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Lymphocyte response to phytohaemagglutinin in the presence of serum from pregnant women: correlation with serum levels of alpha-foetoprotein

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SUMMARY

The effect of sera from women in different stages of pregnancy on cultures of human lymphocytes stimulated with phytohaemagglutinin (PHA) has been studied and correlated with alpha-foetoprotein (AFP) levels.

Sera taken before the twentieth week of gestation showed low levels of AFP and failed to inhibit lymphocyte proliferation. Inhibition was obtained with 38·5% of sera taken from weeks 20 to 30 and with 51·7% of sera after the thirtieth week of pregnancy. Average serum levels of AFP also increased with advanced gestation, but a consistent correlation was not found between AFP concentration and inhibitory activity for every individual serum tested. Lymphocytes from pregnant women or from normal blood donors behaved in the same way in these tests. The mechanisms of inhibition and the role of AFP are discussed.

INTRODUCTION

The conceptus may have histocompatibility antigens different from those of the mother and can thus be considered as an intrauterine allograft.

Several hypotheses have been suggested to explain why the foetus is not rejected by its mother. Evidence of a diminished immune responsiveness during pregnancy includes the prolonged survival of skin homografts, (Andresen & Mourve, 1962), depressed *in vitro* lymphocyte response to mitogens and in mixed cultures (Hsu, 1974; Purtilo, Hallgren & Yunis, 1972; Kasakura, 1971), and increased susceptibility to virus infections (Farber & Glasgow, 1968). Impairment of the immune response could be caused by factors such as blocking antibodies, hormones or certain plasma alpha-globulins.

The serum level of alpha-foetoprotein has been found to be raised during pregnancy. Although the physiological role of this globulin is not completely understood, it is generally thought to have 'immunoregulatory' activity (Murgita & Tomasi, 1975; Yachnin, 1976; Murgita, 1976).

This study deals with the response of lymphocytes from healthy blood donors and pregnant women to mitogens *in vitro* in the presence of serum from women in different stages of pregnancy, and its correlation with the serum level of alpha-foetoprotein (AFP).

MATERIAL AND METHODS

Heparinized blood samples were obtained from healthy normal donors and pregnant women in different stages of pregnancy. Blood diluted 1/2 in buffered saline, pH 7·2, was layered on Lymphoprep and centrifuged at 400 *g* for 25 min. The lymphocytes, separated in the interphase, were washed twice in Hanks' balanced salt solution and once in RPMI 1640

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culture medium containing glutamine 2 mM, penicilline 50 iu/ml, and streptomicine 50 μg/ml. Cell viability was 95–100% by trypan blue exclusion. Lymphocytes were resuspended to a final concentration of $1{\cdot}3 \times 10^6$ cells/ml in RPMI 1640 medium, to which 20% normal donor or pregnant woman serum had been added. Cell cultures were set up in triplicate in 0·25 ml volumes. One set of triplicate samples served as a control, and PHA (Grand Island Biological Company) was added to another triplicate set to a final concentration of 8·0 μl/ml. The cultures were incubated for 96 hr at 37°C in 5% CO_2 in air atmosphere. Tritiated thymidine (1·6 μCi/ml) was added 16 hr before the end of the incubation period.

The cells were collected on Whatman GF/C filters in a Sampling Manifold (Millipore) and consecutively washed twice each with buffered saline pH 7·2, 5% trichloroacetic acid and methanol. The cell pellets were allowed to dry, and then were dissolved for 30 min in 'NCS' solvent (Amersham Searle) and finally transferred to 3·5 ml of scintillation fluid containing PPO (4·0 g/l) and POPOP (0·05 g/l) in toluene. Radioactivity was measured in a B Mark IV (Nuclear, Chicago) scintillation counter, and the average counts per minute (mean ct/min $\pm$ s.e.) of the triplicate cultures was calculated. Inhibition occurred when the mean ct/min of a triplicate set was 80% or less of the highest count of triplicate cultures of the same cell preparation stimulated with mitogen in the presence of normal (non-pregnant) serum.

Statistically significant differences in the inhibition frequencies were calculated by χ^2 test.

The serum concentration of AFP was measured by RIA on CNBr-activated paper discs (Johansson, Kjessler & Sherman, 1976).

RESULTS

Mean serum levels of AFP tend to increase during pregnancy (Table 1), although individual values for women in a given gestational period are highly variable.

Table 2 shows the changes occurring in the inhibitory activity of pregnant sera as a function of the length of pregnancy. No inhibition was found with sera taken before the twentieth week of pregnancy, whereas it occurred with more than one third of sera taken between weeks 20 and 30 and with over a half of the sera obtained beyond week 30. The difference in inhibition frequencies was statistically significant ($P = 0{\cdot}016$) for the first two groups, but not significant ($P = 0{\cdot}32$) for the last two groups of sera.

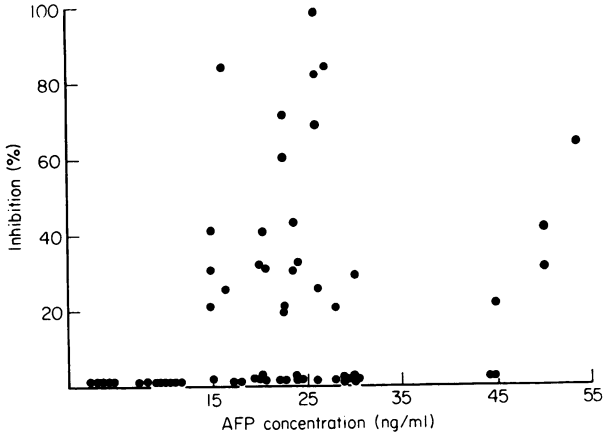

FIG. 1. Comparison between AFP concentration and grade of inhibition of PHA-stimulated lymphocyte cultures.

TABLE 1. Serum levels of AFP during pregnancy

Weeks of gestation	Number of cases	AFP (mean $\pm$ standard error) (ng/ml)
8–20	21	30·047 $\pm$ 6·55
20–30	22	103·046 $\pm$ 21·97
30–45	20	173·701 $\pm$ 38·70

TABLE 2. Effects of serum of pregnant women in different stages on lymphocyte transformation *in vitro* and its relation with the mean concentration of AFP present in the cultures

Weeks of gestation	Number of cases	AFP in cultures (mean) (ng/ml)	Inhibition Number of cases (%)
< 20	11	7·0	0 (0)
20–30	26	19·1	10 (38·5)
> 30	29	36·84	15 (51·7)

As serum AFP levels increase during pregnancy, the concentrations of this protein in the pregnant serum-enriched cell cultures were higher when sera from later gestational periods were used. This can be seen in third column of Table 2; differences in this respect between any two groups were statistically highly significant. The inhibitory effect of advanced pregnancy sera might, therefore, be due to a higher content of AFP.

A more direct comparison between AFP concentration and the inhibition of PHA-stimulated lymphocyte cultures appears in Table 3, where the results are not grouped according to the time of pregnancy at which the sera were obtained, but according to the AFP concentration attained in the culture fluid. When this was less than 15 ng/ml, no inhibition occurred (and this result was statistically significant, $P = 0{\cdot}007$), whereas at progressively higher concentrations inhibition was obtained with increasing frequencies (although the difference between the last two groups was not significant, $P = 0{\cdot}58$). An AFP concentration in excess of 15 ng/ml of culture medium appears to be a necessary, but not a sufficient requirement for inhibition.

The source of lymphocytes does not appear to affect the results of inhibition tests. Table 4 shows the

TABLE 3. Comparison between AFP concentration and inhibition of PHA-stimulated lymphocyte cultures

AFP in cultures (ng/ml)	Number of cases	Inhibition Number of cases (%)
< 15	13	0 (0)
15–20	14	6 (42·8)
> 20	37	19 (51·3)

TABLE 4. Comparative susceptibility of lymphocytes from blood donor or pregnant woman to inhibition of PHA-induced proliferation by pregnant serum

Lymphocyte source	Serum	Week	Control (mean ct/min ± s.d.)	PHA (mean ct/min ± s.d.)	Percentage inhibition
Donor	Donor	—	3193 ± 663	36132 ± 2615	
Donor	Pregnancy	33	3010 ± 373	24188 ± 936	33·05
Pregnancy	Donor	—	1292 ± 394	47133 ± 4196	
Donor	Pregnancy	33	2694 ± 728	27614 ± 2520	41·41

results of a typical experiment: the serum from a 33-week pregnant woman inhibited, to approximately the same degrees, the PHA-induced proliferation of lymphocytes either from a pregnant woman (autologous cells) or from a normal blood donor.

DISCUSSION

Despite the amount of work done in this field in recent years, the biological role of AFP during pregnancy remains obscure. While murine AFP has been shown to bind to maternal oestrogens (Uriel, Nechaud & Dupiers, 1972), thus protecting the foetus against these hormones, a similar effect has not been proved in man (Savu *et al.*, 1974). Murgita & Tomasi (1975) suggested an immunoregulatory role for AFP in the rat. Yachnin & Lester (1976) observed inhibition of human lymphocyte transformation *in vitro* by human AFP, the degree of inhibition varying according to the protein source; foetal AFP was three times more potent than hepatoma AFP. More recently, Charpentier *et al.* (1977) found a stimulatory effect of human AFP.

In this study we have confirmed the presence in pregnant serum of a factor(s) able to inhibit PHA-induced human lymphocyte transformation *in vitro*. The lymphocytes of pregnant women in this respect behave in essentially the same way as the lymphocytes of normal blood donors, thereby ruling out a possible intrinsic defect of the maternal lymphocyte (Carr, Stites & Fudenberg, 1973).

The inhibitory effect could be due (a) to the formation of a protein–mitogen complex which would prevent the mitogen from acting on cells; (b) to an alteration in the membrane receptors for the mitogen, (c) to some change in the metabolic sequence necessary for the elaboration or transmission of the signal for cell proliferation. Our experimental results are equally compatible with any of these mechanisms.

The role AFP might play in the inhibitory action of pregnant woman serum is by no means clear Although sera taken during the first half of pregnancy, when AFP levels are on average at their lowest, were not inhibitory, sera obtained later, after the twentieth week of gestation, failed to show a clear relationship between their inhibitory power and AFP contents. The inhibitory effect became more frequent in the last quarter of pregnancy when the average values of AFP were also highest, but individually higher serum concentrations of AFP did not invariably correspond to more marked inhibitory effects on lymphocyte cultures, and vice versa. Of course, the different sera had to be assayed on lymphocyte preparations from different individuals, which may have contributed to variations in the results. However, while the experiments reported here do not prove that AFP is the factor in pregnant serum responsible for inhibition of PHA-induced proliferation of human lymphocytes, they point to the possibility that AFP may be one among a number of factors whose balanced action occasionally results in inhibitory effects.

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