LEUKEMIA AND CYSTS IN MICE OF THE AFB LINE

V. A. Parnes

From the Leukemia Group of the Institute of Epidemiology and Microbiology
(Dir.—Prof. S. N. Muromtsev of the Akad. Med. Nauk SSSR, Moscow
(Presented by Active Member of the Akad. Med. Nauk SSSR N. N. Zhukov-Verezhnikov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 53, No. 1,
pp. 86 - 92, January, 1962
Original article submitted November 28, 1960

The investigations of Schwartz and co-workers [17] showed that the injection of mice from the highly leukemic AKR line with preparations from the brain of humans who died of leukemia leads to the development of leukemia at an early age in a large majority of the mice, an age at which leukemia does not arise spontaneously. The mice of this line appear to be carriers of a mouse leukemia virus, which is present in the organism for a long time in the latent state, and is activated under natural conditions only when the mice reach a certain age. The experiments of N. P. Mazurenko [2] with the virus of smallpox vaccine support the possibility of activation of latent mouse leukemia viruses under the influence of other viruses.

In our work, we started from the hypothesis that similar activation can occur under the influence of tumor viruses, in particular the hypothetical virus of human leukemia.

In the experiments of N. P. Mazurenko, activation of the leukemic process by the smallpox virus only occurred in mice of a certain subline. It is known that many of the mouse leukemia viruses described in the literature also cause leukemia only in certain lines of mice (Gross' virus, Frend's virus). Some lines are especially resistant to the action of these viruses. Using mice from these lines in investigations on the production of leukemia by means of injecting material from human blood, one would hardly expect positive results. In our experiments on the inbred mice of the "low-cancer" lines C57 black, CC57 white, and CC57 brown, over a period of 2 years after they were injected with material from humans who died from acute leukemia (hemocytoblastosis), we failed to observe even a single case of leukemia.*** A comparison of the results of these experiments and the experiments with mouse leukemia viruses led us to realize the importance of correctly selecting the mice-recipients. In our investigations we decided to use mice in which, according to the data in the literature and our own observations, it was relatively easy to activate the leukemic process, namely mice of the low-leukemic C3HA and highly-leukemic Afb lines.

The purpose of this work was to elucidate the possibility of activating the leukemic process in mice of the highly-leukemic Afb line under the influence of fluid from a monkey kidney tissue culture in which material from the blood of hemocytoblastosis patients was serially passed.

In the course of this work, we isolated a leukemia strain, which we named the Gamaleya No. 1 strain.

EXPERIMENTAL METHOD

The blood of patients with acute leukemia (hemocytoblastosis) served as the starting material for passage in the tissue culture.

Patient K., 17 years old. The diagnosis of hemocytoblastosis was established in the Semipalatinsk Regional Hospital, and confirmed in the hematology clinic of the Central Institute for Blood Transfusion. Blood was drawn on May 8, 1959 into Olsver's solution,* and was stored in a refrigerator at 4 deg prior to its injection into the monkey kidney tissue culture.**

* The leukemic material was submitted to the director of the hematology clinic of the TsOLIPK Prof. M. S. Dul'tsin and to Doctor O. D. Ramonova-Tskhovrebova.

** Cultivation was carried out by the head of the laboratory of the Division of Immunology and Oncology of the IÉM of the Akad. Med. Nauk SSSR I. S. Irlin.

*** D. M. Levina and V. A. Parnes, Vopr. onkol., No. 12 (1961).

Order of footnotes as in Russian.

The intervals in the passage of the material are presented in Table 1. For inoculation of the Afb line mice, we used the culture fluid from the 7th passage. Prior to injection, the latter was centrifuged at 3000 rpm for 20 minutes while refrigerated at 4 deg. For the injection, we took 6 newborn mice of the Afb line, born on the day of the experiment. Each mouse was injected intraperitoneally with 0.05 ml of the supernatant fluid, and another 0.05 ml was injected subcutaneously. The mice were then kept under observation. Passage of leukemias that arose in the experimental mice was carried out subcutaneously and intraperitoneally to young mice of the Afb line. Table 2 shows the intervals of the passages and the method of administration of the inoculate. The latter consisted of a suspension of spleen, thymus, lymph nodes and brain from the leukemic mice. Transplants were made from the mice of the Afb line to newborn mice of other lines: C3HA, C3H(f), C57 black, CC57 white, CC57 brown, A, Asn, and BALB.

TABLE 1. Intervals of the Serial Passages of Blood from Patients with Acute Leukemia (Hemocytoblastosis) in Monkey Kidney Tissue Culture

Passage No.	Date of initiation and end of passage	Use of cultural fluid
1	May 8-25, 1959	For passage No. 2
2	May 25-June 1	For passage No. 3
3	June 1-10	For passage No. 4 and inoculation of newborn mice from the CC57W low-leukemia line
4	June 10-23	For passage No. 5
5	June 23-July 7	For passage No. 6 and inoculation of newborn mice from the CC57W low-leukemia line
6	July 7-22	For passage No. 7
7	July 22-28	For passage No. 8 and
8	July 28-Aug. 11	inoculation of newborn mice from high- and low-leukemia lines

EXPERIMENTAL RESULTS

Two months after injection of the material into mouse No. 718, swelling of the upper portion of the chest was noted; the inguinal and axillary lymph nodes were slightly enlarged. Swelling of the thorax gradually increased, and the lymph nodes continued to enlarge. After 80 days from the time of inoculation, October 26, 1959, the mouse died. On autopsy, marked enlargement of the lymph nodes was observed—submaxillaries, axillaries, inguinals, mesenterics, retroperitoneals; there was considerable enlargement of the spleen, and a very large thymus gland, adjacent to which was a cyst, filled with hemorrhagic fluid and about the size of a pea.

After another month, at the end of November, a comparable swelling of the upper portion of the thorax was observed in mouse No. 719. Palpation disclosed an enlargement of the lymph nodes. The mouse was sacrificed on December 10th (at the age of 125 days). At autopsy, we observed generalized lymph node enlargement, and enlargement of the liver. The spleen was very large, and occupied almost the entire left half of the peritoneal cavity. In the thoracic cavity, adjacent to the markedly enlarged thymus gland, lay a huge cyst, filled with serous fluid. On histological investigation,* the diagnosis of leukemia of the hemocytoblastic type was made for both mice. The remaining mice died on January 1—2, 1960, and were autopsied on January 3 in a decomposed state.

A female mouse, having borne and nursed a litter, was sacrificed on June 16 at the age of 13 months. No unusual changes were observed at autopsy.

Material from mouse No. 719 was used for serial passages in young mice of the same line. Data are presented in Table 2 on the nature of the pathological changes observed in the mice of the first 18 passages. Leukemia of the hemocytoblastic type developed in all the animals, with no tumor nodules at the site of the subcutaneous injection (Figs. 1,2). The exception was one mouse from the 12th passage, which had a reticulosarcoma. We did not observe the appearance of a cyst in a single mouse of the Afb line from the 1st—18th passages. When the leukemic tissue was transplanted from mice of the Afb line to newborn mice of other lines (C3HA, C3H, C3H(f), CC57 white, CC57 brown, C57 black, BALB, A, Asn), leukemia developed only in two mice of the C57 black line, and in mice of the A and C3HA line (Fig. 3). In the latter, it was the same type of hemocytoblastic leukemia as that in the mice of the Afb line.

* The histological investigations were carried out by Prof. A. G. Varshavskii.

DISCUSSION OF THE RESULTS

The cases of leukemia in our experiment were placed in fixative on the 80th and 125th days of life of the mice, but the leukemic process began earlier, as indicated by the enlargement of the lymph nodes that was determined by palpation. Such early development of leukemia is atypical for mice of the Afb line. The clinical

TABLE 2. Conditions of the Serial Passages in Mice of the Afb Line, Using Acellular Fluid from the 7th Consecutive Passage in Monkey Kidney Tissue Culture of Blood Originally Drawn from a Patient with Acute Leukemia (Hemocytoblastosis)

Passage No.	No. of mice with leukemia (numerator) to the total number of mice (denominator)	Date of birth of the mice	Date of the passage	Method of introduction	Pathological changes
1	4/4	Oct. 25	Dec. 10	Subcutaneous	Leukemia
2	8/8	Nov. 15	Jan. 6	"	"
3	4/4	Jan. 6, 1960	Feb. 4, 1960	"	"
4	3/3	Jan. 5	Feb. 23	Subcutaneous and intraperitoneal	"
5	4/4	Jan. 25	Mar. 12	The same	"
6	3/3	March 5	Apr. 9	"	"
	3/3	March 26	Apr. 22	Subcutaneous	"
7	4/4	Apr. 2	May 10	Subcutaneous and intraperitoneal	"
8	4/4	Apr. 30	May 23	Subcutaneous	"
9	5/5	May 10	June 13	Subcutaneous and intraperitoneal	"
10*	5/5	May 24	June 27	Subcutaneous	
11	8/8	June 4	July 11	"	Leukemia, hair loss
12	6/6	June 14	July 26	Subcutaneous and intraperitoneal	Leukemia (in mouse 3250-reticulosarcoma)
13	6/6	June 25	Aug. 10	The same	Leukemia
14*	6/6	July 18	Aug. 22	"	"
15	5/5	Aug. 9	Sept. 4	"	"
16	8/8	July 10, 1960	Sept. 17, 1960	"	"
17*	6/6	Oct. 8	Oct. 8	"	"
18*	10/10	Oct. 26	Oct. 26	Subcutaneous	"

Note. * Using passages 10, 14, 17 and 18, transplants of cellular suspensions were made from the mice of the Afb line to newborn mice of other lines (A, Asn, BALB, C57 black, CC57 white, CC57 brown, C3HA, C3H, C3H(f); transplants were also made from the Afb mice to newborn mice of the same and other lines, using acellular centrifugates and filtrates.

picture of leukemia in the animals was clearly manifested; the diagnosis also was confirmed by histological investigation in the experiments with serial transplants to young and newborn mice of the same line.

All the mice that were inoculated with the material died with symptoms of developing leukemia; all this eliminates any doubt as to the true nature of the leukemia.

A large percentage of mice from the Afb line spontaneously contract leukemia. The leukemia that arose in the experimental mice differs in the earlier appearance of its first signs, the early deaths of the animals, the absence of strict line specificity, very typical of spontaneous leukemia in mice of this line, and the development of serous and hemorrhagic cysts, which are never observed in mice of this line with spontaneous leukemia. We also

failed to find descriptions in the literature of cysts in mice of high-leukemia lines. It has been proven that there is a carrier relationship between the latter and the virus of mouse leukemia. It is felt that the leukemia virus in the organism of the mice from the high-leukemia lines exists in the "infectious" form, and thus, radiation of these mice does not lead to an increase in the rate at which leukemia develops in them, or to a rise in the percent of

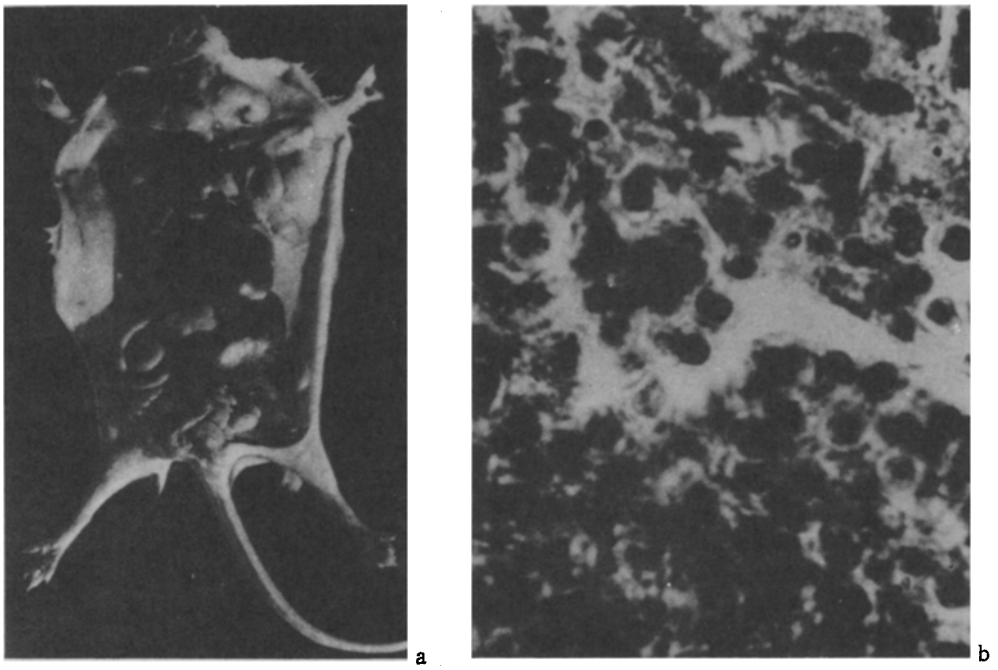

Fig. 1. Leukemia in a mouse of the Afb line subsequent to subcutaneous transplantation of leukemic material. a – Mouse No. 3422/275 from the 14th passage, born July 18, 1960; material injected subcutaneously on August 22nd. The mouse died on September 12th with symptoms of leukemia, without a tumor nodule at the side of injection. Clusters of markedly enlarged, hemorrhagic, submaxillary and axillary lymph glands were seen, along with an enlarged inguinal lymph node and a greatly enlarged spleen. The thymus gland covered almost the entire anterior wall of the heart; b – liver of the same mouse, sharply demonstrating infiltration by leukemic cells. Stained with hematoxylin–eosin. Magnification 1100 × (A.G. Varshavskii's preparation).

cases of leukemia, as happens with irradiation of mice from low-leukemia lines, where the leukemia virus exists within the organism in a noninfectious form [13].

In addition, the injection of mice from the AKR line with acellular extracts of isologous leukemic tissue, as well as filtrates of isologous leukemic brain and filtrates of brain from humans who died of leukemia, accelerates the appearance of leukemia and increases the percent of cases of the disease in the mice [14, 16, 17].

In our experiments, the injection of newborn mice of the Afb line, within the first hours of their life, with fluid from the 7th consecutive passage of monkey tissue culture, in which blood from a patient with acute hemocytoblastosis was initially introduced, led to early development of leukemia in the mice. It is interesting to note that in mice of the low-leukemia line C3HA, who were injected with the same culture fluid, we also observed the development of leukemia. It is difficult to decide immediately on the nature of the injected material's mechanism of action. In the experiments of Logothetis et al.[13], it was shown that activation of the leukemia process in mice of the AKR line, under the influence of filtrates from leukemic human brain, is related to an action with a specific character, which is suppressed by antiserum against the filtrate of leukemic human brain, obtained by immunization of volunteers, but is not suppressed by normal human serum.

At the present time we lack the data necessary for a deeper analysis of the mechanism by which the leukemic process was activated in our experiment.

Another question arises: what led to the development of serous and hemorrhagic cysts in the thoracic cavity of the experimental animals? In the experiments of G. Ya. Svet–Moldavskii and A. S. Skorikova [3], the injection

of Raus's virus into a heterogeneous organism caused the development of cysts. In the experiments of L. A. Zil'ber and I. N. Kryukova [1], analogous results were obtained. In our experiment, we also injected mixed heterogeneous material, which either by itself, or as a result of interference with latent viruses in the organism of the animal-recipients, led to the development of cysts. If we disregard the fact that blood from a patient with acute hemocytoblastosis was injected into the monkey kidney tissue culture, then we cannot ignore the possible presence of latent monkey viruses in it [4, 8, 11, 15].

In the experiments of Dmochowski [6] on cultivation of leukemic material from human blood in monkey kidney tissue culture, a cytopathogenic effect was observed in vitro; the culture fluids did not agglutinate guinea pig erythrocytes, but in mice of the Swiss line who were injected with the fluids, the presence of antibodies against the polio virus was indicated in high titer by the reaction of hemagglutination. In our experiments we did not observe any cytopathogenic effect in the tissue culture, or agglutination of guinea pig erythrocytes by the culture fluids under refrigerated conditions. The injection of newborn mice from the CC57 white line, within the first 24 hours of their life, with culture fluids from the 3rd and 5th consecutive passages of monkey kidney tissue, did not cause the development of leukemia or other neoplasms. It should be taken into consideration that our mice were obtained from the animal stocks of the Division of Immunology and Oncology of the IÉM of the Akad. Med. Nauk SSSR, directed by L. A. Zil'ber, in which work is being carried out on the SE–poliomyelitis virus. Special investigations, carried out by N. Medvedev, T. I. Biryulina, and I. S. Irlin, showed that from 45 to 80% of the mice

Fig. 2. Leukemia in a mouse of the Afb line, subsequent to intraperitoneal injection of leukemic material. Mouse No. 3780/316, from the 15th passage, born August 9, 1960, was injected with the material intraperitoneally on September 4th. The mouse died on September 17th, with symptoms of leukemia. We observed marked enlargement of the lymph nodes, thymus gland, liver and spleen.

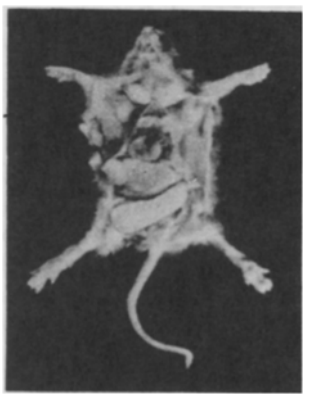

Fig. 3. Leukemia in a mouse of the C3HA line, from a passage of leukemic material taken from a mouse of the Afb line. Mouse No. 3183/245. The material was injected subcutaneously into a newborn mouse. It died at the age of 24 days, with symptoms of leukemia, without the development of tumor nodules at the site of injection. We observed enlargement of the lymph nodes, thymus gland, liver and spleen.

from this animal supply center were infected with the SE–polio virus. The possibility cannot be excluded that the mice which we took for the experiment were also carriers of latent polio virus, and the injection of culture fluid in the first hours of their life led to activation of this virus. In the experiments of Gross, Dulaney, Stewart, Dmochowski and many others, activation of the polio virus was manifested, primarily, by the development of tumors of the salivary glands, tumors of the adrenals, and fibrosarcomas [6, 7, 9, 18]. In our experiments we never observed the development of such tumors in mice of the Afb line. The development of cysts was also observed by us only with direct injection of the culture fluid into the mice, and none were seen in association with the serial passages to newborn and young mice of the same line, or with injection of the material from mice of the Afb line to newborn

mice of other lines (A, Asn, C3HA, C3H, C3H(f), C57 black, CC57 white, CC57 brown, BALB).

Aside from the contingency related to the role of polio virus in the development of cysts within mice of the Afb line, we cannot blame it for the appearance of leukemia in the animals, since the data in the literature definitely indicates that polio virus by itself does not cause leukemia [5, 12].

The question of a factor being responsible for the earlier development of leukemia, as well as the development of cysts by the mice of the Afb line in our experiment, all requires further study.

SUMMARY

Culture fluid from the 7th successive passage of human leukemic material through monkey kidney culture was injected into 6 mice of the high-leukemic strain Afb during the first day after birth. All the mice died between the 80th and 148th days after injection with systemic enlargement of the lymph glands. Serous and hemorrhagyc cysts were observed in 2 mice. Material from one of these mice sacrificed on the 125th day after birth was serially passaged through mice of the same strain. All of them developed leukemia. The leukemic line isolated, which was called Gamaleya No. 1, is hemocytoblastic in type. It is characterized by the absence of local tumor growth and by transplantability to mice of the low leukemic strain C3HA. The possible role of various factors in the development of early leukemia and casts in Afb mice is discussed.

LITERATURE CITED

1. L. A. Zil'ber and I. N. Kryukova, Vopr. virusol., No. 4 (1957). p. 239.
2. N. P. Mazurenko, Vopr. onkol., No. 6 (1960). p. 76.
3. G. Ya. Svet-Moldavskii and A. S. Skorikova, Acta virol., Vol. 4 (1960). p. 47.
4. L. Brown, Am. J. Hyg., Vol. 65 (1957). p. 189.
5. C. Dawe et al., J. Nat. Cancer Inst., Vol. 23 (1959). p. 717.
6. L. Dmochowski et al., Proc. Soc. Exp. Biol. (N. Y.), Vol. 101 (1959). p. 686.
7. A. Dulaney et al., Cancer Res., Vol. 17 (1957), p. 809.
8. J. B. Emery and C. J. York, Virology, Vol. 11 (1960). p. 313.
9. L. Gross, Proc. Soc. Exp. Biol. (N.Y.), Vol. 83 (1953). p. 414.
10. L. Gross et al., Cancer Res., Vol. 19 (1959), p. 316.
11. Hull et al., Am. J. Hyg., Vol. 63 (1956), p. 204.
12. L. Law et al., J. Exp. Med., Vol. 111 (1960). p. 517.
13. C. Logothetis et al., Proc. Soc. Exp. Biol. (N.Y.). Vol. 103 (1960). p. 420.
14. G. Rudali et al., C. R. Acad. Sci. (Paris), Vol. 242 (1956). p. 837.
15. R. Rustigian et al., Proc. Soc. Exp. Biol. (N.Y.) Vol. 88 (1955). p. 8.
16. S. Schwartz et al., Cancer. Res., Vol. 16 (1956). p. 559.
17. S. Schwartz et al., Ibid, Vol. 17 (1957). p. 218.
18. S. Stewart et al., Virology, Vol. 3 (1957). p. 380.
