Binding of Rabbit Antibodies to a Mouse Myeloma Protein by the Myeloma Cells *in Vitro*[1] (34556)

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Specific binding of heterologous antibodies to tumor cells is an objective that has interesting therapeutic possibilities (1–3). In this study, purified rabbit antibodies have been prepared against a protein produced by a plasma cell tumor (myeloma) of BALB/c mice (4). The extent to which these antibodies bind *in vitro* to different types of viable lymphocytes and of tissue residues is reported.

Materials and Methods. Plasma cell tumor. Plasma cell neoplasm MOPC-21A of BALB/c mice was obtained in generation 89 from Michael Potter, M.D., National Cancer Institute. The tumor was transplanted subcutaneously at 3-week intervals in the co-isogenic Jackson Laboratory strain of BALB/c mice.

Purification of myeloma protein. Normal mice and mice bearing large plasma cell tumors were bled. The two pools of sera were subjected to acrylamide gel disc electrophoresis (Fig. 1) in the Canalco model 12 apparatus (Canal Industrial Corp., 5635 Fisher Lane, Rockville, Md.). Both normal mouse γG-globulin and myeloma protein (M) were purified by chromatography on DEAE-cellulose (Fig. 2).

Antisera. Antisera were prepared by injecting rabbits intramuscularly with three portions of 5–10 mg protein, incorporated in complete Freund-McDermott adjuvant, at 3-week intervals. After the first adjuvant injection, four intravenous injections of a saline solution of protein (approximately 5 mg each time) were given, spaced over a 2-week period. Three rabbits were used for immunization with each protein. Rabbits were bled

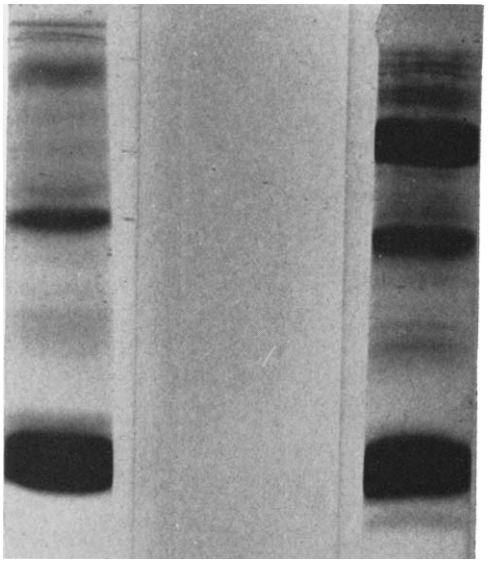

FIG. 1. Acrylamide disc electrophoresis of sera from normal mice and mice bearing the plasma cell tumor MOPC-21A. The myeloma protein is prominent in the right-hand tube.

10 days after the final injection of adjuvant and intermittently following subsequent booster immunizations.

Antibody purification. One portion of antiserum to the myeloma protein was absorbed by incubation for 30 min at room temperature with one-fifth volume of DBA/2 mouse serum, and the precipitate was discarded. Both this absorbed antiserum and unabsorbed antiserum were purified by adsorption and subsequent elution from an insoluble polymer prepared by cross-linking the myeloma protein with glutaraldehyde at pH 6.1 (5). Respectively 0.26 mg and 0.35 mg of absorbed and unabsorbed antibody were recovered per mg myeloma protein initially employed in the cross-linking procedure, fol-

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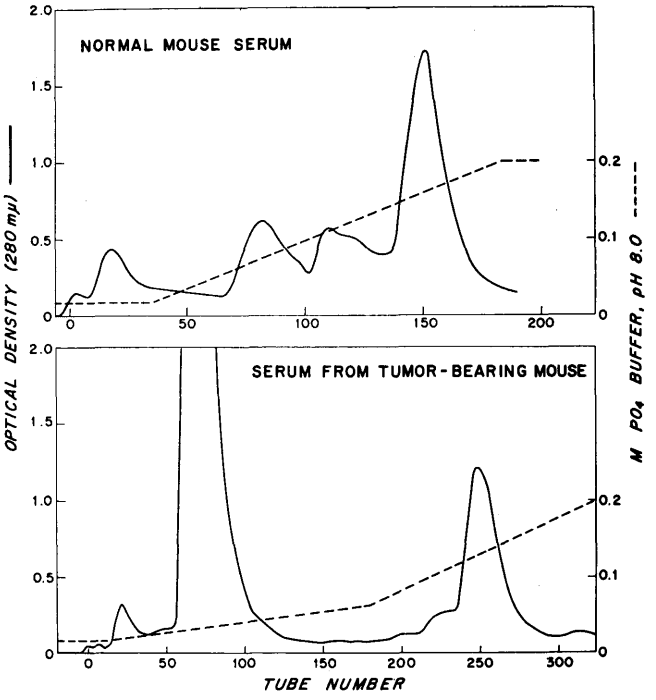

FIG. 2. Chromatography of sera from normal and plasma cell tumor-bearing mice on DEAE-cellulose with an ascending gradient of phosphate buffer, pH 8.0 (dotted lines).

lowing elution of adsorbed antibody with 0.1 *M* glycine-HCl buffer at pH 2.8. Antibody to normal mouse γG-globulin was similarly purified by adsorption and elution from cross-linked mouse γG-globulin.

Radioiodination. Purified antibodies to the myeloma protein or to normal mouse γG-globulin were trace-labeled with ^{125}I by a modification of the iodine monochloride method (6). Normal rabbit γG-globulin was batch-purified with DEAE-cellulose equilibrated with 0.01 *M* phosphate buffer, pH 8.0 (7), then trace-labeled with ^{131}I. All radiolabeled preparations were dialized against isotonic saline buffered with phosphate at pH 7.4. The radioactivities of ^{125}I and ^{131}I in mixtures were determined simultaneously on two channels in a Packard series 5000 gamma-ray spectrometer with a well-type crystal scintillation detector.

Cell suspensions and tissue residues. Fragments of the plasma cell tumor were suspended in "fortified buffer", which was isotonic saline buffered at pH 7.4 with phosphate and contained 1 % rabbit albumin (Pentex, Inc., Kankakee, Illinois). The fragments were minced with scissors and filtered through a 60-mesh stainless steel screen. Suspensions of thymocytes, splenic lymphocytes and lymph node lymphocytes (8) were prepared in the same fortified buffer. All cell suspensions were washed twice and filtered through 60-mesh screen to remove clumps prior to standardization in a hemacytometer chamber and subsequent use in experiments.

For preparation of tissue residues, a weighed piece of tissue was homogenized in four volumes (v/w) of pH 7.4 phosphate-buffered saline in a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 5° for 30 min at 3,000*g*. The residue was resuspended in buffer, washed twice under the same conditions of centrifugation and finally resuspended in fortified buffer.

Determination of antibody binding. The paired label radioantibody technique (9) was used in all experiments. Equal quantities of protein from the following two radiolabeled protein solutions were mixed: (a) purified rabbit antibody labeled with ^{125}I and (b)

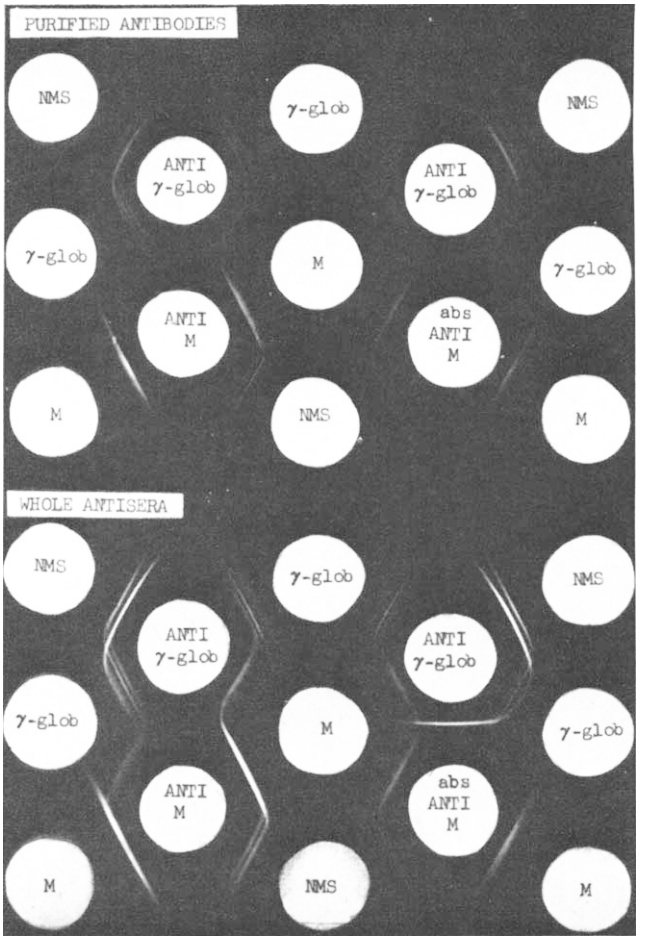

FIG. 3. Agar gel precipitin tests on whole antisera (bottom) and antibodies purified from them by adsorption onto insoluble glutaraldehyde-linked antigen preparations (top). *Antigens:* normal BALB/c mouse serum *(NMS)*, normal BALB/c mouse γ-globulin *(γ-glob)*, purified myeloma protein *(M)*. *Rabbit antibody preparations:* antinormal mouse γ-globulin *(ANTI γ-glob)*, antimyeloma protein M *(ANTI M)*, antimyeloma protein M absorbed with normal BALB/c serum *(abs ANTI M)*.

normal rabbit γG-globulin labeled with ^{131}I. The diluent was isotonic saline buffered at pH 7.4 with phosphate. Aliquots of this radioactive solution were incubated, with occasional mixing for 30 min at room temperature, with known numbers of viable tissue cells or with washed residues derived from a known wet weight of tissue. Cells or tissue residues were suspended in fortified buffer and subsequently washed three times with this buffer. Centrifugations of viable cells were done at 5° for 15 min at 105*g* and of tissue residues at 5° for 30 min at 3,000*g*.

Results. Specificity of antisera. Whole antisera to the myeloma protein M and to normal mouse γ-globulin both reacted with protein M, as seen by an arc of identity (bottom center, Fig. 3). Consonantly, whole antiserum to protein M reacted weakly with normal mouse γ-globulin, and gave two precipitin lines with normal mouse serum (bottom left, Fig. 3). One of these lines was removed by absorption with one fifth volume of normal BALB/c serum (bottom right, Fig. 3). Antibodies purified from these whole antisera by adsorption and elution from insoluble antigens showed identical specificities, except for a decreased reactivity of purified

TABLE I. Specificity of antibody binding to suspensions of myeloma cells and lymphocytes.

BALB/c cell type	No. of cells (10^6)	Ratio[a] of antibody-^{125}I/normal γG-^{131}I after a stated number of washes								
		Unabsorbed antibody to myeloma protein M			Absorbed antibody to myeloma protein M			Antibody to normal BALB/c γ-globulin		
		1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Myeloma	4	11.6	20.7	20.6	11.4	25.0	26.3	6.3	16.6	16.8
	20	10.7	25.1	27.6	3.3	9.5	10.9	5.4	13.3	14.4
Splenic lymphocytes	38	3.6	6.8	6.6	1.9	3.0	2.8	16.8	38.8	39.1
	150	6.8	17.0	16.5	4.1	9.6	10.0	17.2	41.1	42.8
Lymph node lymphocytes	8	1.8	3.0	3.0	2.0	1.7	2.0	4.6	14.3	14.2
Thymic lymphocytes	60	1.5	2.0	1.9	1.1[b]	1.4[b]	1.5[b]	3.0	6.6	6.3

[a] This ratio is corrected to the basis that equal counts of antibody-^{125}I and normal γG-^{131}I were added.
[b] Single values only. All other results, mean of two separate determinations.

antiprotein M to normal mouse γ-globulin (top left, Fig. 3).

In additional experiments, absorption of antisera to protein M with one-half volume of normal BALB/c serum removed all precipitin reactivity. Hence, myeloma protein M is antigenically similar to or identical with a minor component of normal BALB/c serum (10, 11).

Binding of radiolabeled antibodies to cells and tissue residues. The three purified antibody preparations used in immunodiffusion experiments (top, Fig. 3) were labeled with ^{125}I, and normal rabbit γG-globulin was labeled with ^{131}I. Mixtures containing approximately equal protein concentrations of an ^{125}I-labeled antibody preparation and ^{131}I-labeled normal γG-globulin were adsorbed onto washed suspensions of various types of BALB/c lymphocytes or onto washed residues derived from a known wet weight of tissue (see Methods). The specificity with which antibodies bound to the cells or tissues is expressed as the ratio ^{125}I count/^{131}I count (Table I).

Considerably more normal rabbit γG-globulin-^{131}I than antibody-^{125}I was removed from the cell surface between the first and second washes, and consequently, the specificity of binding antibody-^{125}I increased (Table I). For all cell types, the specificity of antibody binding remained remarkably constant between the second and third washes (Table I), even though the absolute counts decreased (Table II). Absorbed antibody to the myeloma protein M was bound most avidly by myeloma cells; in contrast, antibody to normal BALB/c γ-globulin was bound most strongly by splenic lymphocytes (Table 1).

The percentage of added antibody-^{125}I to normal mouse γ-globulin that bound to splenic lymphocytes was surprisingly high (Table II). However, similarly high percentages of this antibody bound to residues obtained from 30 mg wet weight of kidney and liver (23.6 % and 27.5 %, respectively). Even higher percentages (44.8 % and 46.2 %, respectively) were bound by residues derived from 100 mg wet weight portions. Moreover, the specificity of this binding was relatively high (Table III). Hence, the high specificities seen with splenic lymphocytes (Table I) do not prove that these cells produce γ-globulin, but only that γ-globulin is present on their surface.

Discussion. The present *in vitro* study precedes *in vivo* attempts to localize radiolabeled antibodies to the myeloma protein within transplantable myelomas. If adequate localization is obtained, therapy will be attempted with antibodies labeled at high activity levels (6). Since the myeloma cells secrete their characteristic protein into the circulation, much of the injected antibody may be inactivated before it can reach the

TABLE II. Percentage of a mixture of antibody-^{125}I and normal γG-^{131}I bound to cell suspensions.

BALB/c cell type	No. of cells (10^6)	Isotope	% Added counts bound to cells after a number of washes: Absorbed antibody-^{125}I to myeloma protein M plus normal γG-^{131}I (%) 1st	2nd	3rd	Antibody-^{125}I to normal mouse γ-globulin plus normal γG-^{131}I (%) 3rd Wash
Myeloma	4	^{125}I	13.4	9.5	7.9	4.8
		^{131}I	1.18	0.38	0.30	0.29
	20	^{125}I	6.4	4.4	3.7	7.6
		^{131}I	1.96	0.47	0.34	0.53
Splenic lymphocytes	38	^{125}I	3.5	2.4	1.98	23.4
		^{131}I	1.88	0.79	0.70	0.60
	150	^{125}I	8.0	6.2	5.1	28.4
		^{131}I	1.98	0.65	0.51	0.66
Lymph node lymphocytes	8	^{125}I	2.5	0.56	0.51	3.2
		^{131}I	1.24	0.33	0.26	0.22
Thymic lymphocytes	60	^{125}I	1.37[a]	0.48[a]	0.47[a]	1.94
		^{131}I	1.22[a]	0.35[a]	0.32[a]	0.31

[a] Single values only. All other results, mean of two determinations.

tumor. To minimize trapping of antibody, work will be done with small (early) myelomas, with preinjection of unlabeled antibody, and with perfusion of the tumor-bearing limb with labeled antibody. Cross-reaction of antibody with normal serum proteins will also present difficulty. A more attractive therapeutic approach would utilize radiolabeled antibodies to nonsoluble specific tumor antigens. Unfortunately, the most suitable tumor-specific antigen as yet discovered, the carcinoembryonic colon antigen, is soluble and present in the circulation of patients with metastatic cancer of the colon, although in very low concentration (12). Therefore, *in vivo* work with the highly soluble protein M may well help to solve some of the problems which are likely to be present when radiolabeled antibody experiments of similar type are attempted with antibody prepared against the

TABLE III. Specificity of antibody binding to residues of tissue homogenates.

BALB/c tissue homogenate residue	Initial wet weight of tissue (mg)	Ratio[a] antibody-^{125}I/normal γG-^{131}I after the 3rd wash: Unabsorbed antibody to protein M	Absorbed antibody to protein M	Antibody to normal mouse γ-globulin
Kidney	30	4.1	3.0	13.8
	100	7.0	5.0	23.1
Liver	30	5.8	4.3	14.6
	100	9.2	7.1	19.7
Heart	30	2.2	1.6	5.8
Brain	30	3.2	1.3	7.2

[a] This ratio is corrected to the basis that equal counts of antibody-^{125}I and normal γG-^{131}I were added. All results are the mean of two separate determinations.

carcinoembryonic antigen of colon. The latter work is in progress in our laboratory.

The present data show that large numbers of myeloma cells bind purified radiolabeled antibody to the myeloma protein M with *lower* specificity than small numbers of cells (Table I). The reason for this anomalous behavior may be the continuous release of myeloma protein into the medium: myeloma protein would then react with antibody, leaving smaller amounts of antibody free to react with the myeloma cells. In the absence of protein production, the opposite situation would be expected; this was seen for localization of absorbed antibody to protein M on splenic lymphocytes, and for antibody to normal BALB/c γ-globulin on splenic lymphocytes (Table I).

The present study has shown that antibody to a purified myeloma protein adsorbed avidly onto myeloma cells *in vitro*. Likewise, purified antibody to mouse γ-globulin was avidly adsorbed by splenic lymphocytes. These findings indicate presence of the respective proteins on the cell surfaces but do not prove that these proteins are produced within the respective cells. In contrast, the relatively low localization of radiolabeled antibodies to normal mouse γ-globulin on thymic lymphocytes (Table I) and on brain residues (Table III) is of interest.

Summary. Rabbit antibodies were prepared to a purified mouse myeloma protein and to normal mouse γ-globulin. Paired radiolabeled antibody studies were done *in vitro* with purified antibodies to these two antigens. The antibody to the myeloma protein bound to the myeloma cells with greater specificity than to the other lymphoid cells tested. Purified antibody to normal mouse γ-globulin bound far more strongly to splenic than to thymic lymphocytes.

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