It was thus shown by three immunologic reactions that BCG microorganisms and *L. monocytogenes* cells have a common antigen with human malignant tumors, characterized by either partial or complete immunologic identity. Complete immunologic identity was found between antigens of different specimens of tumors from the same organ (carcinoma of the breast, for example) or from similar tumors taken from different species (carcinoma of the human and rat ovaries, for example).

These data can be used to study how malignant cells are affected by immune sera specific not against the antigens of those cells, but against antigens which they contain in common with certain strains of microorganisms.

LITERATURE CITED

1. N. N. Zhukov-Verezhnikov and G. Guseva, Zh. Mikrobiol., No. 3, 14 (1944).
2. N. N. Zhukov-Verezhnikov, I. I. Podoplelov, N. M. Mazina, et al., Usp. Sovrem. Biol., 74, No. 1 (4), 54 (1972).
3. M. S. Lomakin, I. N. Maiskii, T. N. Mamaeva, et al., Byull. Éksp. Biol. Med., No. 1, 65 (1977).
4. E. E. Pogosyants, E. L. Prigozhina, and N. A. Egolina, Vopr. Onkol., No. 11, 29 (1962).
5. C. Bucana and M. J. Hanna, J. Natl. Cancer Inst., 53, 1313 (1974).
6. B. A. McErlean, Nature, 197, 507 (1963).

INDEPENDENCE OF ALPHA-FETOPROTEIN EXPRESSION OF SERUM PROTEIN PRODUCTION IN ADULT RATS WITH HEPATOMA McA-RH7777

G. I. Abelev, D. A. Él'gort, and T. L. Éraizer

UDC 616.36-006.6-008.939.6:616.153.96]-092.9-074

KEY WORDS: alpha-fetoprotein; serum proteins; immunoisotachophoresis.

Many hepatocellular carcinomas synthesize the embryo-specific serum protein alpha-fetoprotein (AFP) together with other blood serum proteins [1]. However, some hepatomas both in man and in animals do not produce AFP [1]. Moreover, even in those hepatomas which do produce AFP this protein is not synthesized by all cells [5]. Isaka et al. [7] showed that ascites rat hepatoma is genetically heterogeneous for this trait, and that clones differing by as much as 50 times in their level of AFP production can be isolated from it.

This paper describes an attempt to determine whether AFP expression in a hepatoma is connected with the production of other serum proteins or whether it is regulated discretely and is independent of the synthesis of proteins of the "adult" type.

EXPERIMENTAL METHODS

A culture of rat hepatoma McA-RH7777 cells was generously provided by Dr. Becker and Professor Van Potter (McArdle Laboratories on Cancer Research, Wisconsin, USA) under the terms of the Soviet-American agreement on collaboration on tumor immunology. The cells were cultured in L15 medium (Flow) with 10% embryonic calf serum (Gibco, USA).

Cloning was carried out in methylcellulose M-281 (Fisher, USA). The final methylcellulose concentration in the mixture was 1% and of embryonic calf serum 30%. In each tube 10,000 cells were cultured in 2 ml of the methylcellulose solution. Clones were isolated on the 10th-14th day. The clones grown in methylcellulose were separated under a "Diawerth" microscope (from Leitz, West Germany). The clones were then cultured in 96-well microplates (from Falcon Plastics, USA).

Laboratory of Immunochemistry and Diagnosis of Tumors, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 9, pp. 333-335, September, 1981. Original article submitted March 12, 1981.

0007-4888/81/9209-1237$07.50

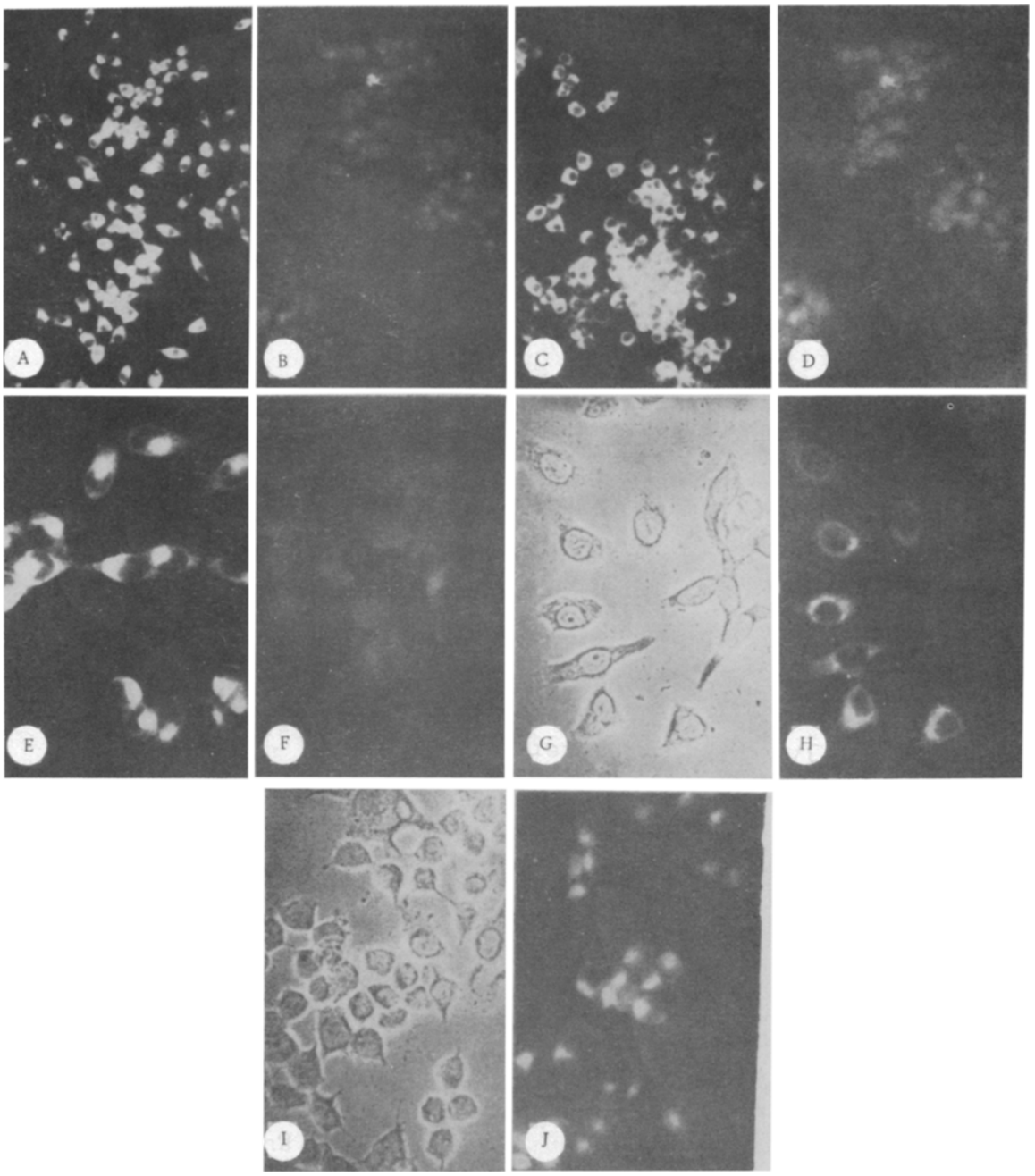

Fig. 1. Immunofluorescence analysis of AFP content in clones E3 (left) and H1 (right) during culture. A and B) 1st passage; C and D) 4th passage; E and F) 12th passage; G-J) 17th passage (G and I — phase-contrast photographs). Opton microscope. Magnification: A-D) 16 ×; E-J) 40 ×.

The cells were fixed in 4% formalin solution in physiological saline and AFP was determined in the cells by indirect immunofluorescence [4] or immunoperoxidase [9] methods, using rabbit antibodies against AFP, purified on an immunosorbent, and donkey antiserum (AS) against rabbit globulins labeled with fluorescein isothiocyanate (from the Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow), or with a peroxidase—antiperoxidase complex (PAP; from Dako, Denmark).

AFP in the culture media was determined by the aggregate-hemagglutination (AHA) method [3], using sheep's red blood cells conjugated with antibodies against rat AFP.

The spectrum of serum proteins secreted by the cells was analyzed by immunoisotachophoresis [2], using an apparatus for electrophoresis of the "Gelman" (USA) type. For this

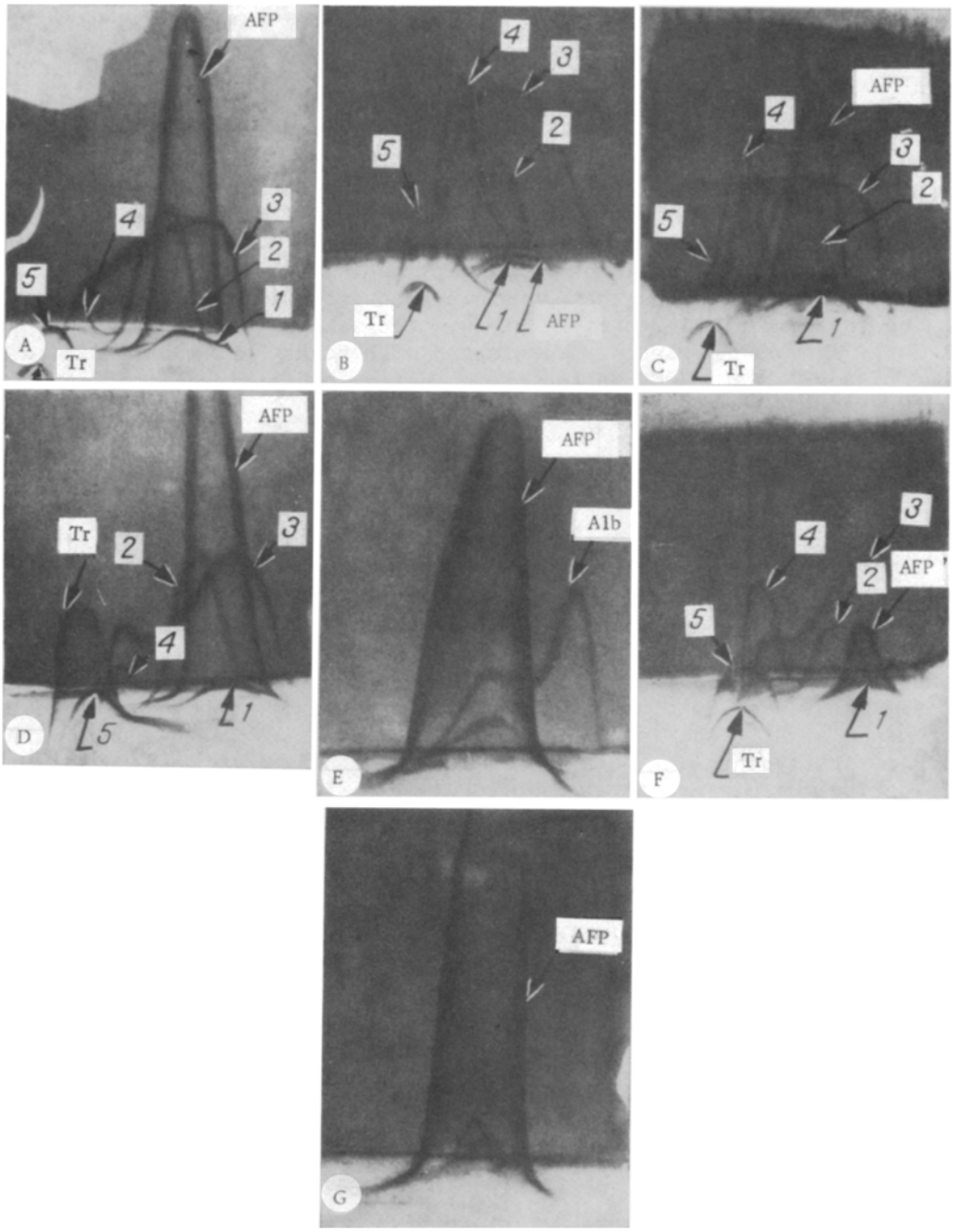

Fig. 2. Immunoisotachophoretic analysis of culture media of clones E3 and H1. Reagents used: A) 50 µl of medium of clone E3 at 11th passage; B) 200 µl medium of clone H1 at 11th passage; C) 25 µl of medium of clone E3 at 11th passage mixed with 100 µl of medium of clone H1 at 11th passage; D) 50 µl of medium of clone E3 at 11th passage + 10 µl transferrin-containing solution; E) 200 µl of medium of clone E3 at 11th passage with addition of 5 µl of solution containing 30 ng of purified rat albumin; F) 200 µl of medium of clone E3 at 11th passage; G) 50 µl of medium of clone E3 at 18th passage. The following reagents were used for detection in crossed immunoelectrophoresis: A-D, G) AS against ARSP in dilution of 1:5 mixed with AS against AFP in dilution of 1:10; E) AS against ARSP in dilution of 1:320 mixed with AS against AFP in dilution of 1:10; F) AS against ARSP in dilution of 1:320 mixed with AS against AFP in dilution of 1:5. 1-5) Nos. of peaks corresponding to their electrophoretic mobility; Tr) transferrin.

purpose 25-200 µl of the sample was mixed with 5 µl of a 10% solution of ampholines ("Servolyt," pH 4.0-6.0) and subjected to electrophoresis on a "Cellogel" cellulose acetate membrane (from Serva, West Germany) in a heterogeneous buffer system ("leading" buffer 0.06 M Tris-HCl, pH 6.7; "closing" buffer 0.012 M Tris-glycine, pH 8.3). The film containing the concentrated and separated proteins was transferred to a thin agarose plate (60 × 90 × 0.5 mm), the cathodal half of which consisted of "pure" 1% agarose in Tris-barbital buffer, pH 8.6 (ionic strength about 0.05), whereas the anodal part contained AS against AFP or against adult rat serum proteins (ARSP), or a mixture of both AS. The film was placed on the "pure" agarose at a distance of 0.5 cm from the agarose containing AS, and crossed immunoelectrophoresis was performed [6]. The plates were then washed, with "pressing": After incubation for 15 min in physiological saline the plates were covered with several layers of dry filter paper and placed for 10 min under a light press, then washed repeatedly with physiological saline for 1-2 h. The washed plates were treated with donkey antirabbit AS (to strengthen the precipitates) for 2 h at 37°C, washed again, dried, and stained with Coomassie Blue R-250 (Serva, West Germany).

EXPERIMENTAL RESULTS

Clones grown in methylcellulose were isolated and the AFP levels in their culture media were determined by the AHA method. Clones with maximally high and maximally low AFP levels (eight clones) were selected for further culture and analysis.

Two "contrasting" clones (E3 and H1), in which the AFP levels in the medium and the fraction of AFP-positive cells remained unchanged in the medium longest were studied in most detail (Fig. 1). In the medium of one of these clones (E3, 7th and 11th passages) the AFP concentration was about 20 µg/ml, whereas the AFP level in the medium of the other clone (H1, 9th and 11th passages) was 100 times lower. The clones compared thus differed not in the presence or absence of AFP, but in the degree of its expression, i.e., they were variants for regulation of AFP synthesis. This conclusion was confirmed by experiments with recloning of the "negative" H1 clone: In all subclones of this culture tested (four first-order subclones and four second-order subclones) ADP was detected by AHA and immunofluorescence methods, thus ruling out the possibility that clone H1 could be contaminated by cells of the AFP-positive clone.

The culture media of these clones were studied by immunoisotachophoresis. This method showed that clones E3 and H1 differed not only in the absolute AFP content, but also in the ratio between AFP and the other serum proteins (Fig. 2A, B). Spectra of the ARSP secreted by these clones were similar, although the over-all level of protein synthesis was about four times higher in clone E3 than in clone H1. When AS against ARSP were used in a dilution of 1:5, six peaks were detected clearly and reproducibly in the culture media of both clones. The pattern was unchanged if a mixture of culture media of these clones was used in the experiment, thus confirming the identity of the antigens secreted by clones E3 and H1 (Fig. 2C). One of these antigens was identified as transferrin by the addition of purified rat transferrin (generously provided by A. K. Yazova) to the medium: This led to a sharp increase in size of the corresponding peak (Fig. 2D). Addition of purified rat albumin to the medium led to the formation of an independent peak, which did not correspond to any of the peaks detected in the culture media (Fig. 2E). Albumin was not detected in the media of clones E3 and H1, whatever dilution of AS was used, confirming data in the literature [8] indicating that no albumin is secreted in cultures of McA-RH7777 cells. When high dilutions of AS against ARSP were used two additional peaks were discovered in the media of clones E3 and H1 (Fig. 2F).

The picture described above was observed in media of clone E3 at the 7th and 11th passages and in media of clone H1 at the 9th and 11th passages. In the media of clone E3 at the 18th and 19th passages there was a marked decrease in the AFP content while the other serum proteins remained at their previous level (Fig. 2G).

The reasons for this equalization are not yet clear. It could be due to the rapid accumulation of genetic variants differing in their level of AFP expression or to the control of this expression by nongenetic mechanisms. This problem is being studied by the writers at present. Whatever the case, however, it will be evident that the level of AFP production does not correlate with synthesis of other serum proteins which we detected and it is evidently controlled by an independent mechanism.

LITERATURE CITED

1. G. I. Abelev, Prog. Exp. Tumor Res., 7, 104 (1965).
2. G. I. Abelev and É. R. Karamova, Byull. Éksp. Biol. Med., No. 9, 334 (1979).
3. A. M. Olovnikov, Dokl. Akad. Nauk SSSR, 169, 1180 (1966).
4. N. V. Éngel'gardt, in: Immunochemical Analysis, L. A. Zil'ber (ed.) [in Russian], Moscow (1968), pp. 165-188.
5. N. V. Engel'gardt, V. S. Poltoranina, L. Ya. Shipova, et al., in: Alpha-Fetoprotein, R. Masseyeff (ed.), Paris (1974), pp. 217-229.
6. N. H. Axelsen, J. Krøll, and B. Weeke, A Manual of Quantitative Immunoelectrophoresis. Methods and Applications, Oslo (1973).
7. H. Isaka, S. Umehara, H. Yoshii, et al., Gann, 67, 131 (1976).
8. L. A. Sternberger, Immunocytochemistry, New York (1974).

CELL CYCLE AND PROLIFERATIVE POOL OF HUMAN TUMOR STRAINS TRANSPLANTED IN NUDE MICE

E. S. Revazova and A. S. Petrova

UDC 616-006.04-018.15:616-006.04-089.843.092.9

KEY WORDS: cell cycle; proliferative pool; human tumor strains.

To determine optimal schemes of treatment the cell cycle of many strains of mouse and rat tumors has been studied. However, the cell kinetics of strains of human tumors transplanted in nude mice, which are nowadays used to study methods of treatment of human tumors, has not yet been investigated.

This paper describes a study of the duration of periods of the cell cycle and the proliferative pool of human tumor strains obtained previously by serial transplantations in nude mice [3]: melanoma, Ewing's sarcoma, carcinoma of the kidney, and Wilms' tumor.

EXPERIMENTAL METHODS

Nude BALB/c mice bred by ourselves and aged 1-1.5 months, underwent subcutaneous transplantation of strains of human tumors in 0.5 ml Eagle's medium in the ratio of 1:3. When tumor nodes measuring 1000 mm^3 had formed the animals were given an intraperitoneal injection of ^{3}H-thymidine (1 μCi/g body weight, from "Izotop," specific activity 19.8 Ci/mole). To avoid differences due to circadian rhythms of mitosis in the tumors, the thymidine was injected always at the same time of day — between 11 a.m. and noon. Animals were killed 1 h later, and every 3 h thereafter for 36 h after injection of the labeled thymidine, 2-4 mice at a time for all transplanted strains. Altogether 130 nude mice were used.

Material was fixed in Carnoy's fluid and embedded in paraffin wax; sections were cut to a thickness of 5 μ and autoradiographs prepared. Light-sensitive type "M" emulsion (Photographic Chemical Research Institute), heated to 41°C, was applied to the sections in darkness. The sections were exposed to the emulsion for 5-6 weeks. They were then stained with hematoxylin and eosin. Cells were taken to be labeled if they had four granules or more. The number of labeled mitoses was counted in the superficial actively proliferating zones of tumor (not deeper than 300 μ), for it was only in that case that a curve of labeled mitoses with two distinct waves could be obtained. On the basis of the results of counting labeled mitoses graphs were plotted and the duration of periods of the cell cycle determined relative to the 50% level of values of the labeled mitosis curve [1]. To calculate the proliferative pool in the tumors the label saturation method [6] was used. ^{3}H-thymidine was injected into the mice seven times at intervals of 6 h and the animals were killed 1 h after the last injection. The proliferative pool (Pc) was determined as the number of cells per 1000 cells for the whole tumor, expressed in percent.

Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 9, pp. 335-337, September, 1981. Original article submitted January 22, 1981.

