Androgen Receptors in Human Synoviocytes and Androgen Regulation of Interleukin 1ß (IL-1ß) Induced IL-6 Production: A Link Between Hypoandrogenicity and Rheumatoid Arthritis?

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ABSTRACT. ***Objective.*** To investigate the hypothesis that synoviocytes possess androgen receptors (AR) that could be modulated by the non-aromatizable androgen, dihydrotestosterone (DHT), resulting in altered levels of inflammatory cytokines.

Methods. Using molecular analyses of AR in combination with the multiprobe ribonuclease protection assay and ELISA, we investigated the presence of AR and the effect of DHT on interleukin 1ß (IL-1ß) induced expression of the IL-6 superfamily of cytokines in synoviocytes.

Results. Our studies corroborate the presence of AR in synoviocytes. DHT exerts a suppressive effect on IL-1ß induced IL-6, macrophage-colony stimulating factor (CSF), and granulocyte-CSF production by synoviocytes. This modulatory effect is exerted at both the transcriptional and translational level; 17ß-estradiol, at high concentrations, had a stimulatory effect.

Conclusion. The identification of functional AR in synoviocytes and the modulatory effect of DHT on the inflammatory process in the joint suggest a direct link between hypoandrogenicity and rheumatoid arthritis (RA) disease status. Understanding the complex regulation of inflammatory cytokines by hormones may contribute to the development of new therapeutic targets for clinical intervention in RA. (J Rheumatol 2002;29:1843–6)

Key Indexing Terms:
ANDROGEN ANDROGEN RECEPTOR
SYNOVIOCYTES RHEUMATOID ARTHRITIS INTERLEUKIN 6

Although a hypogonadal condition has been reported in autoimmune disorders such as rheumatoid arthritis (RA)[1], the contribution of hypoandrogenicity to chronic inflammation in the joint and the associated pathogenesis of RA has remained mostly unexplored.

Androgens induce gene transcription at least in part by androgen receptors (AR). Androgens regulate cytokine production [interleukin 2 (IL-2), IL-5, and IL-6] by activated T cells[2] and the presence of androgens in the lymphoid tissue during the induction of the immune response alters the development of effector T cells[3].

The proinflammatory cytokine IL-6 is elevated in serum and synovial fluid of patients with RA[4] and its level is inversely correlated with serum androgen levels[5]. In animal models of arthritis, IL-6 knockout mice are found to be resistant to antigen induced arthritis[6]. We hypothesized that synoviocytes possess AR and can respond to androgenic hormones. Thus the endocrine balance could contribute to amelioration or exacerbation of the inflammatory process in RA joints. We utilized an experimental model in which synoviocytes isolated from healthy controls were treated with inflammatory cytokine (thus simulating the status of synoviocytes from RA joints) in the presence or absence of specific hormones.

MATERIALS AND METHODS

Tissue culture. Discarded synovial tissue from 5 women (N148, N149, N150, N137 and N174, median age 34.4 yrs) who underwent joint arthroscopy for sports related injuries was used to grow synoviocytes as described[7]. Cultures of synoviocytes, negative for immune cell markers, were utilized at passage 3–6. Synoviocytes were maintained in hormone depleted, phenol red-free complete medium (24 h), then treated with dihydrotestosterone (DHT) or 17ß-estradiol in the presence or absence of IL-1ß

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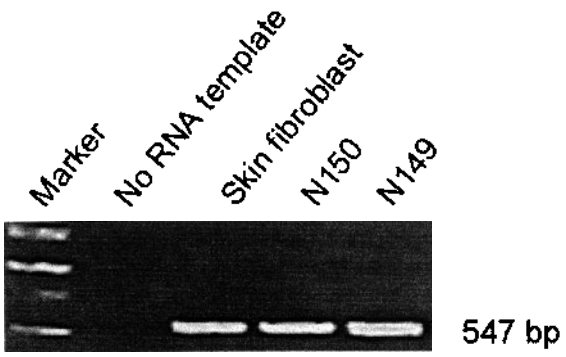

Figure 1. Expression of AR in synoviocytes determined by RT-PCR. Synoviocytes from 2 normal premenopausal women (denoted N150, age 37 yrs, and N149, age 18 yrs) were used for RNA extraction. Total RNA (1 μg) was subjected to RT-PCR using AR-specific primers, with controls receiving either no RT or no template. Human skin fibroblasts served as a positive control.

(Figures 1 and 2). Changes in the mRNA and protein expression of IL-6 related genes were assessed using a Multi-Probe RNase protection assay (RPA) system (Pharmingen, San Diego, CA, USA) and ELISA (R&D Systems, Minneapolis, MN, USA) of the conditioned media before and after treatment of cells with IL-1ß and respective hormones.

Reverse transcription-polymerase chain reaction (RT-PCR). Total RNA (1 μg) from synoviocytes was reverse transcribed using an oligo dT primer and superscript reverse transcriptase (Life Technologies, Gaithersburg, MD, USA). The resulting cDNA was amplified by PCR using gene-specific 3' and 5' primers for AR (Genosys Biotechnologies Inc., Woodlands, TX, USA). The products were separated on a 1% agarose gel containing ethidium bromide.

Ribonuclease protection assay. We utilized RPA specific for the IL-6 gene family [IL-3, IL-7, granulocyte macrophage-colony stimulating factor (GM-CSF), M-CSF, G-CSF, IL-6, leukemia inhibitory factor, stem cell factor, oncostatin M]. The template contained probes for GAPDH and L32 to allow comparison of the levels of individual mRNA species between samples. Briefly, total RNA (2–4 μg) was hybridized to ^{32}P-labeled antisense RNA probe *in vitro* transcribed from a DNA template. The probe and target were resolved on a denaturing polyacrylamide gel and visualized using autoradiography. Images of the autoradiograms were digitally captured using a CCD video camera and Snappy Video Snapshot system and analyzed using Scion Image ß softwear system.

RESULTS

Using RT-PCR we observed AR in normal human synoviocytes (Figure 1). DNA sequencing analysis of the product confirmed the amplification of AR transcripts (data not shown). In addition, DHT (10^{-8} M) treatment of synoviocytes for 0–24 h resulted in downregulation of AR after 4 h, followed by a gradual increase, returning to normal levels after 24 h.

The RPA indicated low basal levels of IL-6, GM-CSF, M-CSF, and G-CSF in these synoviocytes. IL-1ß treatment induced the mRNA levels of IL-6, GM-CSF, G-CSF and M-CSF. DHT treatment of control synoviocytes had minimal effect on basal levels of the IL-6 related genes (data not shown). However, prior exposure of synoviocytes to DHT (24 h) exerted an inhibitory effect on some of the IL-1ß induced genes (mostly G-CSF, M-CSF, and IL-6) (Figures 2A and B). Concurrent addition of DHT with IL-1ß was not as effective. 17ß-estradiol exerted stimulatory effect on GM-CSF, G-CSF, and IL-6 levels (Figures 2C and D). ELISA analysis of the CM from control and treated synoviocytes confirmed the RPA results indicating that DHT exerts an inhibitory effect, while 17ß-estradiol has a stimulatory effect on IL-1ß induced IL-6 production (Figure 2E). Comparable results were obtained from the different synoviocyte cultures used in the study.

DISCUSSION

Our data confirm the presence of AR in normal human synoviocytes and complement our previous findings of estrogen receptor-α (ER-α) in these cells[7]. These observations and the reported presence of the same receptors in macrophage-like synoviocytes[8] underscore the significance of sex hormones in the homeostasis of normal synovial membrane.

Our studies indicate low basal levels of the IL-6 cytokine superfamily in quiescent synoviocytes. IL-1β treatment induces the majority of these genes (IL-6, G-CSF, and GM-CSF), thus simulating the status of synoviocytes from RA joints. Indeed, elevated levels of some of these cytokines have been reported in patients with RA[9]. These cytokines contribute to the inflammatory process either by inducing the production and activity of matrix metalloproteases by synoviocytes or chondrocytes, or alternatively, by recruiting macrophages and lymphocytes into the joint[10]. Our observation that DHT could repress some of the IL-1ß induced genes in synoviocytes could have important implications in RA. A hypogonadic condition, characterized by low serum androgen levels reported in RA patients[1,11,12], could contribute to the ongoing inflammatory process in the joint. This is further supported by the increased incidence of RA in men with advancing age and concomitant decreasing sex hormones[13,14].

Elevated serum and synovial fluid levels of IL-6 in juvenile and adult patients with RA and the inverse correlation between serum androgen level(s) and IL-6 concentration have been reported[4,5]. Our studies indicate that DHT suppresses, while 17ß-estradiol enhances, the production of IL-6 in synoviocytes. This cytokine contributes to the activation and differentiation of autoreactive T cells. This premise is supported by the observation that IL-6 knockout mice are resistant to antigen induced arthritis[6]. Our observations could provide a biological link between hypoandrogenicity and the inflammatory process in RA synovial tissue. In addition, our data regarding the proinflammatory effect of estrogen complement findings reported by Kawasaki and colleagues[15], and give further credence to the significance of sex hormones in RA disease progression.

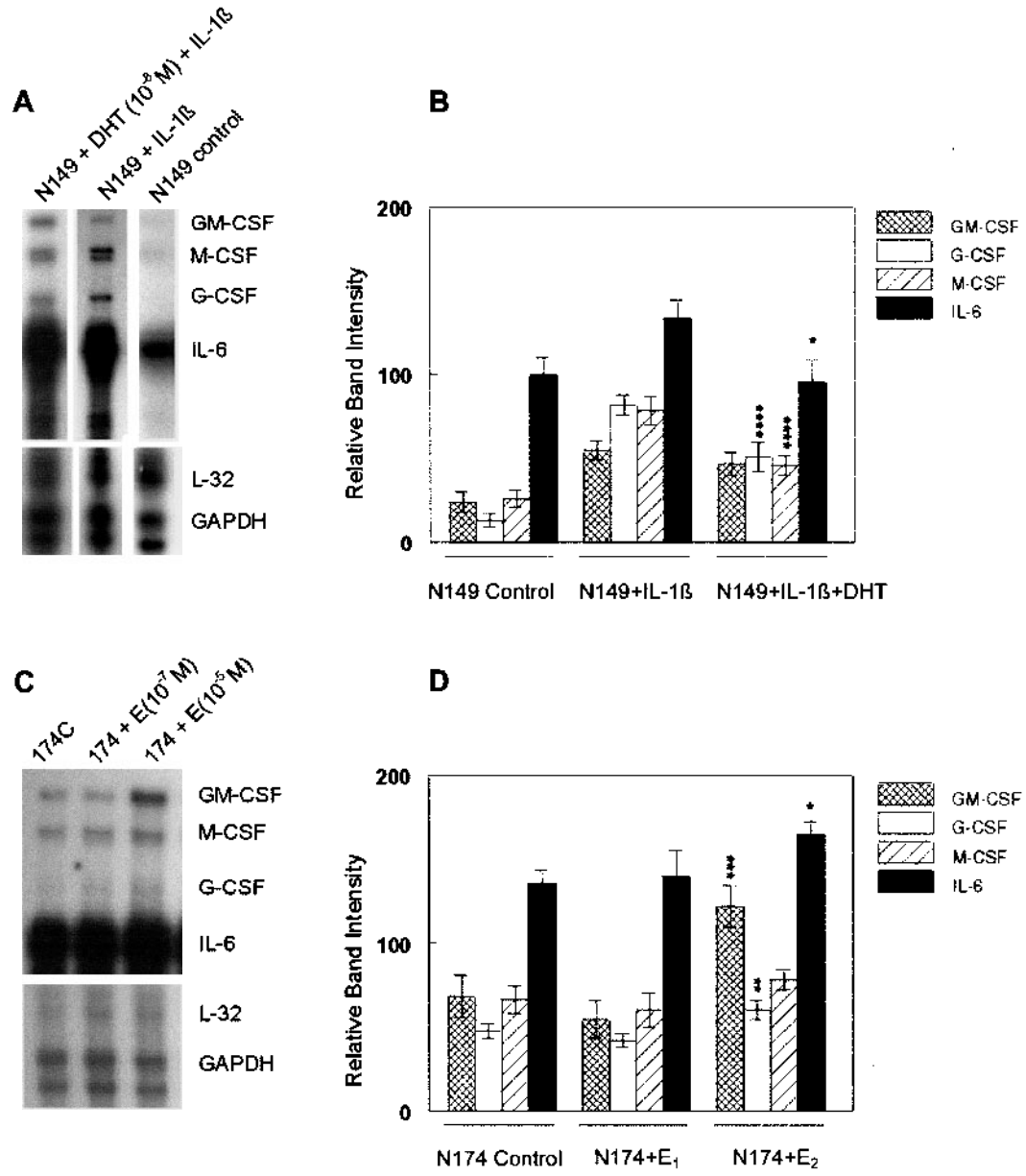

Figure 2. Effect of DHT and 17ß-estradiol on IL-1ß induced genes in normal synoviocytes. Synoviocytes were treated with DHT or 17ß-estradiol for 24 h, followed by a further 10 h incubation in the presence of IL-1ß. Total RNA was extracted (2–4 μg) and subjected to Multi-Probe RPA for detection of the IL-6 gene family. Representative data from an 18-year-old woman (N149) and a 49-year-old woman (N174) are depicted in (A) and (C), respectively. Images of the autoradiograms were analyzed using Scion Image ß softwear (B and D). Each experiment was done in triplicate and one way ANOVA was performed to determine the differences among groups. For the multiple pairwise comparisons, Tukey's studentized range test was used; p values are given for comparison between N149 + IL-1ß and N149 + IL-1ß + DHT, N174 control, and N174 + estradiol. *$p < 0.03$, **$p < 0.01$, ***$p < 0.003$, ****$p < 0.0001$. (E) ELISA analysis of conditioned media from control and treated synoviocytes for the IL-6 content. Each conditioned medium was tested in triplicate and experiments were performed twice with analogous results. Comparable data were obtained when different synoviocytes were tested. Data presented are from N149 synoviocytes; differences between treatments were analyzed by 2 sample t test. $p < 0.05$ was considered significant. *$p = 0.046$, **$p = 0.025$. E: 17ß-estradiol.

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