INHIBITION OF PHA-INDUCED LYMPHOCYTE STIMULATION BY THE PREGNANCY ZONE PROTEIN

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1. Introduction

Mother and child are genetically different and the fetus could be looked upon as an allograft. The reason why this allograft is accepted is unknown. A physiological barrier between mother and fetus [1], and the presence of blocking antibodies [2] and hormones [3] have been suggested. A depression of the maternal cell-mediated immune reactivity [4–6] is another explanation supported by the report of Kasakura [7] that maternal plasma during pregnancy suppressed the mixed leucocyte reaction.

The concentration in plasma of many proteins is altered during pregnancy but some proteins are produced in extensively increased amounts. The pregnancy zone protein (PZ) [8–16] is an α_2-globulin which is absent or present in only small amounts in the sera of non-pregnant women. During pregnancy the serum level of this globulin is increased in most women from 0–4 mg/100 ml to values between 50–200 mg/100 ml. The maximum is generally reached in the third trimester and after delivery there is a rapid decrease to normal levels [17]. In women with early spontaneous abortion PZ is found rarely or in small amounts [18].

The PZ protein has been isolated and found to have a mol. wt. of 326 000 [19].

In the present study PZ was found to inhibit the phytohaemagglutinin (PHA)-induced lymphocyte stimulation, believed to be an in vitro correlate of cell-mediated immunity.

2. Materials and methods

PZ was isolated as described earlier [19]. Serum samples were obtained from pregnant women and the amounts of PZ were measured by single radial immunodiffusion [17].

Venous blood was obtained from healthy non-pregnant blood donors. Lymphocytes were prepared from 300 ml blood by filtration through a nylon fibre column [20, 21] followed by sedimentation in glass tubes placed at an angle of 45° for 1 hr at room temperature. The supernatant fluid was collected and centrifuged at 500 *g* for 10 min.

The resulting cell pellet was resuspended in a synthetic medium 199:HEPES [22] and cultures were established in Microtest II-tissue culture plates (Falcon Plastics, Los Angeles, California), each culture containing 0.2 million lymphocytes.

In one experiment serum samples from ten pregnant women with more than 100 mg pregnancy zone protein per 100 ml of serum were compared to serum samples from eight pregnant women with less than 4 mg per 100 ml of serum. Each culture contained 100 µl of 199:HEPES and 70 µl of the serum sample to be tested. Every individual serum sample was tested in duplicate and the results were expressed as the mean of the two measurements.

In a second experiment the purified pregnancy zone protein was tested. Each culture contained 80 µl of 199:HEPES and 20 µl of autologous plasma. Each culture also contained 70 µl of either: a) saline only

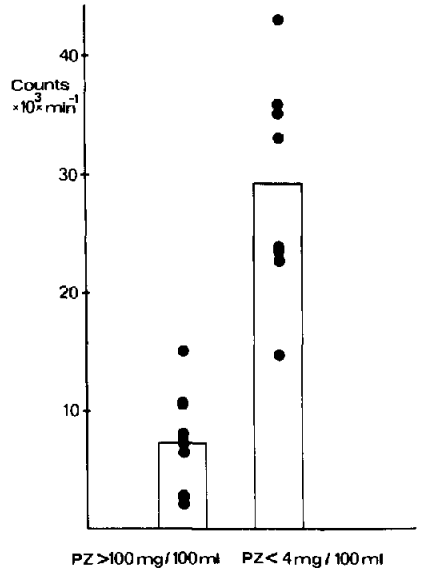

Fig. 1. The lymphocyte response to PHA with sera from ten women with more than 100 mg per 100 ml of PZ and in eight women with less than 4 mg per 100 ml. The incorporation of [^{3}H]thymidine into DNA was measured.

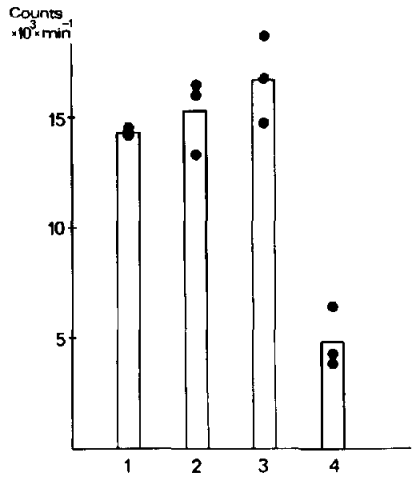

Fig. 2. The lymphocyte response to PHA with 70 μl samples of 1) saline 2) bovine albumin (0.55 mg/ml) 3) bovine albumin (0.22 mg/ml) and 4) purified PZ protein (0.22 mg/ml). The incorporation of [^{3}H]thymidine into DNA was measured.

(0.9% NaCl); b) bovine albumin in saline to a final concentration of 0.55 mg/ml; c) bovine albumin in saline to a final concentration of 0.22 mg/ml or d) pregnancy zone protein in saline to a final concentration of 0.22 mg/ml. Cultures were established in triplicate.

After 2 hr at 37°C 2 μg PHA (Bacto-phytohaemagglutinin P, Difco Laboratories, Detroit, Michigan) in 30 μl saline were added to each culture. The cultures were incubated for 62 hr at 37°C in humidified air supplemented with 5% (v/v) CO_2. Five hours before the end of the culture period 0.4 μCi of [^{3}H]thymidine (specific activity 2.0 Ci/mmol, The Radiochemical Centre, Amersham, Buckinghamshire, England) in 20 μl of saline was added to each culture. The cultures were harvested in a multiple-sample processor [23] and the radioactivity incorporated into DNA was measured in a liquid scintillation spectrometer. Quenching, estimated by the external standard method, was very similar throughout each experiment and therefore results were expressed as counts per min.

3. Results

The lymphocyte response to PHA was found to be lower in cultures containing serum rich in PZ protein than in cultures poor in this protein (fig. 1).

The lymphocyte response to PHA was lower in the presence of PZ protein than in the saline and albumin controls (fig. 2).

4. Discussion

The pregnancy zone protein has been found in increased amounts not only in pregnant women, but also in women taking oral contraceptive drugs [14, 24–26] and in men treated with oestrogen for prostatic cancer [13, 27]. The hypothesis that PZ might function as a steroid carrier has not been supported by recent experimental evidence [28, 29]. The observation in this investigation that the PZ protein inhibits the PHA stimulation of lymphocytes raises the question whether this protein may function as an immunosuppressor during pregnancy.

Since the first report of the pregnancy zone protein [8] there have been several investigations on various α_2-globulins with characteristics more or less similar to those of PZ. Whether Xh [30], SP_3 [31], α-Pregno-

globulin [32] and the pregnancy associated globulin (PAG-Pa1) [33] represent the same protein as PZ is not clear. Stimson [34] recently described the purification of an α_2-glycoprotein from pregnancy serum with a mol. wt. of 506 000. Horne et al [35] were unable to demonstrate a depressive effect on the response of lymphocytes to PHA and PPD of sera containing measurable quantities of PAG.

The mechanism behind the effect of PZ on the lymphocyte response to PHA is unknown. One possibility is that the pregnancy zone protein binds to the lymphocytes, thereby preventing the reaction between PHA and the lymphocytes. Another possibility is that PHA reacts with and becomes inactivated by the pregnancy zone protein.

The effect of PZ on the lumphocyte response to PHA may be related to the finding by Kasakura [7] of immunosuppressive properties of pregnancy serum. This inhibitory activity was more pronounced in pregnancy serum of longer gestation time, reached a maximum at the time of delivery, and then disappeared rapidly during the puerperium. The results presented in fig. 1 with the difference between sera high and low in PZ concentration also reflect the difference between sera from late and early pregnancy.

Another observation of interest in this context is the recently reported depressed lymphocyte response in women taking oral contraceptives [36].

Various homologous glycoproteins are known to inhibit the PHA-induced lymphocyte stimulation. However, none of these glycoproteins seems to be identical with PZ. The immunoregularatory alpha globulin described by Cooperband et al. [37] occurs in non-pregnant healthy subjects, and can be distinguished by electrophoresis from PZ. A placental glycoprotein [38] and a factor in amniotic fluid [39] both depress the lymphocyte response to PHA but differ from PZ which is not found in cord blood, nor in amniotic fluid.

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