tion of KC into the resected liver is an important adaptive mechanism, creating favorable conditions for subsequent proliferation of hepatocytes.

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COMPARATIVE IMMUNOCHEMICAL AND PHYSICOCHEMICAL CHARACTERISTICS OF CHRONIC α_1- AND α_2-MICROGLOBULINS OF THE HUMAN PLACENTA

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It was shown by methods of immunochemical analysis that chorionic α_1-microglobulin is immunologically different from chorionic α_2-microglobulin. Some of the physicochemical properties of these proteins were studied and their differences from each other were established in relation to several parameters.

KEY WORDS: *human placenta; chorionic microglobulin.*

In 1976 the writers [1] identified an organ-specific antigen of the human placenta with electrophoretic mobility of α_2-globulins, which was present in large amounts in the tissues of the early chorion and amniotic fluid during the first half of pregnancy. A little later [2] we identified an organ-specific antigen in the placenta with the electrophoretic mobility of α_1-globulins, and although it was present in comparatively small amounts in the tissue of the chorion, it was found in large quantities in the amniotic fluid during the first 3 months of pregnancy. These two antigens were immunologically different from chorionic gonadotropin, placental lactogen, trophoblastic β-globulin, α-fetoprotein, and the α_2-globulin of the "pregnancy zone" already known, and they were evidently hitherto unknown placental proteins.

The object of this investigation was a comparative immunochemical and physicochemical investigation of these two organ-specific antigens of the human placenta.

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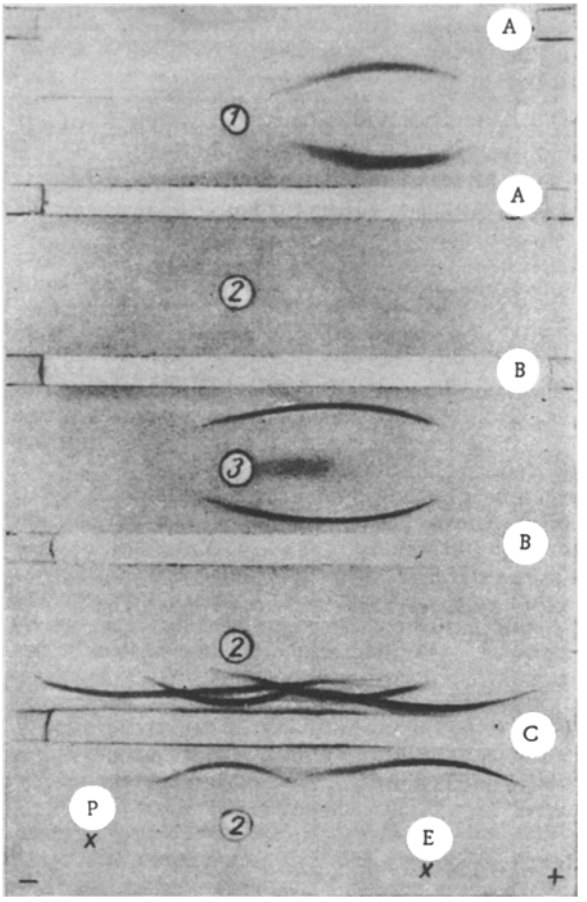

Fig. 1. Comparative immunoelectrophoretic characteristics of chorionic α_1-microglobulin (ChAG-1) and chorionic α_2-microglobulin (ChAG-2). A) Monospecific antiserum against ChAG-1; B) monospecific antiserum against ChAG-2; C) antiserum against human blood serum proteins. 1) Amniotic fluid during first 3 months of pregnancy; 2) donor's serum; 3) chorionic extract. P) Pyronine; E) Evans' blue ("witnessess").

EXPERIMENTAL METHODS

Preparation of chorionic tissue extracts and of monospecific antisera against the organ-specific placental antigens was described previously [1, 2]. Immunoelectrophoresis was carried out by the method of Grabar and Williams [5] and immunodiffusion analysis by Ouchterlony's method in the modification of Khramkova and Abelev [4]. To determine the carbohydrate component in the antigen molecules, the precipitation lines were stained with Schiff's reagent under the control of the serum antigen spectrum and of known glycoproteins (carcinoembryonic antigen, orosomucoid); the neuraminidase test for changes in electrophoretic mobility also was carried out (for this purpose amniotic fluid was first incubated for 24 h at room temperature with neuraminidase in the proportion of 100 units enzyme activity to 1 ml). The molecular weight of the antigens was determined by thin-layer chromatography on Sephadexes G-100 and G-200 with immunodevelopment, for which the layer of Sephadex was removed in successive 1-cm strips perpendicular to the direction of migration of the antigens and placed in a well in agar gel, where the antigens were determined with the aid of appropriate standard monospecific test systems. The electrophoretic mobility of the antigens relative to albumin was determined by Uriel's method [3]. The thermolability of the antigens was determined by heating amniotic fluid or chorionic extract for 30 min within different temperature intervals.

The behavior of the identified antigens with respect to the action of trypsin (1 mg/1 ml amniotic fluid), deoxyribonuclease, and ribonuclease also was determined. The behavior of the antigens also was tested to salting out with ammonium sulfate, and precipitation by rivanol and by perchloric and trichloracetic acids. The concentrations at which precipitation of the antigens occurred were determined with the aid of the corresponding standard test system; the fraction for testing was adjusted to the initial volume of the native material.

EXPERIMENTAL RESULTS

Comparative immunoelectrophoretic analysis of the organ-specific α_1- and α_2-globulins of the placenta is illustrated in Fig. 1 and comparison with the corresponding standard test

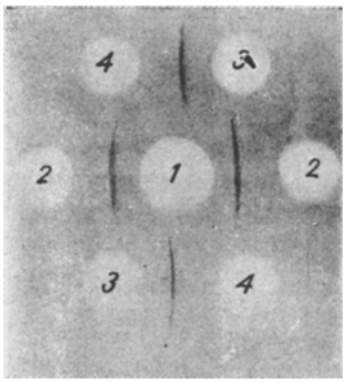

Fig. 2. Comparative immunodiffusion analysis of ChAG-1 and ChAG-2. Test system for ChAG-1: 1) antiserum; 2) solution of antigen. Test system for ChAG-2; 3) antiserum; 4) solution of antigen.

TABLE 1. Comparative Physicochemical Characteristics of ChAG-1 and ChAG-2

Properties	ChAG-1	ChAG-2
Molecular weight	20,000±2000	25,000±2000
Relative electrophoretic mobility	0.85±0.01	0.65±0.01
Staining for glycoproteins	Not stained	Not stained
Change in electrophoretic mobility under influence of neuraminidase	No change	No change
Resistance to action of trypsin	Destroyed	Destroyed
Resistance to action of deoxyribonuclease	Not destroyed	Not destroyed
Resistance to action of ribonuclease	Not destroyed	Not destroyed
Salting out with ammonium sulfate, % saturation	30-50%	25-60%
Precipitation by rivanol	0.4%	0.4% (by 90%)
Precipitation by perchloric acid	0.6% M (by 85%)	0.3 M
Precipitation by trichloracetic acid	2%	2%
Resistance to temperature	Complete loss of antigenic activity at 85°C	Preservation of 10% of antigenic activity at 100°C

system in Fig. 2. Clearly these two antigens were immunochemically identical with one another. Consequently, we were dealing with two antigenically independent organ-specific components of the human chorion.

A comparative analysis of the physicochemical properties of these antigens is shown in Table 1, and they clearly differ from one another in relation to several parameters. Considering that these antigens are proteins in nature (they are destroyed by the action of a proteolytic enzyme) and have low molecular weight (20,000 and 25,000), it was decided to call the α_1-organ-specific placental antigen [2] chorionic α_1-microglobulin (ChAG-1) and the α_2-organ-specific placental antigen [1] chorionic α_2-microglobulin (ChAG-2).

Several of the physicochemical parameters of ChAG-1 and ChAG-2 determined in these experiments can be used, it is suggested, to purify these proteins. With purified preparations of these antigens tests can be carried out for their biological activity, for with this low molecular weight, it seems that they may perhaps have hormonal effects, and the placenta is known to be a producer of several substances with biological activity [6].

The substances ChAG-1 and ChAG-2 identified by the writers are thus two independent antigens, which differ from each other in several physicochemical parameters.

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ACTIVATION OF PRECURSORS OF T KILLERS OF HEMATOPOIETIC STEM CELLS BY THYMOSINE

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Lymph node cells from normal CBA mice, from CBA → CBA syngeneic radiation chimeras, and B mice were incubated *in vitro* with fraction 5 of thymosine, and transplanted into sublethally irradiated (CBA × C57BL)F_1 recipients, and the number of endogenous colonies in the recipients' spleen was determined. Thymosine was shown to potentiate the killer activity of lymph node cells of normal CBA mice and of CBA → CBA syngeneic radiation chimeras, but not of B mice. It is suggested that the target for the action of thymosine is the subpopulation of T_1 lymphocytes.

KEY WORDS: *thymosine; T cell; stem cell.*

It has been firmly established that lymphocytes carrying markers of T cells differentiate from bone marrow precursor cells *in vitro* under the influence of thymosine, a humoral factor of the thymus [3, 5, 7]. Expression of the markers of the T lymphocytes during incubation with thymosine has been shown to take place very quickly (in the course of 1-2 h), and to require transcription of DNA and translation of RNA, but not replication of DNA [8].

The object of this investigation was to study the effect of thymosine on precursor cells of T lymphocytes, exhibiting killer activity against allogeneic hematopoietic stem cells.

EXPERIMENTAL METHODS

CBA and (CBA × C57BL)F_1 mice aged 8-10 weeks were used. To obtain T-deficient animals, normal or thymectomized mice were irradiated in a dose of 750 R and protected with syngeneic bone marrow (10 cells).

Thymosine (fraction 5) was obtained by Goldstein's method with certain modifications [4]. Calf thymus was freed from capsule and homogenized at 12,000 rpm in 0.15 M NaCl (weight:volume = 1:3) in a type 302 Mechanika Precyzyna (Poland) homogenizer. The homogenate was incubated for 16-18 h at 4°C. The insoluble part of the homogenate was removed by centrifugation at 13,000 rpm (Beckman J-21B centrifuge, JA-14 rotor). The resulting supernatant was heated to 30°C for 15 min and the residue of thermolabile components was removed by centrifugation at 13,000 rpm (Beckman centrifuge). The supernatant was treated with 5 volumes of 90% acetone cooled to −20°C. The residue insoluble in acetone was collected by centrifugation at 2500 rpm (Mistral Type 6L centrifuge, J-344 rotor) and dissolved in 10 mM Na-phosphate buffer, pH 7.0 for treatment with ammonium sulfate. The active material was precipitated by ammonium sulfate in saturations from 25 to 50%. The preparation was kept at 4°C in ammonium sulfate (50% saturation, pH 7.0). For the biological tests the preparation was dialyzed against physiological saline. Activity of the thymosine was verified in the test of restoring the sensitivity of rosette-forming cells of thymectomized mice to the inhibitory action of azathioprine [6].

Killer activity was estimated by the ability of T lymphocytes to inactivate endogenous hematopoietic stem cells during the graft versus host reaction [1]. Lymph node cells from CBA mice were transplanted into sublethally irradiated (600 R) (CBA × C57Bl)F_1 mice, and the

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