Inhibition of PHA-Induced Lymphocyte Stimulation by Factors in Maternal Serum during Late Pregnancy

TAKUYA SAITO, MICHIYA TAKADA and NAKAO ISHIDA

Department of Bacteriology, Tohoku University School of Medicine, Sendai

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Saito, T., Takada, M. and Ishida, N. Inhibition of PHA-Induced Lymphocyte Stimulation by Factors in Maternal Serum during Late Pregnancy. Tohoku J. exp. Med., 1975, 116 (1), 77-80 —— Immunosuppressive effect of pregnant serum obtained in the second and third trimesters was demonstrated in the PHA-induced homologous lymphocyte stimulation system, where ³H-thymidine incorporation into DNA was measured. Such an inhibitory factor was not present in the first trimester serum. The immunosuppressive principle detected in the second trimester serum was characterized by a potent inhibitory activity even at an increased concentration of PHA up to 75 µg, whereas the inhibitory activity found in the third trimester serum at optimum PHA concentration (15 µg) was not detectable when the PHA concentration was raised to 75 µg. Thus, the second and third trimester sera must contain different immunosuppressive principles. —— immunosuppression; pregnancy; PHA-blastformation

The mechanism(s) by which an antigenically foreign fetus survives in the pregnant uterus has been studied by many authors and different hypotheses have been proposed (Beer and Billingham 1971). Although the immunological implications of mitogen reactivity are not entirely clear, when the immunosuppressive effect of sera derived from pregnant women and cancer patients was examined on PHA-induced blastformation of human lymphocytes, inhibitory effect of pregnant serum was shown to depend consistently upon when or in which trimester the serum was drawn.

MATERIALS AND METHODS

Immunosuppressive effect of pregnant women sera was tested in vitro by means of inhibition of PHA-induced blastformation of human lymphocytes. Lymphocytes were obtained from male donors. For the separation of lymphocytes, 3 ml of 3% gelatine solution in saline were added to 10 ml of heparinized blood (20 units/ml) and left for 45 min at 37°C. Obtained buffy coat (5-7 ml) was washed twice with an excess amount of gelatin veronal buffered saline (GVBS), followed by centrifugation at 1,000 rpm for 10 min. The sedimented leucocytes from one donor was pooled and suspended in an aliquot amount (usually 10 ml) of RPMI 1640 medium and adjusted to a cell density of 2.4×10⁶/ml. Each test tube with Morton cap received 0.5 ml of this suspension. Pregnant serum

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specimens to be tested were obtained in the first (16 weeks after gestation), second (28 weeks) and third (40 weeks) trimesters. Individual serum or pooled serum specimens in 0.6 ml of RPMI 1640 medium were added to the above culture to make the final concentration of serum 20%, 30% and 50%, respectively, 1 hr before the addition of 0.1 ml solution of PHA-P.

For the induction of blast formation, 6 different concentrations of PHA-P (Difco) in 0.1 ml medium were added to make the final concentrations of 1.5, 3.75, 7.5, 15.0, 37.5 and 75.0 μg/ml in 1.2 ml total volume. Each triplicate tube containing pregnant or non-pregnant serum was incubated for 66 hr at 37°C in humidified air supplemented with 5% (v/v) CO₂. Eighteen hr before the end of the culture period, 0.1 μCi of ³H-thymidine (specific radioactivity 14.5 Ci/mMole, obtained from Daiichi Pure Chemicals, Tokyo) in 0.1 ml of RPMI 1640 medium was added. With each culture, the radioactivity incorporated into DNA was measured in an Aloka LSC 601 liquid scintillation spectrometer.

RESULTS

The main result was illustrated in Fig. 1. The lymphocyte response to 15 μg PHA was found to be lower in cultures containing 50% individual sera of the second and third trimesters. No inhibitory effect was detected with the first trimester serum and non-pregnant female serum. When the PHA concentration was raised

![Fig. 1. Effect of pregnancy sera on the PHA response at optimum dose (15 μg/ml). Cultures contained 1.0×10⁶ leucocytes, serum from non-pregnant or pregnant (final 20% and 50%), and 0.1 μCi ³H-TdR in 1.2 ml RPMI 1640 medium. ••• with PHA-P (Difco) and ○○○ without.](image-url)
Inhibition of PHA-blastformation by Pregnant Serum

In Fig. 2, the effect of pregnancy sera on the PHA response at high dose (75 µg/ml) is shown. The conditions were the same as in Fig. 1.

To 75 µg, serum obtained from the third trimester was no more inhibitory (Fig. 2). The difference between the second and third trimester sera was more clearly illustrated when a pooled serum was examined at 6 different PHA concentrations (Fig. 3). With the second and third trimester serum specimens, the inhibitory effect was apparent when the PHA response containing 30% and 50% serum was compared with that containing 20%. Moreover, the difference in the inhibitory character was noticed between these two specimens (Fig. 3). The second trimester serum contained an inhibitor which inhibits the blastformation even at high concentrations of PHA (37.5 and 75 µg). Conversely, the inhibitory effect of the third trimester serum diminished when the PHA concentration was increased to 37.5 and 75 µg. These experiments were repeated twice with different pools and almost identical results were obtained.

**DISCUSSION**

Mother and child are genetically different, and the fetus could be looked upon as allograft. The reason why this allograft is accepted is the main concern of our research. A depression of the immune reactivity by a factor or factors in the serum of pregnant women here demonstrated was shown by Kasakura (1971), Leikin (1972) and Walker et al. (1972). In their cases, however, the inhibitory activity reached a maximum at the time of delivery. In our test, the second trimester...
Fig. 3. Effect of pooled pregnancy sera obtained in the first, second and third trimesters on the response of lymphocytes to PHA at 6 different concentrations. In each plate, final concentration of serum was 20% (○–○), 30% (●–●) and 50% (▲–▲). The other conditions are the same as in Fig. 1.

serum was more inhibitory than the third trimester serum. The difference, however, is probably not due to the different assay system. Rather a more critical approach to characterize each principle contained in the second and third trimester serum should be necessary. In fact, the second trimester serum contained an inhibitor on the growth of Raji cells, whereas the third trimester serum did not (unpublished data). This is in support of the idea that these 2 sera should contain different immunosuppressive principles.

References