DETECTION OF GROUP-SPECIFIC ANTIGEN OF MOUSE VIRUSES IN NORMAL MICE OF HIGH AND LOW LEUKEMIC LINES

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A method of obtaining a monospecific rabbit precipitating serum against group-specific antigens of mouse leukemia viruses is described. The presence of group-specific antigen in mice of high and low leukemic inbred lines is demonstrated. The results indicate an extremely widespread carrier state of leukemia viruses in mice.

* * *

Group-specific mouse leukemia antigen (GSA), discovered in 1966 [4], is an internal component of the virus identical for all mouse leukemia viruses. The antigen is present in leukemia cells and in the plasma of leukemic mice not only as a component of the virus, but also in a free state [4], and it can evidently play a role in immunity to leukemia.

Determination of GSA by precipitation in agar is done with the aid of precipitating rabbit antisera obtained by immunizing rats of line W/Fu with material obtained from syngenic animals with leukemia induced with Gross virus [4]; mice are evidently tolerant to GSA [4]. Recently, a method of obtaining rabbit antisera containing antibodies against GSA and suitable for determining GSA in the complement fixation reaction [9], by immunizing rabbits with purified Rauscher's virus, has been described. Serologic detection of GSA is a simple test for the presence of mouse leukemia viruses in the tissues to be examined. This antigen has been found by means of rat and rabbit antisera against GSA in lines of mice carrying Gross virus and also in stable strains of mouse fibroblast cultures [9].

This paper describes a simple and economical method of obtaining a precipitating rabbit antiserum against GSA, which was used to investigate the presence of GSA in the spleen of normal mice of both high and low leukemic lines.

EXPERIMENTAL METHOD

Rauscher virus was obtained by ultracentrifugation in a sucrose density gradient from plasma of BALB/c mice with developed leukemia [3, 6]. Plasma, in a volume of 50 ml, was clarified by centrifugation at 10,000 g for 20 min, and then recentrifuged for 90 min at 30,000 g (Spinco L centrifuge, rotor Sw= 25) The residue was resuspended in 1 ml Ringer's solution and ultracentrifuged in a stepwise sucrose density gradient (densities 1.12, 1.14, 1.16, 1.18, 1.22) for 90 min at 105,000 g. Two zones containing virus corresponded to densities of 1.14 and 1.16. These zones were removed, diluted with Ringer's solution , and again sedimented by ultracentrifugation at 105,000 g. Each residue was resuspended in 0.25 ml Ringer's solution and kept at -20°.

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TABLE 1. GSA of Mouse Leukemia Viruses in Spleen and Tumors of Mice of Various Lines and of Rats

Line of mice	Spleen						Tumors			
	newborn mice		mice aged 10-14 days		adult mice (1.5 months)		leukemias		sarcomas	
	presence of GSA	no. of extracts	presence of GSA	no. of extracts	presence of GSA	no. of extracts	presence of GSA	no. of extracts	presence of GSA	no. of extracts
AKR					+++	2	Gross ++++	2		
BALB/c	+	3	+	2	++	3 1	Rauscher ++++	2		
C57BL/6	+	1	+	2	+	4				
C57BL/He	++	1	++	1	+	3	VL = 15		MCh = 11	
C57BL/10Sn	±	2	±	1	—	1	+ KL - 2 —	1 1	+++	1
B10. D2	—	1	—	1	—	2			MCh = 26 +++	1
Af	+	3	+	1	+	3			Rous ±	2 1
C3H/Sn	+	2	+	4	+	4			Rous ++	1
CC57Br	+	1			+	1	Mazurenko +++	1	Polyoma —	1
							Bergol'ts +++	1	MCh = 16 +	1
									MCh = 17 +++	1
									MCh = 18 +++	1
									MCh = 22 +++	1
CC57W	+	3	+	2	+	2				
Noninbred rats	—	1			—	1			Rous —	2

Legend: −absence of GSA; +, ++, +++ presence of GSA in different amounts.

Two rabbits were immunized by injection into the popliteal lymph glands. Virus from both zones was mixed in equal volumes (0.15 ml of each), 0.15 ml of the preparation was added to 0.6 ml of Freund's complete adjuvant (Difco) and the material was injected into rabbits in doses of 0.125 ml into the exposed popliteal lymph glands of both hind limbs. One month later the remaining 0.15 ml of the preparation was diluted with 0.5 ml Ringer's solution and each rabbit was injected with 0.1 ml intravenously and 0.2 ml subcutaneously into the hind limb in the region of the lymph gland. Blood was taken on the 10th, 12th, 14th, and 18th day after reimmunization.

A test system for GSA, obtained from Dr. L. Old (Sloan-Kettering Institute for Cancer Research in New York), was used in the investigation. The test system consisted of rat antileukemic serum (Gross leukemia) and Gross virus destroyed with ether [4].

Mice of lines AKR and C57BL/6 were obtained from the Nursery of the Academy of Medical Sciences of the USSR, and mice of lines C57Bl/He, C57Bl/10Sn, C3H/Sn, BALB/c, and B10D2 from the Nursery of

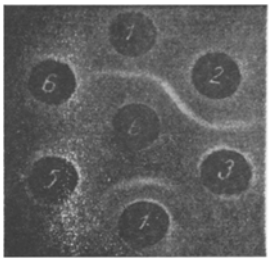

Fig. 1.

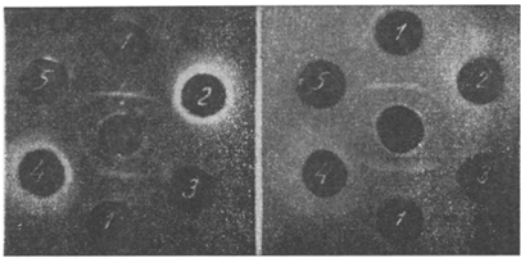

Fig. 2.

Fig. 1. Comparison of rabbit anti-GSA antiserum in precipitation reaction in agar with rat test system for GSA. 1) Rat antiserum against GSA; 2) rabbit antiserum against GSA; 3, 4) splenic extract from mouse with Rauscher leukemia; 5) rabbit antiserum not containing antibodies against GSA; 6) Gross virus 1/200, destroyed with ether.

Fig. 2. Detection of GSA by precipitation reaction in agar in spleen of high-leukemic (AKR), low-leukemic (BALB/c), and leukemia-resistant (C57BL/6) lines of mice. Center: rabbit antiserum against GSA; 1) splenic extract of mice with Rauscher leukemia; 3, 5) physiological saline. Left: 2) splenic extract of 14-day BALB/c mice; 4) splenic extract of adult AKR mice. Right: 2) concentrated splenic extract of adult C57BL/6 mice; 4) unconcentrated splenic extract of adult C57BL/6 mice.

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Rauscher leukemia virus was transmitted by passage through BALB/c mice in the Laboratory of Immunochemistry of Cancer, and mice with Friend, Moloney, Stepina-Zil'ber, and Mazurenko leukemias were obtained from the Laboratory of Etiology of Leukemias, Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR from V. N. Stepina, spleens of mice with carcinogenic leukemia KL-2 and VL-15 from Z. A. Postinkova, polyoma tumors from I. S. Irlin, methylcholanthrene sarcomas (MCh-sarcomas) from Yu. M. Lezhneva and B. D. Brondz, Rous sarcomas of mice from I. N. Kryukova and I. B. Obukh, and sarcomas induced by DMBA from the Laboratory of Virology, Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR from G. Ya. Svet-Moldavskii, to whom the writers are sincerely grateful. All the investigated tumors were taken between the 8th and 44th generations.

The test extracts were prepared by triturating the tissues in a mortar in Ringer's solution in the ratio 1:2. The homogenate was irradiated with ultrasound (6 times, 20 sec each time, with intervals of 1 min, in ice, in the MSE-100 apparatus, 18-20 kHz). The cells were completely destroyed; large fragments were removed by centrifugation at 9000 g for 20 min (TsLN-2 centrifuge). The supernatant was used for estimation of GSA. If the antigen content was low, the extract was concentrated 2-2.5 times with Sephadex G-50 (medium), in the ratio of 1:6 to the volume of extract. To obtain extract from the mouse spleens, not fewer than 10 newborn mice of the same age or 3-4 adult mice were used (corresponding extract). Precipitation in agar was carried out in a semimicro-modification by the method of Gusev and Tsvetkov [1].

EXPERIMENTAL RESULTS

Antibodies against GSA were found in the antiserum of one of the two rabbits immunized with Rauscher virus. Their titer was highest on the 10th and 12th days after reimmunization, but they persisted until the 18th day.

The antiserum was not monospecific. It contained antibodies against mouse serum γ- and α_2-globulins. One further antigen was found in the antiserum, and was present in high concentration in the spleens and plasma of mice with Rauscher and Friend leukemias, but not in extracts from organs from animals with other leukemias. It was also detected in traces in the sera of normal mice. To neutralize antibodies against

this antigen and against the components of mouse serum, lyophilized sera of noninbred mice dissolved in one-fifth of the original volume were used.

Rabbit antiserum neutralized with mouse sera (ratio 1:1 or 1:1.5) usually contained precipitating antibodies against GSA only and was used as a monospecific anti-GSA serum in the precipitation reaction in agar. Splenic extract of mice with Rauscher leukemia in the equivalent dilution (1:3) was used as antigen. Antibodies of the rabbit antiserum detected the same antigen as the rat test system for GSA obtained from Dr. L. Old (Fig. 1).

The results of determinations of GSA in the spleens of mice belonging to different lines of different ages are given in Table 1. They show that GSA was found in mice of nearly all the lines tested, whether with high susceptibility (AKR) or low susceptibility to leukemia (BALB/c, Af, CC57BR, CC57W), or even resistant to leukemia (C57BL/6 and C57BL/He). GSA was certainly not detected only in one coisogenic pair: C57BL/10Sn and B10D2. However, it should be stressed that GSA was found in high concentration in MCh sarcomas induced and reinoculated in these lines, indisputable evidence of the presence of leukemia virus in the mice of these lines also.

In these experiments, in contrast to the results obtained by Nowinski and co-workers [7], GSA was found in low-leukemic and resistant lines of mice. The only difference was in its concentration (Fig. 2a, b). Possibly the very strong test system used by Nowinski and co-workers did not detect low concentration of GSA which were easily demonstrated by the test system used in the present investigation. It must be emphasized that GSA was also detected by the writers in splenic extracts of C57BL/6 mice by means of the test system obtained from Dr. L. Old, taken in the corresponding dilution. Possibly, however, the presence of GSA in mice of the lines studied was due to the higher infectivity with leukemia viruses of the mice now studied than of the mice studied by Nowinski.

The presence of GSA in mice of different lines indicates, in the writers' opinion, the extremely widespread carrier state of leukemia virus among mice with low susceptibility to tumors, in agreement with data for the isolation of virus from normal mice [5] and for detection of GSA in mouse tissue cultures [9]. The possibility is not, however, ruled out that GSA synthesis in mice of low-leukemia lines may be due to the activity of part of the virus genome or that this antigen is present in mice also in cells not infected by the virus. Such a situation is apparently found in hens containing group-specific antigen or viruses of the sarcomo-leukemia group [2, 8].

It cannot be asserted on the basis of these results that GSA is present in every individual of the investigated lines, because a mixture of spleens was used to prepare the extract. The study of GSA by the immunofluorescence method in sections would shed light on this problem.

The wide distribution of GSA in mice at all periods of their life would seem to explain the tolerance of mice to this antigen, in contrast to rats, which contain neither leukemia virus nor GSA under normal conditions, and which form antibodies against GSA extremely easily.

The results obtained afford considerable promise for the study of indirect leukemogenesis, of the pathways of spread of leukemia viruses, and the fate of leukemia viruses in the host organism.

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