STUDY OF TISSUE ANTIGENS OF CC57BR MICE WITH LEUKEMIA (RETICULOSARCOMATOSIS) IN THE GEL DIFFUSION REACTION

B. É. Chechik and V. M. Bergol'ts

UDC 616.155.392-092.9-008.9-097.2-078.734

No specific leukemic antigens could be detected by the gel diffusion reaction in the tissues of line CC57BR mice with a transplanted reticulosarcomatosis induced primarily by a surviving tissue culture infected with human leukemic material. An antigen was detected in all organs of mice with reticulosarcomatosis, which under the experimental conditions used was detected most clearly in the spleens of all healthy mice and less regularly in the spleens of mice with other virus-induced and transplanted leukemias.

* * *

We have obtained a strain of mouse leukemia (reticulosarcomatosis) induced primarily by injection of the culture fluid of a surviving tissue culture infected with human leukemic material [1]. The leukemia is inoculated both by cellular and cell-free material. The mechanism of development of these leukemias is not clear; induction of leukemia could be the result either of activation of a latent virus of mouse leukemia or or adaptation of human leukemia virus in the infected animals.

During recent years several systems of leukemic antigens have been detected in the tissues of mice with virus leukemias by means of the transplantation test [5, 8], the method of fluorescent antibodies [6], and the cytotoxic reaction [7, 9].

Results have been obtained indicating that immunodiffusion methods can be used to detect antigens in mice with virus leukemias which cannot be detected in the corresponding normal mouse tissues [3, 4].

The object of the present investigation was to use antisera against the tissues of mice with our strain of leukemia in the gel diffusion reaction to detect leukemic antigens in them and for the comparative study of tissues of mice with other transplanted and virus-induced leukemias.

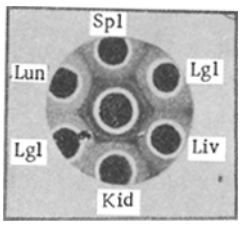

Fig. 1. Gel-diffusion reaction of reticulosarcomatosis antiserum with organ extracts of mice with reticulosarcomatosis. Central well contains reticulosarcomatosis antiserum, peripheral wells tissue extracts of mouse with reticulosarcomatosis: Spl, spleen; Lgl, leukemic lymph gland; Liv, liver; Kid, kidney; Lun, lung.

EXPERIMENTAL METHOD

Antigens for the gel-diffusion reaction and for immunization of the rabbits were prepared as saline extracts by the method described previously [3]. Saline extracts intended for immunization were prepared on the day of injection from a mixture of the spleen, liver, thymus, and neoplastic lymph nodes of mice of line CC57BR with our strain of reticulosarcomatosis maintained by cell inoculations. The extracts were mixed with an equal volume of mineral oil and inactivated BCG vaccine (3 mg vaccine for each rabbit weighing 2.5-3 kg). Altogether 2 or 3 cycles of immunization were carried out. Antigens were injected subcutaneously at 4 points each time. The first cycle consisted of three such injections of increasing doses of antigen (from 2 to 4 ml) at weekly intervals. The second and third cycles consisted of a single injection of 4 ml antigen 1-2 months after the end of the pre-

Laboratory of Immunology of Tumors and Laboratory of Experimental Therapy of Tumors, P. A. Gertsen Moscow Oncologic Research Institute (Presented by Academician of the AMN SSSR N. A. Fedorov).
Translated from Byulleten' Éksperimental'no Biologii i Meditsiny, Vol. 67, No. 5, pp. 53-55, May, 1969. Original article submitted June 8, 1968.

TABLE 1. Reaction of Reticulosarcomatosis Antiserum with Organ Extracts of Healthy Mice and Mice With Transplanted and Virus-Induced Leukemias

Strains of leukemias and mouse lines	Method of inoculation of leukemia	Reticulosarcomatosis antiserum					
		spleen	liver	kidney	lung	leukemic lymph glands	mixture of organs
Strain of reticulosarcomatosis* (CC57BR)	With cells†	+	+	+	+	+	
" " (CC57BR)	"	+	+	+	+	+	
" " (CC57BR)	"	+	+	+	+	+	
" " (CC57BR)	"	+	+	+	+	+	
" " (CC57BR)	"	+	+	+		+	
" " (CC57BR)	"						+
" " (CC57BR)	"						+
Pujman strain (C57BL)	"	±	±	+	±		
" " (C57BL)	"	±	+	±	±		
Zil'ber–Postnikova strain (C57BL)	"	±	±	±			
" " " (C57BR)	"	+	+	±			
" " " (CC57W)	With cell-free filtrates	±	–	–			
" " " (CC57W)	"	+	–	–			
Mazurenko strain	"	±	–	–			
Moloney strain	"	+	–	–			
Rauscher strain	"	–		–			
Friend strain	"	+					
Stepina–Zil'ber strain	"						+
LIO-1 (noninbred)	With cells	±	+	–		+	
NK-Li (noninbred)	"	±	±	±			
TsOLIPK-8 (AKR)	"	+	±			±	
Gorer strain (C57BL)	"	+	±	–		±	
Healthy (CC57BR)		+	±	±	±		
" (CC57BR)		+	±	±			
" (C57BL)		+	±	±			
" (C57BL)		+	±	±			
" (AKR)		+		–			
" (AKR)		+	–				
" (C3HA)		+	±	±			
" (noninbred)							

* Strain obtained in our laboratory (V. M. Bergol'ts).
† Also inoculated with cell-free filtrates in newborn mice; + clear precipitation line; ± weak precipitation line or bending of line of test system near well with this antigen.

vious cycle of immunization. Blood was taken from the rabbits on the 8th day after the last injection. Tissue extracts from mice with reticulosarcomatosis were used to immunize 4 rabbits. The antisera were exhausted with a mixture of extracts of liver, kidneys, lungs, and heart of CC57BR mice [2]. Total globulin fractions were separated from antisera not reacting with extracts of normal mouse tissues by semisaturation with ammonium sulfate. The globulins were concentrated by 5-6 times relative to the initial volume of antisera. The gel-diffusion reaction of Ouchterlony was carried out in a micromodification using agar made up in physiological saline. As a rule systems of individual organs were studied, consisting of the spleen, liver, kidneys, lungs, and affected lymph glands (in the case of leukemias), taken from the same mouse; in some experiments extracts of mixtures of organs were used. Protein was determined in the antigens by the quantitative biuret reaction. The list of virus-induced and transplanted mouse leukemias studied is given in Table 1.

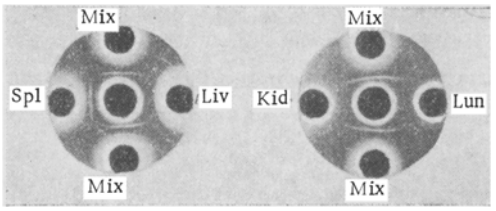

Fig. 2. Gel-diffusion reaction of reticulosarcomatosis antiserum with organ extracts of healthy mice. Central wells contain reticulosarcomatosis antiserum, peripheral wells tissue extracts: Mix, mixture of organs of mice with reticulosarcomatosis; Spl, spleen of healthy mouse; Liv, liver of healthy mouse; Kid, kidney of healthy mouse; Lun, lung of healthy mouse.

EXPERIMENTAL RESULTS

Two exhausted concentrated antisera obtained after the second cycle of immunization were most active. If antiserum was poured into the central well and antigens prepared against organs of line CC57BR mice with our strain of leukemia were poured into the peripheral wells, a common precipitation line was formed opposite the wells containing extracts of all investigated organs (Table 1; Fig. 1). Less definite results were obtained by the study of organ systems of healthy mice and mice with other transplanted and virus-induced leukemias. For example, reticulosarcomatosis antiserum reacted with spleen extracts from healthy mice of lines CC57BR, C57BL, C3HA, and AKR, forming a precipitation line identical with the line opposite wells with organ extracts of mice with reticulosarcomatosis (Fig. 2). This line usually bent either near the wells with extracts or other organs of healthy mice or changed into a very weak line opposite these wells (this reaction was designated ±). The antigen corresponding to this line was detected less regularly in the spleens of mice with transplanted and virus-induced leukemias (in addition to those with our strain of reticulosarcomatosis). The reticulosarcomatosis antiserum either failed to react or reacted by ± with extracts of other organs of these mice. Since no qualitative antigenic differences were detected between the tissues of healthy mice and of mice with leukemias, studies of the individual strains of leukemias were limited to one or two systems of organs.

No specific leukemic antigens were thus found by the gel-diffusion reaction in tissues of line CC57BR mice with our strain of reticulosarcomatosis. An antigen was found in all the organs of these mice which, under the same experimental conditions, was detected most clearly in the spleens of healthy mice of the lines investigated.

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