ONCOGENIC PROPERTIES OF TYPE 12 HUMAN ADENOVIRUS FOR CERTAIN SPECIES OF MAMMALS

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UDC 616-006-02:576.858.5.06]:599.32-092.9

During the last two years the oncogenic action of human type 12 adenovirus has been demonstrated in relation to certain species of laboratory animals [5, 6, 8]. The oncogenic activity of adenoviruses of types 7 and 18 has also been established in experiments on newborn hamsters [2, 3].

The morphological picture of the resulting tumors has been described as undifferentiated [6] and polymorphocellular sarcomas [5].

The object of the present investigation was to reproduce tumors in certain mammals experimentally by inoculating them parenterally in the neonatal state with human type 12 adenovirus.

EXPERIMENTAL METHOD

Experiments were carried out on 20 litters of Syrian hamsters, 21 litters of Wistar rats, 4 litters of August rats, 6 litters of BALB mice, and 2 litters of $C_{57}B1$ mice (from the "Stolbovaya" nursery), on 12 litters of guinea pigs reared in Sukhumi, and on 18 newborn monkeys—7 baboons (Papio hamadryas), 7 macaques (Macacus rhesus), 2 green guenons, 1 mandrill, and 1 brown macaque.

A strain of adenovirus type 12 isolated by Huebner was used for inoculation. The virus was subjected to passage in the authors' laboratory through a series of cultures—HeLa, A-1, FK, and MS—using Medium No. 199 and 2% horse serum. In all the cultures the virus produced a specific cytopathogenic effect on the 5th-7th day after inoculation. To liberate the virus from the infected cultures the method recommended by R. S. Dreizin and V. M. Zhdanov (1962) was used. Material for inoculation of the animals had an activity of not less than 10,000 TCD_{50} of virus/0.1 ml. Culture fluid of uninfected HeLa cells was used as control material.

The animals were inoculated when not more than 24 h old. The rodents were inoculated intraperitoneally in a dose of 0.1 ml and intracerebrally in a dose of 0.02 ml. The monkeys were inoculated intraperitoneally in a dose of 5 ml. Some monkeys were inoculated intravenously with 2 ml of virus and also received 0.5 ml of virus by injection into the medullary canal of the humerus.

In periodic examinations of the animals the peripheral blood and bone marrow were investigated. Blood was taken from the eye of the hamsters at intervals of 2 weeks for subsequent determination of virus-neutralizing antibodies. Blood was also taken from the animals before sacrifice for serological investigation.

The content of virus in the tumors induced by means of the adenovirus was investigated by injecting tumor material into HeLa cultures and subsequently observing the appearance of the specific cytopathogenic effect. Some tumors were tested for their transplantability into adult animals. Some animals died during the observation, and some hamsters were sacrificed at various times after inoculation. All the dying and sacrificed animals were investigated morphologically. Histological examinations were made of all the organs and tissues, which were fixed in 10% formalin. Histological and histochemical staining methods were then used. Blood films and also impressions of the bone marrow, lymph glands, spleen, and tumor were stained by the Romanovskii—Giemsa method.

EXPERIMENTAL RESULTS

During observations on the animals inoculated with type 12 adenovirus, tumors regularly appeared in the hamsters and in both lines of rats (see table). The times of development of the tumor in the hamsters and rats differed significantly. In most hamsters neoplasms were seen from the 30th to the 75th day. In some animals tumors were

Institute of Experimental Pathology and Therapy, Academy of Medical Sciences of the USSR, Sukhumi (Presented by Academician V. V. Parin). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 62, No. 12, pp. 82-87, December, 1966. Original article submitted February 12, 1965.

Oncogenic Activity of Type 12 Adenovirus for Various Species of Animals

Animals	Materials for inoculation	Number of animals			Time when tumors were discovered (in days)
		inoculated	surviving after inoculation	with tumors	
Syrian hamsters	Culture fluid of infected HeLa cells with a titer of 10,000 TCD_{50} virus/0.1 ml	160	101	72	14-120
Wistar rats		142	113	6	74-120
August rats		29	18	1	110
BALB mice		60	47	0	0*
Mice $C_{57}Bl$		11	6	0	0*
Guinea pigs		24	20	0	0*
Monkeys		25	18	0	0*
Syrian hamsters	Control culture fluid from uninfected HeLa	24	24	0	0

*Period of observation more than 200 days.

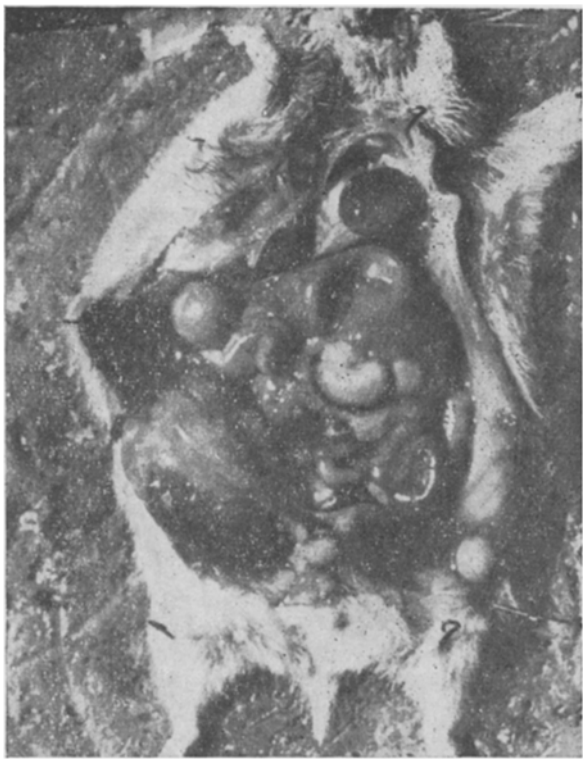

Fig. 1. Multiple tumor nodules in the peritoneal cavity of a hamster inoculated intraperitoneally with type 12 adenovirus.

found only 2 weeks after inoculation. In the rats the tumors mostly appeared 100 days after inoculation. Tumors developed more frequently in the hamsters than in the rats. During the period of observation (about 200 days), tumors developed in 72 of 101 hamsters and in 6 Wistar rats of 113 surviving after inoculation.

Autopsy of the hamsters showed that they developed tumors only at the site of injection of the infecting material, and in some cases the tumors were single. In most animals one or several large nodules were found in the peritoneal cavity, together with numerous tiny nodules (Fig. 1). The lymph glands of the peritoneal cavity were not involved in the neoplastic process. The tumors were well demarcated. They were very soft and encephaloid in consistency. The central parts of the tumors, and sometimes nearly all the tumor tissue showed necrotic changes with many hemorrhages. Liquid blood was constantly found in the peritoneal cavity of the dying and sacrificed animals.

The histological picture of the tumors was not uniform, but was repeated regularly in all the hamsters investigated. In the areas of grayish-pink color the tumor tissue was characterized by a solid structure, containing many tightly packed cells, and many very fine capillaries. The tumor cells possessed large, pale nuclei, as a rule with nucleoli. On staining with Goldman's method, no sudanophilic granules were found in their cytoplasm. The reaction for glycogen and alkaline phosphatase also was negative. By their shape, the nuclei of the cell could be divided clearly into two categories. The large cells of reticulo-endothelial type, with juicy, oval nuclei were situated as a rule along the course of the capillaries, apparently forming part of their wall. The endothelium of the capillaries was either of the usual elongated shape, or indistinguishable from the oval tumor cells. Cells with oval nuclei, situated along the course of the vessels, had a tendency to form plexiform or palisade-like structures. In the spaces between the capillaries, cells of lymphoblast type were predominant, with round and very young nuclei of different sizes, usually containing nucleoli. Only isolated cells had large and intensively stained nuclei, analogous to adult lymphocytes. The parts of the tumor described above were distinguished by very large numbers of mitoses and by the small lumen of their numerous capillaries (Fig. 2).

Together with the changes described above, in the same animals tumors or parts of tumors were found in which most of the cells were round-nucleated elements of different sizes (Fig. 3). The cells in these areas were mostly not so tightly packed. The round-nucleated elements had no tendency to lie mainly along the course of the

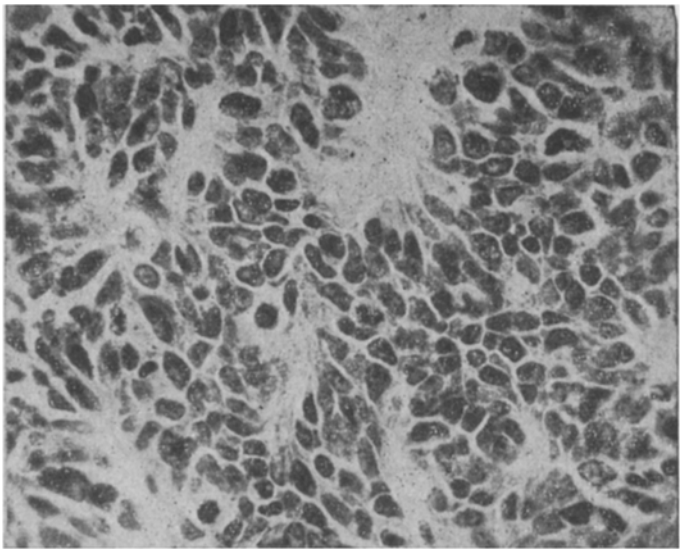

Fig. 2. Part of a tumor consisting mainly of reticulo-endothelial cells. Hematoxylin-eosin. Objective 60x, Gomal 2.

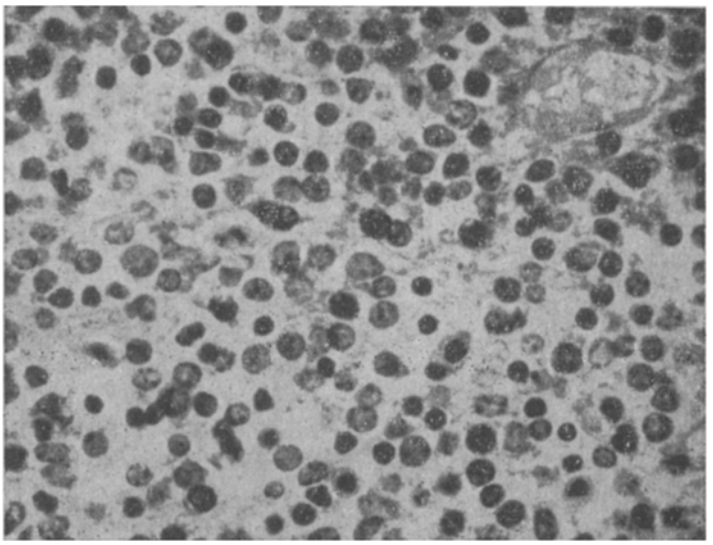

Fig. 3. Part of a tumor consisting mainly of round cells of lymphoblast type. Hematoxylin-eosin. Objective 60x, Gomal 2.

capillaries. Mitoses either were absent or were few in number. As a rule, in the areas with predominantly round-nucleated elements, many greatly dilated capillaries were found. Among the tumor cells, giant malformed cells with oval or irregularly lobular nuclei were constantly found.

Very many sharply dilated capillaries were constantly found in areas of the tumor tissue. As a rule the necrotic tissue was intensively permeated with blood and protein-containing fluid from the round-nucleated elements scattered among it, often having the character of adult lymphocytes. The tumor nodules were surrounded by a capsule.

In the rats of both lines the tumors were indistinguishable from those found in the hamsters.

Growth of the tumors in the animals was accompanied by the development of a progressive hypochromic anemia. Changes of two types were seen in the bone marrow: the number of cells of the erythroid series was greatly increased, and a smaller increase was found in the number of undifferentiated cells with large, pale, irregularly circular nuclei, containing nucleoli. In some hamsters dying from the tumor, young round-nucleated cells appeared in the spleen.

During systematic observations on the guinea pigs and monkeys, no tumors were found to develop. Repeated investigations of the blood and bone marrow of the monkeys revealed no abnormalities in the hemopoietic system throughout the period of observation. Investigation of the content of virus in the tumors of the hamsters and rats by inoculation of HeLa cultures with tumor material in no case revealed a specific cytopathogenic effect in the HeLa culture during observations for 7 days. Later, nonspecific degeneration of the experimental and control cultures was observed. Likewise, no virus was found in the tumors after three or four blind passages of tumor material through HeLa cultures. Transplantation of the tumor material into hamsters lead to survival and development of tumors at the site of injection in the course of 10-20 days. With further passages, the incubation period for development of tumors was shortened. One of the tumors in a hamster has now gone through 15 passages in adult hamsters. No change occurred in the character of the tumor as a result of transplantation.

Inoculation of tumor cells of this strain into HeLa cultures did not lead to the appearance of a specific cytopathogenic effect in these cultures.

The results obtained confirm the findings of Trentin and co-workers [6, 7] concerning the high oncogenic activity of type 12 adenovirus for hamsters. At the same time, the oncogenic action of this virus was demonstrated for rats of two lines—Wistar and August. The only reference which could be found in the literature was to the work of Heubner and co-workers [4], who obtained tumors by injection of this virus into rats of the Sprague—Dawley line in three of ten cases.

Since tumors have also been obtained by means of this virus in mice [5, 8] and in Mastomys natalensis [5], it is clear that type 12 adenovirus is one of the first human viruses found to be oncogenic for several species of animals. Unlike polyoma virus, type 12 adenovirus causes tumors of similar structure in different species of animals [3, 5, 6], as was found in the present experiment.

It is difficult to state precisely to what type the tissue of these neoplasms belong, as is shown by the fact that they are described by various authors as undifferentiated [5, 6]. As a result of morphological investigations, the authors formed the impression that these tumors are derived from hemopoietic and, in particular, from lymphoid tissue. The process of differentiation in the tumors runs from reticulo-endothelial tissue toward cells of the lymphoid series.

It is difficult to interpret the changes in the bone marrow and spleen. They may be a manifestation of a well marked leukocyte reaction of the hemopoietic organs to the development of a widespread neoplastic process. This problem requires further investigation. The absence of tumors in the mice in the present experiments has not yet been explained. There are reports in the literature that mice of lines DBA and A were also resistant to this virus [8].

It is too early to judge the results of the experiments on guinea pigs and monkeys. Possibly the period of observation was too short for tumors to develop in these animals.

LITERATURE CITED

1. R. S. Dreizin and V. M. Zhdanov, Adenovirus Infections [in Russian], Moscow (1962).
2. A. J. Girardi, M. R. Hilleman, and R. E. Zwickey, Proc. Soc., Exp. Biol., New York, 115 (1964), p. 1141.
3. R. J. Huebner, W. P. Rowe, and W. T. Lane, Proc. Nat. Acad. Sci., Washington, 48 (1962), p. 2051.
4. R. J. Huebner, W. P. Rowe, H. C. Turner, et al., Proc. Nat. Acad. Sci., Washington, 50 (1963), p. 379.
5. A. S. Rabson, R. L. Kirschtein, and F. J. Paul, J. Nat. Cancer Inst., 32 (1964), p. 77.
6. J. J. Trentin, Y. Yabe, and G. Taylor, Science, 137 (1962), p. 835.
7. J. J. Trentin, Proceedings of the Eighth International Cancer Congress [in Russian], 3, Moscow-Leningrad p. 89.
8. Y. Yabe, L. Samper, E. Bryan, et al., Science, 143 (1964), p. 46.