HISTOCHEMICAL AND CLINICAL-DIAGNOSTIC STUDY OF PLACENTAL α_1-MICROGLOBULIN USING MONOCLONAL ANTIBODIES

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Placental α_1-microglobulin (PAMG-1) was first identified as an organ-specific placental antigen in 1977 by Petrunin et al. [1], and later Bohn and Kraus [7] described a placental protein 12 (PP-12), which was found to be identical immunochemically with PAMG-1. In turn, Bell and Bohn [4] described an endometrial α_1-globulin (PEG-1), associated with pregnancy, and which was found to be immochemically identical with PP-12. With the aid of monospecific antibodies PEG-1 was found in large amounts in the decidual part of the placenta and the syncytiotrophoblast, in the endometrium in the secretory phase of the menstrual cycle [5], and also in small amounts in the tissues of ovaries, follicular fluid, corpus luteum, and human milk [3]. Pathological secretion of PAMG-1 into the blood serum was recorded in women with pregnancy complicated by toxicosis or in stillbirth [2].

The aim of this investigation was to study the distribution of PAMG-1 in the endometrium in different phases of the mentrual cycle in the placenta at different stages of pregnancy, and also to assess the clinical diagnostic importance of PAMG-1 with the aid of original monoclonal antibodies (MCAB).

MATERIAL AND METHOD

Preparative Isolation of PAMG-1. PAMG-1 was isolated from amniotic fluid (2-10% of all proteins during the second term of pregnancy). The protein was precipitated with lanthanum chloride in a final concentration of 0.5% and the residue was centrifuged at 8000*g*, dissolved in the original volume of a saturated solution of sodium dihydrogen phosphate, carrying the proteins into solution and the lanthanum salt into the residue. PAMG-1 was precipitated with ammonium sulfate at 50% saturation. The residue was dissolved in 1:10 of the original volume. These procedures were followed by chromatography on octylsepharose. PAMG-1 was eluted with 50% ethylene glycol, dialyzed against distilled water, and lyophilized.

Preparation of Fibridomas and MCAB. Female Balb/C rats were immunized by three or four intraperitoneal injections of 100 μg PAMG-1 in phosphate-salt buffer. To hybridize splenocytes with Sp2/O cells, polyethylene-glycol (PEG) 1500 (from "Merck") was used. Antibodies in the primary wells were tested after 12-14 days by ELISA, positive hybridomas were cloned twice by the limiting dilutions method and the clones were maintained in vitro and also in mice receiving pristane. Pure antibodies were isolated on DEAE-cellulose. For isotyping of MCAB in ELISA monospecific rabbit antibodies to classes and subclasses of mouse immunoglobulins (Miles) were used. Enzyme immunoassay was carried out with PAMG-1, fertility α_2-microglobulin (FAMG), trophoblastic β-globulin (TBG), human chorionic gonadotropin (HCG), placental lactogen (PL), and human serum albumin (HSA) [9].

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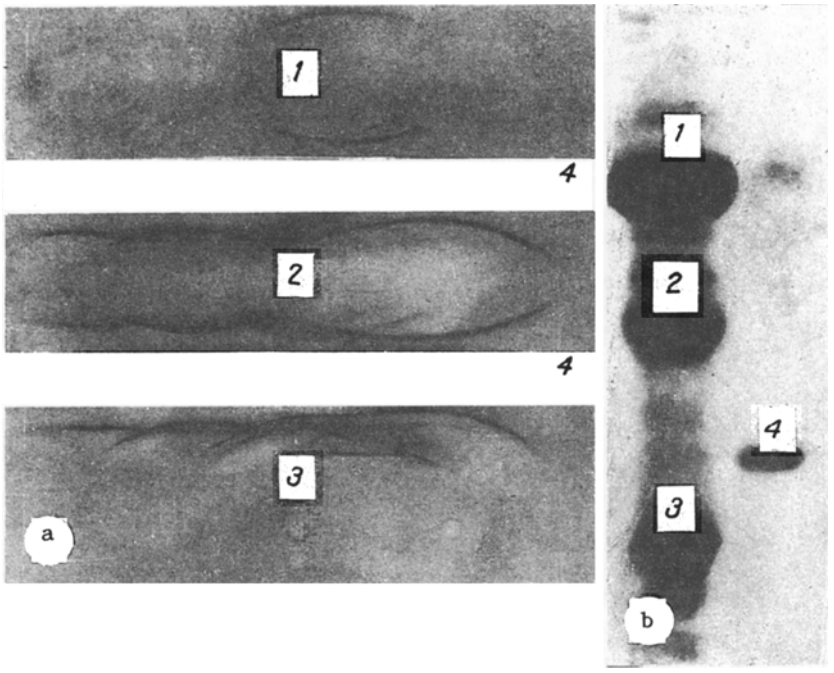

Fig 1. Purity of PAMG-1 preparation: a) immunoelectrophoresis; 1) PAMG-1; 2) amniotic fluid (2nd term of pregnancy); 3) donor's blood serum; 4) antiserum to protein spectrum of amniotic fluid; 5) disk-electrophoresis in 10% polyacrylamide gel with sodium dodecylsulfate; 1) bovine serum albumin (67 kD); 2) ovalbumin (45 kD); 3) chymotrypsinogen A (25 kD); 4) PAMG-1 (35 kD).

Electrophoresis and Immunoblotting. Fractionation of the antigen was carried out by Tsang's method. Proteins were transferred to nitrocellulose ("Pharmacia"). Bovine serum albumin (67 kD), ovalbumin (43 kD), carbonic anhydrase (30 kD), lactalbumin (14.4 kD), and cytochrome c (1225.5 D, all from "Serva"), were used as markers.

Sandwich Version of ELISA. 96-Well planchets ("Costar") were sensitized with MCAB 4B (10 μg/ml) in carbonate-bicarbonate buffer, pH 9.5. Into each well were same volume of the test sera, after which they were incubated for 1 h at 37°C, and then treated with 100 μl of a conjugate of MCAB D3 with peroxidase in a dilution of 1:1000. After incubation of 37°C for 1 h a 0.4% solution of *o*-phenylenediamine in citrate-phosphate buffer, pH 4.7, with 0.1% hydrogen peroxide was added. After 20 min the reaction was stopped by 50% sulfuric acid. The results were read on "Multiscan Titertek" instrument (Flow Laboratories).

Sera from 90 women in the course of pregnancy (5th-40th weeks) and 60 samples of serum from women with habitual abortions were studied. The PAMG-1 concentration also was determined in blood serum from healthy adults (55 sera from men and 51 from nonpregnant women). All samples were kept at −20°C and were tested 3 times.

Immunocytochemical Analysis. Altogether 10 placentas and five samples of decidual tissue after medical abortions and spontaneous miscarriages (3-25 weeks) and after normal labor (39-40 weeks) were tested. Samples of endometrium obtained from seven autopsies not later than 24 h after sudden death were studied. The material was fixed and treated by the method of Sainte-Marie (1962) PAMG-1 in the sections was revealed by the indirect immunoperoxidase method (3-amino-9-ethylcarbasole was used as the chromogen).

RESULTS AND DISCUSSION

By using a combination of salting out and hydrophobic chromatography on octylsepharose a PAMG-1 preparation with a purity of 95% and with an up to 40% yield of the target product was obtained (Fig. 1).

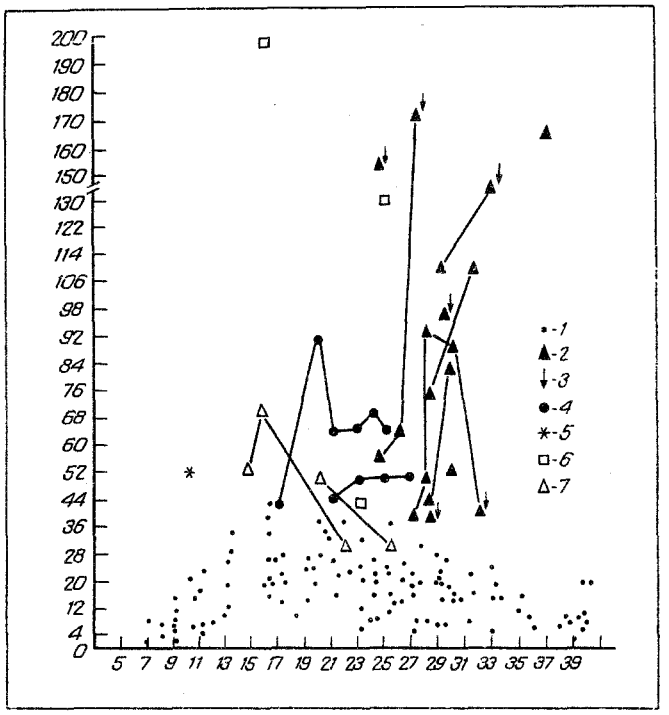

Fig 2. Individual trend of serum PAMG-1 levels during physiological and complicated pregnancy. Abscissa, time of pregnancy (in weeks); ordinate, 1) physiological pregnancy; 2) fetoplacental insufficiency with hypotrophy of the fetus; 3) placental insufficiency with intrauterine death of the fetus; 4) intrauterine infection; 5) anembryony; 6) late abortion; 7) cytomegalovirus infection.

As a result of hybridization and cloning, five stable lines of hybridomas were obtained for the first time: B1, B4, D2, D3, and D5. All MCAB belonged to the IgG_1 class On testing by ELISA they did not give cross reactions with placental proteins – FAMG, TBG, HCG, or PL – and also with human serum albumin In the immunoblotting test, all five MCAB reacted with PAMG-1 (34 kD) and did not react with other proteins of amniotic fluid. The MCAB titer in the culture fluid was 1:1000, and in the ascites fluid 1:100,000, MCAB B4 and D3 revealed two different epitopes on the same PAMG-1 molecule. During adsorption of PAMG-1 on the surface of the well, sensitized by MCAB B4 (binding of the first epitope), subsequent addition of peroxidase-labeled MCAB D3 to the wells revealed the second epitope, If the same MCAB B4 was used as the second antigen, the reaction was negative. We used MCAB B4 and D3 to develop a sandwich version of ELISA for quantitative determination of the serum PAMG-1 level. The sensitivity of the test system was 1 mg/ml and the coefficient of variation about 3.2%.

The results showed that the PAMG-1 level in women varies from 0 to 15 ng/ml and in men from 0 to 19 ng. During physiological pregnancy the PAMG-1 concentration rises up to 40 ng/ml by the second term (Fig. 2). In 23 women with habitual abortion the PAMG-1 concentration was raised by 2-10 times compared with values during physiological pregnancy, and in all these women fetoplacental insufficiency and hypotrophy of the fetus were diagnosed clinically.

Multiple fibroblastlike and epithelioid cells, differing in their PAMG-1 content, were discovered immunochemically with the aid of MCAB B4 in the endometrium during the secretory phase of the normal cycle. It also was found in the cytoplasm of the epithelium and in the lumen of certain endometrial glands In the proliferative stage of the cycle no PAMG-1 was found in the endometrium. At all times of pregnancy PAMG-1 was discovered in the cells of the decidual membrane. These cells differed in shape and in PAMG-1 content. In the early stages of pregnancy (4-7 weeks) MCAB B4 stained the individual glands of the decidual tissue, In the chorionic tissue, no PAMG-1 was found at any stage of pregnancy.

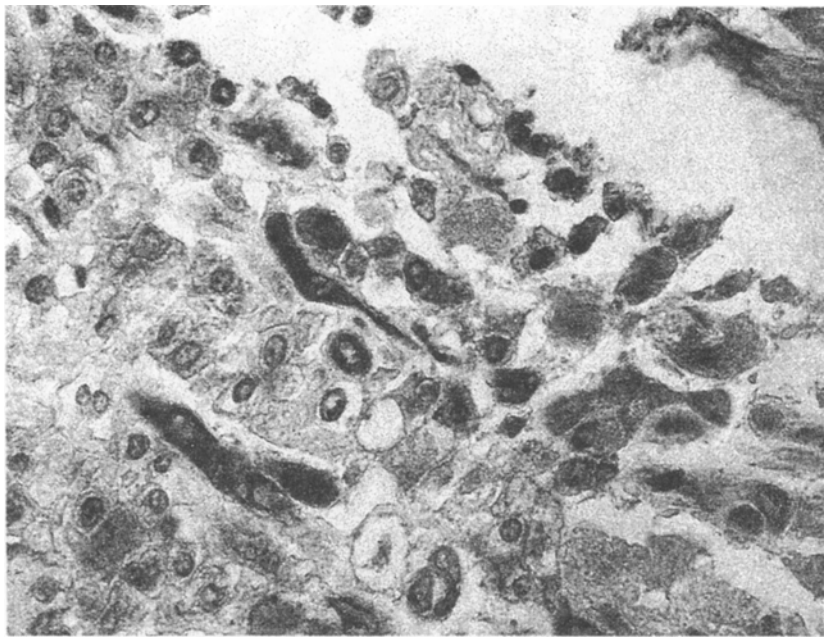

Fig. 3. PAMG-1 in epithelioid and fibroblastlike cells of decidual part of placenta at 24th week of pregnancy. Immunoperoxidase method, 280×.

It is not yet clear whether the PAMG-1 protein is only chemically close to protein PP-12 and PEG-1 or whether it is completely identical with one of them. Judging by data in the literature, PAMG-1 corresponds immunochemically to PP-12 [7]. The latter in turn behaves immunochemically in the same way as PEG-1, but differs from it in its N-terminal amino-acid sequence [6]. The presence of this protein in the decidual cells of the placenta and in the syncytiotrophoblast has been demonstrated with the aid of monospecific antibodies to PP-12. However, antibodies reacting with other placental proteins were found in sera against PP-12 [8]. The MCAB which we obtained reveal PAMG-1 in decidual cells, epithelium, and the lumen of the uterine glands, but not in the trophoblast. MCAB to PEG-1 enables this protein to be localized not only in the decidual cells but also between them, and also in the epithelium of the glands of the decidual tissue [10]. It is evident that data relating to the localization of these proteins and their molecular weight, as well as the incomplete information about their primary structure, call into question their complete identity This is important, for we may be dealing with a family of chemically close, but functionally different proteins. The facts stated above relate both to clinical-diagnostic data which we ourselves obtained and also to the problem of the possible negative action of large quantities of PAMG-1 in the blood on mother and fetus in the presence of placental pathology. In our view, a high PAMG-1 level may be connected with disturbance of the hepatodecidual barrier, but direct immunohistochemical proof of this assumption is necessary.

The investigation showed that the maternal blood PAMG-1 level is raised in fetoplacental insufficiency, including that connected with cytomegalovirus infection and the DBC-syndrome; in half of the cases, moreover, with inadequate treatment the outcome of pregnancy is unfavorable: late abortion, anembryony, intrauterine death of the fetus, and birth of infants with hypotrophy In our cases an interval of 3-6 weeks was noted from the time of discovery of a high PAMG-1 level and the appearance of clinical features.

Our test system can thus widen the field of application of diagnostic sera of this type in obstetric and gynecologic practice with the aim of early detection of intrauterine fetal damage and to predict the course of pregnancy.

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SIMULTANEOUS FLOW CYTOFLUOROMETRIC ANALYSIS OF THE CELL CYCLE AND OF SUBPOPULATIONS OF IMMUNOCOMPETENT CELLS IN WORKERS CLEANING UP AFTER THE CHERNOBYL' ATOMIC POWER STATION DISASTER

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KEY WORDS: immunocompetent cells; irradiation; cell cycle; cytofluorometry.

The study of the cell cycle of subpopulations of immunocompetent cells is both of direct scientific importance as a means of shedding light on the mechanisms of development of immunopathological states and also of practical importance in the search for agents deblocking the cycle and other measures of immunomodulating therapy, exerting a selective action on cells depending on their surface phenotype. A practical solution to these problems became possible as a result of sorting of cells labeled with monoclonal antibodies (MCAB) on a flow cytofluorometer, followed by culture and determination of incorporation of ^{3}H-thymidine and ^{3}H-uridine [8]. Besides the large number of stages involved, with this approach it is almost impossible to prevent the additional activating action of MCAB on peripheral blood mononuclears (PBM). An essential simplification of the technique used in this approach was to use 5-bromodeoxy-uridine (BUdR), followed by conjugation with anti-BUdR MCAB in the indirect immunofluorescence test and with MCAB to surface receptors in the direct test [7]. Recording was carried out on a single-beam cytofluorometer on two-color fluorometry mode. The use of a simple technique of staining cell DNA with the dye Hoechst 3342 in combination with MCAB to differential antigens requires dual-beam cytofluorometry [6]. In recent years the use of MCAB to surface antigens in combination with intercalating dyes for double-stranded DNA (propidium iodide or ethidium bromide), which enables changes to be recorded in the cell cycle in subpopulations of PBM, stimulated by phytohemagglutinin and concanavalin A [5], has been considered the most promising approach. To improve the staining, additional treatment was given with 0.1% saponin solution, or the material is fixed in 2% paraformaldehyde in 70° ethanol [9].

The aim of this investigation was to study the possibility of analyzing the cell cycle of unstimulated PBM from persons exposed to ionizing radiation in direct and indirect immunofluorescence tests.

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