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DISTRIBUTION OF AMNIOTIC α_1-GLOBULIN IN HUMAN FETAL TISSUES

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KEY WORDS: amniotic fluid; amniotic α_1-globulin; antigens of mesenchyme; myeloid series of blood cells; chorionepithelioma of the uterus.

An immonochemical study of human amniotic fluid revealed a protein with the electrophoretic mobility of α_1-globulins that differed from embryonic antigens hitherto known [1]. This protein was not found in the serum of blood donors. Blood serum from pregnant women gave a marked effect of inhibition of the precipitation arc of a standard test system. Considering the electrophoretic mobility of this protein and its discovery in amniotic fluid, it was called amniotic α_1-globulin (α_1-G).

This paper describes the results of an immunofluorescence study of the distribution of α_1-G in fetal tissues and also in some human trophoblastic tumors.

EXPERIMENTAL METHOD

Antisera were obtained by immunization of rabbits with a semipurified preparation of α_1-G isolated from amniotic fluid of human fetuses at the 20th week of intrauterine development [1]. Antibodies against α_1-G were isolated from monospecific antisera with the aid of an immunosorbent prepared on the basis of Ultrogel AcA-34, activated by glutaraldehyde [4], on which the purified preparation of α_1-G was immobilized.

The indirect method of immunofluorescence analysis [2] was used for the immunohistochemical study of α_1-G. Tissues from various organs of 6-10- and 23-24-week fetuses, tissue of a chorionepithelioma of the uterus after fixation with ethanol and acetic acid [5] and embedding in paraffin wax [6], and a suspension of lymphocytes from the blood and various organs of a 23-24-week human fetus, obtained in a Ficoll density gradient (1.073 g/ml) were studied [3]. The resulting suspension contained a small number of cells of the myeloid series. Films were made from the cell suspension, fixed in methanol for 3 min, and then subjected to immunofluorescence analysis.

EXPERIMENTAL RESULTS

The results of immunofluorescence analysis of α_1-G (Table 1) showed the maximal intensity of fluorescence of structures containing this antigen was present in sections of the placenta, where it was located in the syncytio- and cytotrophoblastic cells of the chorion (Fig. 1a). It will be noted that cytotrophoblastic cells exhibited much stronger specific fluorescence than the syncytiotrophoblast. Fluorescence also was observed inside the villi, in cells of the extraembryonic mesenchyme, in Hofbauer–Kashchenko cells and fibroblast-like cells, and

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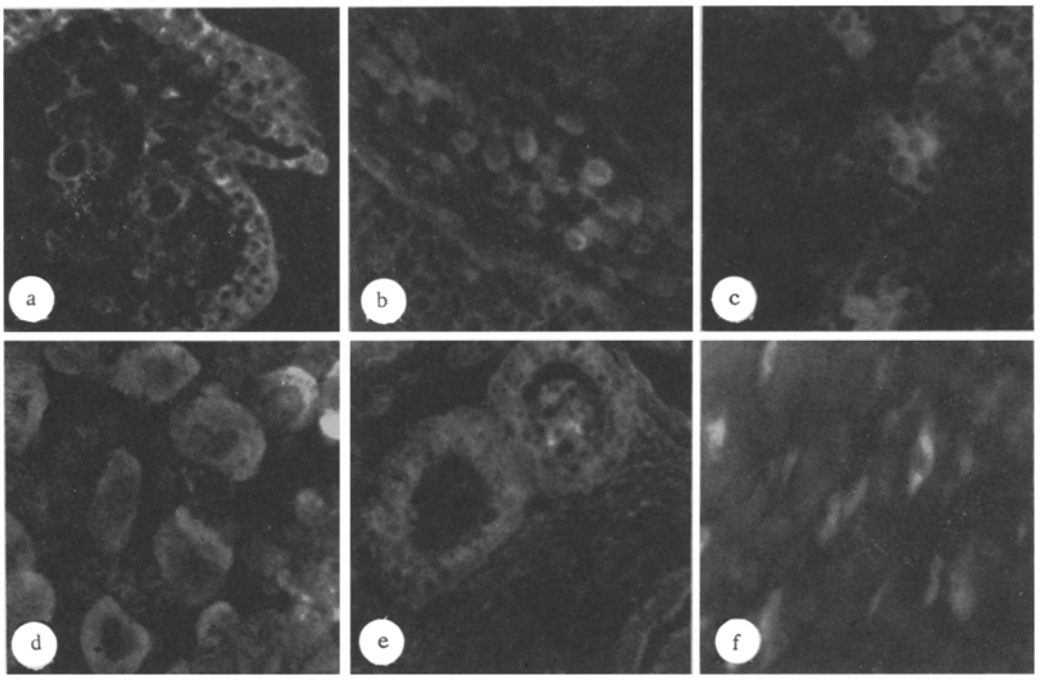

Fig. 1. Immunofluorescence analysis of α_1-G in sections through organs of 6-10-week human fetuses and tissue of a chorionepithelioma of the uterus: a) section through chorion, b) liver, c) red bone marrow, d) chorionepithelioma of the uterus, e) kidney, f) umbilicus. Magnification 5 × 40.

TABLE 1. Results of Immunofluorescence Analysis of α_1-G

Tissue	Period of intrauterine development, weeks	
	6—10	23—24
Placenta	+++	
Stomach	—	—
Small intestine	—	—
Large intestine	—	+
Liver	+	+
Umbilicus	++	+
Bone:		
rib	—	—
femur	—	+
Lung	+	+
Heart	—	—
Brain	—	—
Kidney	+	—
Spleen	Not done	+
Cell suspension:		
from blood	Same	—
from liver	"	+
from spleen	"	+++
from red bone marrow	"	++

Legend. +++) Strong immunofluorescence reaction, ++) average reaction, +) weak reaction, —) no reaction.

also in the vascular endothelium. Specific fluorescence of mesenchymal cells also was found in sections of the fetal umbilicus in the early stages of development, and in the epithelium of the distal and proximal tubules of the kidney; fluorescence was stronger in the distal tubules, probably due to reabsorption of α_1-G from the primary urine of the fetus (Fig. 1e,f).

In the submucosal layer of the large intestine, in tissues of the lung, liver, and spleen, and also in the red bone marrow (Fig. 1b, c) α_1-G was detected in single large cells with a large nucleus.

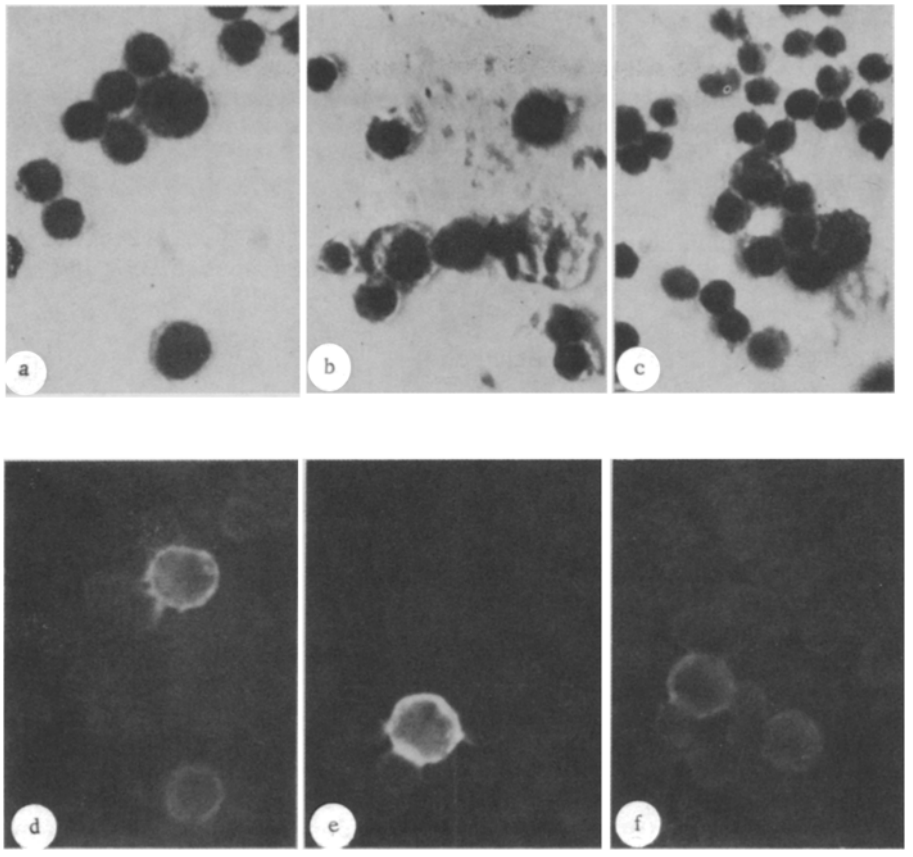

Fig. 2. Analysis of α_2-G in films of cells from a 23-week human fetus. a, d) Liver cells, b, e) spleen cells, c, f) red bone marrow cells. a-c) Stained with azure-eosin; d-f) immunofluorescence analysis. Magnification 5 × 90.

No specific fluorescence linked with the presence of α_1-G was observed in films of a cell suspension isolated from the blood of a 23-week fetus. Bright fluorescence was observed in cells of the myeloid series isolated from the spleen and red bone marrow. Single cells giving weak specific fluorescence were found in films of the lymphocyte fraction from the liver (Fig. 2).

Immunofluorescence analysis of sections through trophoblast tumors revealed a picture of α_1-G distribution similar to that in the human placenta (Fig. 1d). Fluorescence was observed in tumor giant cells (cells of Langhans) and in cells of fibroblast type. The connective-tissue stroma of the trophoblast tumors may perhaps be functionally similar to the extraembryonic mesenchyme of the human placenta.

The discovery of α_1-G in structures of the placenta may thus be indirect evidence that it is synthesized in the placenta and subsequently discharged into the amniotic fluid of the fetus. It is difficult as yet to draw any conclusions on the role of the mesenchymal cells of the placenta and umbilicus in α_1-G synthesis. The fact that α_1-G is found in cells of the myeloid series creates the impression that synthesis of this protein may be connected with the stages of maturity of blood cells of the myeloid series. This is a matter for further study. Investigations using a system of colony-forming cells or blood films from patients with leukemia will evidently help to find the solution.

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EFFECT OF THE DEGREE OF GLUCOCORTICOID SATURATION ON PERIPHERAL BLOOD CFUs LEVELS

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KEY WORDS: recirculation; CFUs; dexamethasone.

It was shown previously that large doses of glucocorticoids reduce migration of hematopoietic stem cells from the bone marrow into the spleen [5]. Moreover, on the basis of the results of a series of investigations into the effect of the pituitary-adrenal system on some aspects of function of CFUs it was concluded that this system has a regulatory role on migration and recirculation of hematopoietic stem cells [1, 2, 4] although the mechanism of action of different doses of glucocorticoids is not clear.

The aim of the present investigation was accordingly to study the action of dexamethasone, administered in different doses, on peripheral blood CFUs, depending on the concentrations of the hormone created by its administration.

EXPERIMENTAL METHOD

Experiments were carried out on (CBA × C57BL)F_1 mice. Dexamethasone phosphate (from Galenika, Yugoslavia) was injected intraperitoneally in doses of 0.005, 0.02, and 0.2 mg per mouse. Animals receiving physiological saline served as the control group. The peripheral blood CFUs level was determined 30 min and 1.5, 5, 24, and 48 h after injection of the hormone or physiological saline, by transplantation of 0.2 ml blood from the injected mice into lethally irradiated recipients. The number of CFUs in the peripheral blood of the donor mice was judged from the number of colonies growing on the spleen of recipient mice on the 8th day after transplantation [8]. The recipients were irradiated on the EGO-2 apparatus in a dose of 850 R with a dose rate of 120 R/min. The degree of saturation of the mice with dexamethasone following injection of the hormone was calculated on the basis of data given in [7]. The endogenous corticosterone level was determined 5 and 24 h after injection of dexamethasone by the method in [6]. The results were analyzed by Student's t test.

EXPERIMENTAL RESULTS

A tendency for the peripheral blood CFUs level to fall, although not significantly, was observed 30 min after injection of dexamethasone in a dose of 0.005 mg per mouse. The number of CFUs after 1.5 h was reduced to 58.1% ($P < 0.05$). After 5 h the number of circulating CFUs was back to normal, after 24 h their level was higher than in the control, and the initial values were reached 48 h after injection (Fig. 1).

A fourfold increase in the dose of dexamethasone (0.02 mg per mouse) was accompanied by a more prolonged fall in the number of circulating CFUs: After 30 min there was a very small decrease in the number of these cells, after 1.5 and 5 h the CFUs level was down to 51.7 and 48% respectively, after 24 h the number of CFUs in the peripheral blood was increased up to 169% of the initial values, and 48 h after injection the CFUs level remained considerably higher than initially, at 222% (Fig. 1).

Injection of dexamethasone in a larger dose (0.2 mg per mouse) gave rise to an intensive and prolonged decrease in the number of CFUs in the peripheral blood. After 30 min the fall in the blood CFUs level was very small, 1.5 h after injection their number had fallen to 25% of its initial value, the level remained low 5 and 24 h after injection of dexamethasone, but after 48 h the number of CFUs was back to the initial level (Fig. 1).

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