

# Induction of IL-1 $\alpha$ mRNA Expression by 17 $\beta$ -Estradiol in Normal Human Keratinocytes

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**Background:** IL-1  $\alpha$  plays an important role in cutaneous inflammation, by the activating of T-lymphocytes. It has been reported that the expression level of IL-1  $\alpha$  was increased or decreased in lupus erythematosus and psoriasis vulgaris, where estrogen undergoes various influences in the clinical course. In human keratinocytes, it is still unclear which estrogen affects the expression of IL-1  $\alpha$ .

**Object and Methods:** To study the effects of estrogen on the expression of IL-1  $\alpha$  by human normal keratinocyte, human normal keratinocytes were cultured with various concentrations of 17  $\beta$ -estradiol.

**Results:** RT-PCR assay specific for IL-1  $\alpha$  mRNA revealed that the expression levels of IL-1  $\alpha$  mRNA were significantly higher under stimulation by 17  $\beta$ -estradiol compared to untreated controls. Also, the effect of 17  $\beta$ -estradiol on the expression of IL-1  $\alpha$  mRNA was higher in middle-aged groups than in old-aged groups and was higher in women's groups than in men's groups.

**Conclusions:** The inducing effect of 17  $\beta$ -estradiol on the expression of IL-1  $\alpha$  mRNA in human keratinocytes provides informations about the pro-inflammatory functions of estrogen in the keratinocytes. (Ann Dermatol 16(4) 163~168, 2004)

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*Key Words:* IL-1  $\alpha$ , 17  $\beta$ -estradiol, Keratinocyte

## INTRODUCTION

Estrogen triggers or improves the inflammation in a complicated way through the regulating of different cytokines. Estrogen change in serum plays a significant role in the development of skin lesions of lupus erythematosus<sup>1</sup> and psoriasis<sup>2</sup>. Although estrogen triggers lupus erythematosus<sup>1</sup> or arouses new psoriasis, it also leads to the improvement of psoriasis<sup>2</sup>. The keratinocyte-T lymphocyte interactions play an important role in the skin inflammation of these

diseases<sup>3</sup>. In this interaction, the keratinocyte-derived IL-1  $\alpha$  is thought to be one of the primary cytokines attracting T cells into the lesions<sup>4</sup>. It appears to be one of a few cytokines present in a considerable amount under normal conditions<sup>5</sup>. The IL-1  $\alpha$  production by keratinocytes is changed in lupus erythematosus<sup>6</sup> and psoriasis<sup>7</sup>. This cytokine is thought to play a central role in inflammatory reactions of the skin, and its release, as an isolated event, would be sufficient to induce inflammation<sup>4</sup>.

More recently, the estrogen regulates IL-1  $\alpha$  production in the different tissues. As such, the IL-1  $\alpha$  production was inhibited by a high dose of estradiol in an immortalized bone marrow cell line<sup>8</sup>. An Estrogen diet resulted in an induced expression of IL-1  $\alpha$  in the uterus<sup>9</sup> and thymocytes<sup>10</sup> of mice. Although estrogen acts variously in the different tissues, the functions in keratinocytes is still unclear. Since the estrogen receptor was found in the epidermal keratinocytes, estrogen is proved to have

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a direct effect on keratinocytes, including its cytokine expression<sup>11</sup>.

In order to explore the function of estrogen in keratinocytes, we examined the effect of 17 $\beta$ -estradiol on the mRNA level of pro-inflammatory cytokine IL-1 $\alpha$  in normal human keratinocytes.

## MATERIALS AND METHODS

### Cell culture

Skin samples from eight healthy volunteers were obtained at Kyung Hee University Medical Center. Volunteers were classified according to sexes (men 4, women 4) and ages (children 2, middle-aged 3, old-aged 3). Human keratinocytes were cultured in KBM, supplemented with 10 g/ml insulin, 1% gentamycin and amphotercin, 1 mg/ml bovine pituitary extract, 0.5 g/ml hydrocortisone and 10 g/ml human epidermal growth factor. The passages 2 and 3 were used for the experiments.

### Estradiol treatment

Cell concentration  $1 \times 10^5$  cell/well was used for each treatment. The keratinocytes were incubated with  $10^{-9}$ ,  $10^{-7}$ ,  $10^{-5}$  M of 17 $\beta$ -estradiol (Sigma, St Louis, MO) for 1, 4, 8, 24 and 48 h, collected and stored at -70°C.

### RNA isolation

Total RNA was isolated by a RNAzol solution kit, following the manufacturers protocol (Tel-test, Inc, Friendswood, Texas). The cells were lysed with 1.0 ml RNAzol B solution and extracted by adding 0.1 volume chloroform to tube. After being centrifuged at 12,000 g (4°C) for 15 minutes, the supernatant was transferred to a new 1.5 ml tube and an equal isopropanol was added. The samples were precipitated for 15 minutes at 4°C. After centrifugation, the pellet was washed with 800  $\mu$ l of 75% ethanol and stored in diethylpyrocarbonate (DEPC) treated water. The total RNAs were measured in 280 nm by the spectrophotometer (Parmacia Bootech, UK)

### Reverse transcription-polymerase chain reaction (RT-PCR)

First-strand complimentary DNA (cDNA) synthesis was performed using a cDNA synthesis kit (Promega, USA) and following the manufacturer's protocol. cDNA synthesis was performed by reverse

transcription in a total volume of 20  $\mu$ l reaction mixture containing 1  $\mu$ g RNA, 2  $\mu$ l of  $10 \times$  reaction buffer (100 mM Tris-HCl, pH 9.0, 500 mM KCl, 1% Triton X-100), 4  $\mu$ l of 25 mM MgCl<sub>2</sub>, 2  $\mu$ l of 10 mM each deoxynucleotide triphosphate (dNTP), 0.5  $\mu$ g Random primer, 20 U of RNase inhibitor, 15 U AMV reverse transcriptase. The mixture was incubated at 25°C for 10 min, 42°C for 1 hr, then heated to 95°C for 5 minutes and chilled on ice.

The PCR reaction was performed in a mixture of 50  $\mu$ l containing 2  $\mu$ l cDNA, 5  $\mu$ l of  $10 \times$  reaction buffer (100 mM Tris-HCl pH 8.3, 500 mM KCl, 15 mM MgCl<sub>2</sub>), 4  $\mu$ l of 2.5 mM each dNTP, 20 pmol of each primer, and 2 U Taq DNA polymerase (Bioneer, Korea). Primers for amplification and the sizes of respective PCR products were as follows: IL-1 $\alpha$ , 5'-GTC-TCT-GAA-TCA-GAA-ATC-CTT-CTA-TG-3' (sense) and 5'-CAT-GTC-AAA-TTT-CAC-TGC-TTC-ATC-C-3' (anti-sense) for 421bp; and beta-Actin, 5'-TAC-CTC-ATG-AAG-ATC-CTC-A-3' (sense) and 5'-TTC-GTG-GAT-GCC-ACA-GGA-C-3' (anti-sense) for 267 bp. The PCR conditions were as follows: denaturation at 95°C-1 min, annealing at 58°C-1min, extension at 72°C-1 min for 25 cycles in a thermal cycler (Perkim Elmer 96000, USA). PCR products were analyzed by electrophoresis on 2% agarose gel, stained with Ethidium Bromide, visualized by image analysis (Gel Doc 1000 gel documentation system, Bio-rad, USA) and intensities of PCR bands were measured by densitometry.

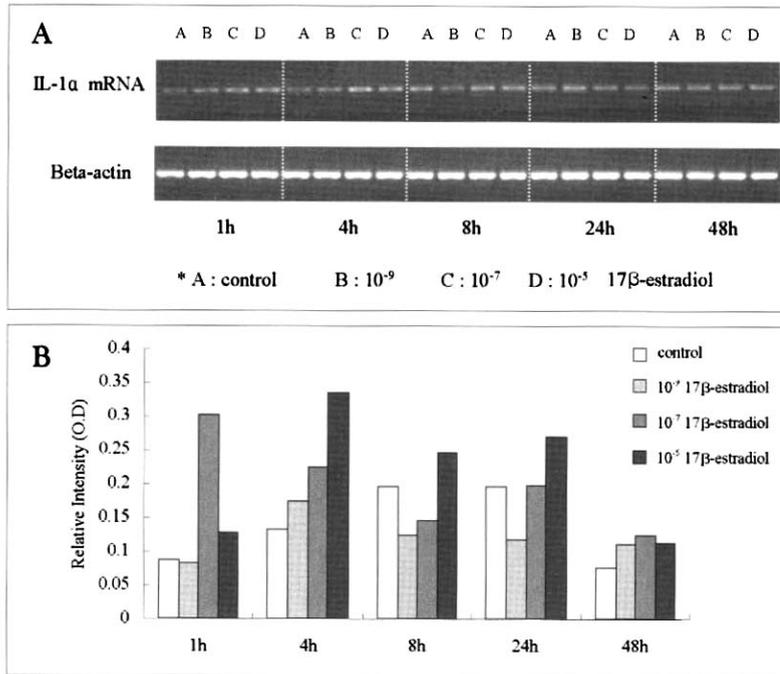
### Statistical Analysis

The statistical significance of differences in the quantity of IL-1 $\alpha$  mRNA levels was tested using the Student's t-test, with a P value of less than 0.05 being considered significant.

## RESULTS

### 1. Expression of IL-1 $\alpha$ mRNA following stimulation by different doses of 17 $\beta$ -estradiol

In this study, we investigated the effects of 17 $\beta$ -estradiol on the expression of IL-1 $\alpha$  mRNA in human keratinocytes. The IL-1 $\alpha$  mRNA level was elevated maximally by treatment with  $10^{-5}$  M of 17 $\beta$ -estradiol after 4, 8, 24 h in comparison with the level in untreated cells. In addition, IL-1 $\alpha$  mRNA levels had a tendency to increase at  $10^{-9}$ M,  $10^{-7}$ M,  $10^{-5}$ M of 17 $\beta$ -estradiol after 4, 8, 24 h (Fig. 1).



**Fig. 1.** Expression of IL-1  $\alpha$  mRNA in the keratinocytes following stimulation by different doses of 17 $\beta$ -estradiol. The human keratinocytes were incubated with 10<sup>-5</sup>M, 10<sup>-7</sup>M, 10<sup>-9</sup>M of 17 $\beta$ -estradiol for 1, 4, 8, 24, and 48 h in a sample of 33 year-old women. Cells harvest were collected for RT-PCR assays of IL-1  $\alpha$  mRNA. (A) Representative RT-PCR showing IL-1  $\alpha$  mRNA and beta-actin mRNA after treatment by 10<sup>-5</sup>M, 10<sup>-7</sup>M, 10<sup>-9</sup>M of 17 $\beta$ -estradiol. (B) Graphical representation of densitometric analyses of IL-1  $\alpha$  mRNA levels. Results are represented as the ratio of expression of IL-1  $\alpha$  mRNA to that of beta-actin in a relative intensity (O.D) (n=3).

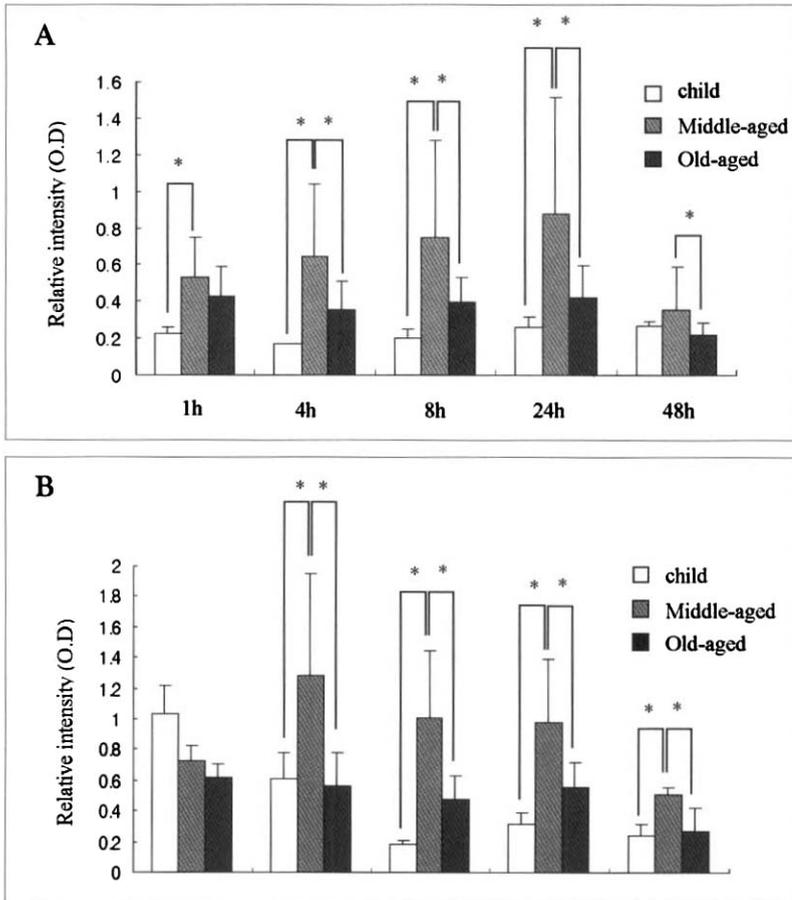
**2. The expression levels of IL-1  $\alpha$  mRNA following stimulation by 10<sup>-5</sup>M of 17 $\beta$ -estradiol according to ages**

We investigated how the expression levels of IL-1  $\alpha$  mRNA differs according to ages. Also, to examine whether the 17 $\beta$ -estradiol-mediated induction of IL-1 $\alpha$  mRNA expression is correlated with ages, the keratinocytes were incubated with 10<sup>-5</sup>M of 17 $\beta$ -estradiol for 1, 4, 8, 24, and 48 h in three groups according to ages (children, middle-age adults, and old-age adults). As it has been shown that the expression of IL-1 $\alpha$  mRNA following stimulation by 10<sup>-5</sup>M of 17 $\beta$ -estradiol was elevated maximally in this study, we used 10<sup>-5</sup>M of 17 $\beta$ -estradiol to stimulate the expression of IL-1  $\alpha$  mRNA. The basal expression levels of IL-1  $\alpha$  mRNA in the middle-age adult groups at all conditions were significantly higher than those of children and old-age groups in unstimulated states (Fig. 2A). The expression of IL-1 $\alpha$  mRNA was upregulated following stimulation by 10<sup>-5</sup>M of 17 $\beta$ -estradiol in all groups. In middle-aged groups, the expression levels of IL-1 $\alpha$  mRNA were significantly higher than in the control groups at 1, 4 and 8 h post stimulation. Also, in both the children and old-age groups, the expression levels of IL-1  $\alpha$  mRNA were significantly higher than in the untreated groups at 1 and 4 h post stimulation. At the stimulation with 17 $\beta$ -

estradiol, the expression levels of IL-1  $\alpha$  mRNA in the middle-age adult groups at all conditions were significantly higher than in the children and old-age groups, except the stimulation with 17 $\beta$ -estradiol for 1 h in the children's groups (Fig. 2B).

**3. Comparison of expression levels of IL-1  $\alpha$  mRNA between men and women**

We investigated how the expression levels of IL-1  $\alpha$  mRNA is different according to sex. To examine whether the 17 $\beta$ -estradiol-mediated induction of IL-1  $\alpha$  mRNA expression is correlated with sex, the keratinocytes were incubated with 10<sup>-5</sup>M of 17 $\beta$ -estradiol for 1, 4, 8, 24, and 48 h in two groups according to sex (men and women). The basal expression levels of IL-1 $\alpha$  mRNA in the women's groups at all conditions were significantly higher than in the men's groups in unstimulated states (Fig. 3A). In the women's groups, the expression levels of IL-1 $\alpha$  mRNA were significantly higher than in the control groups at 1 and 4 h post stimulation. Also, in the men's groups, the expression levels of IL-1  $\alpha$  mRNA were significantly higher than in the untreated groups at 1, 4, and 24 h post stimulation. At the stimulation with 17 $\beta$ -estradiol, the expression levels of IL-1  $\alpha$  mRNA in the women's groups were significantly higher than in the men's groups at 4, 8 and 24 h post stimulation (Fig. 3B).



**Fig. 2.** Expression levels of IL-1α mRNA in the keratinocytes according to ages. The human keratinocytes were incubated in the absence or presence of indicated doses (10<sup>-5</sup>M) of 17β-estradiol for 1, 4, 8, 24, and 48 h in three groups according to ages (children, middle-age adults, and old-age adults). Cells harvest were collected for RT-PCR assays of IL-1α mRNA. Graphical representation of densitometric analyses of IL-1α mRNA levels in the untreated cells (A) and in the cells treated with 10<sup>-5</sup>M of 17β-estradiol (B). Results are represented as the ratio of expression of IL-1α mRNA to that of beta-actin in a relative intensity (O.D) (n=3). \*P < 0.05 as compared with the value in cells of children and old-age adults groups.

**DISCUSSION**

In the present study, it was shown that 17β-estradiol up-regulates the expression of pro-inflammatory cytokine IL-1α mRNA by epidermal keratinocytes in vitro. The treatment with 17β-estradiol at supra-physiological doses resulted in a significant induction in IL-1α mRNA expression in all male and female keratinocytes. The inducing effect of 17β-estradiol on IL-1α mRNA level has been equivocal in vivo studies with uterus cells<sup>9</sup> and mice thymus<sup>10</sup>. Our results support the effect of 17β-estradiol on induction of the expression of IL-1α mRNA in human keratinocytes.

In contrast to our study, it was shown that the estrogen replacement therapy in post-menopausal women reduced the level of IL-1α in serum, which was statistically not significant<sup>11</sup>. Another in vitro study with human immortalized bone marrow cell line showed the inhibitory effect of 17β-estradiol on IL-1α production<sup>8</sup>. Such various results show the

diverse estrogen effects on different types of cells in vivo or in vitro studies.

Estrogen has both anti-inflammatory and pro-inflammatory effects on skin. Kanda et al.<sup>12</sup> postulated that 17β-estradiol suppressed TNF-α and IL-1β-induced RANTES secretion in keratinocytes. Thus, decreased levels of estrogen may lead to increased RANTES production and result in exacerbation of psoriasis at menopause. Estrogen has a pro-inflammatory function in skin. The elevated level of estrogen during pregnancy, puberty, and taking the oral contraceptives are associated with new arousalment and worsening of psoriasis or pustular psoriasis<sup>2,13</sup>, whereas anti-estrogen tamoxifen improves the symptoms<sup>14</sup>.

In the present study, it shows that the expression levels of IL-1α mRNA in middle-aged adults and in women's groups were significantly higher than in the children's, old-aged and men's groups, respectively. 17β-estradiol in high doses of 10<sup>-7</sup>M and 10<sup>-5</sup>M, which correlates to serum estrogen levels in

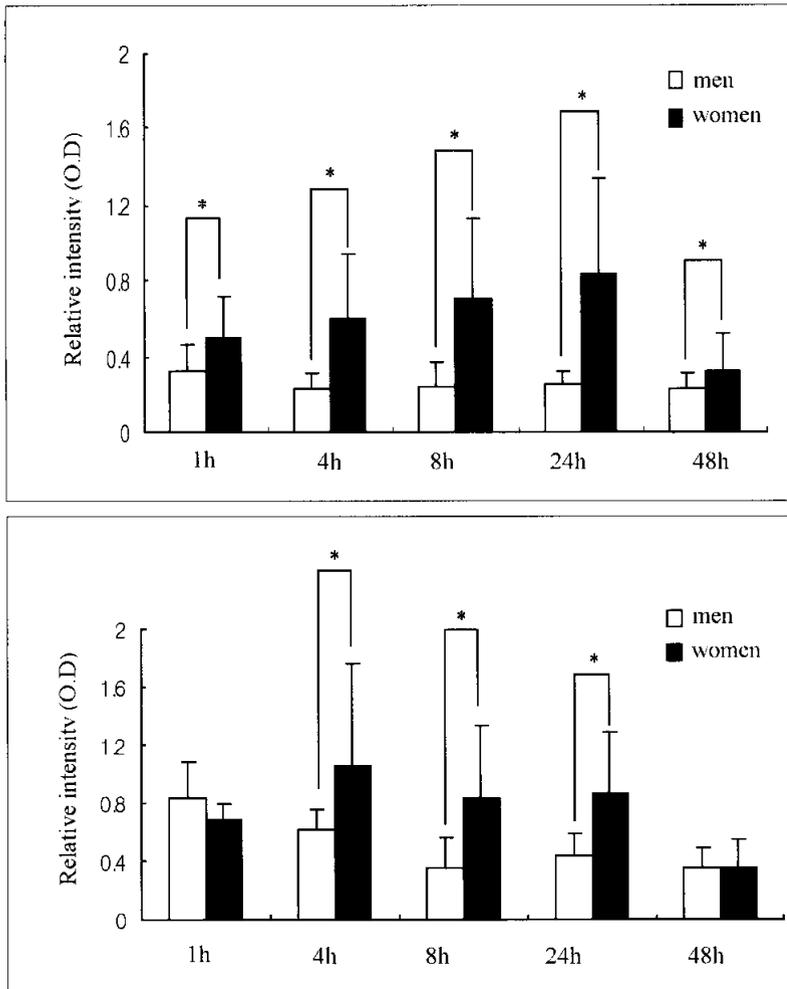


Fig. 3. Comparison of expression levels of IL-1 $\alpha$  mRNA between men and women. The human keratinocytes were incubated in the absence or presence of indicated doses ( $10^{-5}$ M) of 17 $\beta$ -estradiol for 1, 4, 8, 24, and 48 h in two groups according to sex (men and women). Cells harvest were collected for RT-PCR assays of IL-1 $\alpha$  mRNA. Graphical representation of densitometric analyses of IL-1 $\alpha$  mRNA levels in the untreated cells (A) and in the cells treated with  $10^{-5}$ M of 17 $\beta$ -estradiol (B). Results are represented as the ratio of expression of IL-1 $\alpha$  mRNA to that of beta-actin in a relative intensity (O.D) (n=3). \*P<0.05 as compared with the value in control cells.

pregnant women or those taking oral contraceptives<sup>15</sup>, had the most inducing effect on IL-1 $\alpha$  mRNA expression. These results support that the incidence of either psoriasis or pustular psoriasis, in which IL-1 $\alpha$  production by keratinocytes is increased, is high during pregnancy, puberty, and when taking oral contraceptives<sup>2,7</sup>. Also, estrogen has the disease-accelerating effect on lupus erythematosus, where the release of IL-1 $\alpha$  is greatly exaggerated<sup>6</sup>. Furukawa et al.<sup>1</sup> demonstrated that estradiol in oral contraceptives plays a significant role in the development of skin lesions in lupus erythematosus. So, the upregulation of IL-1 $\alpha$  mRNA by 17 $\beta$ -estradiol in our study may be one part of the triggering function of estrogen in this disease. The balance of such functions of estrogen may determine its total effect on the keratinocyte immunity, showing the necessity of further *in vivo* studies, which is yet to be done.

Interestingly, our results showed the ineffective-

ness of estrogen on the expression of IL-1 $\alpha$  mRNA in keratinocytes taken from old-aged volunteers. Hestiantoro et al.<sup>16</sup> has reported the change in characteristics of estrogen receptors in the hypothalamus of elderly women. This change was due to the shift from more nuclear staining of estrogen receptors in young female subjects to more cytoplasmic staining in older female subjects. Katzbur et al.<sup>17</sup> demonstrated that the response to 17 $\beta$ -estradiol declined significantly with age in human bone cells. This may be due to the changes in the level of estrogen receptors, coactivators or repressors in these cells. Inactivation of the estrogen receptors was associated with methylation of the related gene in aging colorectal mucosa, which increases as a direct function of age<sup>18</sup>. Although there is lack of studies about age-related changes in the activity of the estrogen receptors in the keratinocytes, we may consider that ineffectiveness of 17 $\beta$ -estradiol on the keratinocytes

of the elderly in our study could be due to the age-associated inactiveness of the estrogen receptors.

In conclusion, this study suggests that the inducing effect of 17 $\beta$ -estradiol on the expression of IL-1 $\alpha$  mRNA in the keratinocytes provides a further piece of information about the pro-inflammatory function of estrogen in the skin. In this regard, estrogen may represent a tool for further investigations of its role in the skin disease.

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