

ESTRADIOL INDUCES EXPRESSION OF HTLV-I ANTIGENS IN LYMPHOCYTES OF HTLV-I ANTIBODY POSITIVE WOMEN

YOSHINARI MATSUMOTO¹⁾, YOSHIYA ANDO²⁾, CHIAKI MARUYA³⁾,
MASAHIRO ENOMOTO⁴⁾, TETSUNORI MATSUDA⁵⁾

1) *Department of Obstetrics and Gynecology, Osaka City University, Medical School*

2) *Department of Obstetrics and Gynecology, Wakayama Medical University, Kihoku Hospital*

3) *Department of Obstetrics and Gynecology, Ohyodo Municipal Hospital*

4) *Department of Obstetrics and Gynecology, Saisei-kai Chyuwa Hospital*

5) *Department of Obstetrics and Gynecology, Shingu Municipal Medical Center*

Received January 28, 2004

Abstract : Pregnant women tested positive for antibodies to human T cell leukemia virus-I (HTLV-I), even though they had tested negative during a previous pregnancy. We investigated the correlations between production of HTLV-I antibodies (HTLV-I Ab), expression of HTLV-I antigen (HTLV-I Ag), and proliferation of cells due to estrogen in vitro. Peripheral blood lymphocytes (PBLs) were separated from five HTLV-I sero-positive non-pregnant women and titration of HTLV-I Ab in sera undertaken. Cultured PBL were stained by indirect immunofluorescence for detection of HTLV-I Ag expression. It was shown that 17- β estradiol (E2) increased HTLV-I Ab production and expression in cultured PBL from sero-positive women. These results suggest that pregnancy stimulates the production of HTLV-I Ab in women infected with low concentrations of HTLV-I, but who appear sero-negative, and that this is the reason why some pregnant women tested positive even though they had tested negative during a previous pregnancy.

Key words : HTLV-I, antibody, antigen, sero-conversion, human sera

INTRODUCTION

Though blood transmission and other contacts, such as sexual, can cause HTLV-I transmission^{1,2)}, the main route of HTLV-I transmission is thought to be breast feeding from HTLV-I seropositive mothers to their infants^{3,4)}. We have reported that some pregnant women tested positive for the antibody to HTLV-I, even though they had tested negative during a previous pregnancy⁵⁾. Thus, we postulated that delayed sero-conversion of HTLV-I would rarely be observed after HTLV-I infection, but we were suspicious due to the vertical transmission from mother to child. To analyze this mechanism, we investigated the correlations between the production of HTLV-I antibodies, expression of HTLV-I antigen, and the proliferation of cells due to E2 in vitro.

MATERIALS and METHODS

After obtaining informed consent, 10ml of peripheral blood was drawn from five HTLV-I sero-positive non-pregnant women. PBLs were separated by the Ficoll-Conray centrifugation method and washed twice with phosphate-buffered saline (PBS). For assay of HTLV-I antibody production, PBLs were resuspended in ASF102 supplemented with 5 μ g/ml of phytohemagglutinin (PHA) (Sigma, St. Louis, MO, USA), 30 μ g/ml of E₂ (Sigma, St. Louis, MO, USA), or in ASF102 supplemented with 5 μ g/ml of PHA for 8 days at 37°C in 5% CO₂ in air. For assay of the expression of the HTLV-I antigen p19, PBLs were resuspended in RPMI 1640 supplemented with 10% crude IL-2, 10% FCS, 30 μ g/ml of E₂, or in RPMI 1640 supplemented with 10% crude IL-2, 10% FCS for 14 days at 37°C in 5% CO₂ in air. Cell growth was assessed by the trypan blue dye exclusion test using a hemocytometer.

Concentrations of HTLV-I antibodies in cultured media were quantified using specific ELISA kits (Eitest-ATL, Eisai, Tokyo, Japan). But the concentration of HTLV-I antibodies in the cultured media was too low to analyze by the standard procedures of these kits; thus, undiluted cultured media were used for the analyses.

For detection of HTLV-I antigen positive cells, cultured cells were stained by indirect immunofluorescence, washed 3 times with PBS, smeared on 10 hole glass slides and fixed by acetone. The cells fixed on the glass slides were incubated with GIN-14, an anti-HTLV-I mouse monoclonal antibody, for 1 hour at 37°C in a moist chamber[®]. After washing 3 times with PBS, they were incubated with FITC-labeled anti-mouse IgG F(ab)₂ (Cappel Laboratories, West Chester, PA) for 1 hour at 37°C in a moist chamber. After washing 3 times with PBS, positive cell rates were obtained by observation of 10 microscopic fields at 200X.

Full color images were sampled using an automatic fluorescent microscopic picture system with a 450-480 nm light source. Each image was recorded as an inverse gray-scale image using only red channel data. Images were then subjected to analysis by a Scion Image PC (Scion, Fredrick, MD, USA). From each image used we chose 10 standard positive and 10 standard negative cells with the same sized background circles. Positive cells stained whole, but not in ring-like patterns, were chosen for analysis. We calculated the average of the ratios (positive mean average density - background mean average density / negative mean average density - background mean average density).

Data of patients were evaluated by paired T tests and differences were considered significant when $p < 0.05$.

RESULTS

To determine whether E₂ affects increased production of HTLV-I antibodies, the cultured media were analyzed using a specific ELISA. Absorbances of the cultured media with E₂ were higher than those of cultured media without E₂ (Fig. 1).

For the effect of E₂ for total PBL proliferation, we analyzed the average of total cell proliferations when cultured with E₂ or without E₂. The average of the total cell counts cultured with E₂ were 1.3 times more than those without E₂, but the effect of E₂ on total PBL proliferation was not significant (Fig. 2).

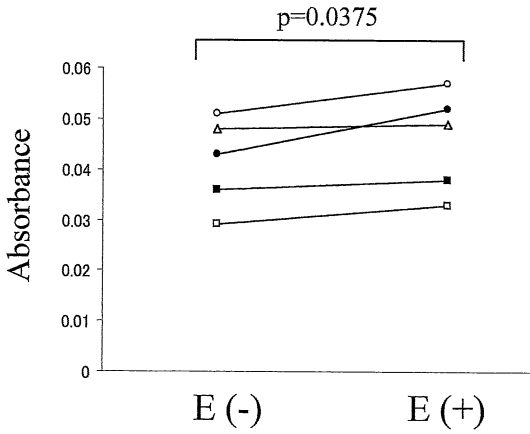


Fig. 1. Alteration of HTLV-I Ab production in cultured media with or without E₂ measured by ELISA.

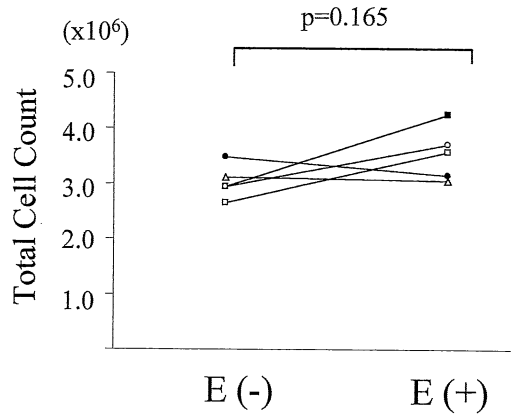


Fig. 2. Cell proliferation in Ag expression media with or without E₂.

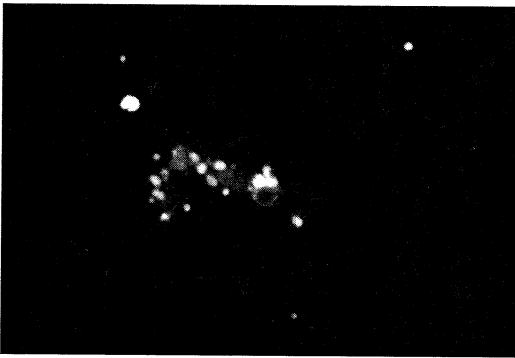


Fig. 3. Immunofluorescence micrograph of acetone-fixed HTLV-I Ag-positive cells. (Some of the positive cells are stained whole, in ring like patterns and as granular patterns on cell surfaces.)

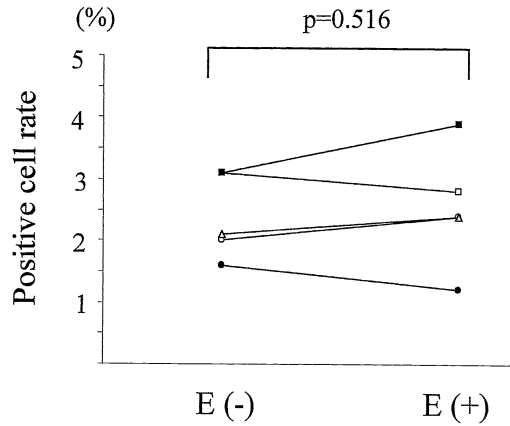


Fig. 4. HTLV-I Ag-positive cells rates in cultured media with or without E₂.

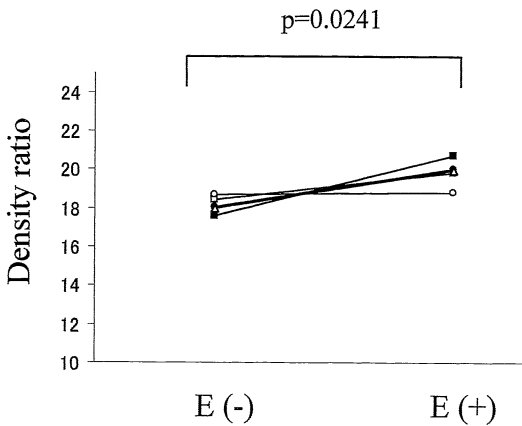


Fig. 5. Densities of the fluorescence intensity of HTLV-I Ag-positive cells in cultured media with E₂ or without E₂.

In the immunofluorescence micrograph of acetone-fixed HTLV-I positive cells shown in Fig. 3, some positive cells are stained whole, in ring-like patterns and as granular patterns on cell surfaces.

For the effect of E_2 on the HTLV-I antigen positive cell rate in total cultivated PBLs, we analyzed the average of the HTLV-I antigen positive cell rates as cultured with E_2 or without E_2 ; effects were not significant (Fig. 4).

We examined whether increased the expression of HTLV-I antigen, or the density of the fluorescence of HTLV-I antigens of positive cells. The average density of the fluorescence of the cultured HTLV-I Ag positive cells with E_2 was higher than those cultured without E_2 (Fig. 5).

DISCUSSION

We have reported that some pregnant women tested positive for the antibody to HTLV-I, even though they had tested negative during a previous pregnancy⁵⁾, but we were then unable to elucidate the mechanism.

In pregnant women, serum free estrogen is mainly E_2 , becoming high in concentrations of 30–40 $\mu\text{g}/\text{l}$ in the third trimester⁷⁾. We examined the effect of this stepped-up serum E_2 for PBLs in non-pregnant HTLV-I sero-positive women, and found a tendency for E_2 to increased production of HTLV-I antibody in vitro. The addition of E_2 did not affect the HTLV-I antigen positive cell rate of cultured PBLs, but did increase HTLV-I Ag expression on HTLV-I positive cultured PBLs. The average of total cell counts cultured with E_2 was 1.3 times greater than that of those without E_2 , but the effects of E_2 on total PBL proliferation were not significant.

This study shows that E_2 affects production of HTLV-I antibodies from HTLV-I sero-positive women's PBLs, and increases HTLV-I Ag expression in HTLV-I positive cultured PBLs. These effects explain why some pregnant women test positive as HTLV-I carriers, even though they had tested negative during a previous pregnancy.

REFERENCES

- 1) Miyoshi, I., Taguchi, H., Fujishita, M., Niiya, K., Kitagawa, T., Ohtsuki, Y. and Akagi, T. : Asymptomatic type C virus carriers in the family of an adult T-cell leukemia patient. *Gann.* **73** : 339–340, 1982.
- 2) Okochi, K., Sato, H. and Hinuma, Y. : A Retrospective study on transmission of adult T cell leukemia virus by blood transfusion: seroconversion in recipients. *Vox Sang.* **46** : 245–253, 1984.
- 3) Kinoshita, K., Yamanouchi, K., Ikeda, S., Momita, S., Amagasaki, T., Soda, H., Ichimaru, M., Moriuchi, R., Katamine, S., Miyamoto, T. and Hino, S. : Oral infection of a common marmoset with human T-cell leukemia virus type-I (HTLV-I) by inoculating fresh human milk of HTLV-I carrier mothers. *Jpn. J. Cancer Res.* **76** : 1147–1153, 1985.
- 4) Nakano, S., Ando, Y., Saito, K., Moriyama, I., Ichijo, M., Toyama, T., Sugamura, K., Imai, J. and Hinuma, Y. : Primary infection of Japanese infants with adult T-cell leukaemia-associated retrovirus (ATLV): evidence for viral transmission from mothers to children. *J. Infect.* **12** : 205–12, 1986.
- 5) Ando, Y., Tanigawa, T., Ekuni, Y., Ichijo, M. and Tohyama, T. : Family study of women showing development of antibody to human T-cell leukemia virus I and assessment of the risk of vertical

transmission of the virus to their children. *J. Infect.* **27** : 151-155, 1993.

- 6) **Tanaka, Y., Koyanagi, Y., Chosa, T., Yamamoto, N. and Hinuma, Y.** : Monoclonal antibody reactive with both p28 and p19 of adult T-cell leukemia virus-specific polypeptides. *Gann.* **74** : 327-330, 1983.
- 7) **Buster, J. E., Chang, R. J., Preston, D. L., Elashoff, R. M., Cousins, L. M., Abraham, G. E., Hobel, C. J. and Marshall, J. R.** : Interrelationships of circulating maternal steroid concentrations in third trimester pregnancies. I. C21 steroids: progesterone, 16 alpha-hydroxyprogesterone, 17 alpha-hydroxyprogesterone, 20 alpha-dihydroprogesterone, delta 5-pregnenolone, delta 5-pregnenolone sulfate, and 17-hydroxy delta 5-pregnenolone. *J. Clin. Endocrinol. Metab.* **48** : 133-138, 1979.