Sex steroid receptors in rheumatoid arthritis

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ABSTRACT

Rheumatoid arthritis (RA) is a disease characterized primarily by chronic inflammatory synovitis and is well-known to be associated with significant sex differences in its prevalence and clinical features. Sex steroids have been proposed to be involved in the pathogenesis of RA, but details pertaining to the expression of sex steroid receptors in RA synovial tissue have yet to be fully characterized. In the present study, we examined oestrogen receptor (ER) α, ERβ, progesterone receptor (PR) and androgen receptor (AR) mRNA expression using real-time reverse transcriptase–PCR (RT-PCR) in eight female RA synovial tissues and six female synovial tissues without inflammation, and determined immunolocalization of ERα, ERβ, PR-A, PR-B and AR using immunohistochemistry in synovial tissues obtained from 22 RA patients. Real-time RT-PCR analysis demonstrated the expression of ER, PR and AR mRNAs in both RA and non-inflamed synovial tissues. Relative abundance of ER mRNAs was significantly higher in RA synovial tissue than non-inflamed synovial tissue (P < 0.05). In addition, the relative ERα/ERβ mRNA expression ratio was significantly lower in RA than non-inflamed synovial tissue (RA, 2.34 ± 1.60; and non-inflamed, 20.7 ± 19.1; P < 0.05). There were no significant differences in relative abundance of PR mRNA. Relative abundance of AR mRNA was significantly lower in RA (P < 0.05). Immunoreactivity for ERα, ERβ, PR-B and AR was detected in the lining cells, inflammatory cells and fibroblasts in all the patients examined. The labelling indices for ERβ and PR-B were more abundant in both lining cells (ERβ, 54.2 ± 12.2 %; PR-B, 73.6 ± 18.9 %) and inflammatory cells (ERβ, 74.6 ± 16.2 %; PR-B, 75.9 ± 16.1 %) than in fibroblasts (ERβ, 36.5 ± 15.6 %; PR-B, 49.4 ± 18.0 %). Labelling indices for ERα and AR were significantly higher in lining cells (ERα, 14.4 ± 8.6 %; AR, 31.2 ± 11.3 %) and fibroblasts (ERα, 12.1 ± 7.5 %; AR, 20.1 ± 9.6 %) than those in inflammatory cells (ERα, 5.7 ± 3.3 %; AR, 9.2 ± 4.4 %). There were significant differences (P < 0.05) in the labelling indices for ERα, ERβ and PR-B between men and women under 50 years of age in fibroblasts of RA synovial tissues. These results indicate that sex steroid receptors are present in RA and non-inflamed synovial tissues, including inflammatory cells in RA, and suggest that sex steroids may play important roles in the regulation of inflammation of RA synovial tissue.

INTRODUCTION

Rheumatoid arthritis (RA) is a disease characterized primarily by a chronic inflammatory synovitis [1]. The aetiology of RA is largely unknown, but there are significant sex differences regarding the prevalence and clinical manifestations of RA. RA is more likely to occur in women, but it is also true that older women and men are almost equally affected by this inflammatory disease [2]. In addition, the clinical severity of symptoms in female

Key words: immunohistochemistry, receptor, rheumatoid arthritis, sex steroid, synovial tissue, real-time reverse transcriptase–PCR.

Abbreviations: AR, androgen receptor; ER, oestrogen receptor; GADPH, glyceraldehyde-3-phosphate dehydrogenase; PR, progesterone receptor; RA, rheumatoid arthritis; RT-PCR, reverse transcriptase–PCR.

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patients diagnosed with RA has been demonstrated to be influenced by changes in sex steroid environment such as pregnancy, delivery and the menstrual cycle [3,4]. The onset of RA symptoms has also been suggested to be often associated with menopausal transition [5]. These epidemiological findings all indicate possible biological roles for sex steroids such as oestrogens in the pathogenesis of this disease.

Several investigators have examined the presence of sex steroid receptors in RA synovial tissues. Ghosh and Seshadri [6] reported that a human cell line derived from the synovial fluid of the knee joint in a patient suffering from degenerative arthritis expressed abundant oestrogen receptor (ER) and progesterone receptor (PR) mRNA. Furthermore, a study by Ushiyama et al. [7] found ER expression to be localized to the synovial lining cells and sublining macrophage-like cells in human RA synovial tissues. Khalihali-Ellis et al. [8] reported the presence of functional ERα in fibroblast-like synoviocytes obtained from RA patients, and Cutolo et al. [9] demonstrated further the expression of oestrogen-binding sites in macrophage-like synoviocytes, sublining macrophages and T-lymphocytes of RA synovial tissues, as well as androgen receptors (ARs) in macrophage-like synoviocytes in RA synovial tissues [10]. In addition, Castagnetta et al. [11] reported the expression of AR in the synovial lining cells of the RA synovial tissue. All of these studies indicated the presence of sex steroid receptors in the cellular constituents of RA synovial tissues, but the expression of sex steroid receptors in non-pathological (i.e. non-inflamed) synovial tissue has not been examined. Therefore, the biological significance of the expression of these receptors in RA synovial tissue has not been fully characterized. In addition, the specific localization of these sex steroid receptors has not been examined in detail in RA synovial tissue.

Histological changes in RA synovial tissue are associated with numerous abnormalities, such as proliferation of the synovial lining layer, infiltration of various subtypes of lymphocytes and plasma cells and the accumulation of fibroblasts and other cell types [12,13]. Sex steroids have been postulated to influence the biological activities of these various cell types. Possible changes occurring in synovial tissue as a result of sex steroid actions are, in general, considered to represent a reflection of the overall hormonal interactions within the inflamed synovial tissue. Therefore, in order to obtain a better understanding of the possible involvement of sex steroids in RA synovial tissue, it is important to study the expression of sex steroid receptors in RA and non-inflamed synovial tissues. In addition, it is also necessary to localize these receptors in each histological component of RA synovial tissue. In the present study, we examined the expression of ERα, ERβ, PR and AR mRNA using quantitative real-time reverse transcriptase–PCR (RT-PCR) and the localization of ERα, ERβ, PR-A, PR-B and AR using immunohistochemistry in RA and non-pathological synovial tissues.

MATERIALS AND METHODS

Tissue samples

Fourteen female synovial tissues were taken from knee joints for real-time RT-PCR study. Eight were RA cases (mean age, 65 years) and six were non-inflamed cases (mean age, 58 years). RA synovial tissues were collected from Japanese RA patients. Non-inflamed synovial tissues were obtained from knees without inflammation; three were from autopsy cases and three were from surgery cases. These tissue samples were dissected into small pieces, frozen in liquid nitrogen and stored at −80°C.

Another 22 synovial tissues obtained from Japanese RA patients who underwent knee joint surgery were used for immunohistochemistry. In the immunohistochemical investigation, RA patients were tentatively classified into three groups: women over 50 years (n = 10; mean age, 61 years), women under 50 years (n = 6; mean age, 37 years) and men (n = 6; mean age, 61 years). All specimens for immunohistochemical examination were fixed in 10% neutral formalin for 18 h at room temperature and embedded in paraffin. These specimens were subsequently sectioned at 3 μm and mounted on silane-coated glass slides (Matsunami Co. Ltd, Tokyo, Japan). None of the patients was treated with glucocorticoids for at least 12 months prior to surgery.

Informed consent was obtained from each patient. All the RA patients fulfilled the American College of Rheumatology criteria for adult RA [14]. This investigation was approved by the Ethics Committee on Human Study at Tohoku University School of Medicine.

Real-time RT-PCR

Total RNA was extracted from frozen synovial tissue samples using the TRIzol reagent (Invitrogen, Tokyo, Japan), a monophasic solution of phenol and guanidine isothiocyanate. RNA concentrations were determined spectrophotometrically. Total RNA (2 μg) was denatured at 70°C for 10 min and then reverse-transcribed using oligo(dT) primers and SuperScript II reverse transcriptase (Invitrogen) in a final reaction volume of 20 μl at 42°C for 60 min. The reaction was terminated by heating at 70°C for 15 min. Real-time PCR amplification was performed on a LightCycler (Roche Diagnostics, Mannheim, Germany) using the LightCycler FastStart DNA Master SYBR Green I (Roche Diagnostics). A master mix of the following reaction components was prepared to the indicated final concentrations: 13.4 μl of water, 1.6 μl of MgCl2 (3 mM), 1.0 μl of forward primer (1.0 μM), 1.0 μl of reverse
primer (1.0 µM) and 2.0 µl of the LightCycler FastStart DNA Master SYBR Green I. A portion (19 µl) of master mix was loaded into glass capillaries and 20 ng of cDNA in 1 µl was added as the PCR template. The capillaries were closed, centrifuged in a microcentrifuge and placed into the cycling rotor. The primer sequences and optimal variables used in the present study are listed in Table 1 [15–19]. Gene-specific primers for human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used as an internal control. To avoid amplification of any genomic DNA, the forward and reverse primers for each gene were chosen from different exons. An initial denaturing step at 95 °C for 10 min was followed by 40 cycles of denaturing at 95 °C for 15 s, annealing at optimal temperature (ERα, 62 °C; ERβ, 66 °C; PR, 70 °C; and AR, 66 °C) for 10 s and extension at 72 °C for 8 s (PR) or 10 s (ERα, ERβ and AR). Real-time PCR monitoring was achieved by measuring the fluorescent signal at the end of the annealing phase for each cycle. Each run consisted of five external standards, a negative control without a template and patient samples with unknown mRNA concentrations. External standards for these receptor mRNAs were prepared by 5-fold serial dilutions of positive control cDNA. As a positive control, frozen tissues of breast carcinoma were used for ERα, ERβ and PR and those of normal testis were used for AR. After PCR, the LightCycler software program plotted logarithmic values of fluorescence against cycle number and a standard curve was produced by measuring the crossing point of each standard and plotting them against the logarithmic values of the concentrations. Relative mRNA abundance in each sample was then calculated automatically by reference to the standard curve constructed each time by the LightCycler software. The level of expression of ERα, ERβ, PR and AR was normalized by GAPDH expression. Following RT-PCR, the reaction products were resolved on a 2 % (w/v) agarose gel by electrophoresis. Gels were stained with ethidium bromide to visualize PCR product size. Negative control experiments lacked the cDNA substrate to check for the possibility of exogenous contaminant DNA. No amplified products were detected under these conditions.

**Immunohistochemistry**

The monoclonal antibody for ERα (NCL-ER-6F11) was obtained from Novocastra Laboratories (Newcastle, U.K.), and the polyclonal antibody for ERβ (66-629) was obtained from Upstate Biotechnology (Lake Placid, NY, U.S.A.). Monoclonal antibodies for two isoforms of PR-A (hPRa7) and PR-B (hPRa2) were purchased from NeoMarkers (Fremont, CA, U.S.A.). These antibodies were raised in mice against PR isoforms obtained from a human endometrial carcinoma (EnCa 101). The hPRa2 antibody recognizes only the PR-B isoform, whereas the hPRa7 antibody recognizes both PR-A and PR-B in immunoblot analysis [20]. However, Mote et al. [21] reported that hPRa7 did not recognize PR-B during immunohistochemistry in fixed tissues even following antigen retrieval, as evidenced by the absence of immunostaining by this antibody in the PR-B-expressing MDA-MB-231/PR-B cell line. This is currently considered to be due to the inaccessibility of the epitope on PR-B recognized by hPRa7 in 10 % formalin-fixed and paraffin-embedded tissue specimens [21]; this, in turn, may be due to an alteration in the conformation of the molecule in which hPRa7 epitope is located in such a way as to reduce its accessibility during immunohistochemistry. The monoclonal antibody for AR (AR411) was obtained from Dako (Carpinteria, CA, U.S.A.).

Immunohistochemical staining was performed employing the streptavidin–biotin amplification method using a Histofine Kit (Nichirei, Tokyo, Japan). Details of immunohistochemical procedures used in the present study have been described previously [22]. Antigen retrieval was used for all the receptors by autoclaving at 120 °C for 5 min. Dilutions of the primary antibodies used in the present study were as follows: ERα, 1:50; ERβ, 1:50; PR-A, 1:200; PR-B, 1:200; and AR, 1:100. The antigen–antibody complex was visualized with 3,3-diaminobenzidine solution [1 mM 3,3-diaminobenzidine, 50 mM Tris/HCl (pH 7.6) and 0.006 % H2O2] and counterstained with haematoxylin. Tissue sections of an invasive ductal carcinoma of the breast were used as positive controls for ERα, ERβ, PR-A and PR-B and normal prostate tissue was used as a positive control for AR. As a negative control, normal rabbit or mouse IgG was used instead of the primary antibodies. No specific immunoreactivity was detected in these tissue sections.

**Scoring of immunoreactivity**

The immunoreactivity of sex steroid receptors was evaluated separately in three major components of inflamed RA synovial tissue, i.e. proliferating lining cells, infiltrating inflammatory cells and accumulating

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**Table 1** Oligonucleotide primer sequences used for real-time RT-PCR analysis

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Primer</th>
<th>Sequence</th>
<th>GenBank&lt;sup&gt;®&lt;/sup&gt;/EMBL accession number</th>
<th>Nucleotide number</th>
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<tr>
<td>ERα</td>
<td>Sense</td>
<td>AGAGCTGCACGCTCTGAGC</td>
<td>M12674</td>
<td>995–1161</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>TTGGAGCTCTCTTCAGTTTTT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERβ</td>
<td>Sense</td>
<td>GTCTAATGGTGAGTATGAT</td>
<td>AB006590</td>
<td>1313–1554</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>CAGAGCTCTGACTTCTG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>Sense</td>
<td>AACACAAAACCTGACACCT</td>
<td>M15716</td>
<td>2288–2486</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>GGACTCATTTTGGCAGT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AR</td>
<td>Sense</td>
<td>CTACCACAGCTCTGGACTC</td>
<td>M23263</td>
<td>3103–3349</td>
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<tr>
<td></td>
<td>Antisense</td>
<td>CAGAGCGGACGACAGTCTG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td>Sense</td>
<td>TGACGGGAGCTACTGG</td>
<td>M32197</td>
<td>731–1038</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>TCCACACTGCTGGTGTTG</td>
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</table>
fibroblasts. Statistical analyses were performed on the findings of immunolocalization in target cells for sex steroid receptors. Two of the authors initially determined the field of view simultaneously using a double-headed light microscope. Scoring for ERα, ERβ, PR-A, PR-B and AR in these cells was performed on high-power fields (×400) using a standard light microscope. Only distinctive intranuclear immunoreactivity was considered positive. In each case, more than 500 cells of each of three components were counted and the percentage of immunoreactivity, i.e. labelling index, was independently determined. When inter-observer differences were greater than 5%, the immunostained slides were re-examined simultaneously using a double-headed light microscope and the labelling index was determined. When inter-observer differences were less than 5%, the mean value was obtained as the labelling index. All results are expressed as the means ± S.D.

**Statistical analysis**
Statistical significance of differences in mRNA expression of sex steroid receptors and ERα/ERβ mRNA ratio between RA and non-inflamed synovial tissue was assessed by Student’s t test. We used Bonferroni’s test for comparison of labelling indices among the three groups [23], and Student’s t test was used for comparison of the labelling indices between ERα and ERβ in three synovial components. P values less than 0.05 were considered significant.

**RESULTS**

**Real-time RT-PCR**
Expression of ERα, ERβ, PR and AR mRNA was detected in all 14 cases examined as a specific single band (168 bp for ERα, 228 bp for ERβ, 199 bp for PR and 246 bp for AR). A band corresponding to the expected size of GAPDH (307 bp) was detected in all samples examined (Figure 1). The level of ERα mRNA expression was significantly higher in RA synovial tissues than in non-inflamed synovial tissues (Table 2). The level of ERβ mRNA was higher in RA synovial tissues, but the differences did not reach statistical significance. The ERα/ERβ expression ratio was significantly higher (P = 0.018) in non-inflamed synovial tissue than in RA synovial tissue [RA, 2.34 ± 1.60 (n = 8); non-inflamed, 20.7 ± 19.1 (n = 6)]. The expression of PR mRNA was not statistically different between the two groups. AR mRNA expression was 10-fold higher in non-inflamed synovial tissue compared with RA synovial tissue.

**Immunohistochemistry**
ERα, ERβ, PR-B and AR immunoreactivity was detected in lining cells, inflammatory cells and fibroblasts in all 22 samples examined (Figure 2). The labelling indices for ERα, ERβ, PR-B and AR are shown in Figure 3 and Table 3. ERα immunoreactivity was found predominantly in lining cells and fibroblasts, and ERβ immunoreactivity was detected in lining cells and

<table>
<thead>
<tr>
<th>Table 2</th>
<th>ERα, ERβ, PR and AR mRNA values corrected for GAPDH mRNA values and measured by real-time RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RA synovial tissue (n = 8)</td>
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<tr>
<td>ERα/GAPDH</td>
<td>0.103 ± 0.060</td>
</tr>
<tr>
<td>ERβ/GAPDH</td>
<td>0.099 ± 0.098</td>
</tr>
<tr>
<td>PR/GAPDH</td>
<td>0.025 ± 0.013</td>
</tr>
<tr>
<td>AR/GAPDH</td>
<td>0.069 ± 0.022</td>
</tr>
</tbody>
</table>

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Sex steroid receptors in rheumatoid arthritis

Immunohistochemistry for sex steroid receptors in RA synovial tissue

RA synovium showed inflammatory changes (a). Marked nuclear immunoreactivity for ERα (b), ERβ (c), PR-B (e) and AR (f) was detected in lining cells, fibroblasts and inflammatory cells, especially in ERβ and PR-B. Immunoreactivity for PR-A (d) was not detected.

Labelling indices for sex steroid receptors in lining cells, fibroblasts and inflammatory cells in 22 RA cases

Significant differences were identified between the labelling indices (LI) for ERα, ERβ, PR-B and AR in all the synovial cell types examined. No significant differences were identified for ERα labelling indices in lining cells and fibroblasts, and PR-B labelling indices in lining cells and inflammatory cells.

Comparison of labelling indices between women under 50 years, women over 50 years and men

Multiple significance tests were used for comparisons among the three different groups. * and †, P < 0.05 compared with the values among the three groups.

<table>
<thead>
<tr>
<th></th>
<th>Under 50 years of age (n = 6)</th>
<th>Over 50 years of age (n = 10)</th>
<th>Men (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ERα</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lining cells</td>
<td>9.2 ± 8.3</td>
<td>14.1 ± 4.6</td>
<td>20.3 ± 12.0</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>5.8 ± 5.7*</td>
<td>12.6 ± 6.5</td>
<td>17.4 ± 7.7*</td>
</tr>
<tr>
<td>Inflammatory cells</td>
<td>3.3 ± 2.9*</td>
<td>5.4 ± 2.3</td>
<td>8.6 ± 3.6*</td>
</tr>
<tr>
<td><strong>ERβ</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lining cells</td>
<td>48.0 ± 18.5</td>
<td>55.1 ± 9.6</td>
<td>58.0 ± 8.8</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>21.2 ± 7.0*</td>
<td>36.3 ± 14.3†</td>
<td>52.1 ± 9.4*†</td>
</tr>
<tr>
<td>Inflammatory cells</td>
<td>64.9 ± 22.4†</td>
<td>81.0 ± 14.9</td>
<td>73.5 ± 7.9</td>
</tr>
<tr>
<td><strong>PR-B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lining cells</td>
<td>54.5 ± 24.8*†</td>
<td>76.6 ± 11.1*</td>
<td>87.7 ± 6.2†</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>35.0 ± 24.2*</td>
<td>51.1 ± 13.9</td>
<td>61.0 ± 8.7*</td>
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<tr>
<td>Inflammatory cells</td>
<td>61.9 ± 24.6</td>
<td>79.9 ± 10.2</td>
<td>83.4 ± 4.0</td>
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<tr>
<td><strong>AR</strong></td>
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</tr>
<tr>
<td>Lining cells</td>
<td>29.5 ± 16.8</td>
<td>33.6 ± 11.2</td>
<td>29.0 ± 5.8</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>21.9 ± 14.6</td>
<td>17.6 ± 6.9</td>
<td>22.5 ± 9.0</td>
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<tr>
<td>Inflammatory cells</td>
<td>7.2 ± 6.3</td>
<td>9.3 ± 2.0</td>
<td>11.0 ± 5.5</td>
</tr>
</tbody>
</table>

Table 3

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fibroblasts, rather than in inflammatory cells. PR-A immunoreactivity was not detected in any of the samples examined. The ERβ labelling index was significantly higher (P < 0.0001) than the labelling index for ERα in each histological component of RA synovial tissue examined. The PR-B labelling index was significantly higher than the labelling indices for ERα and AR in each synovial component examined. There were significant differences between the labelling indices for these receptors in all the components examined, except for the ERα labelling index in lining cells and fibroblasts (P = 0.2659) and PR-B labelling index in lining cells and inflammatory cells (P = 0.6685). The ERα labelling index for fibroblasts (P = 0.0068) and inflammatory cells (P = 0.0045) was significantly higher in men than in women under 50 years of age (Table 3). With the exception of the ERβ labelling index for fibroblasts, there were no significant differences in the labelling indices for ERβ in lining and inflammatory cells between men and women. The PR-B labelling index in lining cells in men and women over 50 years of age was significantly higher than that of women under 50 years of age (P = 0.0105 and 0.0012 respectively; Table 3). The PR-B labelling index in fibroblasts of men was significantly higher than that of women under 50 years of age (P = 0.0123). There were no significant differences in AR labelling index in all the cell components of the RA synovial tissue between male and female cases.

**DISCUSSION**

It is generally considered that all components of synovial tissue in patients diagnosed as having RA contribute to the overall inflammatory activity of the synovial tissue [24]. In the present study, we have demonstrated the expression of sex steroid receptors in the RA synovial tissue using both real-time RT-PCR and immunohistochemistry. Previous studies [5,6,8] have demonstrated that cultured synoviocytes express mRNA for sex steroid receptors, but the present study is the first to demonstrate the presence of mRNA for sex steroid receptors in synovial tissues from RA patients and subjects without inflammation. We have also demonstrated that the levels of expression of these receptors in non-inflamed synovial tissues were different from RA synovial tissues. Synovial tissue may be the target organ of sex steroids and participate in the modulation of synovial inflammation. Immunoreactive proteins for ERα, ERβ, PR-B and AR were also detected in all cellular components of RA synovial tissues. However, our present study has identified some differences in the expression of these receptors in the different components of synovial tissue.

ERβ immunoreactivity was more abundant than ERα in all three components of RA synovial tissue, including the lining cells, fibroblasts and inflammatory cells. The lower ERα/ERβ mRNA expression ratio in RA synovial tissues suggests ERβ was more abundant than ERα. ERα and ERβ are structurally related subtypes of ER, encoded by two distinct genes [25,26] and exhibit significant functional differences [27,28]. Recent studies [6–8] have demonstrated that ERα is present in lymphocytes, macrophages and fibroblast-like synoviocytes in RA synovial tissue, but the expression of other ER subtypes has remained largely unknown in RA synovial tissue. Oestrogens have been demonstrated to act via ERβ in tissues of the central nervous system, cardiovascular system, immune system, urogenital tract, gastrointestinal tract, kidney and lungs [29]. Results from our present study demonstrated a predominant expression of ERβ in RA synovial tissues, suggesting that the oestrogenic actions are mediated predominantly through ERβ. Furthermore, oestrogen signalling, via its binding to ERβ, may play important roles in the regulation of inflammation in RA synovial tissues, but further investigations are required to clarify this.

Results from our present study have also demonstrated that ERα and PR-B, but not ERβ, were more abundant in men than in women under 50 years of age. Pregnancy has been known to ameliorate the clinical severity of RA patients, as shown in the pioneering work of Hench [30]. However, recent studies [8,31] have demonstrated that oestrogen contributes to the exacerbation or enhancement of inflammatory activity in RA synovial tissues. In addition to up-regulating interleukin-1β-induced interleukin-6 production [31], oestrogen has also been shown to exert stimulatory effects on the expression of matrix metalloproteinases in cultured fibroblast-like synoviocytes [8]. In addition, van den Brink et al. [32] have reported that adjuvant oestrogen therapy did not improve disease activity in postmenopausal women with RA. There have been controversies as to the possible clinical value of administering oral contraceptives for the prevention and/or development of RA [33,34]. In addition, oestrogens are well-known to regulate a large number of physiological processes in ER-positive cells. Further investigations are required to clarify the exact roles of oestrogenic actions through ERβ in the various cell components of RA synovial tissue.

The possible roles that progesterone plays in the pathogenesis of RA remains unknown. In the present study, immunoreactive PR-B protein was abundantly present in RA synovial tissue, in contrast with PR-A. PR-A and PR-B differ in that the PR-B protein contains an additional sequence of amino acids at its N-terminus, which has not been detected in PR-A [20,35]. The predominance of PR-B detected in RA synovial tissue in the present study is consistent with similar findings that have recently been reported in various tissues and their disorders, including ovarian endometriosis [36], metastatic lesions of gynaecologic cancers [37], human
chordomas [38] and astrocytomas grades III and IV [39]. PR-B is generally considered more efficient as a transcriptional regulator than PR-A [40,41]. In addition to their function as opposing transcription factors, PR-A and PR-B have also been demonstrated in previous studies [42] to be regulated by progesterone in both a tissue- and cell-specific manner. These results also suggest that progesterone can regulate PR isoforms in progesterone-responsive tissues, but progesterone-dependent transcription is mediated by the PR isoform that is bound to progesterone and/or related ligand. In RA synovial tissue, PR-B is considered to play an important role in the regulation of local inflammation, and progesterone has been shown to exert anti-inflammatory effects in fibroblast-like cells [8,43]. In the present study, we detected a significant difference in the relative level of expression of PR-B in RA synovial tissue between women under 50 years of age and men. These results suggest that progesterone may act through PR-B in RA synovial tissue as an anti-inflammatory steroid. Despite a higher circulating progesterone concentration, the relative paucity of PR-B in RA synovial tissue of premenopausal women may be related to the higher risk of developing RA in women than in men. In addition, ERβ and PR-B were both markedly abundant in all three components of RA synovial tissue, especially in inflammatory cells. Some interaction between ERβ and PR-B, therefore, may regulate inflammatory cells locally in the RA synovial tissue.

AR has been shown to be expressed in macrophage-like synoviocytes in RA synovial tissue [10]. Androgens, in general, exert anti-inflammatory effects in RA [44,45], which is consistent with the presence of AR immunoreactivity in lining cells, inflammatory cells and fibroblasts of RA synovial tissues in the present study. We detected no differences in the pattern of AR expression with regard to sex and age of the patients. Results of recent studies [45] suggest that the anti-inflammatory effects of androgens may be mediated by peripheral cytokine production. Lower expression of AR mRNA in RA synovial tissue detected in our present study suggests that AR expression is decreased and androgens may play a less significant role in RA synovial tissue. In addition, low serum androgen levels have been reported in RA patients [34]. However, further investigations are required to clarify the exact roles of androgens in the pathogenesis and/or development of RA.

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Received 24 September 2003; accepted 21 October 2003
Published as Immediate Publication 21 October 2003, DOI 10.1042/CS20030317