# THE DEVELOPMENT OF LEUKEMIA AND TUMORS IN AN EXPERIMENT ON MICE OF THE C3HA LINE

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Translated from Byulleten' Éksperimental'noi Biology i Meditsiny,
Vol. 53, No. 3, March, 1962
Original article submitted December 28, 1960

Successes in the study of viruses of animal tumors led certain investigators to think that they were on the road to quick resolution of the problem represented by the etiology of malignant neoplasms in man. However, immunological investigations, which demonstrated differences in the antigenic properties of human tumor and normal tissue, did not reveal the nature of these differences. Searching for antibodies to the tumor antigen in cancer patients led to positive results only in a few investigations; vaccination of people with tumor antigens did not increase our knowledge of their nature at all.

No less complicated to deal with are the rather contradictory results provided by biological tests on animals with tumor material taken from humans.

Attention is called to the fact that in many investigations, performed on inbred low-cancer lines of mice, negative data were obtained, while experiments on sensitive animals demonstrated a definite effect of tumor material, taken from humans, on the development time and percent incidence of tumors in the animals. Thus, in the experiments on the high-leukemia mice AKR, Schwartz and co-workers [6] showed a specific activating effect of the material from human leukemia patients, manifested by the early appearance of leukemias. The injection of this same material into the resistant Swiss mice did not cause the development of leukemia.

In experiments involving the injection of mice from the three low-cancer lines, C57 black, CC57 white, and CC57 brown, with human leukemia material for a course of 2 years, we did not observe even a single case of leukemia.

Of interest are the experiments of Schwartz and co-workers, in which the method of "blind" consecutive passages was used, employing human leukemia material in mice of a low-leukemia line. Although leukemia did not develop in these mice, the active agent was retained and even reproduced, and upon injection of the material into mice of the high-leukemia line AKR, the development of leukemia was accelerated [7].

We also undertook a series of blind consecutive passages of material from human leukemia patients, using tissue cultures in this method. The cultures fluids were tested in experiments on mice of the low-cancer line CC57 white, high-cancer line C3HA and high-leukemia line Afb.

In this report we describe the experiments on mice of the C3HA line. The results again demonstrate the difficulties encountered by the investigator in the interpretation of experiments with human neoplastic tissues, where animals or cells of animal derivation are used as the test-subject, and yet may be carriers of latent oncogenic viruses.

## EXPERIMENTAL METHOD

Blood from a patient with acute leukemia (hemocytoblastosis) was injected into a monkey kidney tissue culture. After a series of passages*, the culture fluid was used for injection into newborn mice subcutaneously or intraperitoneally,

*Passage of the material in tissue cultures was carried out by Head Laboratory Technician of the Division of Immunology and Oncology of the N. F. Gamaleya Institute of Epidemiology and Microbiology of the Akad. Med. Nauk SSSR I. S. Irlin.

using a dose of 0.05-0.15 ml, and directly into the tissue of the spleen, visible through the fine skin covering of the newborn mice, in a dose of 0.02 ml. Observations were carried out on the mice. Every mouse that died was autopsied When a mouse showed clear signs of tumor or leukemia or suspicion of it, it was sacrificed and material was transferred to animals of the same line. In making passages, a suspension of brain tissue was added to the tumor tissue in all cases.

## EXPERIMENTAL RESULTS

Culture fluid from the 3rd consecutive passage of blood from a patient with acute leukemia (hemocytoblastosis) in monkey kidney tissue culture was injected directly into the spleen of newborn mice of the low-cancer line CC57 while on the first day of their life, and repeated on the following day in the same dosage. Out of 8 mice that received the injection of culture fluid, 6 survived the injection. Over a course of one year of observations they remained healthy, and were sacrificed at the age of 1 year and 7 days. On autopsy, no pathological changes of any kind were observed.

Culture fluid from the 5th consecutive passage of blood from the same patient was injected into 8 mice of the CC57 white line intraperitoneally, using a dose of 0.15 ml. All the mice survived the injection of material, but died after 2 weeks without signs of leukemia or other neoplasm.

Culture fluid from the 7th passage was injected into mice of the high-leukemia line Afb (3) and two groups of mice of the low-leukemia line C3HA of different age (10 mice were one day old and 6 were 4 days old). All mice of the C3HA line in the first group, having received the material intraperitoneally at the age of 1 day, died after 2 months without signs of malignant neoplasms; 4 of the 6 mice in the second group, which received the material subcutaneously and intraperitoneally at the age of 4 days, died after 6 weeks with signs of enlargement of the lymph nodes. Two mice were sacrificed for passage 7 weeks after their injection with culture fluid. In both there was considerable enlargement of the lymph nodes. Tissue from the hyperplastic lymph nodes and the brain of these mice was transferred subcutaneously and intraperitoneally into mice of the same line.

The passage mice, who were injected subcutaneously with the material on the 22nd day of their life, did not become sick and were sacrificed after $8\frac{1}{2}$ months; no signs of neoplasm were observed in them. On the other hand, in all three one month old passage mice of the same line, that received the material intraperitoneally, neoplasms developed at the approximate ages of 4, 5 and 11 months (mice Nos. 830, 831 and 832 respectively). A mouse No. 832, 3 months and 11 days after injection in the back, a little below the neck, it was possible to feel a freely lying tumor nodule, which was removed by means of an operation, and successfully passed into newborn and young mice of the C3HA line. After 2 months and 7 days, a tumor again appeared in the same mouse, which gradually grew; enlargement of the belly was observed at the same time, as though the mouse were pregnant. The mouse soon died. On autopsy, marked enlargement of the liver and spleen was observed. The latter was very suggestive of a leukemic spleen and in its consistency and color. In the peritoneal cavity, in addition, we observed an enormous cyst with hemorrhagic contents. Analogous changes were observed in mouse No. 831, which died 4 months and 6 days after the injection of material. In the peritoneal cavity of this mouse we observed 3 enormous cysts.

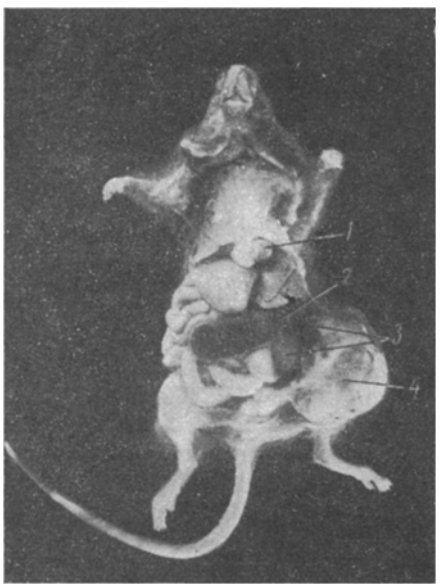

Fig. 1. Mouse of line C3HA, No. 2824/197, from the 5th passage, born 4/4/60, injected with the material 4/4/60, sacrificed on 7/8/60. One can see the small cyst with its serous contents at the processus xiphoideus (1), the markedly enlarged spleen (2), under it, two cysts with hemorrhagic contents (3), further down, the subcutaneous tumor nodule (4).

One of the cysts bore a markedly distended bud. The wall of the cyst and brain tissue were used for subsequent passages to newborn mice of the C3HA line. Mouse No. 830 died $9\frac{1}{2}$ months after the injection. On autopsy, a marked enlargement of the spleen and liver were noted, as well as enlargement of the lymph nodes and the presence of an enormous cyst with hemorrhagic contents and another cyst of considerably smaller size with serous contents in the peritoneal cavity. Material from mice Nos. 831 and 832 were put through 16 consecutive passages in newborn mice

of the same line. The development of cysts and tumors was observed in the mice (Figs. 1-3). Tumor tissue from the 6th passage was transferred to newborn mice of the Afb line. After $1\frac{1}{2}$ months the mice died with symptoms of generalized leukemia and tumor nodules at the site of injection. Material from the 10th passage was successfully transferred to mice of the line CC57 brown and C3H (f), and from the 16th passage, to mice of line CC57 brown. Reverse transfer from mice of line C3H (f) and CC57 brown to mice of the C3HA line was successful.

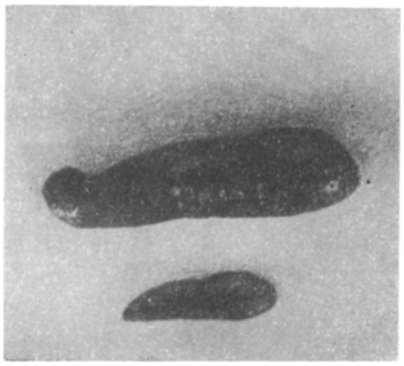

Fig. 2. Normal (lower) and leukemic (upper) spleens. The leukemic spleen is from a mouse of the C3HA line, No. 2430/147, from the 3rd passage. The normal spleen is from a mouse of the same line and same age.

Fig. 3. Mouse of the line C3HA, No. 2661/178, from the 5th passage, born on 3/10/60. The material was injected subcutaneously on 3/10, and the animal sacrificed on 6/14/60. A hemorrhagic cyst and markedly enlarged liver and spleen are apparent.

Transfer of tumor to the mice of line C57 black and BALB has not yet yielded any results, just as the transfers with acellular material (centrifugates and filtrates) directly from mice with tumors to newborn mice of the same line.

With histological study, the preparations of tissues from mice of the C3HA were diagnosed as leukemic, characterized by the prevalence of hemocytoblasts and reticular cells. Tumors at the site of injection showed a glandular-cystic structure, and sometimes there was solid growth.

## Discussion of the Results

In mice of the line C3HA, discovered by E. E. Pogosyants (2, 4), tumors are observed in a large percent of the cases, but rarely do leukemias develop spontaneously. In our experiment, the animals became sick at $1\frac{1}{2}$-2 months after being injected with the culture fluid. On histological investigation of their organs and tissues, the presence of leukemia of the hemocytoblastic type was established. Transfer from the experimental animals to young mice of the same line led to the development in the latter of leukemia and serous and hemorrhagic cysts (and tumors in one mouse); with subsequent transfers to young and newborn animals of the same line, there developed single and multiple cysts, tumors at the site of injection, and metastases of these tumors to other organs.

Since the culture material was injected on the first days of life of the mice, the question arose as to whether the injection of culture fluid into newborn mice is, by itself, a provoking force, leading to activation of the latent viruses in the organism of the animals, and to the development of cysts, leukemia and tumors in them. We observed an additional 12 groups of mice from other experiments, wherein the animals had received injections of culture fluid on the first days of their lives; out of these animals, 23 mice belonged to the C3HA line, 54 to the lines CC57 white, 15 to the line C57 black. Not counting 5 mice of the line CC57 white, which died 8 days after the injection, the remaining mice survived from $2\frac{1}{2}$ months to 2 years. If not a single one of the mice that died or were sacrificed did we note the development of leukemia or other neoplasms. It should be pointed out that all the mice used in these experiments were obtained from the Division of Immunology and Oncology of the N. F. Gamaleya Institute of Epidemiology and Microbiology of the Akad. Med. Nauk SSSR, which is under the direction of L. A. Zil'ber; in this division, work is being carried out with the SE-polio virus, and 45-80% of the mice there have antibodies against this virus. The failure of any

type of neoplasms to develop subsequently to injection of the newborn mice of three different lines (C3HA, CC57 white, and C57 black) with culture fluid indicates that this injection, in the first days of life of the mice, was not enough to disrupt the equilibrium of the organism and lead to activation of the polio virus or any other latent oncogenic viruses which could be found in the organism of the animals.

Another hypothesis arises: were the mice injected with oncogenic virus from the cultured tissue? In the process of in vitro cultivation it might be possible to activate latent viruses if they were in the cells of the cultured tissue, and with prolonged cultivation one might observe the development of malignancies of the tissue itself. In our experiment, we used material from cultures in which no changes of any kind were noted in the structure of the cultured tissue from the monkey kidney, where there was no cytopathogenic effect, and where the culture fluid gave negative cold agglutination reactions to erythrocytes from guinea pigs, mice, chicken and rabbits. This justifies excluding the possibility that viruses with cytopathogenic and hemagglutinating properties were present in the culture. It is known that certain latent monkey viruses do not possess these properties. We cannot exclude the possibility that they multiplied and accumulated in the tissue culture. Injection of culture fluid from the 3rd "blind" consecutive passage in vitro in monkey kidney tissue culture, into newborn mice of the low-leukemia line CC57 white, did not lead to their developing leukemia or other neoplasms over the course of more than a year. Newborn mice of the same line, injected with fluid from the 5th consecutive passage in vitro in monkey kidney tissue culture, also died without signs of tumors or leukemia. Nevertheless, the absence of a direct control, involving injection of the newborn mice of the same line C3HA with fluid from the 7th consecutive passage in vitro in monkey kidney tissue culture, prevents us from rejecting the hypothesis of a possible role for the latent monkey viruses, which, like any other viruses, can enter into an interactive balance with latent viruses (including oncogenic ones) in the organism of the animal-recipient, disturbing the established equilibrium and leading to development of a neoplastic process [1]. The possible role of monkey viruses must especially be taken into account in light of the latest data from Eddy and co-workers [5], according to which the injection of newborn hamster with extracts from certain monkey kidney tissue cultures caused the development of a neoplasm at the site of injection in 70% of the animals. This fact prevents us from postulating anything about the possible role of human leukemic material injected into, and passed through, tissue culture. Our subsequent investigations are devoted to elucidating the factor that caused activation of the leukemic and tumor process in the mice of the C3HA line.

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