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Are pregnancy-associated serum proteins responsible for the inhibition of lymphocyte transformation by pregnancy serum?

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SUMMARY

Certain pregnancy-associated serum proteins, namely pregnancy-associated α_2-glycoprotein, pregnancy-specific β_1-glycoprotein, α-foetoprotein, human placental lactogen and human chorionic gonadotrophin, have been proposed as immunosuppressive factors. A pregnancy serum was constructed from a number of such sera to produce high serum levels of these compounds. Each of the proteins was then removed sequentially from the serum by affinity chromatography and the remaining materials examined for inhibitory activity on lymphocyte transformation. Only the removal of pregnancy-associated α_2-glycoprotein decreased suppression by the serum. However, a large proportion of the serum inhibitory activity could not be accounted for, indicating the presence of other suppressor factors.

INTRODUCTION

Over the past few years the number of pregnancy-associated serum proteins identified has increased tremendously, but the actual biological functions of the majority of these remain obscure. However, a number of investigators have proposed that certain of the pregnancy proteins—pregnancy-associated α_2-glycoprotein (α_2-PAG), pregnancy-specific β_1-glycoprotein (PSβ_1G), human placental lactogen (hPL), alpha-foetoprotein (AFP) and human chorionic gonadotrophin (hCG)—have non-specific immunosuppressive properties, and it has been suggested that these compounds may contribute to the regulation of the maternal immune response to the foetus (Stimson, 1976; Damber *et al.*, 1975; Johannsen *et al.*, 1976; Cerni, Tatra & Bohn, 1977; Contractor & Davies, 1973; Murgita *et al.*, 1978; Yachnin & Lester, 1976; Morse *et al.*, 1976; Caldwell, Stites & Fudenberg, 1975).

It is recognized that pregnancy serum can possess an inhibitory effect on lymphocyte transformation *in vitro* (Kasakura, 1971). In the present study those pregnancy proteins which previously have been proposed as immunosuppressive agents were removed sequentially from serum and the remaining materials examined for inhibitory activity on lymphocyte stimulation by phytohaemagglutinin (PHA) and allogeneic cells (MLR).

MATERIALS AND METHODS

Blood samples. Heparinized peripheral venous blood (20 u/ml) was obtained from healthy males (aged 23–28 years). This was allowed to sediment for 90 min at 37°C and the leucocyte-rich plasma

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collected. Peripheral venous blood was also acquired from normal males and pregnant women (primigravidae; aged 17–25 years) and the serum separated. All sera were complement-inactivated by heating at 56°C for 45 min.

Affinity chromatography and assays. Normal rabbit serum and rabbit antisera to α_2-PAG, hCG, hPL, AFP and PSβ_1G (Dako Immunoglobulins) were precipitated with ammonium sulphate (2 M, pH 6·5, 4°C) and the IgG fractions prepared by chromatography on DEAE-cellulose (Whatman DE-52). The specificity of each antiserum was checked by two-dimensional immunoelectrophoresis over a wide range of antigen/antiserum concentrations. The IgG fractions were then coupled to cyanogen-bromide activated Sepharose 4B (Pharmacia). Following preparation the immunoabsorbants were packed into columns (1·6 × 40 cm) and equilibrated with culture medium. Serum samples were diluted with culture medium (40% v/v), incubated in the columns for 90 min at 18°C and the effluents examined for the presence of inhibitory factors. The affinity chromatography removed > 98% of the pregnancy protein under test and < 4% of the other antigens in the study.

The concentrations of the pregnancy-associated proteins were estimated by enzyme immunoassay procedures using antibody–horse-radish peroxidase conjugates, as described previously (Stimson & Sinclair, 1974; Stimson, 1978; Macdonald & Kelly, 1978; Macdonald *et al.*, 1979).

Lymphocyte transformation. Mononuclear cells were isolated from the leucocyte-rich plasma by Ficoll–Isopaque separation (Böyum, 1968), washed twice and resuspended in culture medium (RPMI 1640 containing 20 mM HEPES buffer, 5 mM $NaHCO_3$ and antibiotics). PHA-induced stimulation of cells (8 μg/ml PHA-P, Difco; $1{\cdot}5 \times 10^5$ cells/0·3 ml) was carried out in flat-bottomed microtitre plates (Flow) for 3 days. The 'two-way' MLR ($1{\cdot}5 \times 10^5$ cells from each individual/0·2 ml) was performed in round-bottomed microtitre plates (Flow) over 5 days. On completion of the culture periods 1·0 μCi/ml ^{3}H-thymidine (Radiochemical Centre) was added and the radioactivity taken up was determined after incubation for 5 hr. All cultures contained 20% v/v serum (pooled male, pregnancy or treated-pregnancy) and the viability of cells was assessed, on completion of the culture periods, by trypan blue exclusion.

RESULTS AND DISCUSSION

A pregnancy serum was constructed from a number of individual pregnancy sera in order to give a sample with extremely high levels of each pregnancy protein under investigation. It was obviously not possible to produce a serum which, when incorporated into cultures, gave concentrations of all the pregnancy proteins suggested as being inhibitory by previous studies. However, very high serum values of each were obtained (amongst the highest reported) considering the restrictions imposed by such a remit. The sample was made up from seven sera (432 sera were examined) and the concentration of each protein was as follows: α_2-PAG—1,608 μg/ml; PSβ_1G—381 μg/ml; hPL—8·6 μg/ml; AFP—502 ng/ml; hCG—81 i.u./ml.

The 'constructed' pregnancy serum was added to lymphocyte cultures in an intact form and following removal of the specified pregnancy proteins by affinity chromatography. The lymphocytes were stimulated by PHA and allogeneic cells, and the effects of the samples on the incorporation of ^{3}H-thymidine are shown in Fig. 1 compared with a control serum from normal males. The pregnancy serum reduced lymphocyte transformation by > 50% both in the MLR and PHA-stimulated cell cultures, as expected (Kasakura, 1971; Stimson, 1976). If any one of the pregnancy proteins under investigation was responsible for this inhibition, its removal should have diminished the suppressive effect of the serum. However, in both types of culture employed the elimination of PSβ_1G, hPL, AFP and hCG caused little effect. The removal of α_2-PAG alone resulted in increased lymphocyte transformation, but even in this case only a partial return to normal values was observed.

The results obtained indicate that PSβ_1G, hPL, AFP and hCG are not responsible for the inhibition of lymphocyte transformation *in vitro* which can occur in the presence of pregnancy serum. Apart from hCG, the suppressive properties of which are now believed to be due to a contaminant in commercial preparations (Caldwell *et al.*, 1975; Morse *et al.*, 1976), the data do not exclude PSβ_1G, hPL and AFP from possessing immunosuppressive roles. These proteins are

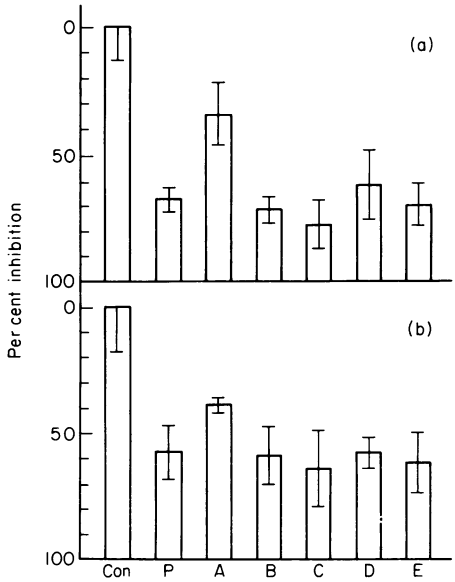

Fig. 1. Effect of removal of pregnancy-associated serum proteins on the inhibition of lymphocyte transformation by pregnancy serum. The pregnancy proteins were eliminated from the serum by employing affinity chromatography with immunoabsorbants. (a) PHA-induced stimulation, (b) mixed lymphocyte reaction. Results expressed as the mean ±s.d. of five determinations from three separate experiments. Con=control pooled male serum, taken as 0% inhibition (stimulation indices, PHA—94·3; MLR—15·6); P=pregnancy serum; A to E=pregnancy serum minus α_2-PAG, PSβ_1G, hPL, AFP, hCG respectively.

synthesized by the foeto-placental unit and they could be present in the placenta at concentrations sufficiently high to be inhibitory.

Alpha$_2$-PAG has previously been shown to inhibit a variety of *in vitro* tests of immune reactivity, especially those pertaining to cell-mediated immunity, and to be associated with subpopulations of T lymphocytes and monocytes (Stimson, 1977). This study confirms the involvement of α_2-PAG in the 'pregnancy serum effect' but indicated that another factor(s) of major importance must also be present to account for the observed inhibitory effects.

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