The results show that besides known populations of cytotoxic effector cells, such as lymphocytes and monocytes, platelets also can carry out destruction of tumor cells. It was thus shown for the first time that platelets of lung cancer patients possess specific cytolytic activity and cause lysis of freshly isolated and transplantable lung cancer cells. Elucidation of the mechanisms of the cytolytic action of platelets, including the study of receptors and structures participating in interaction with tumor cells, isolation of the cytotoxic factor from platelets, investigation of the specificity of their action in other groups of cancer patients also, are tasks of fundamental importance for characterization of the cellular systems involved in interaction with malignantly transformed cells.

LITERATURE CITED

1. S. N. Bykovskaya, M. S. Iobadze, T. A. Kupriyanova, et al., Byull. Éksp. Biol. Med., No. 1, 86 (1987).
2. D. B. Amos, Ann. N. Y. Acad. Sci., 87, 273 (1960).
3. J.-C. Cerottini and K. T. Brunner, Adv. Immunol., 18, 67 (1974).
4. M. I. Grethen, N. E. Kay, R. J. Johnson, et al., Blood, 65, 1252 (1985).
5. R. B. Herberman, J. Y. Djeu, and H. D. Kay, Immunol. Rev., 44, 43 (1979).
6. I. R. Pasternac, R. J. Johnson, and H. S. Shin, J. Immunol., 120, 1560 (1978).
7. P. Skov Madsen, P. Hokland, and M. Hokland, Acta Path. Microbiol. Scand., Sec. C, 94, 193 (1984).
8. A. Uchida and M. Moore, Cancer Immunol. Immunother., 20, 29 (1985).

HUMAN TUMOR CELL LINES OBTAINED BY TISSUE CULTURE PASSAGE FROM STRAINS TRANSPLANTED INTO NUDE MICE

T. V. Yudicheva, T. I. Milovanova, Yu. N. Solov'ev, and E. S. Revazova

UDC 616-006-018.15-092.4

KEY WORDS: carcinoma of the uterus, Wilms' tumor.

It has rarely proved possible to obtain a continuous cell line of a human tumor by passage in tissue culture from biopsy or operative material. A more promising method is to obtain cell lines from strains of human tumors maintained by long-term passage through nude mice. As a result of repeated serial transplantations, cells with shortened cell cycles accumulate in tumors [1, 2]. Cells of transplantable strains are capable of unlimited multiplication, and for that reason the possibility of obtaining immortalized cell lines from them is much greater.

Two human cell lines are described in this paper: carcinoma of the body of the uterus and Wilms' tumor, obtained from strains transplanted into nude mice.

EXPERIMENTAL METHODS

The strain of carcinoma of the body of the human uterus was obtained from operative material by serial transplantations in nude mice. This strain was transplanted subcutaneously and has undergone 60 passages at intervals of 7-8 days. A uterine tumor with dimorphic structure of adenocarcinoma and undifferentiated carcinoma was used as original material. Throughout the period of passage the tumor basically preserved its stereotyped structure and cell composition and corresponded to the pattern of an undifferentiated carcinoma. No sign of a glandular structure (adenocarcinoma), present in the original material, could be detected in the transplants [3]. The strain of Wilms' tumor has undergone subcutaneous transplantation into nude mice at intervals of 22-24 days. For 57 generations it has maintained the structure of a nephroblastoma, with some predominance of the epithelial over the mesenchymal component. In all passages the strain has corresponded in principle to the original tumor

---

All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 6, pp. 710-713, June, 1988. Original article submitted March 13, 1987.

0007-4888/88/0006-0844$12.50

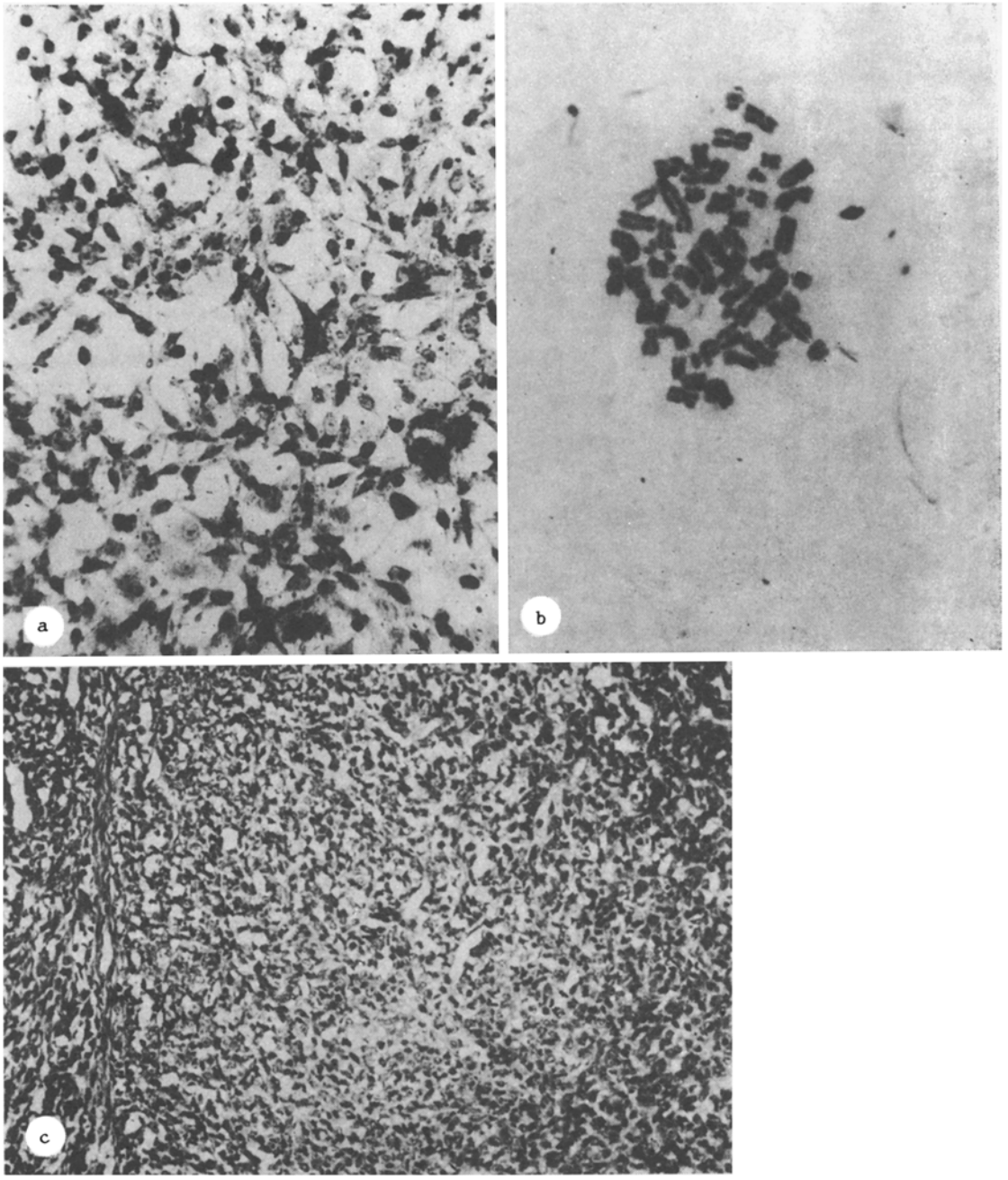

Fig. 1. Cell line (a), karyotype of cell line (b), and transplant (c) of carcinoma of the body of the uterus. a) Stained with azure and eosin (450×); b, c) stained with hematoxylin and eosin. Magnification: b) 900, c) 160×.

[4]. The nude mice, based on a line bred by the writers, were used at the age of 1-2 months for subcutaneous transplantation of 10^6-10^8 cells from tissue culture. Primary cell cultures were obtained by trypsinization of tumor transplants. The seeding dose was $6 \cdot 10^5$ cells/ml. The cells were grown on Eagle's medium with 10-15% fetal calf serum, 2 mM glutamine, and antibiotics. The cultures were grown at 37°C in closed flasks. The cells were removed from the glass at room temperature in the course of 2-3 min with Versene and chymotrypsin (0.1 mg chymotrypsin to 1 ml of Versene). The cell lines were transplanted daily with a multiplicity of seeding of 1:2-1:3. Monolayer cultures were stained for 3 min with Leishman's reagent, washed, and counterstained for 20 min with 0.1% eosin.

Colchicine was added to the culture, which were incubated at 37°C for 3 h. Next, a hypotonic solution consisting of one part of Eagle's medium and three parts of distilled water, was added to the sedimented cells, and the sample was incubated for 30 min at 37°C. The cells were then washed three times (15 min each time) with a mixture consisting of 1 part

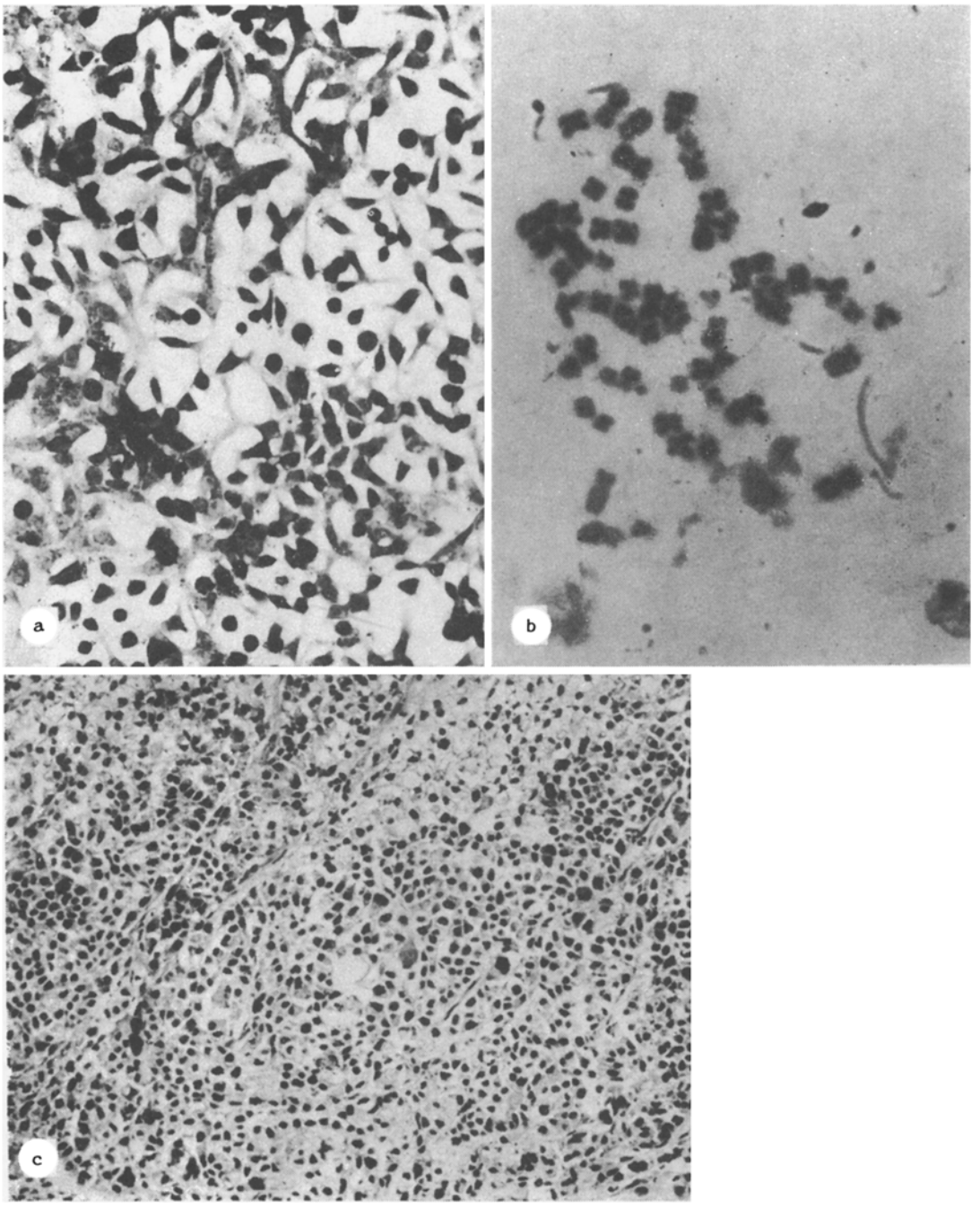

Fig. 2. Cell line (a), karyotype of cell line (b), and transplant (c) of Wilms' tumor. a, c) Stained with hematoxylin and eosin; b) stained with azure and eosin. Magnification: a) 450×, b) 900×, c) 160×.

of glacial acetic acid and 3 parts of methyl alcohol. The cell suspension was dropped on to cold wet slides. The preparations were heated, flamed, and then stained by the Romanovsky–Giemsa method, and with azure and eosin for 30-40 min, and dried at room temperature. Pieces of the tumor transplants were fixed in Carnoy's fluid and embedded in paraffin wax. Sections of the tumors 5-7 μ thick were stained with hematoxylin and eosin and with picrofuchsine. Tissues of the strain of Wilms' tumor were additionally impregnated with silver by Foot's method.

EXPERIMENTAL RESULTS

The cell line of carcinoma of the body of the uterus has gone through 50 passages in tissue culture. The monolayer culture consists of epithelial and polygonal cells with large, round and oval nuclei, and with small inclusions in the cytoplasm (Fig. 1a, b). The cells

have the human karyotype. Most cells contain 41-44 chromosomes, but some have 49-50 chromosomes. There are also cells with a 1.5-fold and double set of chromosomes. On transplantation of the cells from culture into nude mice, the animals developed tumors corresponding to an undifferentiated carcinoma, i.e., the transplanted strain of carcinoma of the body of the uterus from which this cell line was obtained. The cell line of Wilms' tumor has gone through more than 40 passages in tissue culture. The monolayer culture is polymorphic and consists mainly of epitheloid and single fibroblast-like cells. The nuclei are mainly round and oval and the cytoplasm of the cells is relatively wide and vacuolated. The culture has a tendency to grow in islets. The cells have the human karyotype. Most cells contain 39-42 chromosomes but some cells have 55-60 chromosomes. About 30% of the cells have a hypodiploid set of chromosomes, although polyploids also are found (Fig. 2a, b). During transplantation of the cells from culture into nude mice the animals developed tumors with a morphological picture identical to the strain of Wilms' tumor from which this cell line was obtained. The tumors consist of two tissue components: eipthelioid, consisting of polygonal cells, forming tubular-trabecular structures, and mesenchymal cells, which are elongated cells forming interweaving bands.

The cell lines thus obtained and also the strains from which they arise make it possible for experiments to be conducted with cells of the same human tumor under different conditions: both in tissue culture and in animals.

LITERATURE CITED

1. E. S. Revazova and A. S. Petrova, Vestn. Akad. Med. Nauk SSSR, No. 7, 31 (1981).
2. E. S. Revazova and A. S. Petrova, Vopr. Onkol., No. 8, 46 (1981).
3. E. S. Revazova, Yu. N. Solov'ev, and T. V. Yudicheva, Byull. Éksp. Biol. Med., No. 7, 71 (1985).
4. E. S. Revazova and Yu. N. Solov'ev, Byull. Éksp. Biol. Med., No. 2, 191 (1985).

POSSIBLE NEOPLASTIC TRANSFORMATION OF THYROID CELLS DURING THE GRAFT VERSUS HOST REACTION

T. D. Kozlova, V. V. Zarudin, and N. B. Kozlov

UDC 616.441-006-092:612.6.02.017.1

KEY WORDS: graft versus host reaction, thyroid gland, A and B cells, atypical follicular adenoma.

In the graft versus host reaction (GVHR) proliferation of lymphoid tissue is observed, and under certain conditions the tissue may undergo malignant transformation [6]. The possibility cannot be ruled out that in other organs, under GVHR conditions, when their function is disturbed and when immunologic reactivity is altered in the host, tumors may develop. It was shown previously that during GVHR there is marked inhibition of thyroid function [2].

This paper gives data on morphological changes in the thyroid tissue during GVHR (acute and chronic forms).

EXPERIMENTAL METHODS

Experiments were carried out on 60 F_1 hybrid rats. A systemic acute GVHR was induced by intravenous injection of $60 \cdot 10^6$ spleen cells (SC) from the C57Bl/6 parent into (CBA × C57Bl/6)F_1 hybrids. The thyroid gland was investigated at successive stages of development of GVHR on the 3rd, 10th, and 24th days after injection of the donor's cells. The chronic GVHR was studied on the traditional model. (C57Bl/6 × DBA/2)F_1 hybrids were given an injection of $50 \cdot 10^6$ SC of the DBA/2 parent [7]. The thyroid gland was investigated 6 months after induction of the donor's cells. The chronic GVHR was tested, allowing not only for the dura-

Central Research Laboratory, Smolensk Medical Institute.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 6, pp. 713-715, June, 1988. Original article submitted May 26, 1987.

0007-4888/88/0006-0847$12.50