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# Studies on Leukemia in the C3Hf Strain of Mice I. Ori-gin, Transmission and Morphology of Spontaneous Lymphatic Leukemia

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# Studies on Leukemia in the C3Hf Strain of Mice I. Ori-gin, Transmission and Morphology of Spontaneous Lymphatic Leukemia\*

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# Abstract

The ongm and characteristics in transmission and morphology of spontaneous lymphatic leukemia in a low-leukemic strain C3Hf have been described. The leukemia line is being currently subjected to a vigorous search for the presence of a filtrable leukemia agent.

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## STUDIES ON LEUKEMIA IN THE C3Hf STRAIN OF MICE

# I. ORIGIN, TRANSMISSION, AND MORPHOLOGY OF SPONTANEOUS LYMPHATIC LEUKEMIA

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Leukemia in mice of the C3H or C3Hf substrains except C3Hf/Fg is exceptionally rare (Table I)<sup>1-6</sup>, and there has been no detailed description of the disease in these substrains of mice. Lymphatic leukemia in C3Hf mice that we encountered has provided an occasion to study the characteristics and etiology of leukemia in a low-leukemic strain of mice. For the attempted transmission experiments to be reported in the subsequent papers, a brief description of the lymphatic leukemia in C3Hf mice is warranted.

# MATERIALS AND METHODS

Mice of strains C3Hf, C3H/He/Mi/Ky, RF, CBA, DBA, C57BL, and ddN were used. Among them, C3Hf, CBA, DBA, and C57BL mice were raised in the mouse colony of this University by brother-to-sister mating from litters obtained in 1958 from Dr. A. Kirschaum, Baylor University College of Medicine in Houston, Texas. The C3Hf subsrtain, also called Zb, should be designated C3Hf/Bi/Ki<sup>\*</sup>, but for the sake of simplicity only the C3Hf symbol is used in this paper. RF mice were inbred from a nucleus obtained from the

Table I. Incidence of spontaneous leukemia in different substrains of the C3H line

Symbol	Leukemia incidence	Investigator	Reference
C3H/Bi/Gs & C3Hf/Bi/Gs	less than 0.5%	Gross	1
C3Hf/Bi/Gs/Lv	one in 4 years	Levinthal & Buffett	2
C3Hf*	1.8%	Lemonde & Clode	3
C3Hf/He	approx. 1%	Law	4
C3H/He/Mi/Ky	1.6%	Hamazaki	5
C3Hf/Fg	51%	Law	6

\* Substrain is not specified.

\* C3H mice, foster-nursed, originally obtained from Bittner, carried for many generations by Kirschbaum.

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Roscose B. Jackson Memorial Labaratory, Bar Harbor, Maine in 1959. The origin of the C3H/He/Mi/Ky line has been described<sup>7</sup>. DdN mice were purchased from a commercial breeder in Japan. The animals were constantly supplied with Oriental laboratory chow and tap water.

In attempts to transmit leukemia, intraperitoneal inoculations of minced, small fragments of leukemic, mesenteric lymph node and/or spleen were performed by means of a trocar. However, to maintain complete sterility, intraperitoneal or subcutaneous inoculations of 0.2 to 0.3 ml. of a leukemic cell suspension by means of a syringe and a small needle were preferred. The aseptically removed splenic tissue was transferred to a small sterile Petri dish and tweezed in physiological saline solution with sterile scissors to obtain a cell suspension of 20% concentration. Once leukemia developed into an ascites form, intraperitoneal inoculations of 0.2 to 0.3 ml. of ascites were performed for serial cell-graft.

Mice were autopsied, when they appeared moribund or soon after they died.

#### RESULTS

1. Origin of Lymphatic Leukemia in C3Hf Mice

A 19-month-old C3Hf male mouse, one of three mice that had been transplanted intraocularly with methylcholanthrene-induced DBA ascitic lymphoma cells three months previously, developed a palpable spleen. When the mouse was autopsied on August 31, 1961, it appeared sick and emaciated. At postmortem examination there were moderate enlargement of the spleen and mesenteric lymph node and slight enlargement of the peripheral lymph nodes. The liver and thymus were not enlarged. The transplanted right eye was shrunken and the cornea was quite opaque without any evidence of an intraocular lymphoma.

The leukocyte count made of the tail blood at time of autopsy was 12,500 per cu.mm. with 10% lymphoblasts, 67% large lymphocytes, 18% small lymphocytes, 3% monocytes, and 2% segmented neutrophils. There was evidence of anemia.

Microscopically, there was leukemic infiltration of the spleen, liver, bone marrow, kidney, and lung. The normal architecture of the spleen was lost by the leukemic infiltration and focal hemorrhages. The liver showed periportal leukemic infiltration as well as accumulation of leukemic cells in the sinusoids.

2. Isologous Transmission of C3Hf Leukemia by Cell-graft

Intraperitoneal inoculations of minced tumor fragments, lymphoid tumor emulsions or ascitic lymphoma cells have resulted in the development of leukemia in mice of the C3Hf line in which the leukemia originated. The leukemia has

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been carried through 20 generations by successive cell-grafts, and over 80 adult C3Hf mice of both sexes became leukemic as the result of transplantation. Grafts were successful in 100% of the cases irrespective of the age and sex of the animals and route of inoculations. On rare occasions, transplantation was made into 1 mouse only because of shortage of mice, and at other times I had to transplant leukemia from a mouse which died earlier than was expected and yet this transplantation succeeded. In the beginning of the serial cell-graft, leukemia developed in approximately 4 weeks after inoculation of the leukemic cells. After successive cell transfers, the tumor has grown faster and the killing time has been gradually shortened to 9 days by the present 20th transplant generation (Fig. 1). Around 6 days after transplantation, the spleen becomes



Fig. 1. Gradual shortening of the survial time of transplanted mice during serial cell-grafts through 20 transfer generations over approximately one year.

palpably enlarged. A marked leukocytosis with appearance of many immature cells in the peripheral blood was observed terminally within one to two days before mice succumbed to the disease. Presently the leukemia has been maintained as an ascites form, although concomitant enlargement of the spleen and lymph nodes occurs.

Subcutaneous inoculation of ascitic leukemic cells was followed by a local growth, splenomegaly, axillary and inguinal lymphadenopathy with a terminal leukemic blood picture. But there was no ascites and the degree of involvement

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of the visceral lymph nodes was only slight.

Homologus transplantation into adult mice of strains C3H/He/Mi/Ky, RF, CBA, DBA, C57BL, and ddN did not result in the development of leukemia.

3. General Morphology of the Transplanted Disease

Leukemia developing in C3Hf mice following inoculation of leukemic cells resembled the primary leukemia (Fig. 2). In fully developed cases, there were a markedly enlarged spleen and a moderately enlarged, whitish, oblong mesenteric lymph node. Usually the liver and thymus showed slight or no enlargement. Retroperitoneal and mediastinal lymph nodes were involved more frequently than the cervical, axillary or inguinal groups. The spleen was reddish brown with whitish mottling. The liver was light brown in color. Splenomegaly was associated with intrasplenic hemorrhages or hemorrhagic infarcts until the 13th transfer generation, after which hemorrhagic lesions of the spleen became less conspicuous, and a small amount of ascitic fluid began to accumulate. The amount of ascites varied from 0.5 to 2.0 ml. and it was either milky or sanguinous. The ascites contained 526,000 tumor cells per cu. mm. on an average.

The peripheral leukocyte count made of the tail vein at time of autopsy ranged from 24,300 to 303,000 per cu. mm. with an average of 85,000 per cu. mm. The number of lymphoblasts in the peripheral blood varied from 2 to 66% with an average number of 24%. Smudge cells in the peripheral blood smears ranged from 13 to 136 and averaged 56 per 100 leukocytes. Erythroblasts were rare.

As an example to show the degree of leukemic infiltration in the hematopoietic organs, differential counts were made of the peripheral blood, bone marrow, spleen, lymph node, and thymus of a leukemic adult male mouse that appeared terminally ill (Table 1). Differentiation of the myeloid cell series was done according to the schematic representation of BARMES and SISMAN<sup>8</sup>. The mouse was transplanted with a splenic cell suspension on March 11 and autopsied on March 31. At the time of autopsy the leukocyte count was 126,000 per cu. mm., and the mouse showed an enormously enlarged spleen with hematomas but enlargement of the thymus, lymph nodes, and liver was slight. In transplanted leukemias, although enlargement of the thymus and peripheral lymph nodes was usually minimal as in this case, cell suspension smears of these organs invariably disclosed leukemic cells of varying numbers. The degree of leukemic infiltration of hematopoietic organs including thymus seemed to depend, among other factors, on the duration of illness.

On histologic examination, the liver, spleen, kidney, lymph nodes, bone marrow, and lung showed infiltration by leukemic cells. In the liver the leukemic infiltration was chiefly perivascular but sinusoidal accumulation of leukemic cells was also present (Fig. 3). Interesting enough, in some cases,

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		Blood	Marrow	Spleen	Node	Thymus
Lymph. series	Lymphoblasts	20%	39%	76%	70%	82%
	Large lymphocytes	74%	21%	5%	3%	6%
	Small lymphocytes	4%	7%	3%	27%	12%
Myelo. series	Myeloblasts		-			
	Promyelocytes		1%			
	Myelocytes		1%			
	Metamyelocytes		17%			
	Neutrophils	2%	3%	1%		
M	lonocytes					
Eosinophils						
Erythroblasts			11%	15%		

Table 1. Differential counts of a peripheral blood smear and cell suspension smears of bone marrow, spleen, lymph node, and thymus of a mouse with transplanted leukemia

sections of liver revealed a few microabscesses or focal necrosis of the hepatic cells (Fig. 4). Both spleen and mesenteric lymph node were completely replaced by leukemic cells (Fig. 5). In the former there was an association of hemorrhagic areas. In the latter the leukemic infiltration extended to the surrounding fat tissue through the capsule. The kidney showed leukemic infiltration in the parenchyma, under the capsule, and in the perirenal adipose tissue (Fig. 6). In the lung there was a peribronchial leukemic infiltrate (Fig. 7). The brain, heart, and testes were free from leukemic infiltration.

# 4. Cytological Observations

Stained smear preparations revealed that the nucleus of the neoplastic cell was round or slightly indented, and was surrounded by a narrow rim of nongranular, basophilic cytoplasm (Fig. 8). Some of the large leukemic cells in the peripheral blood and ascites showed a coarse chromatin pattern, differing from normal lymphocytes primarily in their cytoplasmic basophilia. Smudge cells were numerous in the peripheral blood smears. All the leukemic cells were peroxidase-negative.

Phase contrast microscopy of the ascitic neoplastic cells revealed their nuclei to be round or oval, often showing a deep indentation or lobulations. There were usually one or two nucleoli which varied in size. The nuclear membrane was conspicuous. There were not many dot- or rod-shaped mitochondria around the nuclear membrane or in the area of the nuclear indentation. The cytoplasm occasionally contained one or two highly refractile vacuoles. Ascitic tumor cells showed more variations in size and more nuclear lobulations than leukemic cells in the peripheral blood (Figs. 9 and 10). 298

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#### DISCUSSION

The described neoplastic process in C3Hf mice fulfills the criteria of a lymphoid neoplasm of mice9. The C3Hf mouse which developed lymphatic leukemia had been transplanted, 3 months previously, with methylcholanthreneinduced DBA ascitic lymphoma cells in the anterior chamber of the right eye. The other two C3Hf male mice which received a similar intraocular transplantation died without evidence of leukemia. The origin of the C3Hf leukemia may be questioned as to a possible relationship to the intraocular transplantation. The leukemia, however, showed a strict strain specificity, and proved not transplantable to other strains of mice tested including DBA mice. This transplantation experiment excludes a possibility of DBA leukemia growing in C3Hf mice. Absence of a neoplastic growth in the anterior chamber of the transplanted eve also speaks against this possibility. Induction of leukemia in the intraocularly transplanted C3Hf mouse by a possible viral agent of DBA ascitic tumor cells is another possibility. This idea, again, is unlikely in view of our negative experiment to isolate a filtrable agent from the DBA leukemia (unpublished data). The primary leukemia in the C3Hf mouse was, therefore, considered a spontaneous disease that could occur sporadically in this strain of mice.

Enhancement of virulency of neoplastic cells by repeated passages appears to be a common phenomenon of cancer<sup>10-12</sup>. Also repeated intraperitoneal inoculations of tumor cells usually result in the development of an ascites form of cancer<sup>13</sup>. In C3Hf leukemia, whether transplanted subcutanously or intraperitoneally, the neoplastic process remains as lymphosarcoma throughout most of the inoculated period until it terminally invades the blood circulation, assuming a leukemic picture. Thus, basically there is no difference between lymphosarcoma and leukemia and both are essentially the same disease<sup>11</sup>.

Following RICHTER and McDOWELL's<sup>14</sup> first successful transmission of mouse leukemia, numerous transplantable leukemias and lymphoid tumors in mice, both spontaneous and induced, have been described, and transplantation of tumors has become an important tool in all phases of cancer research. DUNHAM and STEWART<sup>15</sup> reviewed all kinds of transplantable animal tumors including leukemias and lymphosarcomas in mice and presented them in a tabular form. In recent years many more murine leukemias of proven viral etiology have been

Fig. 2. A C3Hf male mouse bearing transplanted lymphatic leukemia of the 18th transfer generation. Bloody ascites has been removed.

Fig. 3. Section of liver of the mouse shown in Fig. 1. Heavy leukemic infiltration in the periportal space and diffusely infiltrating leukemic cells in the sinusoids.

Fig. 4. Section of liver of the same mouse showing a microabscess and leukemic infiltration.

Fig. 5. Section of spleen of the same mouse showing a sheet of leukemic cells replacing the normal architecture.



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reported<sup>16-22</sup>

Hereditary nature of mouse leukemia was first considered by SLYE<sup>23</sup> and the high incidence of leukemia in successive generations of mice of Ak or C58 was explained by genetic factors<sup>11,21</sup>. At least several factors such as genetic, hormonal, and viral appear to play a role in the causation of spontaneous leukemia. LAW<sup>6</sup> re-evaluated non-viral factors in the light of a viral theory of leukemia. LAW and MOLONEY<sup>25</sup> recently reported that otherwise low-leukemic strains C3Hf/Lw and C3Hf/Gs can be made high-leukemic simply through foster-nursing of litters. Yet the nature and etiology of leukemia that occurs uncommonly and sporadically in these low-leukemic strains of mice remain obscure. Attempts to isolate a leukemia agent from the C3Hf leukemia line are being made, and their results will be reported in a separate paper.

## SUMMARY

The origin and characteristics in transmission and morphology of spontaneous lymphatic leukemia in a low-leukemic strain C3Hf have been described. The leukemia line is being currently subjected to a vigorous search for the presence of a filtrable leukemia agent.

# ACKNOWLEDGEMENT

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#### REFERENCES

- 1) GROSS, L.: High susceptibility of 1 to 14 days old C3H mice to "Passage A," leukemic filtrates. *Proc. Soc. Exp. Biol. Med.* 97, 300, 1958
- 2) LEVINTHAL, J. D. and BUFFETT, R. F.: Thymectomy and thymic grafts in mouse viral leukemia. *Ibid.* 106. 426, 1961
- 3) LEMONDE, P. and CLODE, M.: In vitro culture of a leukemic agent from spontaneous mouse leukemia. Nature 193, 1191, 1962
- 4) LAW, L.W.: Increase in incidence of leukemia in hybrid mice bearing thymic transplants from a high leukemic strain. *Nat. Cancer Inst.* 12, 789, 1952
- 5) HAMAZAKI, Y.: Personal communication
- 6) LAW, L. W.: Present status of nonviral factors in the etiology of reticular neoplasms of the mouse. Ann. N. Y. Acad. Sci. 68, 616, 1957
- 7) OKADA, K., MIYOSHI, I., KAWAMURA, N. and SATO, M.: Studies on leukemogensis by cell-free filtrates of AKR mouse leukemia. Acta Haemat. Jap. 25, 1, 1962

- Fig. 8. A lymphoblast appearing in the peripheral blood.
- Fig. 9. Ascitic neoplastic cells as observed by phase contrast microscopy.
- Fig. 10. Ascitic neoplastic cells stained with May-Grünwald-Giemsa.

Fig. 6. Section of kidney of the same mouse showing subcapsular and focal, parenchymal leukemic infiltration.

Fig. 7. Section of lung of the same mouse showing a small peribronchial aggregate of leukemic cells.

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- 8) BARNES, W. A. and SISMAN, I. E.: Myeloid leukemia and non-malignant extramedullary myelopoiesis in mice. Am. J. Cancer. 37, 1, 1939
- 9) DUNN, T.B.: Normal and pathologic anatomy of the reticular tissue in laboratory mice. With a classification and discussion of neoplasms. J. Nat. Cancer Inst. 14, 1281, 1954
- 10) RICHTER, M. N. and MACDOWELL, E. C.: Studies on mouse leukemia III. A comparison of four lines of leukemia transmitted by inoculation. J. Exp. Med. 52, 823, 1930
- 11) FURTH, J., SEIBOLD, H.R. and RATHBONE, R.R.: Experimental studies on lymphomatosis of mice. Am. J. Cancer 19, 521, 1933
- DE BRUYN, W. M., KORTEWEG, R. and VAN WAVEREN, E. K.: Transplantable mouse lymphosarcoma T 86157 (MB) studied in vivo, in vitro and at autopsy. Cancer Research 9, 282, 1949
- 13) OKADA, K., MIYOSHI, I., KAWAMURA, N. and SATO, M.: Cell-free transmission of AKR mouse leukemia. Arch. Hist. Jap. 22, 25, 1961
- 14) RICHTER, M. N. and MACDOWELL, E. C.: The experimental transmission of leukemia in mice. Proc. Soc. Exp. Med. 26, 362, 1929
- 15) DUNHAM, L. J. and STEWART, H. L.: A survey of transplantable and transmissible animal tumors. J. Nat. Cancer Inst. 13, 1299, 1953
- 16) FRIEND, C.: Cell-free transmission in adult Swiss mice of a disease having the character of a leukemia. J. Exp. Med. 105, 307, 1957
- 17) GRAFFI, A.: Chloroleukemia of mice. Ann. N.Y. Acad. Sci. 68, 540, 1957
- GROSS, L.: Development and serial cell-free passage of a highly potent strain of mouse leukemia virus. Proc. Soc. Exp. Biol. Med. 94, 767, 1957
- 19) SCHOOLMAN, H. M., SPURRIER, W., SCHWARTZ, S.O. and SZANTO, P.B.: Studies in leukemia. VII. The induction of leukemia in Swiss mice by means of cell-free filtrates of leukemic mouse brain. *Blood* 12, 694, 1957
- 20) GROSS, L.: Serial cell-free passage of a radiation-activated mouse leukemia agent. Proc. Soc. Exp. Biol. Mcd. 100, 102, 1959
- 21) LIEBERMAN, M. and KAPLAN, H.S.: Leukemogenic activity of filtrates from radiationinduced lymphoid tumors of mice. *Science* 130, 387, 1959
- MOLONEY, J. B.: Biological studies on a lymphoid leukemia virus extracted from sarcoma 37. I. Origin and introductory investigations. J. Nat. Cancer. Inst. 24, 933, 1960
- 23) SLYE, M.: The relation of heredity to the occurrence of spontaneous leukemia, pseudoleukemia, lymphosarcoma and allied diseases in mice: Preliminary report. Am. J. Cancer 15, 1361, 1931
- 24) MACDOWELL, E.C. and RICHTER, M.N.: Mouse leukemia IX. The role of heredity in spontaneous cases. Arch. Path. 20, 709, 1935
- 25) LAW, L. W. and MOLONEY, J. B.: Studies of congenital transmission of a leukemia virus in mice. Proc. Soc. Exp. Biol. Med. 108, 715, 1961

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