ON CYSTS IN MICE OF THE LOW CANCER LINE C57BL

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 55, No. 4, pp. 82-91, April, 1963
Original article submitted August 1, 1962

Rudali and co-workers [8] described cystic changes in the lymphatic nodes of mice of the C57BL line that had not undergone experimental treatments. These changes developed in the mice at a late age (an average of 14 months).

In 1957, we set up an experiment on mice of the C57BL line, investigating the leukemogenic activity of a brain filtrate from a man who died of acute leukemia (hemocytoblastosis). Twenty months after the injection of the filtrates not a single case of leukemia was noted in the mice [3], but in 3 of the mice we observed a significant enlargement of the axillary lymph nodes. The mice were sacrificed at the age of $20\frac{1}{2}$ months. In all 3 mice the axillary lymph nodes had become cysts, filled with light, slightly opalescent fluid. In one of them (No. 20), the cystic changes also occurred in the submaxillary and inguinal lymph nodes; in addition, under the skin on the left, we detected a small (size of a chicken feed granule) tumor nodule, lying closely against the wall of the cyst in an inguinal lymph node. The fluid from the cystically altered lymphatic nodes, and the walls of the cyst, were divided up and used for transplantation to other mice of the same line. Newborn mice which were injected with the cystic fluid remained healthy for $1\frac{1}{2}$ years of observations. On autopsy, no cysts or tumors were found in them.

Fig. 1. Multiple cysts of the lymphatic nodes in a mouse of the C57BL line.

In the mice who were injected with a suspension of the walls of the cystically altered lymph nodes, tumors appeared at the site of transplantation, and with subsequent serial passage to newborn mice of the same line, plus the addition of a suspension of brain tissue, we noted the development of numerous tumor nodes (under the skin, in the lungs, on the mesentery and peritoneum, in the liver, spleen, kidneys, and, in one case, in the urinary bladder), and cysts containing serous or hemorrhagic contents (Fig. 1). In many mice we observed hyperplasia of the splenic tissue [5]. The tumors were glandular – cystic in structure. The material was carried through to the present time, via 34 passages.

Cellular Transplantations to Mice of Other Lines and to Rats. The material from C57BL mice of the 2nd and subsequent passages was used for injection into newborn mice of other lines (CC57BR, CC57W, BALB, A, ASn, Afb, C3HA, C3Hf) and into newborn rats of the Wistar line and of the non-pedigreed type. Transplantation of the tumor by cell suspensions succeeded only in mice of the CC57BR and CC57W lines, and in non-pedigreed white mice. In the CC57BR and CC57W mice, the tumors began to take beginning with the 6th consecutive passage in newborn mice of the C57BL line, and with the 14th passage for the non-pedigreed mice. Reverse transplantations to the original line were always successful. The strain yielded 100% transplants in mice of the CC57BR and CC57W lines, but it was only possible to maintain it in the non-pedigreed mice for 2-3 consecutive passages. Transplants of the tumor to rats did not take. Development of cysts from heterologous transplants was observed only in the passage mice of CC57BR and CC57W lines and in rats. Upon passage to newborn mice cysts were observed in the animals at the age of $1\frac{1}{2}$ months and older, but with injection of analogous material into newborn rats they were seen only after 14-17 months. In mice of the CC57BR and CC57W lines, as well as in mice of C57BL, we sometimes observed multiple cysts.

TABLE 1. Quantity and Intervals of the Development of Tumors, Leukemias, and Cysts in Mice of Various Lines, Injected at an Early Age with Acellular Preparations from Experimental Mice of the C57BL Line

Line of the mice	Experimental number	Age of the mice taken in the experiment (in days)	Method of injection	Maximum period of observation (in months)	Number of mice			Numbers of tumors				Number of leukemias	Number of mice with cysts	Number of mice with both tumors and cysts	Total number of mice with cysts	Number of mice with hyperplasia of the spleen and lymph nodes
					maculated	surviving the raising period	with tumors and leukemias	of the lungs	of the mammary glands	of the thymus	of different sites					
C57BL	98	1	Subcutaneously	19	6	5	0									
	279	7	Intraperitoneally	14	6	4	0									
	371	1	Subcutaneously	12½	5	3	1				1 (1½)		1 (12½)			
	428	2	Subcutaneously	16	5	3	1	1 (16)						1 (16)		
	478	10	Intraperitoneally	18	6	3	0									1 (5)
	479	7	Intraperitoneally	10	5	3	3	2 (11; 13)				1 (9)				
Total . . .						21	5	3			1	1			2	
CC57BR	430	1	Subcutaneously	18	14	14	3	2 (4; 4)				1 (16)				3 (10; 13; 14)
	486	9	Intraperitoneally	16	11	10	7	2 (14; 14)			3 (13—15)	2 (13; 16)		3 (13; 14; 15)		2 (7; 7)
	487	4	"	7	7	5	1	1 (6)								2 (7; 7)
	488	4	"	17	6	6	2	1 (17)			1 (9)					
	500	1	"	3	15	2	1				1 (3)			1 (3)		
Total . . .						37	14	6			5	3			4	
CC57W	280	1	Intraperitoneally	14	11	9	1	1 (13)								
	289	1	Subcutaneously	7	3	3	0									
	348	1	Intraperitoneally	15	6	6	1	1 (15)					2 (13; 14)	1 (15)		
	473	5	"	14	6	4	1	1 (14)						1 (14)		
	470	1	"	14	8	5	4	3 (11; 12; 12)		1 (14)				1 (12)		

TABLE 1. Continued

Line of the mice	Experimental number	Age of the mice taken in the experiment (in days)	Method of injection	Maximum period of observation (in months)	Number of mice: inoculated	Number of mice: surviving the raising period	Number of mice: with tumors and leukemias	Numbers of tumors: of the lungs	Numbers of tumors: of the mammary glands	Numbers of tumors: of the thymus	Numbers of tumors: of different sites	Number of leukemias	Number of mice with cysts	Number of mice with both tumors and cysts	Total number of mice with cysts	Number of mice with hyperplasia of the spleen and lymph nodes
CC57W	497	1	Subcutaneously	14	13	2	1				1 (14)			1 (14)		
	509	3	Intraperitoneally	18	5	4	1					1 (12)				
Total . . .						33	9	6		1	1	1			6	
C3HA	99	30	Subcutaneously	7	4	4	0									2 (5; 8)
	281	1	Subcutaneously and intraperitoneally . . .	14	6	6	2	2 (14; 14)								
	421	2	Subcutaneously	19	6	6	4		2 (12; 15)		1 (18)	1 (19)		1 (12)		1 (12)
	431	3	"	19	7	7	2	2 (19; 19)								4 (19)
Total . .						23	8	4	2		1	1			1	

Note. The age of the mice in months is shown in parentheses.

TABLE 2. Distribution of Neoplasms in the Different Age Groups

Line of the mice	Experiment No.	Number of surviving mice (according to months)																		Total number of mice surviving 7 months
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th	17th	18th	19th	
C57BL	98	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5			
	279	4	4	4	4	4	4	4	4	4	4	4	4	4	4						
	371	3	3 (1)	2	2	2	2	2	2	2	2	2	2	1 (к)							
	428	3	3	3	3	3	3	3	3	3	3	2	1	1	1	1	1 (1; к)				
	478	3	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2			
	479	3	3	3	3	3	3	3	3	3 (1)	2	2 (1)	1	1 (1)							
Total . . .		21						19		(1)		(1)		(1; к)			(1; к)				19 (4; 2к)
CC57BR	420	14	14	13	13	9	8	3	2	2	2	2	2	2	2	2	2 (1)	1	1		
	486	10	10	10	10	10	10	10	8	8	7	7	7	7 (2; к)	5 (3; к)	2 (1; к)	1 (1)				
	487	5	5	5	5	5	5 (1)	2													
	488	6	5	5	5	5	5	3	3	3 (1)	2	1	1	1	1	1	1	1 (1)			
	500	2	2	2 (1; к)																	
Total		37						18		(1)				(2; к)	(3; к)	(1; к)	(2)	(1)			18 (10; 3к)
CC57W	280	9	9	9	9	9	8	8	8	8	8	8	8	8 (1)	7						
	289	3	3	3	3	2	1	1													

TABLE 2. Continued

Line of the mice	Experiment No.	Number of surviving mice (according to months)																		Total number of mice surviving 7 months
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th	17th	18th	19th	
CC57W	348	6	6	6	6	6	6	6	6	3	3	3	3	2 (к)	2 (к)	1 (1; к)					
	473	4	4	4	4	4	4	4	4	4	4	4	4	4 (1; к)	4 (1; к)	2	2	2	2		
	470	5	5	5	5	5	5	5	5	4	4	4 (1)	3 (2; к)	1	1 (1)						
	497	2	2	1	1	1	1	1	1	1	1	1	1	1	1 (1; к)						
	509	4	4	4	4	4	4	4	4	4	4	4	4 (1)	3	3	3	3	3	3		
Total . . .	- -	33						29				(1)	(3; к)	(1; к)	(3;3к)	(1; к)					29 (9; 6к)
C3HA	99	4	4	4	3	1	1	1	1												
	281	6	6	6	6	6	6	6	6	6	6	6	6	6	6 (2)						
	421	6	6	6	6	6	6	6	6	5	5	5	5 (1; к)	4	4	4 (1)	3	3	3 (1)	2 (1)	
	431	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	6	6 (2)	
Total . .		23						20					(1; к)		(2)	(1)			(1)	(3)	20 (8; к)

Note. The figure in parentheses designates the number of mice with tumors and leukemias; the letter "k" indicates mice with cysts.

Transplants with Acellular Material. Transplants of acellular Seitz filters and centrifugates were made from newborn mice of the C57BL line, 2nd, 3rd, 6th, and subsequent passages, to mice of homologous and other lines (CC57BR, CC57W, C3HA). As can be seen from Table 1, which presents the results of 22 experiments in this series, a portion of the mice showed development of tumors, leukemia, and cysts, primarily at a late age. Out of 114 mice that survived the raising period (1 month), tumors, leukemias, and cysts occurred in 30, 6, and 13 mice, respectively. The greatest number of cysts were seen in mice of the CC57W line.

The total percent of animals with tumors and leukemias was not high. In experimental mice of the CC57BR and C57BL lines, it exceeded appreciably the percent of spontaneous tumors occuring in the untreated mice of these low-cancer lines [1, 4]. According to the data of N. N. Medvedev [4] for the years 1957-1960, in mice of the CC57W and CC57BR lines living to an age of seven months, the development of cysts was not noted, and the total percent of neoplasms was equal to 27 and 5.6, respectively, of which leukemias represented 0.9 and 0.

Table 2 shows that in our experimental mice the proportions were different. Out of 18 mice of the CC57BR line surviving to 7 months of age, tumors and leukemias developed in 10, and out of 29 mice of the CC57W line – in 9. The difference in the amount of neoplastic pathology between these lines was smaller in the experiment than in untreated mice [4]. In mice of the CC57BR line, the number of tumoral disorders was considerably greater than in the untreated mice of the same line that were observed by N. N. Medvedev. This is undoubtedly of interest, since our experimental mice originate from the N. N. Medvedev breed. Cysts and leukemias developed in the mice of all the lines used in our experiments (see Table 1).

"Zigzag" passages were made from mice that received the tumor filtrates and, in turn, developed tumors and cysts, to newborn mice of the same and other lines. The "zigzag" consisted of alternating the passage between a cellular suspension of the tumor and an acellular Seitz filter. The material was supplemented with extracts or filtrates (in the case of the acellular transplant) of brain tissue from these same mice. Fig. 2 shows the schema for certain of these experiments. It was possible to carry out only 2 or 3 "zigzags". Upon injection of the filtrates, only in 4 experiments did development of tumors, leukemias, and cysts occur in more than half the animals (experiments No. 479, 486, 421, and 470). The total percent of experimental mice with tumors and leukemias was equal to 24 in line C57BL, 27.3 in line CC57W, and 38 in line CC57BR.

Attempts to Obtain a Cystic Strain. Tumor and cyst material from mice injected with the filtrates of tumor and brain tissue were used for cellular transplants to mice of the homologous lines. The majority of transplantations were successful, and in a portion of the passage mice, along with tumors, there appeared single and multiple cysts (Fig. 3). As can be seen from Table 3, in some passages the cysts appeared in more than half of all the animals. In one mouse from the 4th passage development of serous cysts was observed particularly early (on the 18th day of life). The tumor-cyst strain was taken through 5 passages.

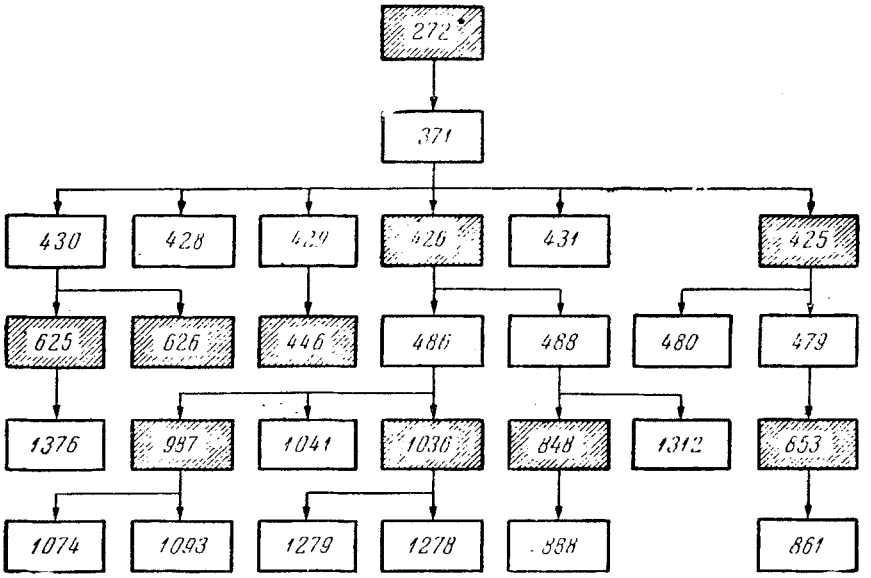

Fig. 2. Schema for passage of the filtrates (light rectangles) and suspensions (dark rectangles) of tumor tissue, passed in "zigzag" fashion.

TABLE 3. Development of Cysts in Mice of Lines CC57BR and C57BL in a Series of Serial Passages of Material from Mouse No. 5922, Which was Injected with a Filtrate of Tumor and Brain Tissue

Passage	Experiment No.	Material for passage	Line of the mice	Number of mice			Latent period (in mo.)	Age of the mice with cysts (in wk)
				taken in the experiment	dying and sacrificed	with cysts		
0	(486)	Seitz filter of tumor and brain tissue	CC57BR	9	9	3	12-15	52-64
1st	(987)	Suspension of tumor from mouse No. 5922 with a kidney cyst from experiment 486	CC57BR	8	8	3	1-1.3	9-10
1st	(987a)	Fluid from kidney cyst of mouse No. 5922	CC57BR	2	0	–	–	–
2nd	(1035)	Suspension of tumor from a mouse of the 1st (987) passage	CC57BR	6	1	0	–	–
2nd	(1083)	Suspension of tumor from a mouse of the 1st (987) passage	CC57BR	12	12	4*	1	8-9
3rd	(1085)	Suspension of tumor and mesentery lymph node from a mouse of the 2nd (1035) passage	CC57BR	10	10	6*	0.8-1	8-10
3rd	(1194)	Suspension of tumor from a mouse of the 2nd (1083) passage	C57BL	13	4	3	1.8	11-12
3rd	(1170)	Suspension of tumor from a mouse of the 2nd (1083) passage	C57BL	5	3	2*	1.8& 2.3	14-17
4th	(1164)	Suspension of cyst wall and tumor from a mouse of the 3rd (1085) passage	CC57BR	5	5	2	1 & 1.5	5-8
4th	(1398)	Suspension of cyst wall + cystic fluid from a mouse of the 3rd (1194) passage	C57BL	11	6	6*	1-1.5	6
4th	(1399)	Suspension of cyst wall with cystic fluid from a mouse of the 3rd (1194) passage	C57BL	6	6	1*	0.6	2.6
4th	(1184)	Suspension of cyst wall and tumor from a mouse of the 3rd (1085) passage	C57BL	4	4	1	1.2	8
4th	(1183)	Suspension of cyst wall and tumor from a mouse of the 3rd (1085) passage	CC57BR	6	6	0	–	–
4th	(1400)	Suspension of cyst wall + cystic fluid from a mouse of the 3rd (1194) passage	C57BL	4	4	3*	1	4.5
5th	(1244)	Suspension of tumor from a mouse of the 4th (1183) passage	CC57BR	9	9	6*	1.4	9-10

*Multiple cysts were observed in the mice

Note. In all cases, a suspension of mouse brain tissue was added to the material.

Cyst material, used to establish the tumor-cyst strain, was taken from a mouse of the C57BL line, which had been injected with a filtrate of brain from a human patient who died of acute leukemia. In this trial, in 2 other experimental animals sacrificed at a late age, cystic changes were observed in the lymph nodes.

As we know, the injection of certain tumorogenic viruses of animals into a heterologous organism leads to the development of cysts in the latter [2, 6, 9]. It was tempting to postulate that the cystic changes in the mice of our

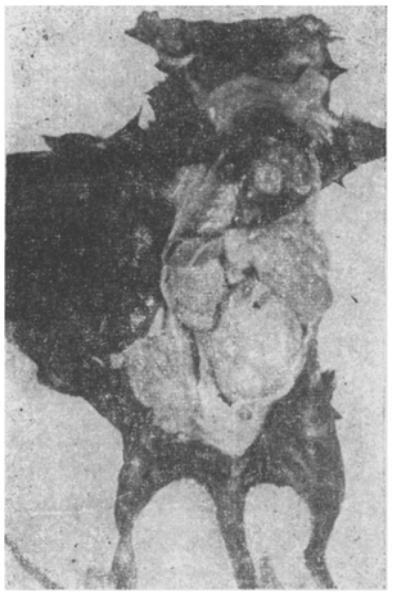

Fig. 3. Intraperitoneal serous cyst in a mouse of the C57BL line.

experiment were connected with injection into the mice of a filterable factor from the tissues of the leukemic patient. In order to determine the soundness of this theory, we analyzed the results of experiments with the injection into mice of inactivated leukemic and normal human material. We observed a group of 20 mice of the C57BL line, injected with a filtrate of brain from a human patient that died of acute leukemia (hemocytoblastosis). Prior to injection into the mice this filtrate was heated at 65° for 70 min. In 2 mice from this group, over 2 years of age, cystic swellings of the axillary lymph nodes were observed at autopsy.

In one of the control experiments, where the mice were injected with a filtrate from normal human tissue, enlargement of the lymph nodes was observed in the animals on palpation. One mouse of this experimental group was sacrificed; the spleen, liver, and enlarged lymph nodes were taken for passage to mice of the same line. In one of the passage mice we noted enlargement of the lymph nodes, marked enlargement and alteration of the tissue in the spleen, and tumor degeneration of the liver. Further passages were performed to mice of both homologous and other lines (CC57BR and C3HA), and to newborn rats. The development of cysts was noted, in individual cases, in mice of all the lines employed. A serous cyst also developed in one of the 3 rats of the Wistar line, at the age of $15\frac{1}{2}$ months (experiment No. 582). The majority of cysts developed in mice of the CC57BR line. These observations on the mice of the control series did not afford us the right to link the development of cysts in the experimental mice with their injection of human leukemic material. It should be pointed out that in the uninoculated F_1 generation of the C57BL mice, which was injected with the filtrate of human leukemic brain, we observed the development of cysts and tumors with the same characteristics as in the inoculated animals: the transplants were successful in the mice of the same and other lines, and in the non-pedigreed mice, and led to the development of multiple tumor nodules and cysts. In addition, in one case we observed the development of a serous cyst in an adult uninoculated mouse of the C57BL line.

In analyzing our experiments we must take into consideration the presence of latent polio injection in the animal rooms of the Division of Immunology and Oncology of the N. F. Gamaleya Institute of Epidemiology and Microbiology of the Akad. Med. Nauk. SSSR, from which our animals were obtained. According to the data of N. N. Medvedev and other authors, 45-80% of the animals of this vivarium possess antibodies against the virus of SE-polio. The selective investigation of sera from our experimental animals (aged 6 months and older) for the presence of antibody against the polio virus yielded positive results.

According to the data of Rowson et al., [7], cysts may be observed in mice inoculated with the polio virus. In the experiments of these authors, there was suspicion of the presence of a mixed infection in the animals injected with the polio virus. The possibility is not excluded that in our experiments the development of cysts was related, in some measure, to a polio infection. However, numerous attempts at isolating the polio virus, using the method of tissue culture (experiments of D. M. Levina), have not yet afforded positive results. Possibly, this is a result of a mixed infection of the mice.

As in the case of Rudali and co-workers [8], we obtained negative results from injection of the mice with cystic fluid, but with transplantation of a suspension from the walls of a cyst, we observed the development of cysts in a portion of the animals. In individual passages they were rather numerous, and they appeared in the mice at an early age.

Further investigations will have to elucidate the conditions and reasons leading to the development of the cysts.

SUMMARY

Autopsy findings revealed cystic changes in the lymph glands observed in old mice of the C57BL line formerly subjected to the test of leukemogenic activity of the brain filtrate of a man who had died of acute leukemia.

The cystic fluid inoculated to newborn mice of the same line gave negative results. In a series of consecutive passages of cellular suspensions there was a development of tumors and cysts in young mice of homologous C57BL line as well as in mice of the CC57BR and CC57W lines. In transplantation of a cell-free material to newborn mice of four different lines (C57BL, CC57BR, CC57W, and C3HA) the cysts usually developed only at a more mature age, whereas in subsequent serial passages of suspensions of cystic walls and tumor from these mice – at the age from 4.5 mo to 18 days.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
