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Moloney Lymphoma Antibodies from Mice; Localization in Spleens of Moloney Lymphoma Bearing Mice* (33728)

ISAAC WITZ,[1] GEORGE KLEIN AND DAVID PRESSMAN

Department of Biochemistry Research,[2] Roswell Park Memorial Institute, Buffalo, New York 14203; and Department of Tumor Biology, Karolinska Institute, Stockholm 60, Sweden

Moloney virus induced lymphomas have been shown to contain tumor specific transplantation antigens (1–3). The existence of these antigens has been demonstrated by transplantation methods (1) and by various tests involving specific humoral antibodies induced by injecting heavily irradiated lymphoma cells, into syngeneic mice (2). This paper describes studies of the *in vivo* localization properties of the latter type antibodies. Antibodies were demonstrated which were fixed in the spleen when injected intravenously into tumor bearing mice but showed no such fixation in normal mice. The fixation was demonstrated using radioactive iodine as a label for the antibodies. This was interpreted as showing that the spleen contains antigens associated with the Moloney tumor or virus.

Materials and Methods. The following Moloney lymphomas were used: (i) YAC (an ascites tumor) in transplant generation 155 in male A/Sn mice and in generation 156 in male A/Sn, A/Hc and A/St mice; (ii) the YBA tumor (solid) in generation 45 in female CBA mice; (iii) the YLI tumor (solid) in generation 31 in male C57 Leaden mice.

The syngeneic antiserum for the detection of antigens associated with Moloney lymphoma was produced by repeated inoculation of irradiated lymphoma cells into syngeneic recipients (2). It had high cytotoxic activity against the Moloney lymphoma cells in the presence of complement *in vitro* and showed a high degree of membrane fluorescence against the same targets in the indirect test. Normal serum of the appropriate mouse strains was used as control.

The globulin fraction of each serum (from either normal or immunized mice) was prepared by precipitation with Na_2SO_4 as described previously (4), but with the modification that the initial Na_2SO_4 concentration was 18% (w/v) rather than 13.5% and the final Na_2SO_4 concentration was 13.5% rather than 12%.

Tumor sediments were prepared as described previously. Washed tissue fragments were homogenized and the insoluble sediment was washed thoroughly with borate buffer (pH 8.0) and water, and subsequently lyophilized (4). The globulin fraction from the Moloney antiserum was iodinated with iodine labeled with ^{125}I and globulin from normal isogeneic serum was iodinated with iodine labeled with ^{131}I was described previously (4). The paired-labeled mixture was purified with the corresponding tumor sediment as described below.

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[1] Present address: Department of Microbiology, Tel-Aviv University, Tel-Aviv, Israel.

[2] A unit of the New York State Department of Health.

Antibodies were purified by treatment of the radioiodinated globulin preparations with the appropriate tumor sediment for 60 min at 37°. The washed sediments were treated with glycine buffer at pH 2.4 to elute bound antibodies as described previously (4).

For the *in vivo* localization assay of radiolabeled isoantibody, volumes of 0.25–0.4 ml of paired-labeled eluates were injected intravenously into normal or tumor-bearing mice. After 18–24 hr, the mice were anesthetized with pentobarbital and perfused thoroughly with saline through the vena cava. The perfused organs were assayed for radioactivity in a γ-ray scintillation spectrometer. The percentage of injected radioactivity localized in organs was determined and the results are expressed both as net percentage of injected radiolabeled globulin from the antiserum localized (percentage of injected immune radioglobulin localized minus percentage of injected normal radioglobulin localized) and as the ratio of the two isotopes (ratio of the percentage of injected immune radioglobulin localized to the percentage of injected normal radioglobulin localized).

Results. In vivo localization studies were carried out with paired-labeled mixtures consisting of ^{125}I-labeled mouse globulin isolated from anti-Moloney antiserum and of ^{131}I-labeled globulin isolated from corresponding normal mouse serum. The mixtures had been purified by absorption on and elution from tumor sediments in such a way that a mixture intended to be injected into mice bearing a certain tumor was purified with the sediment of the same tumor. Three such eluates were obtained and each was injected into mice bearing Moloney tumors and into normal mice.

Assays showed that the spleen of tumor-bearing mice was essentially the only organ which showed preferential uptake of the globulin from the Moloney antiserum. Table I summarizes individual results of the *in vivo* localization pattern of the two globulins in spleens of tumor-bearing mice and in spleens of normal mice. All the other organs including the solid tumors showed no preferential uptake of antibody globulin over normal globulin. The ascitic cells of the YAC tumor showed some questionable fixation.

Normal spleen showed in some cases a low preferential uptake of globulin from the antiserum. This may be a reflection of the presence of some cross reacting antigenic component derived from a naturally carried mouse leukemia virus, such as the Gross virus, in the spleens of normal mice. The frequent existence of this situation has been clearly documented (5).

Discussion. This study, which is based on previous findings concerning specific *in vivo* localization of radiolabeled isoantibodies in mice, involves the injection of radioiodine labeled globulin from specific isogeneic anti-lymphoma antiserum as a reagent for detection and demonstration of these specific antigens which are available to circulating antibodies.

The specific antibody was purified with sediments of Moloney lymphomas, but did not exhibit specific localization in tumors. There are numerous possible reasons for this failure to localize in tumors. The most probable are the following: the vascular circulation through the tumor is poor in the case of the solid tumors, any specific localization hidden by the high nonspecific uptake of macromolecules by tumors (6), or that the pertinent antigens may not have been available to circulating antibody. The fact that we obtained specific antibody localization in the spleen of tumor-bearing animals, which is essentially the only organ in which such localization could be demonstrated, is in accord with the tumor cell content of the spleen and the better circulation of blood through the spleen.

Whether the antibodies being fixed are those directed towards antigens expressed on the outer membrane of virus carrying cells or towards virion antigens remains to be seen. The latter could be present in the cell interior, on budding virus particles and as free virus. It should be borne in mind that the insoluble sediment of tumor homogenates was used as the adsorbent for the purification of the labeled antibodies in the present work and the antibodies must therefore reflect the

antigenic composition of this sediment. This may correspond more to intracellular, than to surface expressed materials. Due to the preferential multiplication of the virus particularly in the megakaryocytes of the spleen and the bone marrow (7, 8), it is conceivable that more antigen of this type is available in the virally infected spleen, than in the

TABLE I. *In Vivo* Localization in Spleen of Mouse Antibodies to the Moloney Lymphoma.

The ^{125}I-labeled IgG from Moloney antiserum was mixed with ^{131}I-labeled globulin from normal syngeneic mice, and the pair was purified with the appropriate tumor sediment.[a] Data are for normal and Moloney lymphoma-bearing mice.

Mice assayed	Anti-Moloney antibodies localized (percentage of injected dose (net))[b]		Percentage of injected antiserum globulin localized/percentage of injected normal globulin localized
	Per organ	Per gram	
CBA			
Normal CBA (Klein)	0.0017	0.0170	1.09
	—0.0002	—0.0022	0.99
Normal CBA/St	0.0034	0.0340	1.15
	0.0016	0.0160	1.05
	0.0018	0.0200	1.06
CBA (Klein) with YBA	0.0482	0.2410	1.96
	0.0692	0.3642	4.30
	0.0481	0.2672	2.64
CBA/CAJ with YBA	0.0529	0.3306	2.22
CBA/St with YBA	0.0366	0.2440	1.72
	0.0595	0.2975	2.53
	0.0612	0.3400	2.93
A			
Normal A/Ha	0.0001	0.0006	1.00
	0.0038	0.0317	1.09
	0.0027	0.0193	1.05
A/Ha with YAC transplanted from A/Sn	0.0477	0.1908	1.85
	0.0441	0.2205	1.43
	0.0340	0.1700	1.28
A/Ha with YAC transplanted from A/Ha	0.0199	0.0995	1.33
	0.0323	0.1794	1.39
	0.0161	0.0805	1.18
A/Sn with YAC	0.0482	0.3213	1.92
	0.0446	0.2478	1.97
C57 Leaden			
Normal C57 Leaden	0.0369	0.4100	2.21
	0.0164	0.1640	1.32
C57 Leaden with YL1	0.4567	2.0805	8.01
	0.3753	1.5012	5.64
	0.3682	1.8410	5.59

[a] The mixture intended to be injected into normal or YBA lymphoma-bearing CBA mice was purified with YBA sediments. The mixture intended to be injected to normal or YAC lymphoma bearing A mice, was purified with YAC sediment and the mixture intended to be injected to normal or YL1 lymphoma-bearing mice was purified with YL1 sediment.

[b] Percentage of injected ^{125}I-labeled globulin localized from antiserum minus the percentage of injected ^{131}I-labeled globulin from normal syngeneic mice.

growing tumor where much of the corresponding antigen would be hidden behind intact cell membranes. Lymphoma cells show a variable degree of virus production by budding and the virus release in transplanted Moloney lymphomas can be quite low (2). Absorption to and elution from intact tumor cell surfaces will be required to clarify this question.

Summary. Radioiodine labeled globulin isolated from isogeneic mice immunized with irradiated Moloney lymphoma cells, and purified by absorption on the elution from the insoluble sediment of a homogenate of the Moloney lymphoma were shown to localize in spleen of Moloney lymphoma-bearing mice. Three different Moloney lymphomas were tested. In all three of them, there was a significant increase in specific localization of the antiserum globulin in spleen of the lymphoma-bearing mice, as compared to normal mice. There was little localization in the tumors however. The tests were done utilizing the paired-labeled antibody technic.

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