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Demonstration of HCG on the Surface of Maternal Lymphocytes and Discrimination of T and B Cells by Esterase Cytochemistry

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Immunological functions pregnant woman in the early fetomaternal relationship were investigated. Maternal T cell population as determined by the standard method gradually decreases as plasma HCG increases from between 5 and 10 weeks of gestation. In this study, the presence of HCG on the surface of maternal lymphocytes was detected by the direct immuno-histochemical method using enzyme-labeled antibodies: (1) HRP-anti human ß-HCG rabbit Fab', (2) HRP-anti α-HCG rabbit Fab', (3) HRP-anti human native HCG rabbit Fab'. Further (4) HRP-normal rabbit Fab', (5) HRP-anti rabbit IgG goat F(ab')$_2$ and (6) activated HRP were used as staining controls. In order to determine whether the maternal lymphocytes masked with HCG were T or B cells, a modified technique of esterase cytochemistry was employed. The reaction of esterase cytochemistry on rosette forming lymphocytes by SRBC exhibited the features of T cells, as the lymphocytes display a dense localized positivity made up of one to four coarse granules.

(Key Words: Masking Factor, T Cell, HCG, Esterase Cytochemistry, α-naphthol Butyrate)

INTRODUCTION

Pregnancy has been regarded as a successful natural allograft, as the effective transplantation antigens are detected very early in embryonic life (1, 3, 9, 10, 14).

We have investigated immunological function of pregnant women in early fetomaternal relationship. Our interpretation of the results is that the apparent depression of T cell levels in pregnant women during first trimester not attributable to a true decrease of T cell population, but is due to transient, simulated decline of T cell characteristics caused by masking factors such as HCG (13, 18, 19).

In this study, the presence of HCG on the surface of maternal lymphocytes during first trimister was demonstrated by the direct immunohistochemical method using enzyme-labeled antibodies. Furthermore whether these maternal lymphocytes were T or B cell was discriminatd by the modified technique of esterase cytochemistry.

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MATERIALS AND METHODS

Samples and Controls:

Five samples of heparinized peripheral venous blood (PVB) were taken from pregnant women with no complication from ten weeks to twelve weeks of gestation. Three healthy nonpregnant women of child-bearing age and two healthy men served as controls.

Methods for enzyme reaction:

[1] Separation of lymphocyte:

2ml of heparinized PVB were mixed with an equal amount of phosphate buffered saline (PBS) (0.01M, pH 7.4), and the lymphocytes were isolated by specific gravity centrifugation using lymphoprep (Nyegaard and Co., A/S, Oslo).

The isolated lymphocytes were washed with PBS three times and subjected to the enzyme reaction.

[2] Enzyme reaction:

Lymphocyte suspensions were smeared on glass slide and allowed to stand at room temperature to dry. The smears of lymphocytes were fixed for 15 minutes at 4°C with 4 percent periodate-lysine-paraformaldehyde (PLP) solution (7). After washing off the fixative with PBS, antigen-antibody reaction were conducted at room temperature for 30 minutes, using each of the antibodies listed below.*-1

The smears of lymphocytes were washed with three times with PBS. The smears of lymphocytes were postfixed with 2% glutaraldehyde in 0.1M sodium phosphate buffer, pH 7.4 for 10 minutes.

The smears of lymphocytes were washed with three times with PBS. The labeled horseradish peroxidase (HRP) was enzyme histochemically stained by the Graham-Karnovskys' method.

[3] Procedure of α-naphthol butyrate (α-NB) staining

1) Samples

The maternal lymphocytes demonstrated by rosette formation with sheep Lymphocytes from pregnant women that formed rosettes with sheep red blood cells (SRBC) sensitized with antibody to HCG were used as samples for cytochemical esterase reaction. (ref. Yamauchi *et al.* 1981)

2) α-NB staining

Fixation: Rosette forming lymphocytes by SRBC sensitized with antibody to HCG were fixed with cold fixative solution*-2 in a small tube for 1 minute. The samples after fixation were washed 3 times with distilled water centrifuged

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*-1 Antibodies

#1: HRP-anti human ß-HCG rabbit Fab'
#2: HRP-anti human ß-HCG rabbit Fab'
#3: HRP-anti human native-HCG rabbit Fab'
#4: HRP-normal rabbit Fab'
#5: HRP-anti rabbit IgG goat $F(ab')_2$
#6: activated HRP
#4, #5 and #6 were used as staining controls.

*-2 Fixative: 10% formalin and 45% acetone in 1/15M PBS. pH 6.3.

at 800 r.p.m. and resuspended. The samples were exposed to the substrate solution*-3 for 30 minutes at 37°C. The samples were washed 3 times with distilled water by centrifugation at 800 r.p.m. and resuspended. Nuclei counterstaining on the samples was done with Mayer's hematoxylin (Muto pure chemical Co. Tokyo) for 20 minutes at room temperature. The samples were washed 3 times 5 minutes each with distilled water by centrifugation at 800 r.p.m. The samples after staining were smeared on glass slides and placed at room temperature to dry. The smears were mounted with glycerol-gelatin.

RESULTS

Demonstration of HCG using enzyme-labeled antibodies:

The brown color deposits, characteristic of 3·3' diaminobenzidine (DAB) reaction products for peroxidase were demonstrated on the surface of maternal lymphocytes incubated with the antibody solution, i.e. HRP-anti human α-HCG rabbit Fab' and HRP-anti human native HCG rabbit Fab'. (Fig. 1 and Fig. 2)

On the other hand, no particular difference was observed when anti human ß-HCG or anti human native HCG, were used in the immunohistochemical method using enzyme-labeled antibodies as shown as Fig. 1 and Fig. 2.

No positive reaction products were observed in the control cases of non-pregnant women, healthy men and in the control stainings with solutions #4, #5 and #6. (Fig. 3 and Fig. 4)

Discrimination of T or B cell by esterase cytochemistry:

The reaction of esterase cytochemistry on rosette forming lymphocytes by SRBC sensitized with antibody to HCG showed dense localised dot like esterase positivity as shown in Fig. 5 and a number of distinct reaction patterns were seen in lymphocytes which were resistant to NaF.

DISCUSSION

In a previous report, the rise and fall of T cell population determined by the standard method reported by Yada *et al* (16) showed an inverse correlation with an increase and decrease in the plasma HCG level (18, 19).

The results of sequential determination of plasma HCG and the demonstration of HCG on the surface of maternal lymphocytes suggested that HCG produced in the syncytialtrophoblast of placenta could have caused depression of the ability of lymphocytes to form resettes as a T cell marker during the first trimester. It is quite conceivable that the reduction of the T cell pupulation determined by the standard method by Yada *et al* (16) does not represent a true decrease in the number of T cells, but would represent masking of T cell characteristics by the adhesion of HCG onto the T cell surface in pregnant women (18, 19).

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*-3 Substrates: a) 10 mg fast garnet GBC (FC-GBC) and 0.5 ml α-NB were dissolved in 9.5 ml 1/15 M PBS and adjusted to pH 6.3. Make just before use.

b) Sodium fluoride (NaF): Dissolved 4.5 mg NaF in 3 ml α-NB and FG-GBC solution. Make just before use.

In this study, the presence of HCG on the surface of maternal lymphocytes was proven by the presence of anti-β HCG and anti-native HCG labeled with HRP, using an enzyme-labeled antibody method as well as by rosette formation of SRBC sensitized with anti-HCG. Furthermore, not all of the maternal lymphocytes were bound with HCG but some were HCG free and others were weakly bound with HCG, as shown in the positive immunostaining cases Fig. 1 and Fig. 2.

The histochemical esterase reaction of various haematological cells has been investigated using different substrates, such as α-naphythyl-acetate (2, 6, 11, 17), naphthol-As-D-acetate (12, 15) and naphthol-As-D-chloroacetate (4, 6, 8, 11, 17).

The technique used in the present investigation was devised by Higgy *et al* (5) and they showed that an esterase reaction pattern is capable of differentiating several varieties of lymphocytes. T cells display a dense localized positivity made up of one to four coarse granules, B cells appear to be negative and null cells show scattered granular positivity. NaF could inhibit the esterase reaction in monocytes but not in lymphocytes. Granulocytes are negative or only faintly positive.

The modified technique has been devised to discriminate T or B cells of rosette forming lymphocytes by SRBC sensitized with antibody to HCG.

The patterns of histochemical esterase activity with our method revealed that most of the maternal lymphocytes coated by HCG exhibited the features of T lymphocytes.

Thus, the findings of this investigation confirmed that the maternal lymphocytes which showed a simulated and temporary reduction in population in pregnant women during first trimester should be T cell.

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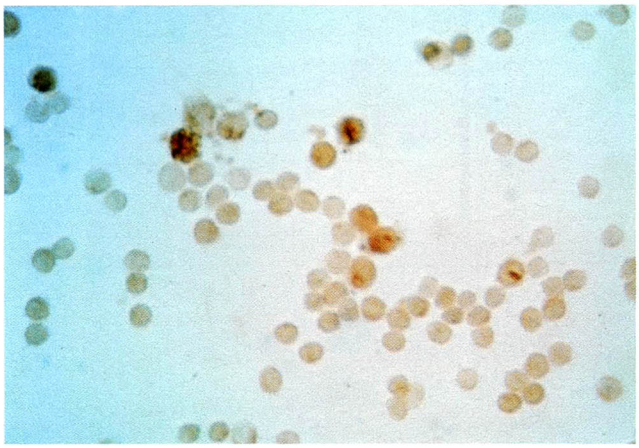

Fig. 1 The localization of ß-HCG was demonstrated on the surface of maternal lymphocytes, using the enzyme-labeled antibody. A case of a 10 weeks pregnancy. (200×)

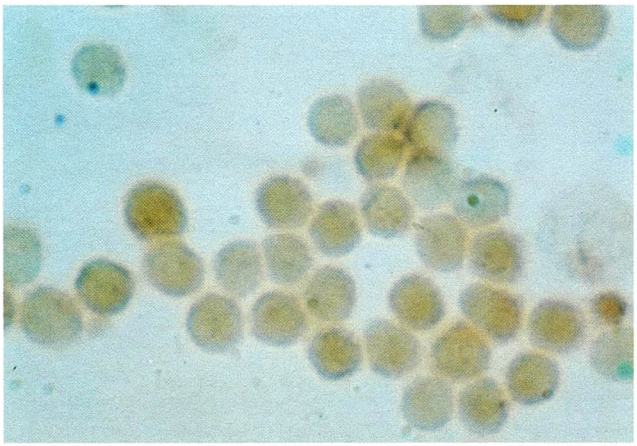

Fig. 2 In the reaction with anti native HCG in cases of 10 weeks pregnancy, development of a brown colour was observed on the surface of maternal lymphocytes. (1,000×)

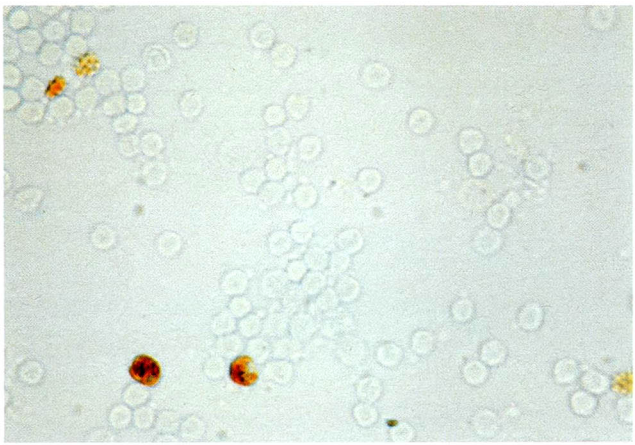

Fig. 3 No brown colour development due to reaction products was observed, in control cases of nonpregnant women and men. (200×)

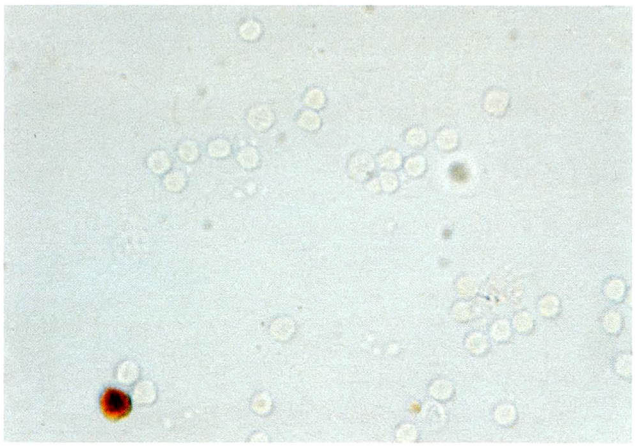

Fig. 4 No brown color development was observed on the surface of maternal lymphocytes, using HRP-normal rabbit Fab' and HRP-anti rabbit IgG goat $F(ab')_2$. (200×)

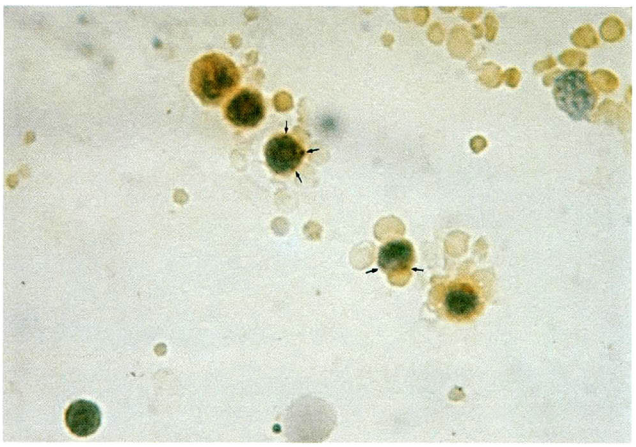

Fig. 5 The pattern of esterase activity in rosette-forming lymphocytes by SRBC sensitized with antibody to HCG showed dense, localized dot-like esterase reaction. (400×)