TYPE-SPECIFIC ANTIGEN OF GROSS LEUKEMIA VIRUS IN TUMORS AND NORMAL TISSUES AND ITS IDENTIFICATION BY IMMUNODIFFUSION METHODS

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A monospecific serum was obtained against type-specific antigen of Gross leukemia virus (AGLV). Tumors of different etiology were investigated with the aid of a test system for AGLV. AGLV was found in a spontaneous kidney tumor (lymphosarcoma) of CC57BR mice and in tumors induced by chemical carcinogens. The results suggest activation of the MuLV virus genome in strains of mice with a low predisposition to leukemia.

KEY WORDS: leukemia; Gross leukemia virus; type-specific antigen; immunodiffusion; immunofluorescence.

The genome of mouse leukemia viruses (MuLV) is present in normal cells of different strains of mice, where it is under host control, and in mice with a low predisposition to cancer it can exist for a long time in an inactive form [1, 10, 14, 15]. The genome of "endogenous" MuLV is usually activated in tumors of varied etiology [4, 12, 15, 16, 18].

The object of this investigation was to obtain a monospecific precipitating serum for the type-specific antigen (TSA) of Gross leukemia virus. Such a serum could be used to detect endogenous leukemia viruses, for endogenous viruses of mouse leukemias, except the S-tropic viruses [11], are antigenically identical with or related to Gross virus [13, 14].

EXPERIMENTAL METHOD

Gross and Rauscher viruses were obtained from Electro-Nucleonic Laboraties Inc. The viruses from the culture medium were twice purified in a sucrose density gradient and the concentration of virus particles was 10^{11}-10^{12}.

Lymphosarcoma virus of CC57BR mice was isolated from the culture medium by ultracentrifugation [8].

To obtain antiserum, rabbits were immunized with intact Gross virus into the lymph glands [3]. At the first injection 0.4 ml of the virus preparation was injected together with an equal volume of Freund's complete adjuvant. The same dose of virus without adjuvant was used for the first revaccination, and during subsequent revaccination 0.2 ml of the virus preparation was given. Blood was taken on the seventh, ninth, and 11th days. After the first revaccination a serum was obtained that could be used for work by the immunoautoradiographic method (IR method) [1], and after the third and fourth reimmunizations the serum was suitable for the precipitation method in agar (PR method).

Extracts of tumor and normal tissues, prepared as described previously [6], treated with two volumes of ether, and then concentrated threefold, were used as antigens. The ether removed lipids from the extract, which would otherwise form opaque haloes in the agar and interfere with the reading of the test.

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TABLE 1. TSA of Gross Virus in Viruses, Tumors, and Normal Tissues of Mice

Material for testing	Disease	Strain of mice	TSA of Gross virus (AGLV)				Antigen gs-1
			extracts		plasma		extracts
			precipitation	IR-method	IR-method	IR-method	precipitation
Tumors:							
induced:							
by viruses							
Gross	Leukemia	AKR	+	+			+
Moloney	"	BALB/c	—	—	—	—	+
Friend	"	"	—	—	—	—	+
Moloney	"	"	—	—	—	—	+
S-3	"	C57BL	—	—	—	—	+
by chemical carcinogens							
7,12-DMBA	Myeloid leukemia	CC57W	±	+			+
methylcholanthrene	Sarcoma MCh-11	C57BL/10Sn	±	+	—	+	+
"	" MCh-26	B10D2	±	+	—	+	+
"	" MCh-36	Af	±	+			+
spontaneous kidney tumor	Lymphosarcoma	CC57BR	+	+	—	+	+
Normal organs:							
spleen		AKR	—	+	—	+	
		C57BL/10Sn, BALB/c, CC57BR, 129	—	—	—	—	
thymus		AKR, C57BL/10Sn BALB/c, CC57BR, 129	—	—			
Viruses:			Sucrose gradients				Sucrose gradients
Gross			—	+			+
Rauscher			—	—			+
endogenous virus of CC57BR lymphosarcoma			—	+			+

The serum was neutralized with splenic extract from mice with Rauscher's leukemia to remove antibodies against gs-1 antigen and with normal mouse serum. The neutralizing dose of antigen was chosen by the PR method. The serum was regarded as neutralized if an excess of neutralizing antigen was determined in it with the corresponding test system. For the PR reaction with virus preparations the antiserum was exhausted additionally with bovine serum. The IR reaction was carried out as described previously [1].

The following tumors were used: 1) lymphoma of AKR mice – this is a spontaneous tumor of the thymus and tumors were used at the eighth passage in strain AKR; 2) lymphosarcoma of CC57BR mice – a spontaneous tumor of the kidney transplanted in CC57BR mice for several years [5]; 3) sarcomas induced by 2,3-methylcholanthrene: MCh-11 in C57BL/10Sn mice, MCh-26 in B10D2 mice – tumors maintained by prolonged passage, and MCh-36 – tumors at the second and 11th passages in Af mice [2]; 4) myeloid leukemia – this was obtained by L. A. Zil'ber and Z. A. Postnikova by injection of 7, 12-dimethylbenzanthracene (DMBA) into newborn mice of the low-cancer strain CC57W and a lyophilized extract of the myeloid leukemia was used at the eighth cell-free passage [4]; 5) leukemia S-3 was induced in C57BL mice [9].

Spleens of mice aged 6-8 weeks and thymus glands from mice aged 3-4 weeks from strains BALB/c, C57BL/10Sn, CC57BR, and 129, with low predisposition to leukemia, and strain AKR, with a high incidence of leukemia, were used as normal organs.

EXPERIMENTAL RESULTS

The serum of a rabbit immunized with intact Gross virus (GLV) formed three bands in the precipitation test with lymphoma extract from AKR mice. Despite immunization with the intact virus, antibodies against gs-1 antigen and also traces of antibodies against one of the antigens of normal mouse serum were found in the serum. Both antigens could be neutralized by the addition of one volume of splenic extract from mice with Rauscher leukemia to eight volumes of antiserum. After neutralization a monospecific serum was obtained which gave a reaction of identity with the lymphoma of AKR mice and with the GLV preparation. This serum, in conjunction with extract of AKR lymphoma was used as the test system for the antigen under investigation (AGLV).

Extracts of different leukemias and normal tissues and also preparations of viruses were tested with

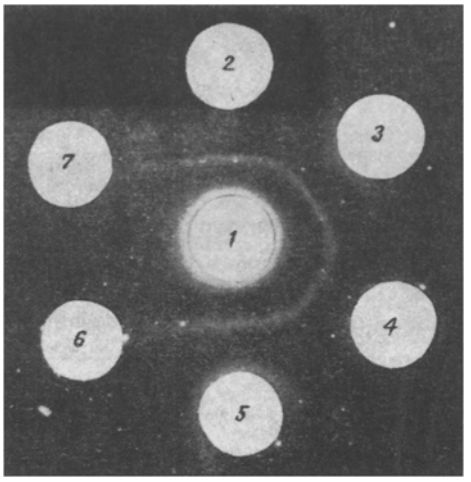

Fig. 1. Detection of type-specific antigen (TSA) of gross virus in different tumors: 1) rabbit antiserum against Gross virus; 2) lymphosarcoma of CC57BR mice (test system for TSA or Gross virus); 3) AKR lymphoma; 4) sarcoma MCh-36; 5) DMBA myeloid leukemia; 6) Rauscher leukemia; 7) Friend leukemia.

the test system by the PR method in agar. Negative cases were retested for AGLV by the IR method with the test system diluted 8 or 16 times. AGLV was found in AKR lymphoma, in the GLV preparation, and in spontaneous lymphosarcoma of CC57BR mice. Later (Fig. 1) the antigen was found in about the same quantity as in AKR lymphoma, and in the subsequent experiments extract of CC57BR lymphosarcoma was used for the test system. AGLV was found in the transplantable MCh sarcomas and the DMBA myeloid leukemia, and in small quantities also in normal serum and plasma of preleukemic AKR mice.

Discovery of the antigen in the GLV preparations and in AKR lymphomas and its absence in the spleens of low-leukemic strains of mice (Table 1) indicate that the antigen was associated with the virus. The absence of antigen in leukemias of the FMR (Friend–Moloney–Rauscher) group is evidence of its type-specificity.

The discovery of this antigen in spontaneous CC57BR lymphosarcoma (Fig. 1) is interesting. The cells of this tumor produce virus in culture [8]. The D-1.18 zone of the sucrose gradient of the culture medium gave a reaction of identity with the test system for AGLV described above. This suggests that the virus of CC57BR lymphosarcoma is an activated endogenous leukemia virus. The discovery of AGLV in tumors induced by chemical carcinogens (Fig. 1) is no less interesting. In the case of DMBA myeloid leukemia, a cell-free extract was found to have leukemogenic activity in newborn CC57W mice [4]. The discovery of AGLV in myeloid leukemia also points to activation of an endogenous virus in this tumor.

The writer's observations show that MCh sarcomas either contain infectious virus, or they synthesize virus proteins, a protein with the type-specific determinant of Gross virus, and protein p-30 with the gs-1 determinant (Table 1). The presence gs-1 antigen in transplantable MCh sarcomas was shown by the writer previously by an immunofluorescence method in sections [7]. Activation of gs-1 antigen in primarily induced MCh sarcomas was reported by Whitmire et al. in 1971 [18]. The present experiments in which AGLV was found in a spontaneous tumor and in tumors induced by carcinogens suggest that the virus genome is activated in mice of low-leukemic strains and this is accompanied by the synthesis of a protein with the type-specific determinant of gross virus.

At the present time the writer is studying the localization of this antigen on the membrane and virus particle by an electron microscopic method.

AGLV is possibly identical with the chief glycoprotein of the Gross virus membrane (gp 69/71), described by Stand and August in 1974 [17]. It will be interesting to compare the test system described by the present writer with the sera of C57BL/6 mice immunized with Gross lymphomas and of NZB mice, using the immunodiffusion method in the modification described during the production of the test system for TSA of Rauscher virus [6].

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