A FETAL CARBOXYLIC ESTERASE OF MOUSE ORIGIN COMMON TO NORMAL EMBRYONIC BRAIN AND MURINE EXPERIMENTAL TERATOCARCINOMA

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1. Introduction

In a previous note [1] we described an esterase E_3, specific for fetal brain from strain A mouse. This enzyme has been shown to be an arylesterase (EC. 3.1.1.2.) which is not inhibited by 10^{-4} M eserine. It is present in the brain of fetuses from the sixteenth day of gestation until 2 days after birth and is absent in adult mouse brain. As this enzyme seemed to be a marker of nervous tissue differentiation we have tried to demonstrate its presence in neuronal tumors. Previous attempts to identify this esterase in neuroblastoma C 1300, transplanted on strain A mouse, were unsuccessful.

We report here some preliminary results on the search for esterase E_3 on experimentally transplanted teratocarcinomas.

2. Materials

2.1. The following experimental teratomas were provided by Dr. L.C. Stevens (Jackson lab., Bar Harbour):

i) OTT 55.68 was obtained in 1966 by grafting a 3 days 129/SV embryo into the testis of an adult 129 mouse.

ii) OTT 60.50 was obtained in 1967 by grafting a 6 days 129/SV embryo into an adult 129 mouse testis.

These tumors were transplanted subcutaneously and differentiated in a variety of tissues (i.e. neural, epithelial, muscle) [2].

iii) OTT 24.66 was obtained in 1968 by grafting a 6 days A/HE embryo into the testis of an F_1 129XA/He male.

The tumor OTT 24.66, also transplanted subcutaneously, differentiates mainly in anaplastic nervous tissue. Embryoïd bodies which are known to be the equivalent of a primitive embryo with its 3 germ layers are not present in this tumor. All these tumors are transplanted every 3 or 4 weeks. The tumors are removed from the mice after a cervical dislocation, washed twice in physiological buffer and pooled at −20°C until used.

2.2. 7, 10, 13 day-old embryos

Mice were allowed to mate overnight and were checked for sperm plugs the next morning. The day on which the sperm plugs are detected is designated as day no. 2. The embryos were carefully removed from the uterus to prevent any contamination by the gestation tissues, washed in (PBS), pooled for extraction and stored at −20°C.

2.3. 18 day-old embryo, newborn brains were obtained as previous described [1].

3. Methods

3.1. Extraction

After thawing, tumors as well as fetal and newborn brains were minced, suspended in 4 vol of cold 50 mM PO_4HK buffer, pH 7.5, disrupted by 6 strokes in a Dounce homogeniser. Triton X-100 (0.5%, w/v) was added to facilitate the release of the esterase E_3 which mainly exists in a membrane bound form.

Solubilisation was then accomplished by ultrasonic treatment at 4°C in a M.S.E. ultrasonic disintegrator (5 pulses of 30 sec each at 30 sec intervals). The homogenate was centrifugated at 100,000 *g* for 1 hr, in a Spinco model L or in a Sorvall centrifuge at 40,000 *g* for 30 min.

The supernatant was dialysed overnight against 50 mM PO_4HK buffer, pH 7.5, without Triton X-100, at 4°C and stored at −80°C in small aliquots.

Alternatively and to avoid any contamination by physiological fluids some experiments were carried out with dissociated cells, prepared according to the technique of Howard et al. [3]. Fresh samples of tissue were incubated with gentle stirring for 30 min at 37°C in 5 vol of Difco 199 medium pH 7.5, containing: 0.10% collagenase, 0.20% hyaluronidase (both Sigma type 1), 0.40 M, 0.01% sodium citrate, and 2×10^{-3} M β-mercaptoethanol.

The dissociated cells were collected after 5 min centrifugation at 150 *g* and washed twice in Difco 199 medium. The cells were homogenised as described above.

3.2. Ammonium sulfate precipitation

The supernatant from the centrifugation at 40,000 *g* was precipitated at 4°C by ammonium sulfate, 0.55 M. The precipitate was gently suspended in 2 ml of buffer and extensively dialysed against 50 mM PO_4HK buffer, pH 7.5.

3.3. Electrophoresis separation and identification of esterase E_3 with β-naphthyl acetate have been carried out as previously described [1].

3.4. Preparations of neuronal and glial fractions

An attempt has been made to determine the origin of the cells containing the E_3 activity. Neuronal and glial enriched fractions from tumor tissue and fetal brain tissue were prepared according to the technique of Packman et al. [4].

4. Results and discussion

As shown in fig. 1, esterase E_3 can be demonstrated in electrophoresed extracts of both 18 day fetal brain and OTT 24.66. The esterase E_3 is present in embryonic brain at 16 days and cannot be detected until 48 hr after birth. The esterase band no. 5 is a serum contaminant. The esterase E_3 is already present in teratoma OTT 24.66 as soon as the tumor can be removed from the mouse (10 to 12 days after transplantation).

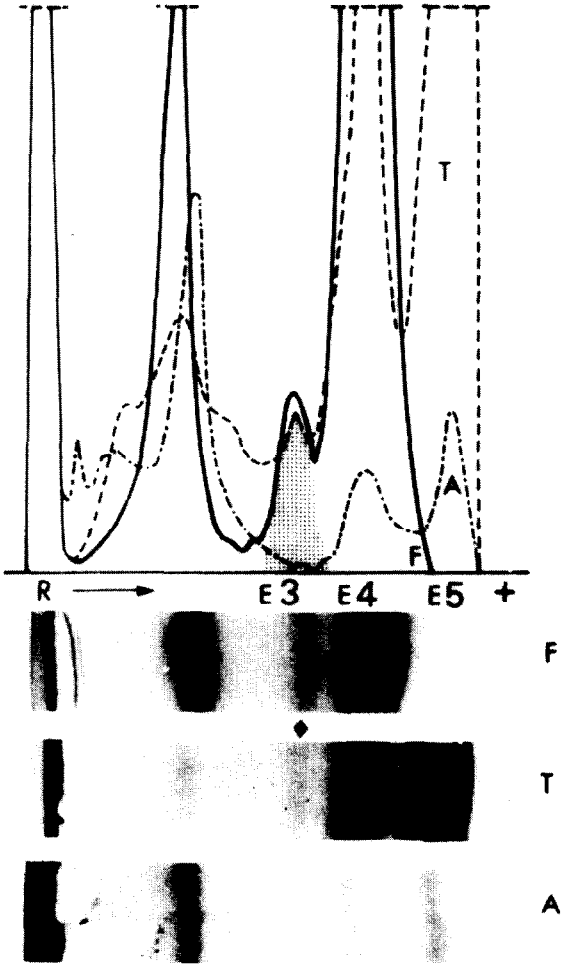

Fig. 1. Electrophoretic patterns of carboxylic esterase in soluble extracts of fetal brain (18 days of gestation) (F), teratocarcinoma OTT 24.66 (T) and adult brain (A). Each reservoir contains 100 μl of soluble extracts (10 mg/ml). Electrophoresis was carried out in 5% acrylamide −0.8% agarose gels mixed in 25 mM barbital buffer, pH 8.2. Electrophoresis was run for 3 hr at 6 V/cm in a cold room (+ 4°C). The slabs were incubated for 45 min in a solution of phosphate buffer, 50 mM, pH 7.5, containing 10 mmol of β naphthyl acetate and 5 mg/10 ml of diazotized orthodianisidine (Diazoblue B) [5].

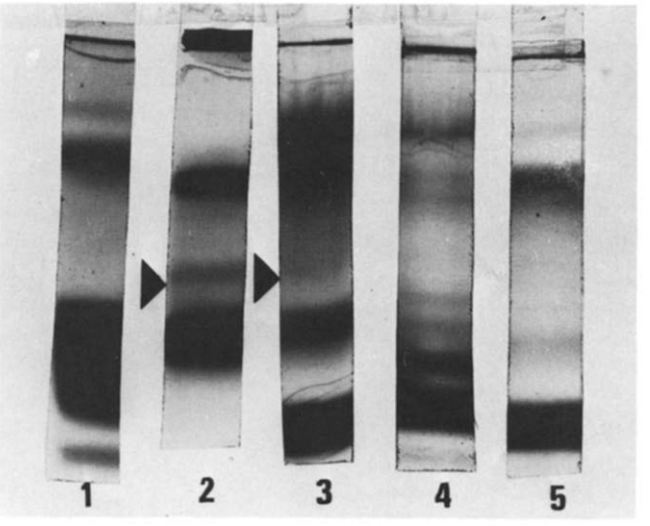

Fig. 2. Comparative electrophoretic patterns of carboxylic esterases of: 1) 7 day-old embryo (whole embryo); 2) brain fetal extract (18 days); 3) OTT 24.66 teratocarcinoma; 4) OTT 60.50 teratocarcinoma; 5) OTT 55.68 teratocarcinoma. In each starting reservoir there is the same amount of proteins (10 to 11 mg/ml).

Fig. 2 shows the absence of esterase E_3 in extracts from embryos less than 16 days, from teratoms OTT 60.50, and from OTT 55.68.

Esterase could not be demonstrated in soluble extracts of liver, kidney and muscle from 18 day-old fetuses.

The possibility of contamination of brain extracts by the mid-gestation tissue derived from both maternal and fetal origin was then considered. In the first half of pregnancy some esterases can be revealed in the trophoblast tissue (foetal placenta), in the decidua (maternal placenta), and in the yolk sac (visceral endoderm) [6, 7]. Sherman showed that a pool of A and B isozymes with esterase activity was common to the embryo proper, while an esterase F activity was present in trophoblast preparations as early in gestation as the eight day, at least. The esterase F was never detected in the amnion or fetus at any stage of gestation.

However, we have never detected esterase E_3 in the soluble fractions of homogenates obtained from extracts of maternal uterus and derived tissue, nor in fetal envelopes, at different times of gestation.

Attempts to study the quantitative differences in esterase activity in soluble extracts were unsuccessful. This was due to the high background produced by non-specific serum esterases which contaminated the extract. The specific activity expressed as μmol of β naphthyl acetate hydrolysed per min per mg of protein varied in the range of 6.50 to 9 μmol in tumors as well as in fetal brain and serum.

Preliminary results on the cell localization of esterase E_3 by electrophoresis show that the enzyme is mainly present in the glial fraction of both fetal brain and teratocarcinoma.

The most important feature of this preliminary work is the demonstration of an esterase of fetal origin in some teratocarcinomas.

The exact significance of esterase E_3 is still unknown but if we assume that esterase E_3 is a transitory marker of neural differentiation, its appearance in teratocarcinoma OTT 24.66 could be explained by the presence of nervous cells passing through this stage of differentiation at the time of removal. This may be true even if this tumor, although normally predominantly neural, also contained epithelial and mesodermic tissue and large numbers of undifferentiated stem cells.

Acknowledgements

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