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A ROLE OF MATERNAL IMMUNE CHANGES IN THE MAINTENANCE OF CONCEPTUS AND IN THE TRIGGER OF LABOR

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Synopsis The changes in maternal cell-mediated immunity were studied throughout the course of pregnancy, delivery and puerperium. The study was conducted using two different indices: lymphocyte response per unit volume of peripheral blood to phytohemagglutinin (PHA) and the immuno-suppressive effect of serum on the response of normal lymphocyte to PHA stimulation. The results showed that decreased lymphocyte response and increased immuno-suppressive effect of serum contribute to the maintenance of the conceptus in utero and that opposite phenomena take place about 20 days before the onset of labor. It was concluded that a purposeful change in the maternal cell-mediated immunity plays a role in the maintenance of the fetus and its appendages and in triggering labor.

Introduction

The changes in cell-mediated immunity during pregnancy are believed to play a key role in elucidating the mechanism of immunologic tolerance in pregnant women. There remains, however, conflict as to whether the response of maternal lymphocytes to T-lectin or other mitogens falls[4,7,9,10,11,15] or remains unchanged[1,2,3,14,17], and no definite conclusions have been reached.

The present authors suspected that the discrepancy in the lymphocyte response lies in the inadequate method of measuring lymphocyte functions and came to believe that the functions measured in terms of per unit volume of whole blood reflect the systemic cell-mediated immunity far more faithfully than the functions measured in terms of per unit count of lymphocytes. This reasoning prompted the authors to verify the usefulness of Park & Good's micromethod of evaluating lymphocyte responses to phytohemagglutinin (PHA).

The present paper aims at elucidating the changes in cell-mediated immunity in healthy women throughout pregnancy, delivery and puerperium by lymphocyte reactivity and by assessing the serum immuno-suppressive effect by a modification of Park and Good's method[12]. The term lymphocyte response or lymphocyte reactivity used in this paper means the in vitro responsiveness of lymphocytes to PHA, which is believed to be a function of the thymus-derived lymphocytes concerned with transplantation and cancer immunity.

Material and Method

1) Maternal lymphocyte reactivity per unit volume of peripheral blood during pregnancy.

We reviewed Park & Good's micromethod of evaluating the T-cell function with a view to determining the optimum conditions of incubating lymphocytes. The outline of our procedure for evaluation of the T-cell function is as follows:

One hundred and fifty pregnant women were subjected to this study. Blood samples

were taken, centrifuged twice, and the serum was displaced with a control AB-serum to eliminate individual difference in the effect of serum against maternal lymphocyte responses. A 0.05 ml aliquot of this blood was added to 0.95 ml of RPMI-1640 medium (Gibco) containing 20 μg of PHA-P (Difco) in each ml placed in a tissue culture tube. The mixture was incubated at 37°C in humidified air with 5% CO_2 for 24 hours. One μCi of ^{3}H-thymidine (specific activity 5Ci/mMol) was added to the mixture, and incubation was continued for another 24 hours. After the second incubation, erythrocytes were lysed by addition of distilled water, and lymphocytes were collected on glass-fibre paper set in a vacuum filter unit and washed with normal saline and 15 ml of cold 5% trichloroacetic acid solution. Thymidine incorporated into the acid-insoluble fraction was assayed with a Nuclear-Chicago liquid scintillation counter to estimate the lymphocyte activity in terms of DNA synthesis expressed in count per minute (cpm). For each experimental group triplicate cultures were tested.

2) T-cell and B-cell counts during pregnancy.

Distinction between T-cell and B-cell was accomplished with Tachibana and Ishikawa (1973)'s microassay kit[13] taking advantage of differences in the capacity of lymphocytes for spontaneous rosette formation. One hundred and fifty-seven pregant women were subjected to this study. A nucleated cell that formed a rosette with four or more erythrocytes around it was taken as a T-cell, while a cell that attracted four or more EACs was considered a B-cell. The percentages of rosette-forming T-cells and B-cells were microscopically determined on 400 lymphocytes. The absolute numbers of T-cells and B-cells per unit volume of blood were calculated by multiplying the count of lymphocytes in peripheral blood by the percentages of T-cells and B-cells.

3) Effect of maternal serum immunosuppression on lymphocyte response during pregnancy.

Group 0 blood obtained from a healthy nonpregnant volunteer was washed three times with a control AB-pooled serum and then restored to its original volume with the same serum. To a 0.05 ml aliquot of this blood a 0.25 ml portion of pooled serum from pregnant women was added. To this mixture a 0.7 ml portion of RPMI-1640 medium containing 20 μg of PHA-P was added. This mixture was subjected to the microassay described in **1) Micromethod of evaluating lymphocyte response.** This test was performed in 40 pregnant women. Since a decrease in thymidine uptake accounts for a decrease in normal lymphocyte reactivity due to serum from pregnant wome, a lower value means greater immunosuppression and vice versa.

4) Individual follow-up of the lymphocyte response and serum immuno-suppressive effect in the last month of pregnancy.

The maternal cell-mediated immunologic function was followed up in 20 individual women who had developed spontaneous labor but received no medicaion. This study aimed at evaluating the lymphocyte response and serum suppressive effect of peripheral blood obtained weekly before breakfast on and after the 37th week until the onset of spontaneous labor.

Results

1) Changes in maternal lymphocyte reactivity per unit volume of peripheral blood during the course of pregnancy.

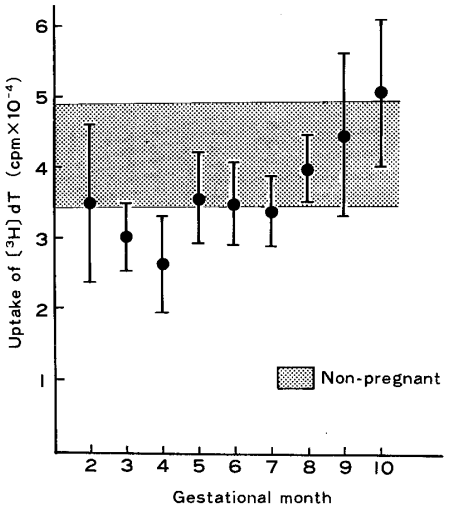
Fig.1 shows characteristic changes in the capacity of lymphocytes for DNA synthesis per unit volume of blood. The thymidine

Table Changes in the percentage and count per unit volume of T-cell and B-cell in the peripheral blood with the progress of pregnancy. Tachibana & Ishikawa (1973)'s microassay kit was used

		T-cell		B-cell	
Gestational month	No	%	Count/mm³	%	Count/mm³
(Non-pregnant)	37	72.8±13.7	2,395± 739	21.7±13.6	710±429
2	13	68.7±10.7	2,245± 558	25.9± 6.7	905±458
3	14	57.1±16.1	1,958± 593	30.2±12.4	997±423
4	13	49.2±16.1	1,482± 332	32.4± 9.7	1,043±386
5	14	55.3± 7.9	2,280± 951	28.1±10.7	1,025±316
6	12	63.0±16.5	2,762± 878	30.1±12.8	1,317±570
7	11	67.8±14.4	2,148± 758	31.4±13.7	1,084±672
8	13	78.3±15.9	3,036±1,255	20.9±16.2	699±379
9	15	89.4± 8.1	3,425± 715	11.5± 5.9	479±228
10	15	78.4± 6.9	2,792± 911	21.3± 9.1	816±575

(mean±s.d.)

Fig. 1 Changes in the maternal lymphocyte response to PHA stimulation per 50μl whole blood during the course of pregnancy



uptake by lymphocytes of non-pregnant women was 41,532±7,359 cpm (mean±s.d.). This value served as the control. The actual uptake proved to decline in the first trimester, recover in the second trimester to the lower limit of the range in non-pregnant women, and increase again in the third trimester.

2) Changes in T-cell and B-cell counts during pregnacy.

In order to determine what factors give rise to the characteristic lymphocyte response, we follwed up the count of peripheral blood lymphacytes and that of T-cell and B-cell in the peripheral blood throughout the course of pregnancy (Table). As a result, the lymphocyte count was found to remain unchanged and changes in the T-cell count per unit volume to follow nearly the same pattern as that of characteristic thymidine uptake shown in Fig. 1. The table shows that changes in the count of B-cell is inversely related to those of T-cell.

3) Changes in the immuno-suppressive effect of maternal serum on lymphocyte response during the course of pregnacy.

Fig. 2 clearly shows a one-sided increase in the suppressive effect of serum, which constitutes a contrast to the time-dependent lymphocyte response shown in Fig. 1. This fact implies that the factor that resides in serum and suppresses lymphocytic DNA synthesis increases during pregnancy.

4) Sequential changes in the lymphocyte response and serum immuno-suppressive effect in the last month of pregnancy until the onset of labor.

Fig. 3 illustrates dynamic changes in both lymphocyte response and the serum effect in two representative cases. For a period of several days about 20 days before

Fig. 2 Changes in the immuno-suppressive effect of maternal serum on the response of normal lymphocyte to PHA stimulation during the course of pregnancy. A decrease in uptake of [^{3}H] dT means greater suppressive effect of maternal serum

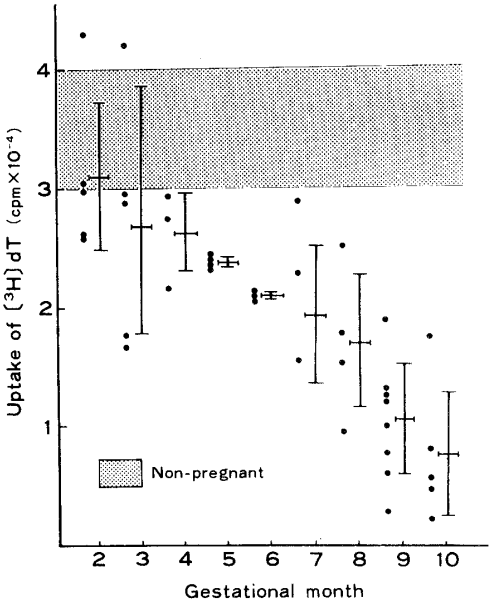
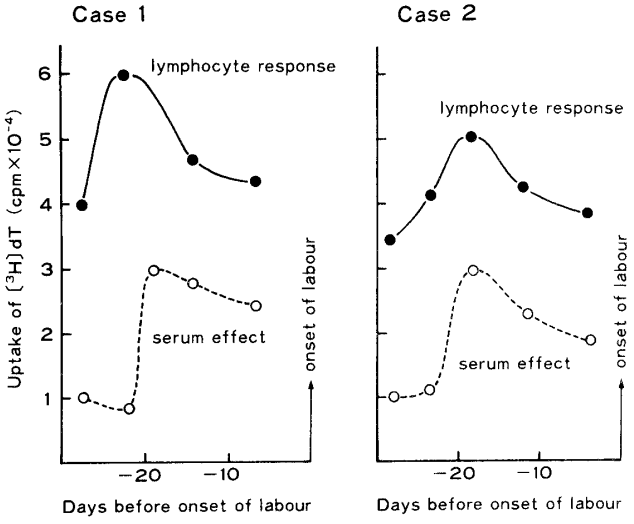


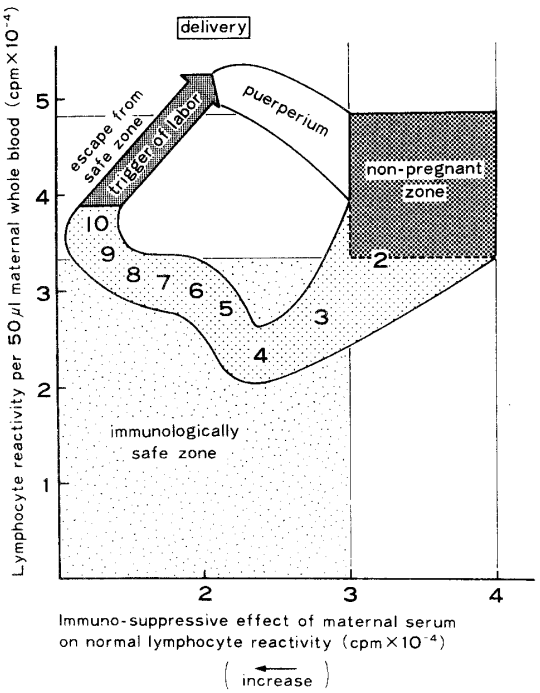
Fig. 3 Changes in maternal cell-mediated immunity during the last month of pregnancy. The lymphocyte response and serum effect were followed up every 7 days



the onset of spontaneous labor the lymphocyte response rises and the serum effect decreases.

5) Changes in maternal lymphocyte response and the immuno-suppressive effect of serum throughout the course of gestation

Fig. 4 Schematic presentation of the changes in maternal cell-mediated immune state in the course of pregnancy, delivery and puerperium, based on the findings in Figs. 1, 2 and 3. Since a decrease in thymidine uptake accounts for a decrease in normal lymphocyte reactivity by serum from pregnant women, a lower value means greater serum effect



(schematic presentation).

Fig. 4 is a schematic presentation of the changes in maternal cell-mediated immune state throughout the course of pregnancy, delivery and puerperium, based on the findings in Figs. 1,2 and 3. The lymphocyte reactivity as the ordinate is plotted against the immuno-suppressive effect of serum as the abscissa. The non-pregnant zone was synthesized from non-pregnant ranges in terms of the two parameters shown in Figs. 1 and 2. The immunologically safe zone is defined as a square enclosed by the lower limits of the two different non-pregnant values shown in Figs. 1 and 2. This figure shows that the cell-mediated immunity in terms of these two indices follows a characteristic circular course during the entire

period of pregnancy, delivery and puerperium, as described in detail below. In the first trimester the maternal immunity travels toward, and enters, the immunologically safe zone; in the second trimester it shifts toward the upper limit of the zone. Meanwhile in the third trimester the immunity crossess the border of the safe zone and, about 20 days before the onset of labor, the immunity rushes forward, supported by increased lymphocyte reactivity and decreased immuno-suppressive effect.

Discussion

The changes in maternal cell-mediated immunity during pregnancy have been dealt with in a great number of papers. These authors are in conflict as to whether the maternal lymphocyte response reduces or remains unchanged and have been unable to come to a definite conclusion. The present authors have recently come to believe that the key factor contributing to this discrepancy is an inadequacy in the conventional methods of assessing the lymphocyte response, and have adopted a modified micromethod of evaluating it. This method requires neither isolation of lymphocytes from peripheral blood nor adjustment of lymphocyte count, and must reflect the systemic cell-mediated immunity more accurately than any of the conventional techniques of measuring blastogenic response per unit count of lymphocytes.

This method has clearly shown that the lymphocyte response during pregnancy follows a characteristic, purposeful pattern as shown in Fig. 1. This pattern turned out to have derived from the changes in the lymphocyte count of maternal peripheral blood but from the changes in the T-cell count. As for the serum immuno-suppressive effect, another factor contributing to the cell-mediated immunity, most researchers[4,5,6,7,8,16] including the present authors are agreed that it increases with the progress of pregnancy and reaches a maximum level at the 10th gravid month (Fig. 2). The follow-up study of cell-mediated immunity during the last month of pregnancy showed that the course of immunity runs a peculiar pattern (Fig. 3). The transitory changes during this month, which consist of elevated lymphocyte reactivity and reduced serum inhibitory effect, become manifest about 20 days before the onset of spontaneous labor. These changes result in enhancement of maternal immunity, and might trigger spontaneous labor.

It was not until the application of this micromethod, which measures lymphocyte functions in terms of per unit volume of whole blood, that maternal cell-mediated immunity during the last month of pregnancy has come to allow individual follow up. Possible causes of the transitory rise in the maternal immune state include an increase in lymphocyte response brought about by the the brisk release of thymo-lymphatic tissue-derived T-cell into the peripheral blood and a decreased immuno-suppressive effect of the serum due to "fading" chorio-decidual function.

The overall findings led to the schematic presentation (Fig. 4) of the changes in maternal cell-mediated immune state from pregnancy through puerperium. This scheme indicates that the maternal immunologic state invariably lies in the safe zone throughout the course of maintenance of the conceptus in utero and that emancipation from this immunological restraint triggers the onset of labor.

This paper presents only data about cell-mediated immunity in the course of pregnancy, delivery and puerperium, and does not extend to the search for the factor that leads to the circular pattern shown in Fig. 4. The authors

have come to believe that the changes in the maternal lymphocyte response must be studied taking into account endocrinologic effect on lymphoid tissues including the thymus. Besides, it must be reasonable to postulate that the serum immuno-suppressive effect at the feto-maternal junction is far more pronounced than that in the peripheral blood to such a degree as to secure local milieu suited for time-dependent physiological changes throughout the course of pregnancy, delivery and puerperium.

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