Stimulation of anti-RNP antibody binding to cultured keratinocytes by estradiol

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Summary. Antibodies to extractable nuclear antigens (ENA) are frequently demonstrated in the serum of patients with cutaneous lupus erythematosus. To gain a better understanding of the influence of sex hormones on cutaneous lupus, we investigated the in vitro binding of anti-ENA antibodies to cultured human keratinocytes. Estradiol-β augmented the binding of anti-RNP, anti-SS-A/Ro, and anti-SS-B/La antibodies to cultured cells, but produced no enhancement of the binding of anti-Sm antibodies. In addition, we examined the effects of 16-α-hydroxyestrone, a highly estrogenic metabolite, on the binding of anti-ENA antibodies to cultured cells. This agent did not produce any augmentation of binding. Based on these experiments, we discuss the influence of estradiol on cutaneous lupus.

Key words: Estradiol — Systemic lupus erythematosus — Anti-RNP antibody — Keratinocytes — Hydroxyestrone

Among the system of antibodies to extractable nuclear antigens (ENA) in SLE, the pathological role of anti-RNP and anti-Sm antibodies in the development of skin disease is not yet fully understood. We recently reported that the binding of anti-RNP antibody, as well as that of the anti-SS-A/Ro and anti-SS-B/La antibodies, to human keratinocytes is induced by ultraviolet (UV) radiation [4]. Based on these in vitro experiments [3, 4], we investigated the effects of estradiol on the binding of anti-RNP and anti-Sm antibodies to cultured keratinocytes. In addition, we examined the effect of 16-α-hydroxyestrone, a highly estrogenic metabolite, because elevation of this metabolite has been reported in both males and females with SLE [8].

Materials and methods

Culture medium

The basic medium for the culture of human keratinocytes used in the present study consisted of MCDB153 (Irvine Scientific, Irvine, Calif., USA) supplemented with 10 ng/ml epidermal growth factor (Collaborative Research, Boston, Mass., USA), 5 μg/ml insulin (Sigma Chemical Co., St. Louis, Mo., USA), 1.4 μM hydrocortisone (Sigma), 0.1 mM ethanolamine (Sigma), and 0.1 mM phosphoethanolamine (Sigma). In some experiments, KGM medium (Clonetics, San Diego, Calif., USA) was used. In primary culture and first-passage cell culture, the basic medium was supplemented with 0.1 mM Ca ++ and whole bovine pituitary extract (60 μg/ml) (Collaborative Research) [1].

Cell culture

Neonatal human keratinocytes were cultured from neonatal foreskins obtained at circumcision by the method of Boyce and Ham [1]. Keratinocytes from neonatal foreskins were plated into 25-cm² plastic flasks (Corning Glass Works, Corning, N.Y., USA). Primary cultures were expanded in the first passage and then frozen in aliquots. In some experiments, EPI-PAK cells (Clonetics) were used.
Reagents

The following reagents were used: 17-β-estradiol (Sigma), 16-α-hydroxyestrone (Sigma), progesterone (Sigma), dihydrotestosterone (DHT, Sigma), and 100% pure glass-distilled ethanol (kindly supplied by the Department of Endocrinology of the University of Colorado School of Medicine).

Serum specimens

Monospecific anti-RNP antiserum, anti-Sm antiserum, anti-SS-A/Ro antiserum, and anti-SS-B/La antiserum were kindly provided by the Department of Rheumatology of the University of Colorado School of Medicine and also by the Department of Internal Medicine of Kyoto University. The specificity of the sera used in this study was determined by the routine indirect immunofluorescence (IF) technique for antinuclear antibodies, double immunodiffusion, counter immunoelectrophoresis, and immunoblotting analysis using methods which have been described previously [3]. Monoclonal anti-ss/ds DNA antibody was obtained from autoimmune-prone mice by the hybridoma technique [2].

Indirect IF method

An aliquot (1.5 ml) of a second-passage keratinocyte suspension (6.25 x 10⁶ cells) was plated into each well of a Lab-Tek chamber slide (Miles, Naperville, Ill., USA) and cultured for 4—5 days in supplemented MCDB153 medium. In most experiments, 48 h after adding the sex steroids, each specimen on the glass slide was washed with PBS and incubated with diluted (1:100) antiserum or normal human serum (NHS) at 4°C for 1 h. The details of the procedure have been described previously [3]. In brief, as the second step, specimens were fixed in acetone at room temperature (RT) for 30 s. Then specimens were incubated with FITC-conjugated rabbit F(ab')₂ anti-human IgG (DAKO, Santa Barbara, Calif.) for 30 min at RT and for 1 h at 4°C. Stained specimens were observed by epifluorescence microscopy (Olympus, Tokyo, Japan). At least 500 cells of each specimen were counted using a x 400 magnification lens. Positivity was determined by subtracting the values for untreated cells from the values for treated cells [3], and each test was run in duplicate.

Table 1. Stimulation of anti-RNP antibody binding to cultured keratinocytes by 17-β-estradiol

<table>
<thead>
<tr>
<th>Monospecific antiserum</th>
<th>Binding of antiserum (%) (mean ± SE)a</th>
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<tbody>
<tr>
<td>Anti-RNP</td>
<td>26.4 ± 2.4</td>
</tr>
<tr>
<td>Anti-SS-A/Ro</td>
<td>23.9 ± 2.6</td>
</tr>
<tr>
<td>Anti-SS-B/La</td>
<td>21.7 ± 2.2</td>
</tr>
<tr>
<td>Anti-Sm</td>
<td>7.4 ± 1.7</td>
</tr>
<tr>
<td>Anti-NHS</td>
<td>5.1 ± 1.7</td>
</tr>
</tbody>
</table>

a Data from three separate experiments

Binding was assayed by indirect IF studies

Flow cytometry analysis

Flow cytometry was performed according to the method described previously [3, 4]. Second-passage cells were cultured for 4—5 days in supplemented MCDB153 medium in a 60-mm plastic dish. When the cells grew to 60—70% confluency, they were treated with various agents and were harvested 48 h later. Cells in culture flasks were harvested by washing twice with solution A and adding 0.05% trypsin (Sigma) and 0.01% EDTA. The harvested cells were washed twice with PBS. After the final wash, 1 x 10⁶ cells were resuspended in cold PBS containing 1% bovine serum albumin and let stand at 4°C for 2 h to block subsequent non-specific protein binding. Then the keratinocytes were incubated at 4°C for 1 h with the diluted specific antisera or NHS (diluted 1/64 or 1/100). After washing twice with PBS, cells were fixed with 2% paraformaldehyde solution at 4°C for 30 s. Next, the cells were incubated at 4°C for 4 h with FITC-conjugated rabbit F(ab')₂ anti-human IgG (Dako) (diluted to 1/100). After the final washing with PBS, these cells were analysed using a Coulter EPICS multiparameter sensor system (Coulter, Hialeath, Fla., USA). As controls for each sample tested, two specimens were prepared from the same lot of cultured foreskin keratinocytes as follows: 1) cultured keratinocytes which were not treated with any agents were stained with the same antisera and second antibody; 2) cultured keratinocytes which were treated with agents were stained with NHS and the same second antibody.