INHIBITION OF THE MIXED LYMPHOCYTE REACTION BY THE PREGNANCY ZONE PROTEIN

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1. Introduction

Serum from pregnant women has been found to inhibit the stimulation in vitro of lymphocytes from non-pregnant donors [1–4]. It has not been clarified which factor(s) in serum are responsible for the inhibition. Various hormones [5–9] and serum proteins [10,11] may be involved.

The pregnancy zone protein (PZ) is a high-molecular-weight serum α_2-globulin (mol. wt 359 000) [12,13]. Non-pregnant women and men usually have concentrations between 1 and 40 μg of PZ per ml of serum [14], whereas serum from women in the third trimester of pregnancy contains around 1000 μg per ml [15]. The origin and the biological role of PZ is unknown. An increased level in serum is induced by oestrogen administration [16]. However, PZ seems not to be a carrier of oestrogen [17]. In a previous study PZ was found to inhibit the PHA-induced lymphocyte stimulation, which suggests that the protein may have immunosuppressive properties [18].

In the present investigation the isolated pregnancy zone protein in physiological concentrations was found to inhibit the mixed lymphocyte reaction.

2. Materials and methods

2.1. *Pregnancy zone protein*

Purification of PZ from serum of pregnant women was performed as previously described [12,13]. In order to remove polyacrylamide monomers remaining after the last purification step (preparative polyacrylamide gel electrophoresis) the protein was rechromatographed on a DEAE-Sephadex ion exchanger under conditions earlier described [12,13]. Aliquots of PZ were dialyzed for 2 days at 4°C against 0.9% NaCl and filtered through a Millex^{TM} filter (0.22 μm, Millipore S.A., France).

PZ was specifically absorbed from a partially purified preparation containing between 40–50% PZ. This preparation contained in order to increase the recovery, a broader part of the protein peak obtained from the last purification step, the preparative polyacrylamide gel electrophoresis. To absorb PZ rabbit anti-PZ immunoglobulin was coated on heat-killed *Staphylococcus aureus* (Cowan I, National Collection of Type Cultures, no. 8530). These staphylococci contain protein A on their surface which combines with the Fc portion of IgG [19]. Rabbit anti-PZ antiserum [20] or rabbit normal serum was precipitated with ammonium sulphate in a final saturation of 33% in order to enrich the immunoglobulins. The precipitates were solubilized and coated on staphylococci essentially according to the technique described by Kronvall [21]. One volume of coated staphylococci was mixed with two volumes of PZ solution. After incubation at 37°C for 2 h the mixtures were centrifuged for 1 h at 3000 *g*. The supernatants were removed, dialyzed against 199-HEPES [24] at 4°C for 2 days and filtered through a Millex^{TM} filter.

2.2. *Mixed lymphocytes culture*

Venous blood from healthy non-pregnant blood donors was defibrinated by gentle agitation with glass beads. The lymphocytes were separated by centrifugation on a layer of Ficoll-isopaque [22]. The cells were washed three times in 199 : HEPES and suspended in

199 : HEPES – 30% inactivated (56°C, 30 min) autologous serum. Streptomycin sulphate was added in 100 μg per ml of medium.

PZ or control solutions were added in 100 μl samples to the wells of a Falcon Micro Test II tissue culture plate (Gateway International, Los Angeles). Equal numbers of lymphocytes from two donors were mixed and 100 μl of the mixture were added to each well. To some wells 100 μl of a suspension of lymphocytes from one donor only were added. The final lymphocyte density was 1.5×10^6/ml.

Each microplate was closed with a film and incubated at 37°C for 6 days. Five hours before the end of the incubation period 0.04 μCi of [^{14}C]-thymidine in 20 μl saline was added to each culture. Cultures were washed and precipitated on glass fibre filters by a semiautomatic multiple-sample processor [23] and the radioactivity was determined in a scintillation counter. Quenching, estimated by the external standard method, was very similar throughout each experiment and therefore results were expressed as counts per min. In one experiment cultures were cyto-centrifuged, fixed in methanol and Giemsa stained. In each culture 1000 cells were counted in order to estimate the frequency of blastlike cells. Dye exclusion test was performed at the beginning as well as the end of the incubation period, using trypan blue [24].

Table 1

The effect of purified PZ on the mixed lymphocyte reaction. The incorporation of [^{14}C]-thymidine into DNA was determined. Different preparations of PZ and lymphocytes from various donors were used in the two experiments. Bovine serum albumin (BSA) and saline were used as control solutions. In non-stimulated cultures with lymphocytes from single individuals 50–300 cpm were recorded

Experiment no 1

Substance added	μg/ml	Counts × 10^3 per min		
		Mean	Range	n
PZ	160	0.5	0.4 – 0.8	4
PZ	40	2.8	2.4 – 3.6	4
BSA	160	5.7	5.1 – 6.4	4
BSA	40	4.2	3.0 – 5.0	4
saline		5.2	4.0 – 6.2	20

Experiment no 2

Substance added	μg/ml	Counts × 10^3 per min		
		Mean	Range	n
PZ	170	2.1	1.6 – 2.9	4
PZ	35	10.9	10.5 – 12.0	3
saline		11.7	8.4 – 14.6	11

3. Results

The incorporation of [^{14}C]-thymidine into DNA was lower in the presence of 160–170 μg of purified PZ per ml of culture than in control cultures containing bovine serum albumin or saline. In cultures containing 35–40 μg of PZ per ml the incorporation was decreased only slightly or not at all (table 1). The frequency of blastlike cells in a culture containing 170 μg of PZ per ml of culture was 16%, compared to 35% in a culture absent of PZ. Blastlike cells as well as small lymphocytes had a similar appearance in both preparations. PZ in a concentration of 100 to 300 μg per ml of lymphocyte suspension did not affect the exclusion of trypan blue from the lymphocytes, which contradicts a cytotoxic effect of PZ on the lymphocytes.

A partially purified PZ preparation was incubated in the presence of staphylococci coated with rabbit anti-PZ immunoglobulin or rabbit normal immunoglobulin, More than 99% of PZ was found to be absorbed by the former and less than 10% by the latter staphylococci. Both of the incubated preparations inhibited the incorporation of [^{14}C]-thymidine into DNA. However, the inhibitory effect was reduced by absorption of PZ from the preparation (table 2).

4. Discussion

In the present study purified PZ in physiological concentration was found to inhibit the mixed lymphocyte reaction. Criteria of purity were the observation of a single band on polyacrylamide gel electrophoresis, homogenous sedimentation behaviour and a straight line obtained after equilibrium sedimentation [12]. When a PZ-containing solution was adsorbed by incu-

Table 2
Effect on the mixed lymphocyte reaction of a partially purified PZ preparation which had been absorbed by immobilized rabbit anti-PZ immunoglobulin or immobilized rabbit normal immunoglobulin. A control solution containing PBS only was treated similarly by immobilized rabbit normal immunoglobulin. The incorporation of [^{14}C]-thymidine was determined. Various blood donors were used in the two experiments. In non-stimulated cultures with lymphocytes from single individuals 50–150 cpm were recorded

Preparation tested	Concentration of PZ (μg/ml)	Radioactivity (counts × 10^3 per min)	
		Exp no 1	Exp no 2
PZ preparation absorbed by rabbit anti-PZ immunoglobulin	0.3	1.7 1.2	2.1 2.0 1.3 1.8 2.1
PZ preparation absorbed by rabbit normal immunoglobulin	600	0.8 0.5	0.7 0.9 0.6 0.8
Control solution	0	3.5 3.2 2.8 3.5 4.1	3.8 3.1 3.0 3.5 3.3

bation with immobilized anti-PZ immunoglobulin its effect on the mixed lymphocyte reaction was reduced. Thus the specificity of the inhibitory effect of PZ was confirmed.

Serum from pregnant women is known to inhibit the lymphocyte stimulation in vitro [1–3,18]. The PHA-induced lymphocyte stimulation has been found to be inhibited by human chorionic gonadothropin [5,6], human chorionic somatomammothropin [5], immunoregulatory α-globulin (IRA) [11] as well as by PZ [18]. However, there are few reports on the effect of these factors on the mixed lymphocyte reaction. Human chorionic gonadothropin has been shown to inhibit the reaction only at concentrations above those normally present throughout most of pregnancy [6]. Kasakura found no close correlation between cortisol concentration and inhibition of the mixed lymphocyte reaction [25].

PZ seems to be one factor responsible for the inhibitory effect of plasma from pregnant women on the mixed lymphocyte reaction. The inhibitory effect [25] as well as the serum level of PZ [15] increase with time during pregnancy and decrease soon after delivery. Although PZ was found to have an immunosuppressive effect in vitro it remains to be shown whether it has such an effect also in vivo. If so, PZ may be a factor of importance for the acceptance of the fetus by the mother.

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References

[1] St. Hill, C. A., Finn, R. and Denye, V. (1973) Brit. Med. J. 3, 513.
[2] Kasakura, S. (1971) J. Immunol. 107, 1296.
[3] Ayoub, K. and Kasakura, S. (1971) Clin. Exp. Immunol. 8, 427.
[4] Purtilo, D. T., Hallgren, H. M. and Yunis, E. J. (1972) The Lancet I, 769.
[5] Contractor, S. F. and Davies, H. (1973) Nature New Biology 243, 284.
[6] Jenkins, D. M., Acres, M. G., Peters, J. and Riley, J. (1972) Am. J. Obstet. Gynecol. 114, 13.
[7] Medawer, P. H. and Sparrow, E. M. (1956) J. Endocr. 14, 240.
[8] Waltman, S. R., Burde, R. M. and Berrlos, J. (1971) Transplantation 11, 194.
[9] Munroe, J. S. (1971) J. Reticuloendoth. Soc. 9, 361.
[10] Riggio, R. R., Parrillo, J. E., Bull, G. F., Swartz, G. H., Stenzel, K. M. and Rubin, A. L. (1971) Transplantation 12, 400.
[11] Glasgow, A. H., Cooperband, S. R., Occhino, J. C., Schmid, K. and Mannick, J. A. (1971) Proc. Soc. Exp. Biol. Med. 138, 753.
[12] Von Schoultz, B. and Stigbrand, T. (1973) Acta Obstet. Gynec. Scand. 52, 51.
[13] Von Schoultz, B. and Stigbrand, T. (1974) Biochim. Biophys. Acta 359, 303.
[14] Damber, M.-G., Carlström, K., von Schoultz, B. and Stigbrand, T. (1975). To be published.
[15] Von Schoultz, B. (1974) Am. J. Obstet, Gynecol. 119, 792.
[16] Damber, M.-G., von Schoultz, B., Solheim, F. and Stigbrand, T. (1975) Am. J. Obstet. Gynec. In press.
[17] Von Schoultz, B., Stigbrand, T., Carstensen, H., Södergård, R. and Shanbhag, V. (1973) J. Steroid Biochem. 4, 483.
[18] Von Schoultz, B., Stigbrand, T. and Tärnvik, A. (1973) FEBS Lett. 38, 23.
[19] Forsgren, A. and Sjöquist, J. (1966) J. Immunol 97, 822.
[20] Beckman, L., von Schoultz, B. and Stigbrand, T. (1973) Acta Obstet. Gynec. Scand. 51, 157.
[21] Kronvall, G. (1973) J. Med. Microbiol. 6, 187.
[22] Böyum, A. (1968) Scand. J. Clin. Lab. Invest. 21 (suppl), 27.
[23] Hartzman, R. J., Bach, M. L., Bach, F. H., Thurman, G. B. and Sell, K. W. (1972) Cell. Immunol. 4, 182.
[24] Rabinowitz, Y. (1964) Blood 23, 811.
[25] Kasakura, S. (1973) Nature 246, 496.