SPECIFIC SURFACE LEUKEMIC ANTIGEN AND MALIGNANT TRANSFORMATION OF CELLS IN CC57BR MICE INFECTED WITH MAZURENKO'S VIRUS

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Specific surface antigen detected by immunofluorescence in CC57BR mice infected with Mazurenko's virus was found before a cytohistological diagnosis of leukemia could be made and before the cells had acquired transplantability. This antigen was determined in the infected mice at about the same times in the spleen, thymus, lymph glands, and bone marrow.

The cells of mice with virus-induced leukemia contain a specific surface antigen which is detectable by the cytotoxic test and by immunofluorescence [1, 2, 8].

The object of the present investigation was to study the time of appearance of specific antigen in the cells of different organs and tissues of mice after infection of the animals with one of the leukemic viruses and to compare these results with the apparance of malignant transformation of the cells as detected by morphological investigation and determination of transplantability of the cells.

EXPERIMENTAL METHOD

To induce leukemia, CC57BR mice aged 2 weeks were injected intraperitoneally with concentrated Mazurenko's virus [4, 5] in a dose of 0.2 ml (1 ml virus-containing fluid is equivalent to 1.5 mg leukemic tissue). The virus was purified and concentrated by differential centrifugation as described by Moloney [9]. Antileukemic sera were obtained by injecting adult male CC57BR mice twice with a 20% virus-containing supernatant and following this by immunizing the animals repeatedly (5-7 times) with increasing concentrations of a homogenate of leukemic tissue. Before injection, the leukemic tissue was irradiated with x rays in a dose of 9600-12,000 R. Specific surface antigen in the cells of organs and tissues of mice infected with virus were detected by the indirect Coons' method as modified by Möller [10]. The reaction was assessed by means of the fluorescence index [7]. A fluorescence index of 0.2 or above was regarded as positive. Cells of the thymus, lymph glands, spleen, and bone marrow were studied. A mixture of cells of the corresponding organs taken from 3 animals was tested. Cells of organs of the same mice incubated with serum from healthy animals of the same line (to calculate the fluoresence index) and cells from healthy mice incubated with antileukemic serum acted as controls. The specificity of the fluorescence thus obtained was confirmed by fluorescence suppression experiments, in which specific antisera exhausted with leukemic cells were used. These mouse organs used in the immunofluoresence test were subsequently examined histologically and cytologically. Starting from the 5th day after infection of the animals with the virus, a suspension of spleen cells taken from 3 mice, as prepared for the immunofluoresence test, was injected intraperitoneally into three CC57BR mice aged 1-1.5 months, in doses of 4-6 million cells for each

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TABLE 1. Results of Study of Specific Surface Leukemic Antigen in Cells from Organs and Tissues of Mice Infected with Mazurenko's Virus and of Malignant Transformation of These Cells

Day after infection	Fluorescence index				Results of investigation		Transplantation of cells*
	of spleen	of thymus	of bone marrow	of lymph glands	histological	cytological	
2	0,16	0	0,1	0	Normal	Normal	—
4	0,13	0	0,08	0	"	Stimulation of reticulo-endothelial tissue	—
5	0,05	0,1	—	0,08	"	The same	0/2
7	0,24	0	0,39	0,09	"	" "	0/3
8	0,06	0,04	0,11	0,07	"	" "	0/2
10	0,21	0,02	0,1	0,06	"	" "	0/3
12	0,30	0,03	0	0,16	"	" "	0/3
13	0,22	0,18	0,17	0,01	"	" "	0/3
14	0,41	0,16	0,21	0,17	Number of undifferentiated cells in spleen and lymph glands increased	" "	0/3
15	0,24	0,25	0,20	0,30		" "	0/3
16	0,32	0,33	0,21	0,40		" "	0/3
18	0,32	0,30	0,20	—		" "	1/3
22	0,41	0,31	0,25	0,43	Leukemia	Leukemia	2/3
28	0,59	0,71	0,44	0,66	"	"	3/3

*Numerator represents number of mice developing leukemia; denominator number of mice in experiment.

animal. Observations were made on the animals for 3.5 months. The diagnosis of leukemia was made on the basis of the typical picture of the disease.

EXPERIMENTAL RESULTS

Altogether 109 CC57BR mice, infected with concentrated virus at the age of between 10 and 14 days, were used in the experiments. The dynamics of detection of specific antigen in the organs of these animals and results of morphological studies and of transplantation experiments are given in Table 1. Examination with the luminescence microscope showed that spleen and bone marrow cells of mice on the 7th day after infection with virus contained specific surface antigen. Later, from the 10th to the 13th day, the immunofluorescence reaction was positive, but only with spleen cells. However, starting from the 12th day, the fluorescence index became positive for tests of the lymph glands, thymus, and bone marrow. Specific surface antigen was detected clearly in all the organs tested after the 15th day, and on subsequent days the fluorescence index gradually increased (Fig. 1).

It will be seen in Fig. 2, which shows the curves of weight of the spleen reflecting the development of leukemia and the results of specific fluorescence in the cells of this organ, that a direct relationship exists between the detection of specific surface antigen and the weight of the spleen in the infected animals. The fluorescence index was always positive when the weight of the spleen exceeded that of the control mice by 1.5 times. The very small increase in weight of the spleen in the group of control mice is explained by growth of the animals (the mice used in the experiment were aged 10–14 days).

An increased number of undifferentiated cells was detected histologically in the spleen and lymph glands only on the 14th day. As a rule these cells were located in the wide germinal centers of the follicles, but they also occurred as separate small foci in the pulp of the spleen. On the 22nd day of investigation and later, the structure of the spleen and lymph glands became less distinct because of diffuse or focal hyperplasia of cells of the reticular type and hemocytoblasts. A similar picture was observed in the thymus. The bone marrow at all stages of investigation preserved its typical polymorphic cell composition. Not until the 22nd day after infection with virus could small, solitary foci of leukemic cells be found in the bone marrow.

Cytological investigation on the 4th day after infection of the animals with virus showed focal proliferation of reticular cells around the follicles and in the red pulp of the spleen, and also in the bone marrow. Degenerative changes in the parenchymatous cells, with micronecroses and hemorrhages

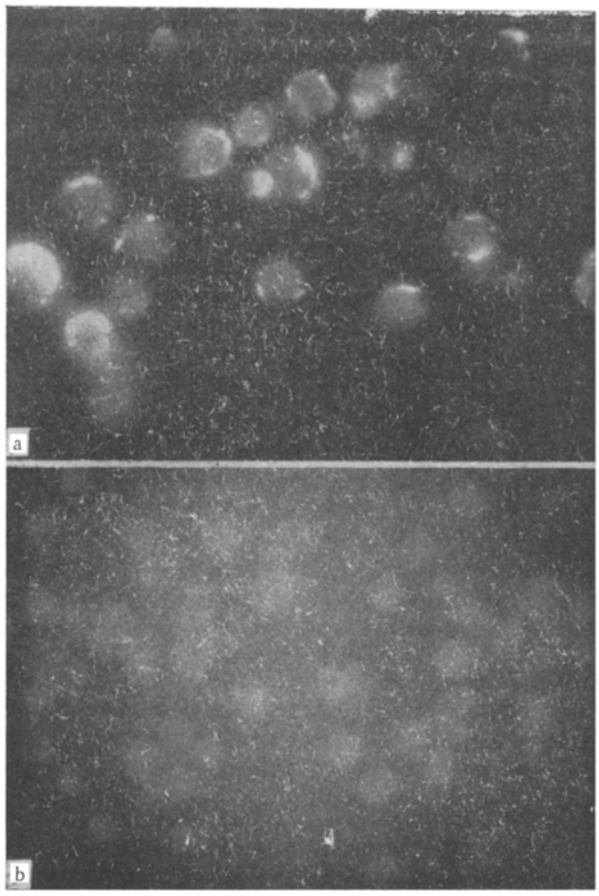

Fig. 1. Immunofluorescence of spleen cells of CC57BR mice infected with Mazurenko's leukemia: a) incubation with leukemic serum; b) incubation with serum of healthy mice of the same line.

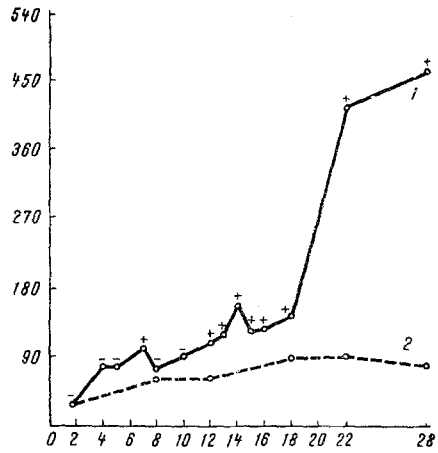

Fig. 2. Correlation between increase in weight of spleen and immunofluorescence of cells of this organ in CC57BR mice infected with Mazurenko's virus. Abscissa, days after infection; ordinate, weight of spleen (in mg); 1) experiment; 2) control; + positive fluorescence index; - negative fluorescence index.

seen in the liver and kidneys. A characteristic change was the appearance of numerous histiocytes, fibrocytes, and reticular cells in the spleen, liver, and kidneys, and the appearance of numerous monocyte-like cells in the circulating blood. This active state of the reticuloendothelial system persisted until the picture of leukemia was fully developed, which occurred on the 22nd day after injection of the virus.

Cell transplantations gave positive results on and after the 18th day after infection of the animals with virus: the recipient mice developed leukemia after 4-5 weeks.

It is clear from the description given above that the appearance of specific leukemic antigen in animals infected with virus preceded malignant transformation of normal cells into leukemic cells, as detected morphologically and by the transplantability of the cells.

The nature of the specific surface antigen detectable by immunofluorescence tests in leukemias is not yet clear. By analogy with the results obtained in model experiments with the DNA-containing viruses, the

appearance of this antigen may perhaps be associated with changes in the cell surface during virus-induced leukemogenesis. From this standpoint, the results of these investigations agree with those obtained by other workers who used the immunofluorescence method in vitro to detect the appearance of specific surface antigen before morphological transformation of cells in a tissue culture infected with OB-40 and polyoma viruses [3, 6]. In these cited experiments, specific fluorescence was associated with the formation of a newly synthesized cell surface antigen only, and was not due to the virus itself or to antigen of the virus particles. In the present experiments, it was impossible to differentiate between cell antigen and antigen of the virus particles, because in mouse leukemias induced by RNA-containing viruses, the virus particle antigens are located on the surface of the cells of tissues and organs of both the sick animals and animals infected with virus. Specific fluorescence on the surface of cells studied in the present experiments was evidently both to the appearance of a new cell antigen and also to antigen of the virus particles themselves.

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