CYTOGENETIC STUDY OF MOUSE LEUKEMIA PRODUCED BY THE MYELOID CHLORLEUKEMIA VIRUS

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The discovery of the characteristic, so-called Philadelphia chromosome in human chronic myeloid leukemia, deserves particular attention in the study of experimental leukemia. According to present data, alteration of karyotype in the viral leukemias of mice and rats is more the exception than the rule [4, 5, 7, 8-11]. However, it must be observed that the number of studies of this question is small; mainly the leukemias of Friend and Gross have been studied.

The present paper was designed to study the changes in karyotype in leukemia induced in mice by the myeloid chlorleukemia virus. The presence of subacute myeloid leukemia among diseases caused by this virus suggests a certain analogy between this process and human chronic myeloid leukemia.

METHODS

The virus of myeloid chlorleukemia, similar to the Graff virus, was isolated in 1958 by E. L. Prigozhina from admixture with Ehrlich's carcinoma [2]. It is passed in mice by infecting them on the first day of life with a cell-free extract from the liver, spleen and lymph nodes of leukemic mice. A total of nine primary leukemias were studied (seven hemocytoblastic and two reticulomyeloid)* inoculated with virus in C57 BL mice and non-pedigreed mice and three transplantable strains obtained in our laboratory by transplanting in vivo leukemias produced by such virus: LZ (48-50th generation), LBL (58-60th generation) and LII (4-5th generation). We took a suspension of leukemic cells from the spleen, lymph nodes, tumor nodes or ascites. Strain LZ was also studied by using a culture of leukemic cells. We have earlier described the cultivation method [3]. The mice were injected intraperitoneally with colchicine in amounts from 20-100 micrograms at two to three hours before sacrifice. In four instances 10 micrograms of colchicine were introduced 18-19 h before death. The use of colchicine during the long period permitted us to obtain a larger quantity of metaphase plates. This method of colchicine treatment we had also used on cell suspensions in vitro [1]. In cultures, colchicine was added in a dose of 0.1 microgram per one ml of medium at 18-19 h. After treatment of the cells with a hypotonic solution (1%) of sodium citrate the material was fixed, stained with acetocarmine and squashed preparations were made according to the accepted method [6, 8].

RESULTS

Induced Leukemia

Study of cells from the spleen and lymph nodes of mice infected with leukemia induced by the myeloid chlorleukemia virus showed that the leukemic cells in all nine instances retained the diploid chromosome number (Table 1). Dispersion around cells of the modal class appeared insignificant in all nine animals. A small number of cells were hypodiploid—closely following the modal class of 39 chromosomes. In all cases single cells were found with 37 and 38 chromosomes. The appearance of hypodiploid cells in some cases, probably, depends on the loss of one or several chromosomes during preparation of the specimen. In a few cases hyperdiploid cells were

*Hematologic and histologic studies of leukemias carried out by E. L. Prigozhina.

TABLE 1. Distribution of Cells According to Chromosome Number in Mice with Leukemia Induced by the Myeloid Chlorleukemia Virus

No. of mice	Chromosome number								Tetraploid	Total cells
	37	38	39	40	41	42	43	46		
274		2	4	14	2				1	23
280			5	14	1		1		1	22
282										
283		1	4	18		1				24
289		1	1	14	3			1	1	21
314	1		1	22	3				2	29
b/ No.	1		7	14					4	26
340 MC*	1	1	7	18	2					29 } 50
340		1	1	18	1					21 }
392	1		3	13	3					20

*Cells with metacentric chromosome.

TABLE 2. Distribution of Cells by Chromosome Number in Mice with Transplanted Leukemia

Strain	No. of animals	No. of cells	Chromosome number							Tetraploid	Hypertetraploid
			38	39	40	41	42	44	45		
LZ in vivo	4	45	3	9	27	2	1			3	
LZ in vivo	4	114		17	65	3				25	4
LBL	4	51	3	7	38	3					
LII Tumor form	2	51	3	10	28	1		1	1	9	
LII Ascitic form	2	54		4	34	1	1	1		13	

encountered with 41 chromosomes and sometimes—single superdiploid cells with 42, 43 and 46 chromosomes. Polyploidy was 4%. Single tetraploid or quasi-tetraploid cells were encountered in almost all instances. In one mouse, in 29 out of 50 leukemic spleen cells studied a large metacentric chromosome was present. The majority of cells containing metacentrics had a diploid chromosome number.

Transplantable Leukemias

The majority of leukemic spleen cells in mice with strict hemocytoplastic LZ leukemia in vivo and in vitro possess the normal diploid complement of chromosomes. The number of cells in the modal class revealed by both investigative methods (directly and after cultivation in vitro), was roughly 60% and cells with 39 chromosomes constituted 15-20%. After cultivation of the leukemic cells in vitro a considerable increase in polyploid cells was observed (Table 2).

Upon study of 51 ascitic cells from four mice with transplanted, poorly-differentiated LBL leukemia no divergence from the normal mouse karyotype was detected (see Table 2).

The same situation occurred with counting of chromosomes in ascitic and in tumor forms of the transplantable myeloid leukemia LII. About a hundred cells in all were studied in these two forms. In this instance diploid cells predominated, but in both forms of leukemia in vivo a significant number of polyploid cells (17 and 24%) were encountered.

Thus, as a result of karyotype studies in induced and transplantable leukemia produced by the virus of myeloid chlorleukemia, it has been established that some of the cells retain a normal karyotype. Our data concur with the results obtained from studies of Graff virus induced myeloid chlorleukemia [5], Gross and Friend leukemias and

viral leukemia. In rat leukemia induced by the Mazurenko virus, diploid chromosome complements were found in two out of three cases studied [4]. Some investigators [7] found in single cases of Gross and Friend leukemia predominance of cells with 41 chromosomes, which we did not succeed in observing. However, isolated cells containing 41 chromosomes were encountered in almost all animals with induced viral leukemias. One out of nine cases had a marker metacentric chromosome. As our studies have shown, after transplantation of viral leukemia the normal karyotype may be retained throughout many generations.

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