The Influence of Inflammatory Cytokines on Estrogen Production and Cell Proliferation in Human Breast Cancer Cells

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Abstract. Estrogens play important roles in the development of breast cancer. Inflammatory cytokines such as interleukin-6 (IL-6) and interleukin-1β (IL-1β) exist at high concentrations in breast cancer tissue. Although these cytokines are thought to exert some effect on cancer growth, their precise mechanism is still unclear. In the present study, we investigated the effects of inflammatory cytokines on aromatase (Arom) and steroid sulfatase (STS), which are estrogen-producing enzymes, and cell proliferation using human breast cancer cell lines (SK-BR-3, MCF-7). IL-6 and IL-1β stimulated the activity of Arom and STS. Estrone sulfate (E1-S) had a stimulus effect on cell proliferation of MCF-7. Although IL-6 did not show significant effect on cell proliferation, cell proliferation was significantly increased when IL-6 and E1-S were simultaneously added to the incubation medium. This cell proliferative effect was apparently stronger than the addition of E1-S alone. Addition of IL-1β in the presence of E1-S also significantly enhanced cell proliferation though IL-1β alone did not show any effect. These results led us to the hypothesis that inflammatory cytokines such as IL-6 and IL-1β regulate proliferation of breast cancer cells through estrogen production by steroid-catalyzing enzymes in the tissue.

Key words: Human breast cancer, Estrogen, Estrogen producing enzymes, Inflammatory cytokines


SEX steroids play important roles in the development of hormone dependent cancers. The frequency of breast cancer among post-menopausal women is high and most of the breast cancers are hormone dependent. Studies have suggested that estrogens such as estradiol stimulate the growth of cells in breast cancer [1, 2] and it is reported that the estrogen concentration in the cancer tissue is generally higher than that in normal tissue [3-5]. However, the mechanism of estrogen accumulation in breast cancer tissue remains obscure. In breast cancer tissue, estradiol levels are regulated by steroid uptake from plasma and by intracellular enzymatic metabolism. Almost all sex steroids in post-menopausal women are synthesized from precursors of adrenal origin except for small amounts of ovarian testosterone and androsterenedione. Many investigators have described enzyme systems for estrogen biosynthesis in breast cancer tissue.

Aromatase (Arom) activity that converts androgens to estrogens has been identified in 50-60% of breast cancers [6]. Although some Arom inhibitors are in clinical use to inhibit Arom activity in breast cancer [7], their in vitro potency is not always reflected in the clinical environment. In addition, the pharmacological effects of second-generation Arom inhibitors on estrogen-dependent cancer growth remain controversial. Steroid sulfatase (STS) converts conjugate estrogens to free potent estrogens and is found in almost all types of breast cancer [6]. Stud-
ies have demonstrated that the activity of STS is significantly higher than that of Arom in breast cancer tissue. The formation of estrone via the sulfatase pathway is the major contributor to estrogen synthesis in breast tumors [1, 3, 6, 8]. The enzyme, 17β-hydroxysteroid dehydrogenase (17β-HSD), which catalyzes the reversible interconversion of estrone (E₁) to the more biologically active estradiol (E₂), is also expressed in breast cancer tissue. This enzyme has both oxidative and reductive activities [2, 12]. Breast cancer tissue thus has the complete enzyme system required for estrogen biosynthesis, which may explain the estrogen accumulation in cancer tissue.

Human breast cancer tissue secretes many biologically active substances that regulate the proliferation of normal and cancer cells. The inflammatory cytokines, interleukin-6 (IL-6) or interleukin-1β (IL-1β), regulate steroid catalyzing enzymes and are secreted by inflammatory cells and fibroblasts in breast cancer tissue. Studies have reported that inflammatory cytokines enhance the activity of steroid catalyzing enzymes [2, 6, 9–11]. Although these inflammatory cytokines are known to inhibit cancer cell growth [6, 12], the effect of cytokines on estrogen-producing enzymes is not clear.

We hypothesized that these cytokines affect the proliferation of breast cancer cells by regulating the steroid catalyzing-enzymes in breast cancer tissue, which led to estrogen production. To test this hypothesis, we evaluated the influence of inflammatory cytokines on steroid catalyzing-enzymes and the proliferation of human breast cancer cell lines.

Materials and Methods

Materials

Materials were purchased from following companies: fetal bovine serum (FBS), Eagle's minimal essential medium (MEM) and McCoy's 5A medium, from Gibco (Grand Island, NY); [1β,3βH]androstenedione (SA = 28.50 Ci/mol), [3H]estrone-sulfate (E₁-S) (SA = 53.00 Ci/mol) and [4-14C]estrone (E₁) (SA = 50.30 Ci/mol), from New England Nuclear Corp. (Boston, MA); non-labeled steroids, from Sigma Chemical Co. (St. Louis, MO); IL-1β, from Stratmann Biotech GMBH; anti-human IL-1 receptor antagonist (IL-1Ra), from Pepro Tech (London, England); IL-6, from Sigma Chemical Co. (St. Louis, MO); IL-6 Receptor, Soluble (IL-6 SR), from Calbiochem-Novabiochem Co. (La Jolla, CA). Cell proliferation assay system (5-bromo-2-deoxyuridine-enzyme-linked immunosorbent assay [BrdU-ELISA]), from Boehringer Mannheim (Mannheim, Germany); 3-O sulfamete was a generous gift from Kanebo Pharmaceutical Corp (Tokyo, Japan).

Cell culture

Human breast cancer cell lines (SK-BR-3, MCF-7) were obtained from the American Type Culture Collection (Rockville, MD, USA). SK-BR-3 was cultured in McCoy’s 5A medium and MCF-7 was cultured in MEM supplemented with penicillin G (40 U/ml), streptomycin (40 μg/ml) and FBS (10%). Cells were cultured in 10 cm-diameter tissue culture dishes at 37°C in 5% CO₂/95% air. When cells reached 80% confluence, the medium was changed to FBS-free medium and the incubation was continued for 24 h.

Both cell lines possess estrogen and progesterone receptors. In our conditions, SK-BR-3 showed higher Arom activity than MCF-7, and MCF-7 showed higher STS activity than SK-BR-3. Therefore, we employed SK-BR-3 for Arom activity study and MCF-7 for STS activity study.

Experiment 1 Effect of IL-1β and IL-6 on Arom activity

To investigate the effect of cytokines on Arom activity, the SK-BR-3 cells were incubated with IL-1β (10 ng/ml) or IL-6 (10 ng/ml) and without them as control for a further 24 h. SK-BR-3 cells were simultaneously cultured with [1β,3βH] androstenedione (20 nM), then Arom activity was measured over 12 h in vitro using the [3H] water assay. Steroids were removed from the medium using charcoal, then the tritium concentration was measured in the medium using a liquid scintillation counter (Aloka LSC-652, Tokyo).

Experiment 2 Effect of IL-1β, IL-1ra and IL-6 on STS activity

To investigate the effect of cytokines on STS activity, the MCF-7 cells were incubated with IL-1β (10 ng/ml), IL-1β (10 ng/ml) + IL-1ra (100 ng/ml) and
IL-6 (10 ng/ml) and without them as control for a further 24 h. MCF-7 cells were simultaneously cultured with [3H]E$_1$-S (2 nM) for 12 h, and the reaction was then stopped by adding ethyl acetate. 5000 dpm [14C] E$_1$ was added into the medium to determine recovery of the extraction process. We separated [3H] estrogen by thin-layer chromatography (TLC) and then measured its radioactivity to determine STS activity.

**Experiment 3 Effect of E$_1$-S, 3-0 sulfamate, IL-6, IL-1β on cell proliferation**

We examined the effect of E$_1$-S, 3-0 sulfamate (STS-inhibitor), IL-6 and IL-1β on the proliferation of MCF-7 cells using a BrdU-ELISA kit. At 80% confluence, MCF-7 cells were plated at a density of 5000 cells/well in FBS-free MEM with E$_1$-S (10$^{-7}$ M), E$_1$-S (10$^{-7}$ M) + 3-0 sulfamate (10$^{-5}$ M), 3-0 sulfamate (10$^{-5}$ M), IL-6 (10 ng/ml), IL-6 (10 ng/ml) + E$_1$-S (10$^{-7}$ M) and IL-1β (10 ng/ml) + E$_1$-S (10$^{-7}$ M), in 96-well tissue culture plates and incubated for 96 h. The fluorescence measured in control wells was set at 100% and the effects of E$_1$-S, 3-0 sulfamate, IL-6, IL-6+E$_1$-S and IL-1β+E$_1$-S on cell proliferation were compared with this value. Absorbance was measured using the ELISA reader at 450 nm (reference wavelength, 690 nm).

**Statistical analysis**

Comparisons between groups were analyzed using Student’s t-test. Differences were considered significant at $P<0.05$.

**Results**

**Experiment 1 Effect of IL-1β and IL-6 on Arom activity**

The amount of Arom activity in SK-BR-3 was 0.51 pmol/mg protein/hr. When IL-1β and IL-6 were added to the media, the activity was significantly ($P<0.05$) enhanced to 120 and 150%, respectively, of the control (Fig. 1).

**Experiment 2 Effect of IL-1β, IL-1ra and IL-6 on STS activity**

The level of STS activity in MCF-7 was 0.40 pmol/mg protein/hr and this activity was significantly ($P<0.05$) enhanced to 130 and 140% of the control value by IL-1β and IL-6, respectively. The activities stimulated by IL-1β were completely inhibited by the addition of IL-1 receptor antagonist (Fig. 2).

![Fig. 1. Effect of IL-1β and IL-6 on Arom activity. Arom activity is expressed as a percentage of the control value. Results are expressed as mean ± SD values from four determinations (*$P<0.05$). The activity was significantly enhanced to 120 and 150% of the control value in the presence of IL-1β and IL-6.](image-url)
**Fig. 2.** Effect of IL-1β, IL-1ra and IL-6 on STS activity.

STS activity is expressed as a percentage of the control value. Results are expressed as mean ± SD values from four determinations (*P < 0.05). The activity was significantly enhanced to 130 and 140% of the control value in the presence of IL-1β and IL-6. The stimulated activities by IL-1β were completely inhibited by the addition of IL-1 receptor antagonist.

**Experiment 3 Effect of E1-S, 3-sulfamate, IL-6, IL-1β on cell proliferation**

Cell proliferation was significantly (*P < 0.01) enhanced by E1-S (10⁻⁷ M) to 200% of the control value. The simultaneous addition of 3-sulfamate (10⁻⁵ M) to the medium neutralized the stimulatory effect of E1-S (Fig. 3). Although addition of IL-6 alone did not show significant influence on cell pro-

**Fig. 3.** Effect of E1-S, 3-sulfamate on cell proliferation.

Cell proliferation is expressed as a percentage of the control count. Results are expressed as mean ± SD values from eight determinations (*P < 0.01). Cell proliferation was significantly enhanced to 200% of the control by the addition of E1-S. When 3-sulfamate (STS inhibitor) was simultaneously added to the medium, the stimulatory effect of E1-S was almost completely inhibited.
liferation, cell proliferation was significantly ($P < 0.01$) enhanced to 450% of the control by the presence of both IL-6 and E₁-S (Fig. 4). Addition of IL-1β in the presence of E₁-S also significantly ($P < 0.01$) enhanced cell proliferation to 160% of the control (Fig. 5).

### Discussion

It has been well documented that estrogen stimulates breast cancer tissue. Speirs et al. reported the stimulatory effect of estrogen on cell proliferation in breast cancer cells [2]. The existence of Arom [13] and STS [14] has also been demonstrated. Both en-

![Graph](image1)

**Fig. 4.** Effect of E₁-S, IL-6 and IL-6 + E₁-S on cell proliferation.
The addition of IL-6 alone did not show significant influence on cell proliferation. Cell proliferation was significantly ($*P < 0.01$) enhanced to 450% of the control by the presence of both IL-6 and E₁-S.

![Graph](image2)

**Fig. 5.** Effect of IL-1β + E₁-S on cell proliferation.
Cell proliferation was significantly ($*P < 0.01$) enhanced to 160% of the control by the presence of both IL-1β and E₁-S.
zymes are known to be important for the formation of estrogen from conjugated androgen/estrogen. The results of the present study demonstrated that internally converted estrogen stimulates the proliferation of breast cancer cell lines. We also demonstrated that IL-6 and IL-1β stimulated cell proliferation in breast cancer cells. These inflammatory cytokines enhance the action of enzymes that produce estrogen (Arom and STS). This stimulation of cell proliferation is probably caused by the enhancement of the estrogen-producing enzyme actions (Arom and STS) by IL-6 and IL-1β, because they increase such enzyme actions as shown in experiments 1 and 2. Estrogen stimulates cell proliferation in breast cancer and Sasano et al. found the estrogen receptor α (ERα) in 18 of 25 breast tumor samples [15]. Thus, estrogen may act through ERα in breast cancer tissue in a genomic manner.

Some investigators have reported that inflammatory cytokines have specific effects upon estrogen-producing enzymes in breast cancer tissue. Turgeon et al. reported that IL-4 and IL-6 have a stimulatory effect on oxidative type 17β-hydroxysteroid dehydrogenase (17β-HSD), which catalyzes the interconversion of E2 into E1 [12]. They also reported that IL-4 and IL-6 inhibit reductive type 17β-HSD, which catalyzes the interconversion of E1 into E2. They concluded that these cytokines inhibit E2 production in breast cancer tissue in an effort to decrease E2-induced cell proliferation. In contrast, Purohit et al. reported that IL-6 stimulates reductive type 17β-HSD [9] and suggested that the IL-6 effect is modified by αM. Opinions about the stimulatory effect of IL-6 upon estrogen production by breast cancer tissue still broadly differ [2, 10, 11, 16].

Purohit et al. demonstrated that IL-6 and IL-1β both stimulate Arom activity and that the STS activity of breast cancer cell line increases in the presence of exogenous IL-6 [6]. The present study corroborates their findings. We also demonstrated that IL-6 and IL-1β stimulate the proliferation of breast cancer cells in the presence of E1-S. Our data show that E1-S stimulated the proliferation of the MCF-7 breast cancer cell line. Although the addition of IL-6 alone did not show significant effect on cell proliferation, it had significant stimulatory effect on cell proliferation when IL-6 and E1-S were simultaneously added to the medium. IL-1β also significantly increased cell proliferation in the presence of E1-S. To our knowledge, this is the first report to demonstrate the proliferative effect of E1-S in breast cancer cells. STS was also stimulated by IL-6. It is interesting to note that IL-1β enhanced cell proliferation in the presence of E1-S. It is known that IL-1β alone inhibits cell proliferation [6]. These cytokines may stimulate the activity of estrogen-producing enzymes that consequently increases the growth of breast cancer tissues. Pasqualini et al. claim that the STS pathway (conversion from E1-S to E2) is more important than the Arom pathway (conversion from androstenedione to E1) for breast cancer growth [1]. IL-6 and IL-1β are biologically active cytokines that are secreted by inflammatory cells in breast cancer tissue. Suzuki et al. reported that tumor-infiltrating lymphocyte negatively correlated with ER [17]. Sasano et al. reported that Arom activity did not show any correlation with ER [18] and it has been reported that STS activity did not correlate with ER [19].

Our data imply that increased cytokine levels in breast cancer tissue enhance the proliferation of cancer cells through stimulating the activity of enzymes such as STS and Arom that produce bioactive estrogens. Although the present results partially support our hypothesis when ER is positive in breast cancer, it is difficult to make a comprehensive theory as some of the results conflict with those of past reports. Further study is needed to clarify the physiological role of inflammatory cytokines on estrogen-producing enzymes.

References

Cytokines regulate estrogen production in breast cancer


