Localization of an ^{125}I-Labeled Rat Transplantation Antibody in Tumors Carrying the Corresponding Antigen[1] (36326)

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Many concepts in the field of immunology postulate localization of antitumor or transplantation antibodies in target tissues. Such highly preferential localization has been surprisingly difficult to demonstrate (1–3). The studies reported here describe the preparation and purification of a labeled rat transplantation antibody and show that after intravenous injection the labeled antibody localizes with considerable specificity in a tumor carrying the corresponding transplantation antigen.

Materials and Methods. Inbred Fischer-344 and Buffalo rats were used in these experiments. A methylcholanthrene (MC) tumor was induced and maintained in Fischer rats. Serum that contained a high titer of hemagglutinating antibodies to Fischer transplantation antigen was obtained by multiple injections of viable tumor into Buffalo rats. These procedures are described elsewhere (4). Described here are the radiolabeling of the γ-globulin isolated from the Buffalo antisera, the purification of the labeled immune γ-globulin by adsorption and elution from Fischer transplantation antigen expressed on Fischer-344 red cells, and the study of the *in vivo* behavior following injection of the labeled, purified preparation into Buffalo immunosuppressed rats each bearing an MC tumor.

Iodination. Gamma globulin obtained by Na_2SO_4 precipitation from immune Buffalo serum was labeled in 1.5 mg quantities with 50 mCi of ^{125}I by the iodine monochloride method (5) with a final specific activity of 15 mCi of ^{125}I/mg of γ-globulin. Normal Buffalo serum, inactivated 30 min at 56°, was used as a protective protein for the labeled preparation in a final concentration of approximately 40%. For paired label experiments normal Buffalo γ-globulin was labeled with trace amounts of ^{131}I.

Purification. ^{125}I-Labeled antibody was separated from ^{125}I-labeled nonspecific γ-globulin by a twice repeated adsorption–elution procedure starting with Fischer-344 red blood cells. In the first adsorption the entire volume of labeled material (4.5 ml) was incubated 1 hr at 37° with an equal volume of packed red cells, previously washed 3 times with 0.85% saline. Following incubation the cells were washed twice with saline. Of the initial ^{125}I γ-globulin, approximately 2% was adsorbed to cells at this stage.

Dissociation of the bound labeled γ-globulin from the red cells was obtained by the method described by Kidd (6) where antibody-coated cells are converted to stroma, and the antibody is eluted into solution by the use of HCl–citrate buffer at pH 3.2. Of the 2% activity, approximately a 25% loss occurred upon conversion to stroma; and, in different preparations, 5–10% of the remaining activity was recovered with elution. On neutralization of the eluate to pH 7.4, additional ^{125}I was lost on a precipitate forming at this point, so that the overall efficiency of this first purification was in the range of 0.06%. However, hemagglutination studies showed that a substantial fraction of the initial Fischer-344 red cell agglutinating activity was still present at this point.

A second purification of this once purified

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preparation was carried out using the same techniques, except that 1.75 ml of packed cells was used to absorb antibody from the 3.5 ml of eluate. Approximately 25% of the starting eluate activity was found adsorbed to the red cells after incubation and washing, and 60% of the adsorbed radioactivity was eluted into solution. Again a loss occurred on neutralization so that the overall efficiency of the second purification was approximately 9%, a 150-fold increase in percentage yield compared with the first purification.

In vivo *studies.* Radioactivity distribution studies were carried out in Buffalo rats immunosuppressed with X-irradiation and cortisone (4) and carrying subcutaneous transplants of the Fischer-derived MC tumor, and in Buffalo rats similarly treated but without tumor implants. Seven days after tumor transplantation the rats were injected in the saphenous vein, under Fluothane anesthesia, with 0.4 ml of a saline mixture containing 0.065 μCi of ^{125}I-labeled twice purified transplantation antibody and 0.08 μCi of ^{131}I-labeled normal Buffalo γ-globulin.

All rats were started on KI water (6.25×10^{-4} *M*) prior to injection and maintained on KI water until time of sacrifice. The rats were sacrificed 48 or 96 hr after injection by saline perfusion by inserting a cannula either in the inferior vena cava or hepatic vein. The amount of saline used was 50–100 ml/rat. One-tenth milliliter of heparin at 1000 units/ml was injected prior to sacrifice. A well-type dual channel gamma scintillation counter was used for radioactivity measurements.

Results. In vitro *assay of specificity.* ^{125}I immune Buffalo γ-globulin at various stages of purification was tested by measuring the degree of binding to Fischer and Buffalo red cells. A mixture of 0.2 ml of washed, packed cells and 0.4 ml of labeled immune and normal Buffalo γ-globulins fortified with 10% normal Buffalo serum was incubated 1 hr at 37°, and the cells were washed twice with saline following the incubation. Of the added activity, the average percentage adsorption on red cells of unpurified, once purified, and twice purified ^{125}I-immune Buffalo γ-globulin was respectively: 5, 25, and 55% on Fischer-

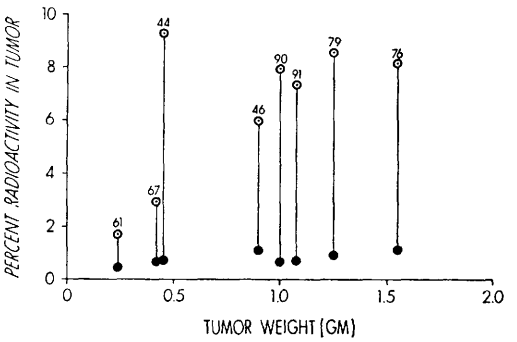

FIG. 1. Percentage of injected radioactivity in whole tumor 48 hr after injection of the paired labeled γ-globulins in 8 Buffalo rats bearing Fischer MC tumors. The ^{125}I-purified immune γ-globulin (⊙); and ^{131}I-normal γ-globulin (●); are plotted against tumor size for each rat. The weight (g) of each rat is indicated.

344 red cells; and 5, 6, and 4% on Buffalo red cells. The adsorption of ^{131}I-normal Buffalo γ-globulin was approximately 2% for both types of cells.

In vivo *distribution studies.* Eight rats with growing tumors were killed 48 hr after injection of the labeled preparation. Figure 1 shows the percentage of injected ^{125}I antibody and ^{131}I-control γ-globulin found in the individual tumors at this time.

Figure 2 shows the average ratio of the fraction of the injected dose of ^{125}I-antibody and ^{131}I-normal γ-globulin present in blood, tumor, and other tissues of these eight tumor-bearing rats, and of 5 control rats without tumors also killed 48 hr after radioactive

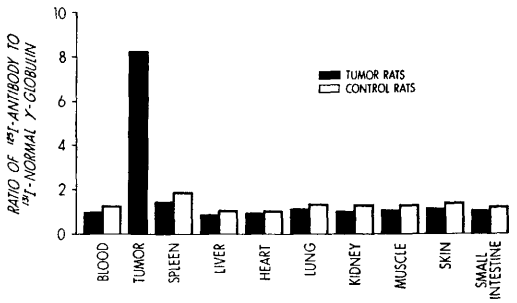

FIG. 2. The average ratio of the fraction of injected dose of ^{125}I-antibody to ^{131}I-control normal γ-globulin present in tissues of 8 tumor-bearing rats and 5 control rats without tumors killed 48 hr after radioactive isotope injection.

TABLE I. Distribution of ^{125}I-Labeled Transplantation Antibody and of ^{131}I-Labeled Normal γ-Globulin in Rats With Growing Tumors and Control Rats Without Tumors.

			% Injected dose/g of tissue/100 g of rat				
			Tumor rats[a]			Control rats[a]	
Time[b]	Tissue	No. of rats	^{125}I	^{131}I	No. of rats	^{125}I	^{131}I
48	Blood	7	1.70 ± 0.51	1.74 ± 0.34	7	2.77 ± 0.20	2.23 ± 0.26
	Tumor	8[c]	5.48 ± 1.92	0.72 ± 0.23			
	Spleen	8	0.59 ± 0.28	0.43 ± 0.20	5	0.99 ± 0.64	0.52 ± 0.21
	Liver	8	0.13 ± 0.24	0.15 ± 0.28	5	0.07 ± 0.05	0.07 ± 0.04
	Heart	8	0.15 ± 0.08	0.17 ± 0.10	5	0.21 ± 0.07	0.23 ± 0.09
	Lung	8	0.48 ± 0.23	0.48 ± 0.30	5	0.44 ± 0.15	0.35 ± 0.07
	Kidney	8	0.22 ± 0.10	0.25 ± 0.14	5	0.36 ± 0.10	0.28 ± 0.06
	Muscle	8	0.16 ± 0.03	0.16 ± 0.04	5	0.22 ± 0.08	0.18 ± 0.05
	Skin	8	0.35 ± 0.09	0.33 ± 0.08	5	0.47 ± 0.05	0.36 ± 0.05
	Small intestine	8	0.17 ± 0.05	0.17 ± 0.05	5	0.27 ± 0.04	0.26 ± 0.12
96	Blood	4	0.68 ± 0.34	0.82 ± 0.01	4	1.46 ± 0.16	1.23 ± 0.35
	Tumor	4	1.80 ± 0.69	0.29 ± 0.03			

[a] Average values ± one standard deviation.

[b] Interval in hours between injection and sacrifice.

[c] One rat injected 9 days after transplantation.

isotope injection. The ratio of isotope localization is approximately 1 in all tissues except in tumors where the ^{125}I is more abundant by a factor of 8. Table I gives in more detail the ^{125}I and ^{131}I distribution in the 8 growing tumor and 5 control rats killed at 48 hr, as well as in 4 tumor-bearing and 4 control rats killed 96 hr after injection of isotopes.

Discussion. These studies demonstrate the preparation of a radioactive rat transplantation antibody in a state of considerable purity, and show that under certain conditions this antibody, injected into an animal in which only tumor carries the corresponding antigen, preferentially localizes to a high degree in this tumor tissue. Ability to prepare such a purified antibody may be useful in studies of immunological enhancement, applied for example to organ transplants. Also these results give renewed hope for success in isolating antibodies to tumor in syngeneic systems with the eventual possibility of using such antibodies for diagnostic or therapeutic purposes.

It should be noted that this MC tumor was rejected within 8 days in Buffalo rats in which no immunosuppression was used, and that the technique of X-irradiation plus cortisone was only marginally effective in promoting tumor growth. A few tumors grew until they killed the host; however, many were ultimately rejected. All tumors on which data is reported here were pearly white, and neither appeared as vascular nor grew as rapidly as in their isogeneic host, the Fischer-344 rat. The ^{125}I-antibody injected into animals with tumors decreasing in size gave tumor uptake values substantially lower than the values for growing tumors reported here.

Summary. A method was developed for preparing antibody to Fischer-344 rat transplantation antigen, using as an antibody source serum from Buffalo rats immunized by repeated transplants of a carcinogen-induced Fischer tumor. Immune γ-globulin was labeled with ^{125}I and radioactive antibody isolated by twice repeated adsorption onto Fischer red blood cells and elution from stroma prepared from these cells. *In vitro* such antibody bound specifically to Fischer red blood cells. *In vivo* this antibody localized preferentially in the Fischer tumor grown in immunologically suppressed Buffalo rats.

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