Studies in Leukemia

VII. The Induction of Leukemia in Swiss Mice by Means of Cell-free Filtrates of Leukemic Mouse Brain

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THE ACCELERATION of the development of leukemia by cell-free filtrates prepared from brains of leukemic AKR mice has recently been reported.[1] By the technics described, leukemia develops in approximately 50 per cent of AKR mice in 3 to 5 weeks after a single intracerebral or intraperitoneal inoculation. Because of the high incidence of spontaneous leukemia in the AKR mouse, it was desirable to attempt to reproduce these results in a strain of mice in which leukemia is rare.

MATERIAL AND METHODS

As a source of agent, filtrates were used from leukemic tissues originating in a Swiss mouse. This leukemia strain is derived from a 33 week old Swiss female mouse that had been caged with AKR males for the purpose of hybrid breeding. She developed a "spontaneous" lymphoblastoma characterized by severe generalized lymphadenopathy, mesenteric tumor, an extraordinarily enlarged thymus and pronounced hepatosplenomegaly (fig. 1). The gross and microscopic characteristics of the lymphoblastoma in this mouse were indistinguishable from the spontaneous lymphoblastoma of AKR mice.

Cell suspensions of tumors of this Swiss mouse were injected intraperitoneally into adult DBA and Swiss mice* in both of which lymphoblastoma was produced. A cell-free filtrate was made of the brain of one of the leukemic DBA mice and nine DBA mice were inoculated intracerebrally. Lymphoblastoma developed in four of these mice. The tissues of these four mice were used as the secondary source material for the experiments described.

The Swiss mice were obtained from the Harlan Small Animal Farms, Cumberland, Indiana. The spontaneous incidence of leukemia in these mice is less than 1 per cent. The AKR mice were either of our stock colony or were obtained from the Jackson Memorial Laboratory, Bar Harbor, Maine. The DBA mice were bred in our laboratory from a breeding colony obtained from Dr. A. Tannenbaum. The incidence of spontaneous leukemia in this strain is less than 2 per cent.

When cell suspensions of tumor were used, approximately 250,000 cells were inoculated subcutaneously and 500,000 cells intraperitoneally (0.25 and 0.5 cc. respectively of a 10^6 per cubic centimeter cell suspension).

Cell-free filtrates were prepared by the technic previously described.[1] For intraperitoneal injections 0.5 cc. were used and for intracerebral injections, 0.075 cc.

Heat inactivation of filtrates was at 65 C. for 45 minutes.

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* All animals used in these experiments were at least four weeks of age at the time of inoculation.

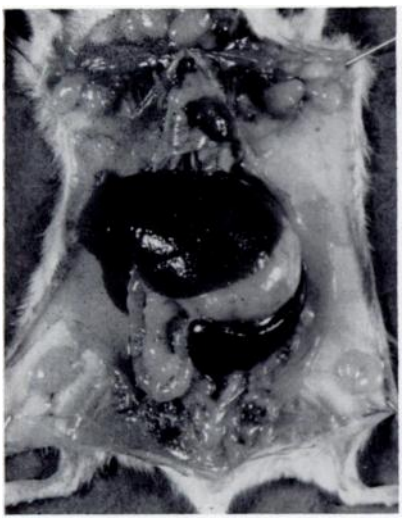

FIG. 1

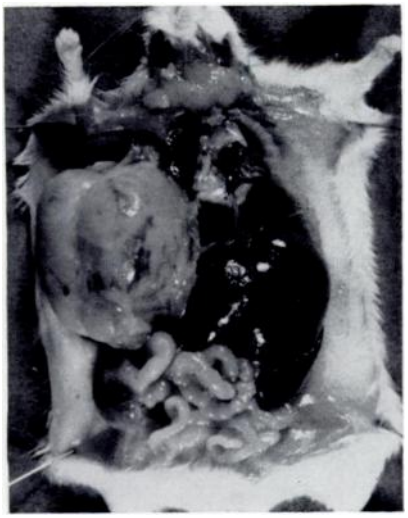

FIG. 2

FIG. 1.—Thirty-three week old Swiss female mouse with "spontaneous" lymphoblastoma. Note enlarged thymus, discrete mesenteric tumor, marked generalized lymphadenopathy, and hepatosplenomegaly.

FIG. 2.—Swiss mouse four weeks after the subcutaneous injection of a tumor cell suspension.

Histologic sections were in each instance made of the following organs: liver, spleen, lung, lymph nodes, tumor mass, and brain, and of the urogenital organs whenever they seemed involved.

RESULTS

The experiments with this lymphoblastoma are summarized in tables 1 and 2.

A. *The Effect of Injection of Tumor Cell Suspensions.* In 22 experiments, 36 of 149 (24 per cent) Swiss mice injected subcutaneously with Swiss tumor suspension showed progressive growth of the tumor (fig. 2). In the remaining 113

TABLE 1.—*Transmissibility of a Swiss Strain Lymphoblastoma by Means of Tumor Cell Suspensions*

Material injected	Recipient strain	Number of experiments	Site of inoculation	Number of animals in which lymphoblastoma developed
Swiss Tumor Cell Suspensions	Swiss	22	S.C.	36/149
		20	I.P.	64/144
	DBA	3	S.C.	5/9
		3	I.P.	2/9
	AKR	5	S.C.	0/14
		5	I.P.	0/10
DBA Tumor Cell Suspensions	Swiss	5	S.C.	11/22
		3	I.P.	12/18
	DBA	2	S.C.	5/5
		2	I.P.	5/5
	AKR	2	S.C.	0/5
		2	I.P.	0/9

S.C. = Subcutaneous. I.P. = Intraperitoneal.

TABLE 2.—*Transmissibility of a Swiss Strain Lymphoblastoma by Means of Cell-free Filtrates*

Material injected	Recipient strain	Number of experiments	Site of inoculation	Number of animals in which lymphoblastoma developed
Swiss Tumor	Swiss	2	I.C.	0/30
		2	I.P.	0/27
Swiss Brain	Swiss	5	I.C.	26/75
		2	I.P.	17/30
	DBA	2	I.C.	4/10
	AKR	3	I.C.	0/18
Swiss Brain Heat Inactivated	Swiss	1	I.C.	1/25
		1	I.P.	0/10
DBA Tumor	Swiss	1	I.C.	0/20
		1	I.P.	0/22
DBA Brain	Swiss	2	I.C.	22/31
		1	I.P.	22/25
	DBA	1	I.C.	4/9
	AKR	1	I.C.	0/10
		1	I.P.	0/10
DBA Brain Heat Inactivated	Swiss	1	I.C.	0/24

I.C. = Intracerebral. I.P. = Intraperitoneal.

animals, either no growth took place or after initial growth during the first 9 to 14 days, the tumor rapidly regressed. In 20 experiments, 64 of the 144 mice (44 per cent) inoculated intraperitoneally showed progressive growth of the tumor.

The Swiss tumor grew in five of nine DBA mice injected subcutaneously and two of nine injected intraperitoneally. It failed to grow in 14 AKR mice injected

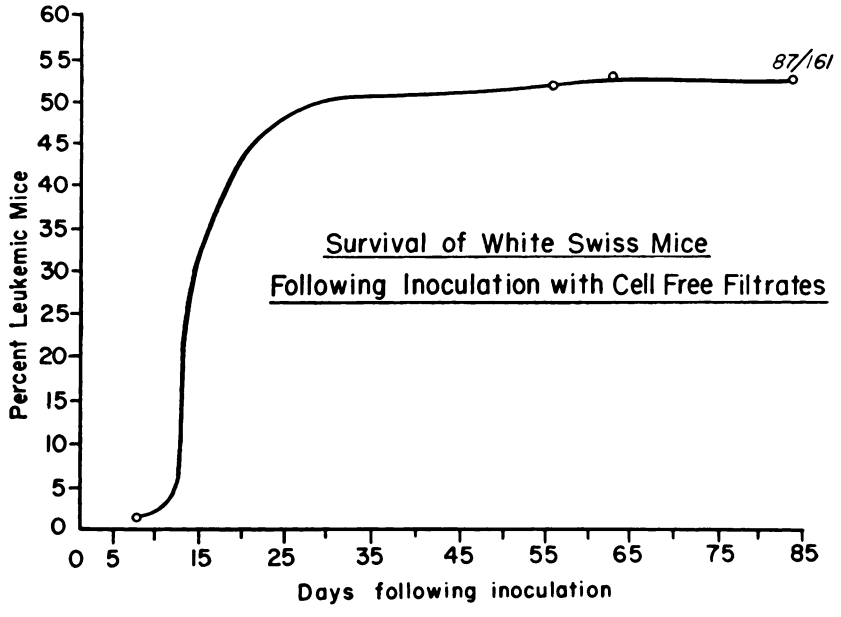

CHART 1

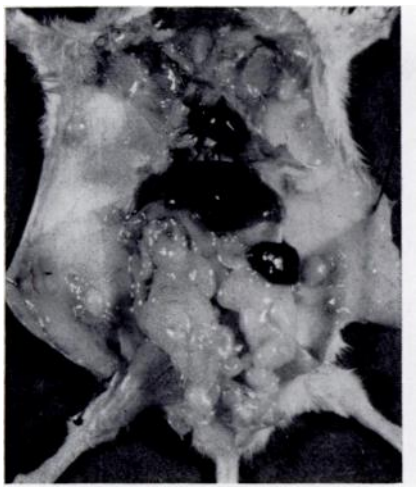

FIG. 3

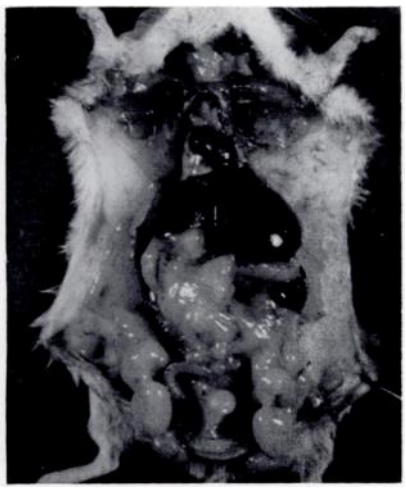

FIG. 4

FIG. 3—Swiss mouse two weeks after the intraperitoneal inoculation of 0.5 cc. of cell-free filtrate of leukemic Swiss brain. Note prominence of mesenteric tumor.

FIG. 4.—Swiss mouse two weeks after the intracerebral inoculation of 0.075 cc. of cell-free filtrate of leukemic Swiss brain. Note infiltration of genital organs and extensive mesenteric tumor.

subcutaneously and 10 AKR mice injected intraperitoneally. When tumor from the DBA leukemic mice was inoculated subcutaneously into five AKR mice, it regressed rapidly after initial growth in the first week. It failed to grow in nine AKR mice injected intraperitoneally.

B. *The Effects of Cell-Free Filtrates of Leukemic Brain.* Filtrates prepared from either Swiss or DBA *tumor* failed to induce lymphoblastoma in the 99 animals inoculated.

Filtrates prepared from Swiss and DBA leukemic *brains* induced a lymphoblastoma in 48 of 106 adult Swiss mice inoculated intracerebrally and 39 of 55 mice inoculated intraperitoneally.

In experiments using heat inactivated brain filtrates as a simultaneous control

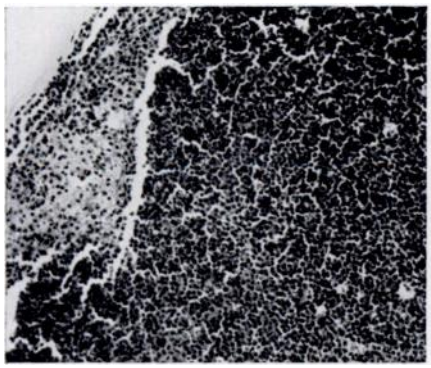

FIG. 5

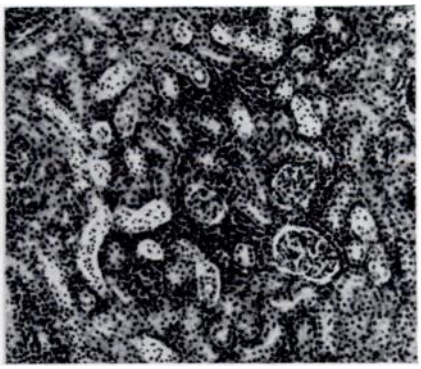

FIG. 6

FIG. 5.—Lymph node. Diffuse proliferation of lymphoblasts resulting in obliteration of the normal architecture and infiltration of the capsule. × 110.

FIG. 6.—Kidney. The interstitial tissue is infiltrated with lymphoblasts. × 110

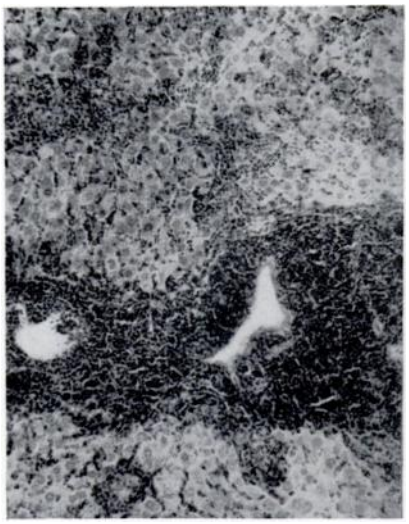

FIG. 7

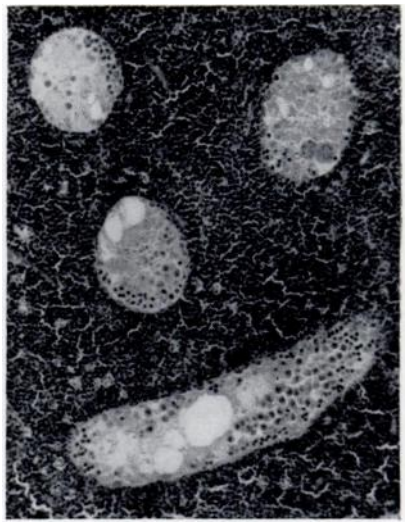

FIG. 8

FIG. 7.—Liver. Note dense tumor infiltration of enlarged portal fields as well as sinusoids. × 80.

FIG. 8.—Testicle. The intertubular stroma is densely infiltrated with tumor. × 90

Alternative Figure—Liver. Infiltration of the sinusoids and replacement of the liver cell plates by mononuclear cells with large nuclei frequently with nucleoli. × 500.

(table 2) only one of 49 animals inoculated intracerebrally developed the lymphoblastoma. None of 10 animals inoculated intraperitoneally manifested the disease.

Regardless of the site of inoculation with the brain filtrate, the animals began to manifest the disease as early as eight to ten days after injection. The survival time after inoculation is shown in Chart 1.

The gross autopsy findings differed significantly from those seen in AKR lymphoma. Only two animals exhibited marked peripheral lymphadenopathy. Although slight enlargement of the thymus occurred in 30 per cent, in only three animals did the thymic tumor approach the proportions commonly seen in the AKR lymphoma. In 50 per cent of the animals splenomegaly was significant, whereas moderate to severe hepatomegaly was present in 75 per cent. The outstanding finding was a massive diffuse infiltration of the mesentery by tumor which frequently invaded the kidneys and genital organs. This was true regardless of whether the filtrate was inoculated intraperitoneally (fig. 3) or intracerebrally (fig. 4). Ascites was present in 25 per cent of animals inoculated intraperitoneally and in 10 per cent of those inoculated intracerebrally.

Primitive lymphoid cells were observed in the blood in 10 of 26 cases, whereas the marrow showed significant involvement in 8 of 31 cases studied.

The histologic findings were similar to those seen in the lymphoblastoma of the AKR strain. These, as well as the extensive involvement of the genital organs, are illustrated in figures 5–8.

The filtrates failed to accelerate the development of lymphoblastoma in the 38 AKR mice injected.

DISCUSSION

The brain of leukemic Swiss mice contains a filterable agent which induces leukemia in the homologous and the DBA strain. The agent present in the

brains of leukemic Swiss mice is not identical with that found in AKR mice, for neither filtrate consistently produces a lymphoblastoma in the heterologous strain.

This lymphoblastoma seen differs from the disease of Swiss mice described by Charlotte Friend[2] in the formation of the large tumors and the local growth resulting from transplant of tumor tissue.

That the cell-free filtrate of the leukemic Swiss mouse brain induces leukemia in this low leukemic strain as well as in DBA mice is interpreted as evidence that the filterable agent is fundamentally concerned with the initiation of the leukemic process rather than with the acceleration of the development of a latent potential.

Although the nature of the agent in these filtrates has not been defined, certain facts are available from the present and previously observed data:

1. The agent will pass a bacterial filter (Seitz or Berkfeld).[1, 3, 4]
2. It is inactivated by heat,[1, 3] formalin and probably ultraviolet irradiation.[4]
3. A single inoculation will produce leukemia in the susceptible strain in 2 to 4 weeks.[1, 3] This interval between inoculation and the development of leukemia is of the same order of magnitude as that resulting from the inoculation of tumor cell suspensions. The interval is remarkably shorter than the interval necessary for the action of repeated administration of known carcinogens.
4. The agent may be serially passed through a resistant strain so that after five blind passages it still produces leukemia when inoculated into susceptible animals.[4]
5. In Swiss mice the incidence of leukemia after the inoculation with a single dose of cell-free brain filtrate is approximately 50 per cent, whereas the incidence of spontaneous leukemia in this strain even at one year of age is no greater than 1 per cent.
6. The agent is found in leukemic animals and is not derived from a source foreign to the animal.

Summary

1. A 33 week old Swiss female mouse that had been caged with AKR males exhibited a "spontaneous" lymphoblastoma resembling the spontaneous disease in the AKR strain.

2. Tumor cell suspensions of this animal did not grow when inoculated into AKR mice.

3. Tumor cell suspensions grew progressively in both Swiss and DBA mice, producing a leukemia.

4. Cell-free filtrates of leukemic Swiss or DBA mouse brain induced leukemia in 48 of 106 adult Swiss mice inoculated intracerebrally and 39 of 55 animals inoculated intraperitoneally. The leukemia appeared in the majority of the animals within one to three weeks from the time of inoculation.

5. Cell-free filtrates of the leukemic tumor failed to induce leukemia under the conditions of the experiment.

Summario in Interlingua

1. Un mus switze feminin de 33 septimanas de etate, que habeva essite incaviate con masculos del racia AKR, exhibiva un lymphoblastoma "spontanee" que resimilava le forma spontanee del morbo in le racia AKR.

2. Suspensiones de cellulas del tumor de iste animal non cresceva post inoculation in muses del racia AKR.

3. Suspensiones de cellulas del mesme tumor cresceva progressivemente e resultava in leucemia tanto in muses switze como etiam in muses del racia DBA.

4. Filtratos discellulate de cerebro de leucemic muses switze o muses del racia DBA induceva leucemia in 48 ex 106 adulte muses switze subjicite a inoculationes intracerebral e in 39 ex 55 animales subjicite a inoculationes intraperitoneal. Le leucemia se manifestava in le majoritate del animales intra un a tres septimanas post le inoculation.

5. Sub le conditiones del experimento, filtratos discellulate de tumor leucemic non induceva leucemia.

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[4] Unpublished data.