgroups, each included 8 rats: Group III-A (control castrated group) which represented the control group of castrated rats, Group III-B (castrated CIA group) in which CIA was induced in the rats, five days after bilateral orchiectomy, Group III-C (testosterone treated castrated- CIA) in which CIA was induced in rats five days after bilateral orchiectomy and the rats were injected with testosterone oenanthate (Cidotestone) at a dose of 6.25mg/kg, and Group III-D (Letrozol and testosterone treated- castrated-CIA group): In which CIA was induced five days after bilateral orchiectomy and treated with testosterone injection and co-supplemented with non-steroidal aromatase inhibitor. After 35 days, starting from CIA induction, immediately before animals were sacrificed, blood samples were withdrawn from retro-orbital plexus for determination of blood total lymphocyte count, T-lymphocyte, B-lymphocyte, and monocyte counts, plasma TNF-α, RF, PGE2, IgG. Also TNF-α in synovial fluid was measured. The severity of collagen-induced arthritis was also histopathologically assessed.

Results: Collagen-induced arthritis in sham operated male albino rats (group II) resulted in significant (p<0.05) increases in the mean values of peripheral blood total lymphocytic count, T-lymphocyte, B-lymphocyte, and monocyte counts, significant (p<0.05) increases in the mean values of plasma levels of TNF-α, PGE2, IgG, RF, and synovial fluid level of TNF-α, and significant (p<0.05) increase in histopathological score of arthritic injury. Plasma testosterone level was insignificantly changed compared to group I.

Testosterone deprivation, in group IIIA, resulted in significant (p<0.05) increases in the mean values of peripheral blood total lymphocytic count, T-lymphocyte, B-lymphocytes, and monocyte counts, significant (p<0.05) increases in the mean values of plasma levels of TNF-α, PGE2, and synovial fluid level of TNF-α, insignificantly (p>0.05) changes of the mean values of plasma levels of RF, IgG and histopathological score of arthritic injury compared to control rats (group I).

Induction of CIA in castrated rats (group IIIB) produced significant (p<0.05) decreases in the mean values of total lymphocytic count, T-lymphocyte, B-lymphocytes, and monocyte counts, significant (p<0.05) increases in the mean values of plasma levels of TNF-α, PGE2, and synovial fluid level of TNF-α while the mean values of IgG and RF were insignificantly (p>0.05) changed, and significant increase in histopathological score of arthritic injury compared to group II.

Induction of CIA in castrated male rats with testosterone replacement (group IIIC) produced significant decreases in the mean values of total lymphocytic count, T-lymphocyte, B-lymphocyte, and monocyte counts, significant (p<0.05) decreases in the mean values of plasma levels of TNF-α, PGE2, and synovial fluid level of TNF-α, while the mean values of plasma levels of IgG and RF were insignificantly (p>0.05) changed, and significant decrease in histopathological score of arthritic injury compared to group IIIB.

Induction of CIA in castrated rats treated with testosterone replacement and aromatase inhibition (group IIID) resulted in significant decreases in the mean values of total lymphocytic count, T-lymphocyte, B-lymphocyte, and monocyte counts significant (p<0.05) decreases in the mean values plasma levels of TNF-α, PGE2, and synovial fluid level of TNF-α while the mean values of IgG and RF were insignificantly (p>0.05) changed compared to group III C. and significant decrease in histopathological score of arthritic injury compared to group IIIC.

Correspondence to: Dr. Mohammed E. Saleh, E-mail: Saleh11@hotmail.com.
Summary and Conclusion:

Androgen deprivation aggravates collagen induced arthritis in male rats. Although androgen replacement therapy improved pro-inflammatory and histopathological changed of CIA in castrated rats, yet androgen therapy for male patients with rheumatoid arthritis are not recommended because of the risks of androgen therapy especially in old patient. Androgen therapy is also not recommended since aromatase-induced estrogens synthesis; include pro-inflammatory and anti-inflammatory metabolites, which are present at relatively low levels in RA synovial fluid. Blockade of TNF-α induced up-regulation of aromatase would particularly increase the level of androgens in males, leading to a better clinical outcome.

Key Words: Collagen – Induced arthritis – Testosterone – Castrated rats – Aromatase inhibitor.

RHEUMATOID arthritis (RA) is a chronic inflammatory systemic autoimmune disease that affects about 1% of the general population and is two to three times more common in women than in men [1]. Although the etiology and pathogenesis of RA is not yet fully understood, the disease is characterized by aggressive synovial hyperplasia (pannus formation) and inflammation (synovitis), which, if left untreated, lead to progressive destruction of joint cartilage and bone. The destructive lesions result from immune responses and non antigen-specific innate inflammatory processes [2]. The factors triggering RA are thought to be a combination of genetic, infectious, environmental, and hormonal factors which are all involved in complex, interrelated ways [3].

Experimental and clinical evidence indicates that immune reactivity is greater in females than in males and suggests that gonadal steroids may play an important role in the regulation of the immune response [4,5]. Many cells of the immune system have been found to possess functional sex hormone receptors, such as CD8-positive T cells, B cells and, notably, monocytes/macrophages [6]. 17β-oestradiol (E2) was found to inhibit cellular apoptosis, to increase antibody production by B cells and to exert dose-related effects on T-cell functions [7]. Androgens seem to exert effects opposite to those of E2 on immune response [8].

Collagen-induced arthritis (CIA) is an experimental autoimmune mediated poly-arthritis that is well accepted in different types of rodents. CIA is induced by immunization with type-II collagen, the major constituent protein of articular cartilage. Compared to other experimental arthritis models, CIA has a similar resemblance to human RA in terms of its clinical, histological and immunological features as well as genetic linkage [9].

The aim of the present work is to study the effects of testosterone deprivation, induced by castration of male albino rats, and inhibition of aromatase-induced conversion of androgen into estrogen on collagen induced arthritis evaluated by peripheral total lymphocytic count, T- and B-lymphocytic counts, plasma levels of tumor necrosis factor-α (TNF-α), prostaglandin E2 (PGE2), immunoglobulin G, (IgG) rheumatoid factor (RF), and the level of TNF-α in the synovial fluid in male albino rats. The severity of collagen-induced arthritis was also histopathologically assessed.

Material and Methods

The study was carried out in the Physiology, Biochemistry, Pathology, and Clinical Pathology departments, Faculty of Medicine, Cairo University. The work was conducted between May and November 2012. 48 adult male albino rats aged about 12 weeks with body weights ranging from 178-200 grams were included in this study. Rats were placed under ordinary living conditions (e.g. humidity, temperature, and dark/light cycles) in the Animal House. They were kept in wire mesh cages, 8 rats per cage, with free access to rat chow and water. Animals were randomly divided into the following groups.

1. Group I (control group, n=8):

This group included 8 rats and represented the control placebo group. They were injected intradermally with 0.1 ml isotonic saline in a single dose at the base of the rat tail.

2. Group II (sham-operated, collagen-induced arthritis, n=8):

This group included 8 rats and represented the sham operated group in which an experimental model of autoimmune rheumatoid arthritis was induced.

3. Group III (castrated group, n=32):  

Bilateral orchiectomy was performed in this group. Castrated rats were further subdivided into the following four groups, each included 8 rats.

Group III-A (control castrated group):

Rats of this group were injected with 0.1 ml isotonic saline five days after bilateral orchiectomy.

Group III-B (castrated arthritic group):

CIA was induced in the rats of this group five days after bilateral orchiectomy.
**Group III-C (castrated testosterone treated arthritic group):**

CIA was induced in the rats of this group five days after bilateral orchiectomy. Rats were injected with testosterone enanthate (Cidotestone; Cid Drug Company, Egypt) at a dose of 6.25mg/kg. The dose was calculated in rats by using Paget table in which the rabbit's dose (25mg/kg) was multiplied by 0.25. The drug was given twice weekly via an intramuscular injection [10].

**Group III-D (castrated testosterone and Letrozol treated arthritic group):**

CIA was induced in the rats of this group five days after bilateral orchiectomy and treated with testosterone injection and co-supplemented with non-steroidal aromatase inhibitor “Letrozol” (Femara; Novartis drug company, Egypt) in a dose of 2.5mg/kg via subcutaneous injection [11]. Group III-D aimed at studying the effects of inhibiting estrogen- derived testosterone formation on the pathogenesis of collagen- induced arthritis.

At day 35 starting from CIA induction [12], immediately before animals were sacrificed, blood samples were withdrawn from retro-orbital plexus using capillary tubes and blood was collected in 10ml Eppendorf heparinized tubes. 0.5ml blood was used for Determination of blood total lymphocyte count, T-lymphocyte count, B-lymphocyte count, and monocyte count. Plasma was separated by centrifugation of the remaining blood and stored at -80°C.

**Induction of collagen- induce arthritis:**

CIA was induced in rats by multiple intradermal injections at the base of the tail and into three or five other sites on the back, of 250µg of bovine CII (type II collagen) in 12.5 µl of 0.1 M acetic acid emulsified in an equal volume of Complete Freund’s adjuvant containing 2mg dry weight of Mycobacterium tuberculosis/ml. Rats were challenged again with the same antigen preparation 7 days later. Disease developed about 11 days after the second immunization [13].

**Surgical procedure of bilateral orchiectomy:**

Rats were anaesthetized by intraperitoneal injection of sodium thiopental (Egyptian International Pharmaceutical Industries Company. EIPICO) at a dose of 40mg/kg body weight. Castration was performed as described previously [14]. Midline scrotal incision was done allowing bilateral access to the hemiscrotal content. After exposing each testicle, the spermatic cord was ligated and the testicle was removed. The skin was then closed with silk sutures and local antibiotic skin ointment [Terramycin] was applied with Anaflex powder, then twice daily for the next 5 days. Sham operated rats were subjected to scrotal incision which is then closed by silk sutures. Finally, the rats were housed, 8 rats in separate cages and allowed free access to rat chow and water.

**Measurements of plasma TNF-α, rheumatoid factor, PGE2, IgG, and TNF-α in synovial fluid in knee joints of hind limbs:**

Plasma and supernatant TNF-α, from synovial fluid, were measured by enzyme-linked immunosorbent assay (ELISA) using an enzyme-linked immunosorbent kit (rat TNF-α ELISA kit, Diaclone, Besançon, France). Rheumatoid Factor (RF) (IgM) levels were determined using ELISA kits (DRG Instruments GmbH, Germany). PGE2 concentrations were determined using the PGE2 ELISA Kit (R&D Systems, Minneapolis, MN) while Immunoglobulin G (IgG) concentrations were determined using the ELISA Kit (Bio Supply, UK). Serum testosterone was measured using Rat Testosterone (T) ELISA kit (DRG Instruments GmbH, Germany). All measurements were performed according to the manufacturer’s protocols. These measures were performed in Biochemistry Department.

**Determination of blood total lymphocyte count, T-lymphocyte count, B-lymphocyte count, and monocyte count:**

Blood sample were analyzed using an automated analyzer in Clinical Pathology Department in Clinical Pathology department.

**Histopathological examination:**

After scarification, both knee joints of hind limbs were separated and trimmed down of the surrounding soft tissues, fixed, decalcified, and paraffin embedded. Sections (5µm) were stained with hematoxylin and eosin and scored according to the following scale: 0, no inflammation; 1, slight thickening of synovial cell layer and/or some inflammatory cells in the sub-lining; 2, thickening of synovial lining, infiltration of the sub-lining, and localized cartilage erosions; and 3, infiltration in the synovial space, pannus formation, cartilage destruction, and bone erosion [15]. Histopathological examination was performed in Pathology department.

**Statistical analysis:**

Data were expressed as mean standard deviation. The difference between two groups was assessed by using student t-test for unpaired data. p<0.05 values are considered statistically significant
and \( p > 0.05 \) values are considered statistically insignificant \([16]\).

### Results

Effects of collagen-induced arthritis (CIA), castration, CIA and castration without and with testosterone replacement, and CIA with testosterone replacement and aromatase inhibition by Letrozol on peripheral blood total lymphocytic count, T-lymphocyte, B-lymphocyte, and monocyte counts (cell/\( \mu \)L\(^3\)).

Table (1) and Figs. (1,2) demonstrated that, compared to control group I, collagen-induced arthritis in sham operated male albino rats (group II) resulted in significant \((p < 0.05)\) increases in the mean values of peripheral blood total lymphocytic count, T-lymphocyte, B-lymphocyte, and monocyte counts.

<table>
<thead>
<tr>
<th>Normal rat</th>
<th>Bilateral orchiectomy</th>
<th>Collagen-induced arthritis</th>
<th>Testosterone</th>
<th>Letrozol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Group IIIA</td>
<td>Group IIB</td>
<td>Group IIIIC</td>
<td>Group IIID</td>
</tr>
<tr>
<td>Total lymphocytic</td>
<td>1792.5±81.3</td>
<td>4658.7±259.6#</td>
<td>3077.5±182.3#</td>
<td>2375.0±157.5©</td>
</tr>
<tr>
<td>T-lymphocytes</td>
<td>1498.8±102.4</td>
<td>3511.8±233.2#</td>
<td>2026.2±147.8#</td>
<td>1293.8±298.8©</td>
</tr>
<tr>
<td>B-lymphocytes</td>
<td>293.8±56.1</td>
<td>1246.8±115.1#</td>
<td>1001.2±108.1#</td>
<td>606.3±55.8@</td>
</tr>
<tr>
<td>Monocytes</td>
<td>363.2±54.4</td>
<td>720.0±44.4#</td>
<td>501.8±33.7#</td>
<td>497.0±28.4©</td>
</tr>
</tbody>
</table>

Results are expressed as mean±S.D.
# : Significant changes \((p<0.05)\) compared to group I.
Ø : Significant changes \((p<0.05)\) compared to group II.
@ : Significant changes \((p<0.05)\) compared to group IIIB.
© : Significant changes \((p<0.05)\) compared to group IIIIC.

Testosterone deprivation, caused by castration (group IIIA), resulted in significant \((p<0.05)\) increases in the mean values total lymphocytic count, T-lymphocytes, B-lymphocyte, and monocyte counts, compared to control rats (group I).

Induction of CIA in castrated rats (group IIIB) produced significant increases in the mean values of total lymphocytic count, T-lymphocyte, B-lymphocyte, and monocyte counts compared to group II. Induction of CIA in castrated male rats with testosterone replacement (group IIIC) produced significant decreases in the mean values of total lymphocytic count, T-lymphocyte, B-lymphocyte, and monocyte counts compared to group IIIB.

Induction of CIA in castrated rats treated with testosterone replacement and aromatase inhibition (group IIID) resulted in significant decreases in the mean values of total lymphocytic count, T-lymphocyte, B-lymphocyte, and monocyte counts compared to group III C.

Effects of collagen-induced arthritis (CIA) castration, CIA and castration without and with testosterone replacement, and CIA with testosterone replacement and aromatase inhibition by Letrozol on plasma levels of tumor necrosis factor-\( \xi \) (TNF-\( \xi \)), prostaglandin E2 (PGE2), immunoglobulin G (IgG), and rheumatoid factor (RF).

Table (2) and Figs. (3-6) demonstrated that, compared to group I, collagen-induced arthritis in sham operated male albino rats (group II) resulted in significant \((p<0.05)\) increases in the mean values plasma levels TNF-\( \xi \), PGE2, IgG, and RF.

Testosterone deprivation, caused by castration (group IIIA), resulted in significant \((p<0.05)\) increases in the mean values of plasma levels TNF-\( \xi \), PGE2 and insignificant \((p>0.05)\) changes in the mean values of plasma levels of IgG, and RF compared to group I.

Induction of CIA in castrated rats (group IIIB) produced significant \((p<0.05)\) increases in the mean values of plasma levels TNF-\( \xi \), PGE2 and significant \((p>0.05)\) changes in the mean values of plasma levels of IgG, and RF compared to group II.

Induction of CIA in castrated male rats with testosterone replacement (group IIIC) produced significant \((p<0.05)\) decreases in the mean values of plasma levels TNF-\( \xi \), PGE2 and insignificant \((p>0.05)\) changes in the mean values of plasma levels of IgG, and RF compared to group IIIB.

Induction of CIA in castrated male rats with testosterone replacement and aromatase inhibition by Letrozol on plasma levels of tumor necrosis factor-\( \xi \) (TNF-\( \xi \)), prostaglandin E2 (PGE2), immunoglobulin G (IgG), and rheumatoid factor (RF).
Table (2): Effects of collagen-induced arthritis (CIA) castration, CIA and castration without and with testosterone replacement, and CIA with testosterone replacement and aromatase inhibition by Letrozol on plasma levels of tumor necrosis factor-α (TNF-α), prostaglandin E2 (PGE2), immunoglobulin G (IgG), and rheumatoid factor (RF).

<table>
<thead>
<tr>
<th></th>
<th>Normal rat</th>
<th>Sham operated-CIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
<td>Group II</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>8.6±1.91</td>
<td>24.3±5.83#</td>
</tr>
<tr>
<td>PGE2 (pg/ml)</td>
<td>30.1±3.8</td>
<td>63.85±11.85#</td>
</tr>
<tr>
<td>IgG (ng/ml)</td>
<td>10.1±2.31</td>
<td>22.44±3.91#</td>
</tr>
<tr>
<td>RF (IU/ml)</td>
<td>2.4±0.45</td>
<td>21.61±4.45#</td>
</tr>
</tbody>
</table>

Bilateral orchietomy

<table>
<thead>
<tr>
<th></th>
<th>Group IIIA</th>
<th>Group IIIB</th>
<th>Group IIIC</th>
<th>Group IIID</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α (pg/ml)</td>
<td>15.9±4.2#</td>
<td>60.2±11.5©</td>
<td>16.6±2.4@</td>
<td>12.6±1.7Ø</td>
</tr>
<tr>
<td>PGE2 (pg/ml)</td>
<td>40.3±5.4#</td>
<td>79.1±13.6Ø</td>
<td>59.33±9.4@</td>
<td>41.28±0.29Ø</td>
</tr>
<tr>
<td>IgG (ng/ml)</td>
<td>10.74±2.11</td>
<td>23.08±3.4</td>
<td>22.86±3.55V</td>
<td>20.43±2.46Ø</td>
</tr>
<tr>
<td>RF (IU/ml)</td>
<td>3.1±0.7†</td>
<td>24.3±5.5</td>
<td>22.96±4.67V</td>
<td>24.55±4.55phenotype</td>
</tr>
</tbody>
</table>

Results are expressed as mean±S.D.

# : Significant changes (p<0.05) compared to group I.
Ø : Significant changes (p<0.05) compared to group II.
© : Significant changes (p<0.05) compared to group III.
∇ : Significant changes (p<0.05) compared to group IIIB.
† : Insignificant changes (p>0.05) compared to group I.
∇ : Insignificant changes (p>0.05) compared to group II.
Φ : Insignificant changes (p>0.05) compared to group III.

Induction of CIA in castrated rats treated with testosterone replacement and aromatase inhibition (group IIID) resulted produced significant (p<0.05) decreases in the mean values of plasma levels TNF-α, PGE2 and insignificant (p>0.05) changes in the mean values of plasma levels of IgG, and RF compared to group III C.

Effects of collagen-induced arthritis (CIA), castration, CIA and castration without and with testosterone replacement, and CIA with testosterone replacement and aromatase inhibition by Letrozol on plasma levels of tumor necrosis factor-α (TNF-α) level in synovial fluid of male albino rats.

Table (3) and Fig. (7) demonstrated that, compared to group I, collagen-induced arthritis in sham operated male albino rats (group II) and testosterone deprivation, caused by castration (group IIIA) resulted in significant (p<0.05) increases in the mean values of TNF-α in synovial fluid.

Table (3): Effects of collagen-induced arthritis (CIA), castration, CIA and castration without and with testosterone replacement, and CIA with testosterone replacement and aromatase inhibition by Letrozol on tumor necrosis factor-α (TNF-α) level in synovial fluid of male albino rats.

<table>
<thead>
<tr>
<th></th>
<th>Normal rat</th>
<th>Sham operated-CIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
<td>Group II</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>1.48±0.44</td>
<td>7.31±1.5#</td>
</tr>
</tbody>
</table>

Bilateral orchietomy

<table>
<thead>
<tr>
<th></th>
<th>Group IIIA</th>
<th>Group IIIB</th>
<th>Group IIIC</th>
<th>Group IIID</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α (pg/ml)</td>
<td>4.5±2.3#</td>
<td>14.8±2.4©</td>
<td>7.9±1.3@</td>
<td>5.1±1.1©</td>
</tr>
</tbody>
</table>

Results are expressed as mean±S.D.

# : Significant changes (p<0.05) compared to group I.
Ø : Significant changes (p<0.05) compared to group II.
© : Significant changes (p<0.05) compared to group III.
@ : Significant changes (p<0.05) compared to group IIIB.
© : Significant changes (p<0.05) compared to group IIIC.

Induction of CIA in castrated rats (group IIIB) produced significant (p<0.05) increases in the mean values of TNF-α in synovial fluid compared to group II.

Induction of CIA in castrated male rats with testosterone replacement (group IIIC) produced significant (p<0.05) decreases in the mean values of plasma levels TNF-α, PGE2 and insignificant (p>0.05) changes in the mean values of plasma levels of IgG, and RF compared group IIIB.

Induction of CIA in castrated rats treated with testosterone replacement and aromatase inhibition (group IIID) resulted produced significant (p<0.05) decreases in the mean values of plasma levels TNF-α, PGE2 and insignificant (p>0.05) changes in the mean values of plasma levels of IgG, and RF compared to group III C.

Effects of collagen-induced arthritis (CIA), castration, CIA and castration without and with testosterone replacement, and CIA with testosterone replacement and aromatase inhibition by Letrozol on histopathological scoring.
Table (4): Effect of collagen-induced arthritis (CIA) on plasma testosterone level in male albino rats.

<table>
<thead>
<tr>
<th></th>
<th>Normal rat</th>
<th>Sham operated-CIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone (pg/ml)</td>
<td>119±10.23</td>
<td>113.63±10.64†</td>
</tr>
</tbody>
</table>

Results are expressed as mean±S.D.
†: Insignificant changes (p>0.05) changes compared to group.

Table (5) and Figs. (9-15) demonstrated significant (p<0.05) increase of arthritic injury in group II compared to control group (group I), insignificant (p>0.05) changes of arthritic injury in group IIIA compared to group I, significant (p<0.05) increase of arthritic injury in group IIIB compared to group II, significant (p<0.05) decrease in group IIIC compared to group IIIB, and significant (p<0.05) decrease in group IIID compared to group IIIC. These results demonstrated the importance of testosterone in ameliorating arthritic injury of CIA. Furthermore these results also denoted to the importance of inhibition of aromatase-induced estrogen production in decreasing arthritic injury of in CIA.

Table (5): Significance of histopathological scoring of arthritic injury in different groups.

<table>
<thead>
<tr>
<th></th>
<th>Bilateral orchiectomy</th>
<th>Collagen- induced arthritis</th>
<th>Testosterone</th>
<th>Letrozol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham operated-CIA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>0.00±0.00</td>
<td></td>
<td>0.38±0.52†</td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>1.88±0.35#</td>
<td></td>
<td>2.75±0.46</td>
<td>1.75±0.46</td>
</tr>
</tbody>
</table>

Results are expressed as mean±S.D.
# : Significant changes (p<0.05) compared to group I.
†: Insignificant changes (p>0.05) compared to group I.
Ø : Significant changes (p<0.05) compared to group II.
@ : Significant changes (p<0.05) compared to group IIIB.
© : Significant changes (p<0.05) compared to group IIIC.

Fig. (1): Effects of collagen-induced arthritis (CIA), castration, CIA and castration without and with testosterone replacement, and CIA with testosterone replacement and aromatase inhibition by Letrozol, on T-lymphocyte count.
# : Significant changes (p<0.05) compared to group I.
Ø : Significant changes (p<0.05) compared to group II.
@ : Significant changes (p<0.05) compared to group IIIB.
© : Significant changes (p<0.05) compared to group IIIC.

Fig. (2): Effects of collagen-induced arthritis (CIA), castration, CIA and castration without and with testosterone replacement, and CIA with testosterone replacement and aromatase inhibition by Letrozol, on B-lymphocyte count.
# : Significant changes (p<0.05) compared to group I.
Ø : Significant changes (p<0.05) compared to group II.
@ : Significant changes (p<0.05) compared to group IIIB.
© : Significant changes (p<0.05) compared to group IIIC.
Fig. (3): Effects of collagen-induced arthritis (CIA) castration, CIA and castration without and with testosterone replacement, and CIA with testosterone replacement and aromatase inhibition by letrozol on plasma levels of tumor necrosis factor-α (TNF-α).

# : Significant changes ($p<0.05$) compared to group I.
@ : Significant changes ($p<0.05$) compared to group II.
© : Significant changes ($p<0.05$) compared to group IIIB.
Ø : Significant changes ($p<0.05$) compared to group IIIC.

Fig. (5): Effects of collagen-induced arthritis (CIA) castration, CIA and castration without and with testosterone replacement, and CIA with testosterone replacement and aromatase inhibition by letrozol on plasma level of immunoglobulin G (IgG).

# : Significant changes ($p<0.05$) compared to group I.
† : Insignificant changes ($p>0.05$) compared to group I.
‡ : Insignificant changes ($p>0.05$) compared to group II.
∇ : Insignificant changes ($p>0.05$) compared to group IIIB.
φ : Insignificant changes ($p>0.05$) compared to group IIIC.

Fig. (7): Effects of collagen-induced arthritis (CIA), castration, CIA and castration without and with testosterone replacement, and CIA with testosterone replacement and aromatase inhibition by letrozol on tumor necrosis factor-α (TNF-α) level in synovial fluid of male albino rats.

# : Significant changes ($p<0.05$) compared to group I.
Ø : Significant changes ($p<0.05$) compared to group II.
@ : Significant changes ($p<0.05$) compared to group IIIB.
© : Significant changes ($p<0.05$) compared to group IIIC.
Study of the Possible Potential Role of Testosterone in Modulating Testosterone (pg/ml) and Histopathological scoring

Fig. (8): Effect of collagen-induced arthritis (CIA) on plasma testosterone level in male albino rats.

†: Insignificant changes ($p>0.05$) changes compared to group.

Fig. (9): Significance of histopathological scoring of arthritic injury in different groups.

# : Significant changes ($p<0.05$) compared to group I.
† : Insignificant changes ($p>0.05$) compared to group I.
Ø : Significant changes ($p<0.05$) compared to group II.
@ : Significant changes ($p<0.05$) compared to group IIIB.
© : Significant changes ($p<0.05$) compared to group IIIC.

Fig. (10): Section in the knee joint in group I (normal), showing normal bone trabeculi, normal cartilage and normal synovial membrane H&E, x250).

Fig. (11): Section in the knee joint in group II (CIA-sham operated rats), showing focal cartilage destruction (H&E, x250).

Fig. (12): Section in the knee joint in group III A (control group of castrated rats), showing normal bone trabeculi, normal cartilage, normal synovial membrane and mild inflammatory cellular infiltration (H&E, x250).

Fig. (13): Section in the knee joint in group IIIB (CIA-castrated rats), showing narrowing of the joint space, extensive cartilage destruction and bone erosion (H&E, x250).

Fig. (14): Section in the knee joint in group IIIC (CIA-castrated rats treated with testosterone), showing focal cartilage destruction and no bone erosion (H&E, x250).

Fig. (15): Section in the knee joint in group IIID (CIA-castrated rats treated with testosterone & letrazol), showing mild cartilage destruction and no bone erosion (H&E, x250).
Rheumatoid arthritis (RA) is a chronic, inflammatory, systemic autoimmune disease that affects about 1% of the general population and is two to three times more common in women than in men [1]. The factors triggering RA are thought to be a combination of genetic, infectious, environmental, and hormonal factors which are all involved in complex, interrelated ways [3].

Rat CIA model is a widely used animal model of inflammatory polyarthritis with similarities to RA, and primarily mediated by an autoimmune response [17]. Injection of native type II collagen (CII) leads to the development of severe polyarticular arthritis in primates and rodents. This model, which relies upon the host's own immune system, is associated with synovitis and erosion of both bone and cartilage leading to severe loss of joint function [20].

The results of the present work demonstrated that induction of CIA in male albino rats resulted in significant increases in the mean values of total lymphocytic count, T-lymphocyte, B-lymphocyte, and monocytes counts.

Systemic inflammation per se affects peripheral blood cell counts, and increased numbers of circulating activated lymphocytes have been detected in almost every autoimmune disease. Consequently, actual peripheral blood cell counts may reflect organ involvement in the underlying disease, systemic disease activity as well as immunosuppressive therapy [19].

Adaptive immune responses are vital for the efficient eradication of infectious agents, although dysregulated adaptive immune responses might also lead to autoimmune and chronic inflammatory diseases. A principal component of the adaptive immune response is the CD4+ T-lymphocytes, which can orchestrate the functional activity of both innate and adaptive immune systems [20].

CIA is induced through immunization with bovine type II collagen (CII) emulsified in Complete Freund's adjuvant. Dendritic cells (DCs) will present the CII to naïve T cells, which will differentiate mainly into interferon-γ (IFN-γ)-producing Th1 cells. Likewise, under the influence of the CII-specific Th1 cells, B cells get activated and start to produce CII-specific antibodies. These anti-CII antibodies are primarily of the IgG2a isotype (Th1-associated IgG isotype) [21]. This IgG isotype is shown to be superior in complement activation [22]. The CII-specific antibodies are shown to be crucial and sufficient for disease induction [23]. Many of the CD4+ T cells in the joint are IL-17-positive [24]. IL-17 is important both for recruitment of inflammatory cells and for joint destruction [25].

The results of the present work demonstrated that CIA produced significant increases in the mean values of plasma levels of TNF-α, PGE2, IgG, and RF, and mean value of TNF-α in synovial fluid of collagen induced-arthritis group (group II) compared to control group (group I). These molecules are thought to interact in numerous ways that can exacerbate disease [26].

T cells produce interferon-γ and other proinflammatory cytokines, which stimulate macrophages, fibroblasts, chondrocytes, and osteoclasts. Activated macrophages and fibroblasts release TNF-α, interleukin-1, interleukin-6, interleukin-15, interleukin-18, and other proinflammatory cytokines that stimulate the production of additional inflammatory mediators (chemokines, prostanoids), proteases, and growth factors and activate neutrophils, B cells, and endothelial cells. TNF-α, a central component in the cascade of cytokines induced in RA, exerts its effects through binding to two receptors, which are found on immune, inflammatory, and endothelial cells [27].

Osteoclasts activated by inflammatory cytokines are thought to be responsible for focal bone erosion. Osteoclasts are often seen in the synovium at sites of cartilage destruction in RA patients [28]. The receptor activator of NF-κB (RANK) and its ligand (RANKL) are essential factors for osteoclastogenesis [29]. IL-6 and soluble IL-6 receptor but not IL-6 alone, induced RANKL expression in RA-fibroblast-like synoviocytes. On the other hand, TNF-α and IL-17 did not induce RANKL expression, although both stimulate cell growth and IL-6 production. IL-6 and soluble IL-6 receptor directly induced osteoclastogenesis by inducing RANKL expression in RA-fibroblast-like synoviocytes [30]. IL-17 can act on synoviocytes to induce inflammatory cytokines such as TNF-α, IL-1β, and IL-6, chemokines such as IL-8, chemokine (C-X-C motif) ligand 1 (CXCL1) and CXCL2, and mediators of bone and cartilage loss such as The receptor activator of NF-κB (RANK) ligand (RANKL) and matrix metalloproteinases [31].

The results of the present work are supported by the work of Wang et al., [32] who reported increased levels of proinflammatory cytokines interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) as well as prostaglandin E2 (PGE2) in blood and expression
of cyclooxygenase-2 (COX-2) in paw cartilage of rats subjected to CIA. The cytokines TNF-α, IL-1 and RANKL are commonly elevated, locally and/or systemically, in inflammatory arthritis. These molecules are thought to interact in numerous ways that can exacerbate disease [26].

High levels of proinflammatory cytokines including TNF-α, interleukin (IL)-1 and IL-17 are detectable in the joint fluids and synovium of RA patients. These proinflammatory mediators have been demonstrated to play a vital role in the initiation and development of RA. In addition, these cytokines are closely associated with the production of biologically active lipid mediators, including prostaglandin E2 (PG E2) [33].

Proinflammatory cytokine-activated cells, including synovial cells, chondrocytes, macrophages and monocytes, are the primary sources of PGE2 in the inflamed joints of RA patients. Biosynthesis of the PGE2 is dependent on sequential enzymatic processes beginning with the release of arachidonic acid from membrane phospholipids by phospholipase. The arachidonic acid is further metabolized to PGH2 by cyclooxygenase (COX), which consists of two major isozymes, COX-1 and COX-2. Under basal conditions, COX-1 is constitutively expressed in most tissues, while COX-2 is significantly up-regulated in specific types of cells and tissues, including the synovial tissue. The arthritic severity in murine arthritis is positively correlated with the IgG autoantibody response to CII [40]. In addition, transfer of CII-specific antibodies elicits synovitis in naive recipients [41]. Autoantibodies form immune complexes (IC) with their cognate antigen. ICs are potent activators of a variety of effector mechanisms including activation of effector cells via cross-linking different receptors for the fragment crystallizable (Fc part) of IgG, Fcγ receptor, expressed on the surface of these cells, and interactions with the complement system. The prominent role of autoantibodies and immune complexes in the pathophysiology of RA has brought Fc receptor and complement in the forefront of interest in arthritis research [42].

One of the earliest identified autoantibodies to be associated with RA is rheumatoid factor (RF), an antibody directed against the constant region of IgG. However, its association with RA is not very specific: only 60-70% of all RA patients are positive for RF, and RF is also found in other disorders [43]. The recently identified anticyclic citrullinated peptide (anti-CCP) antibodies have similar sensitivity, but specificity up to 99% [44]. Citrullination is a posttranslational modification of proteins, in which arginine is converted to citrulline by a specialized enzyme, the peptidyl arginine deaminase. The levels of these autoantibodies are associated with disease severity [45]. In addition, therapeutic approaches targeting autoantibody producing B cells show promising results in arthritis [46]. A number of observations in murine models indicate that B cells and humoral immunity are indispensable for arthritis development. Mice deficient for B lymphocytes are protected against arthritis induced by immunization [47].

The present work demonstrated that induction of CIA in sham operated rats (group II) produced significant (p<0.05) increases in the mean values of plasma levels of IgG and RF compared to control group (group I).

One of the characteristic features of CIA is the presence of high levels of circulating anti-collagen type II (CII) antibody, which specifically targets the cartilage of the joints and exacerbates the pathology of arthritis by formation of collagen-IgG complexes and activation of the complement cascade. The arthritic severity in murine arthritis

PGE2 has been reported to modulate immunologic events including dendritic cell maturation, macrophage activation, B-cell and T-cell function [36]. The levels of type II collagen (CII)-specific IgG subclasses (total IgG, IgG1, IgG2a, IgG2b, IgG2c and IgG3) are significantly reduced in mPGES knockout mice during the development of CIA, when compared to control mice. The reduction of CII-specific antibody production correlates with the reduction in the incidence and severity of arthritis, suggesting an important role of mPGES-1 and its derived PGE2 in the development of acquired immune response in CIA [37]. PGE2 played an important role in inflammation and it triggered an acute inflammatory response characterized by edema, pain and infiltration of leucocytes [38]. TNF-α was shown to be a prominent inducer of COX-2 expression and eicosanoid production [39] and this resulted in an increased PGE2 level.

The present work demonstrated that induction of CIA in sham operated rats (group II) produced significant (p<0.05) increases in the mean values of plasma levels of IgG and RF compared to control group (group I).
The results of the present work demonstrated insignificant \( p > 0.05 \) changes in the mean value of serum testosterone level in group II, in which CIA was induced, compared to control group. On the contrary, Bruot and Clemens \[50\] reported that Leydig cells in crude interstitial cell preparations from arthritic rats secreted significantly less testosterone in response to human chorionic gonadotropin stimulation than cells from non-arthritic animals. They suggested that testicular macrophages secrete a factor that may be important in the regulation of testosterone production in the adjuvant-induced arthritic rat. The difference in results may be related to the method of induction of arthritis, species and age of animals, genetic contribution, or duration of the disease.

The results of the present work (Tables 1-3) demonstrated that castration (group III-A) produced significant \( p < 0.05 \) increases in the mean values of peripheral blood total lymphocytic count, T-lymphocyte, B-lymphocyte and monocyte counts compared to non-castrated control rats (group I). Castration also produced significant \( p < 0.05 \) increases in the mean values of plasma levels of TNF-\( \xi \), PGE2, and insignificant changes \( p > 0.05 \) in the mean values of plasma levels of IgG and RF compared to group I. Synovial fluid level of TNF-\( \xi \) was significantly increased \( p < 0.05 \) while and histopathological score was insignificantly \( p > 0.05 \) changed compared to group I.

These results are supported by the work of Olsen and Kovacs \[51\] who reported an effect of androgenic hormones on parameters of thymic function. In humans, treatment with luteinizing hormone releasing hormone analogs, with resultant hypogonadotropic hypogonadism, has been found to result in increases in total peripheral T-cells, including CD4+ and CD8+ cells, as well as increases in recent thymic emigrants \[50\]. Olsen and Kovacs \[52\] confirmed the finding of increased numbers of naïve T-cells in the circulation of two androgen-deficient men, and demonstrated that restoration of physiologic levels of testosterone results in reversal of this increased thymic output of T-cells.

There is an acceleration of thymic decline after puberty, more pronounced in males compared to females, suggesting that increasing levels of sex hormones contribute to the involution process. Among these hormones, androgens in particular exert considerable influence on the size and composition of the thymus \[53\]. Exogenous administration of androgens in adult rodents results in an altered cell trafficking, reduced thymocyte proliferation, and an increase in thymocyte apoptosis \[53\]. Conversely, removal of androgen by castration results in thymic enlargement and increased thymopoiesis \[54\]. Despite the clinical importance, the exact mechanisms by which thymic homeostasis is regulated by androgens remain incompletely understood \[54\].

Induction of collagen induced arthritis (CIA) in castrated rats (group IIIB) produced significant \( p < 0.05 \) increases in the mean values of peripheral blood total lymphocytic count, T-lymphocyte, B-lymphocyte and monocyte counts and significant \( p < 0.05 \) increases in the mean values of plasma levels of TNF-\( \xi \) and PGE2 while the mean values of plasma levels of IgG and RF are insignificantly \( p > 0.05 \) changed. Moreover, the mean value of TNF-\( \xi \) level in synovial fluid and histopathological score were significantly \( p < 0.05 \) increased compared to sham-operated and CIA-induced group (group II).

The results of the present work also demonstrated that induction of CIA in castrated rats which were treated with testosterone replacement (group IIIC) resulted in significant \( p < 0.05 \) decreases in the mean values of peripheral blood total lymphocytic count, T-lymphocyte, B-lymphocyte and monocyte counts and significant \( p < 0.05 \) decreases in the mean values of plasma levels of TNF-\( \xi \), PGE2, and insignificant changes \( p > 0.05 \) in the mean values of IgG and RF compared to group IIIB. The synovial fluid level of TNF-\( \xi \) and histopathological score were also significantly \( p < 0.05 \) decreased compared to group IIIB. These results demonstrate immunosuppressive modulatory effects of testosterone in CIA.

Testosterone is the predominant androgenic hormone and has a range of biological actions. The greater incidence of immune-mediated disease in women and androgen-deficient men has been attributed to the immunosuppressive effects of androgens compared with estrogens \[55\]. Testosterone has immunosuppressive properties concerning macrophages, T-cells, and B-cells. Reduced testosterone concentrations thus affect the anti-inflammatory ability of the immune system, and a chronic inflammatory state may develop \[56\].

In the present work, the mean values of plasma levels of TNF-\( \xi \) and PGE2 revealed significant \( p < 0.05 \) increases in castrated rats (group IIIA) compared to control rats (group I), and a significant \( p < 0.05 \) decrease in castrated rats with testosterone replacement subjected to CIA (group IIIC), compared to castrated rats in rats subjected to CIA.
Expression of the androgen receptor has been documented in lymphoid and non-lymphoid cells of thymus and bone marrow, but not in mature peripheral lymphocytes. This expression pattern suggests that the major impact of androgens must be on the developmental maturation of T and B lymphocytes rather than on the mature effector cells [49].

The insignificant change in IgG and RF in castrated rats, compared to control, is supported by the work of Viselli et al., [60] who reported that the levels of in vitro antibody synthesis (IgM, IgG, IgA) were not higher in castrated animals compared to controls and suggested that androgen deprivation results in a relative decrease in the number of mature peripheral T-cells.

Increase in circulating B-cells following castration reflects the appearance of large numbers of newly emigrated immature and mature B-cells in the periphery, although the relative increase was considerably greater in newly emigrated population. These newly emigrants normally constitute 10-15\% of the adult B-cells, whereas in castrated animals they represent up to 45\% of total circulating B-cell pool [61]. Similar increase in newly emigrated B-cell is observed in mice with mutation of androgen receptors that ablates the responsiveness to the hormone [62]. Thus, a loss of androgen production or function can singly increase B-cell lymphopoiesis [61]. Immature B-cells differ from their mature counterparts in that they are refractory to stimulation [63].

Androgens exert suppressive effects on both humoral and cellular immune responses and seem to represent natural anti-inflammatory hormones; in contrast, estrogens exert immunoenhancing activities, at least on humoral immune response. Low levels of gonadal androgens (testosterone/dihydrotestosterone) and adrenal androgens (dehydroepiandrosterone and its sulfate), as well as lower androgen/estrogen ratios, have been detected in body fluids (that is, blood, synovial fluid, smears, salivary) of both male and female rheumatoid arthritis patients, supporting the possibility of a pathogenic role for the decreased levels of the immune-suppressive androgens. Several physiological, pathological, and therapeutic conditions may change the sex hormone milieu and/or peripheral conversion, including the menstrual cycle, pregnancy, the postpartum period, menopause, chronic stress, and inflammatory cytokines, as well as use of corticosteroids, oral contraceptives, and steroid hormonal replacements, inducing altered androgen/estrogen ratios and related effects. Therefore, sex hormone balance is still a crucial factor.
in the regulation of immune and inflammatory responses, and the therapeutical modulation of this balance should represent part of advanced biological treatments for rheumatoid arthritis and other autoimmune rheumatic diseases [5].

Aromatase gene (CYP19) encodes aromatase, which catalyzes the aromatization of the androgens androstenedione and testosterone to estrone and estradiol, respectively. Aromatase has been found in synoviocytes [64]. Cutolo et al., [65] suggest an accelerated peripheral metabolic conversion of upstream androgen precursors to 17β-estradiol occurs in RA. The present work studied the effects of aromatase inhibition on CIA in castrated rats supplemented with testosterone replacement.

Results of the present work demonstrated that castrated rats maintained on testosterone replacement and aromatase inhibitor and subjected to CIA (group IIID) revealed significant ($p<0.05$) decreases of the mean values of total lymphocytic count, T-lymphocyte, B-lymphocyte, and monocyte counts compared to castrated rats maintained on testosterone replacement and subjected to CIA (group IIIC). This was associated with significant ($p<0.05$) decreases in the mean values of plasma levels of TNF-α, PGE2, while the mean value of IgG and RF showed insignificant ($p>0.05$) change. Moreover, the mean value of synovial fluid TNF-α and histopathological score of arthritic injury were significantly ($p<0.05$) decreased compared to group IIIC.

Experimental and clinical evidence indicates that immune reactivity is greater in females than in males and suggests that gonadal steroids may play an important role in the regulation of the immune response [4]. Many cells of the immune system have been found to possess functional sex hormone receptors, such as CD8-positive T-cells, B-cells and, notably, monocytes/macrophages [6]. 17β-oestradiol (E2) was found to inhibit cellular apoptosis, to increase antibody production by B cells and to exert dose-related effects on T-cell functions [7]. Androgens seem to exert effects opposite to those of E2 on immune response [8]. Clinical epidemiology clearly confirms a higher prevalence of autoimmune diseases in female subjects when compared with male subjects [66]. The insignificant changes in IgG and RF, observed in group IIID, compared to group IIIC may be related to concentration of aromatase-induced estrogen synthesis in group IIIC or may be related to difference in species, dose of androgen used, or the amount of androgen resulting from aromatase inhibition in group IIID.

The significant decreases in plasma levels of TNF-α, PGE2 and synovial fluid TNF-α level following aromatase inhibition in group IIID can be explained by decreased formation of estrogen in arthritic joints. Increased estrogen concentrations in RA synovial fluid from patients of both sexes likely result from the proinflammatory cytokines TNF-α, IL-1β, and IL-6, which accelerate the metabolic conversion of estrogens from androgens by inducing the synovial tissue aromatase [67]. As a consequence, locally increased estrogen levels might exert activating effects on synovial cell proliferation, including macrophages and fibroblasts, stimulation or further activation of synovial cells to produce cytokines would further reduce the availability of anti-inflammatory androgens in local tissues [68]. In this regard, it may be relevant that male patients with RA seem to derive more benefit from anti-TNF-α strategies than do female patients with RA [69]. Because male patients have higher levels of circulating androgens than do female patients, they probably have relatively higher levels of locally increased estrogen concentrations in arthritic joints. Increased estrogen concentrations in RA synovial fluid from patients of both sexes likely result from the proinflammatory cytokines [71]. Therefore, dose-related conversion of estrogens to pro-inflammatory or anti-inflammatory downstream metabolites might support a dual role of estrogens (pro- or anti-inflammatory), for example, during estrogen replacement therapy, depending on the local concentration of 16β-hydroxyestrone or 2-hydroxyestrogens [72].

Summary and Conclusion:

Androgen deprivation aggravates collagen-induced arthritis in male rats. Although androgen replacement therapy improved pro-inflammatory and histopathological changes of CIA in castrated rats, yet androgen therapy for male patients with rheumatoid arthritis are not recommended because of the risks of androgen therapy especially in old patient. Androgen therapy is also not recommended since aromatase-induced estrogens synthesis; include pro-inflammatory and anti-inflammatory metabolites, which are present at relatively low levels in RA synovial fluid. Blockade of TNF-α, PGE2 and synovial fluid TNF-α level following aromatase inhibition in group IIID can be explained by decreased formation of estrogen in arthritic joints. Increased estrogen concentrations in RA synovial fluid from patients of both sexes likely result from the proinflammatory cytokines TNF-α, IL-1β, and IL-6, which accelerate the metabolic conversion of estrogens from androgens by inducing the synovial tissue aromatase [67]. As a consequence, locally increased estrogen levels might exert activating effects on synovial cell proliferation, including macrophages and fibroblasts, stimulation or further activation of synovial cells to produce cytokines would further reduce the availability of anti-inflammatory androgens in local tissues [68]. In this regard, it may be relevant that male patients with RA seem to derive more benefit from anti-TNF-α strategies than do female patients with RA [69]. Because male patients have higher levels of circulating androgens than do female patients, they probably have relatively higher local aromatase-mediated production of proinflammatory estrogens. Therefore, blockade of TNF-α-induced up-regulation of aromatase would particularly increase the level of androgens in males, leading to a better clinical outcome [70].

Increased concentrations of estrogen in RA synovial fluid are more specifically the hydroxylated forms, in particular 16β-hydroxyestrone, which is a mitogenic and proliferative endogenous hormone. In contrast, the 2-hydroxylated forms inhibit the growth-promoting effects of 17β-estradiol and are present at low levels in RA synovial fluid [71]. Therefore, dose-related conversion of estrogens to pro-inflammatory or anti-inflammatory downstream metabolites might support a dual role of estrogens (pro- or anti-inflammatory), for example, during estrogen replacement therapy, depending on the local concentration of 16β-hydroxyestrone or 2-hydroxyestrogens [72].
induced up-regulation of aromatase would particularly increase the level of androgens in males, leading to a better clinical outcome.

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References


