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Localization of Radiolabeled Antibody in SVT2 Tumor Increases with Immunosuppression of the Host (41477)

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Abstract. The localization of radiolabeled tumor-specific antibodies to an SV40-transformed mouse tumor was analyzed in immunosuppressed (X-ray and cortisone) and nonimmunosuppressed mice. (C57Bl/6 × Balb/c)F_1 mice were immunized with the SVT2 tumor of Balb/c origin. Radiolabeled antibody was isolated from ^{125}I-labeled immune gamma globulin by adsorption onto SVT2 cells, and elution from these cells using citrate buffer. The radiolabeled antibodies were injected into normal (C57Bl/6 × Balb/c)F_1 mice. The purified antibodies present in this serum bound specifically *in vitro* to SV40-transformed cell lines. *In vivo*, the ^{125}I-labeled antibodies localized preferentially in the SVT2 tumor in immunosuppressed mice. Significantly less ^{125}I-labeled antibody localized in the SVT2 tumor in nonimmunosuppressed mice. The localization of ^{125}I-labeled antibody in SVT2 tumor in immunosuppressed mice was reduced significantly by passive administration of anti-SVT2 serum.

Cancer diagnosis or therapy using a tumor localizing radiolabeled antibody has been the goal of several groups of investigators (1–7). Most of the work has been done in xenogeneic systems, which require exhaustive absorptions of immune serum with normal cells to obtain a more tumor-specific serum. It would be desirable to work in a syngeneic system in which the only antibody response elicited is that against unique antigens on the tumor cell surface. Simian virus 40 (SV40)-transformed mouse cells possess an antigen specific for SV40-induced tumors that is absent in non-SV40-induced tumors (8–11). It has been shown that immunization of (C57Bl/6 × Balb/c)F_1 mice with syngeneic SV40 tumor (SVT2) elicits antibodies reacting against the SV40 tumor-associated cell surface antigen (10, 11). We chose this syngeneic system to avoid the cross-reactivity problems often associated with the use of tumor localizing antibodies prepared from xenogeneic serum (2).

Two possible limitations in the use of radiolabeled antibodies are the presence of circulating tumor antigens and host antibody. Tumor antigens which are shed from the solid tumor may combine with injected radiolabeled antibody, and the complexes cleared. However, tumor localization by radiolabeled antibodies has occurred even in the presence of high levels of circulating CEA (4, 5, 12). Antibodies produced by the host to its growing tumor may bind to the tumor, masking the binding sites from the labeled antibody (13, 14). Here we report that immunosuppression of the host prior to transplantation of an SV40-transformed mouse tumor led to significantly increased tumor localization of radiolabeled tumor-specific antibodies. This tumor localization was blocked by passive administration of immune serum.

Materials and Methods. *Mice and immunization.* Eight-week-old male (C57Bl/6 × Balb/c)F_1 mice (Animal Genetics and Production Branch, National Cancer Institute, Frederick, Md.) genetically identical to those used by Ting (10, 11) were immunized with the SVT2 tumor. The SVT2 and SVA31 C14 lines of Balb/c embryo cells

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transformed by SV40 were obtained frozen from a tumor bank (Biotech Research Laboratories, Inc., Rockville, Md.) recommended by Dr. C. C. Ting at the NCI. They were grown as monolayers in 150-cm^2 tissue culture flasks (Corning Glass Works, Corning, N.Y.) in RPMI 1640 medium containing 5% heat-inactivated calf serum (Grand Island Biological Co., Grand Island, N.Y.), 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 m*M* L-glutamine at 37° in a humidified atmosphere with 5% CO_2–95% air. The cells were harvested by exposure to 0.1% trypsin–10 m*M* EDTA in PBS, pH 7.2. The mice were immunized with the SVT2 tumor by inoculation of 1×10^7 cells into the footpad. Antisera were collected by cardiac puncture from mice with progressively growing tumors 4 to 6 weeks after tumor inoculation.

Isotopic antiglobulin technique. Sera were assayed for antibody activity by an isotopic antiglobulin technique (15). The antiglobulin used in this procedure was the IgG fraction of sheep anti-mouse IgG (Cappel Laboratories, Cochranville, Pa.) radiolabeled with ^{125}I (Amersham Corp., Chicago, Ill.) at a specific activity of 0.25 mCi/mg by the iodine monochloride method (16). Briefly, 5×10^5 target cells in 0.1 ml HBSS were incubated first with 0.1 ml of a 1:50 dilution of antiserum or normal serum for 30 min at room temperature. The cells were washed four times with 1 ml HBSS containing 10% γ-globulin-free newborn calf serum (Grand Island Biological Co., Grand Island, N.Y.). A second incubation with 10 μl ^{125}I-labeled sheep anti-mouse Ig containing approximately 3×10^6 cpm was done at 0° for 15 min, and the cells were washed seven times. All tests were done in duplicate. The variation among duplicates did not exceed 10%. Activities of the antisera are expressed as an absorption ratio (AR):

$$AR = \frac{\begin{array}{c}\text{^{125}I cpm on cells incubated with}\\ \text{mouse antiserum}\\ \text{+ ^{125}I-antiglobulin}\end{array}}{\begin{array}{c}\text{^{125}I cpm on cells incubated with}\\ \text{normal mouse serum}\\ \text{+ ^{125}I-antiglobulin}\end{array}}$$

A reaction was considered positive when the AR was ≥ 3.

Gamma globulin preparation. Gamma globulin was prepared from pooled anti-SVT2 antiserum by three precipitations with neutral 50% saturated ammonium sulfate. The final precipitate was dissolved in a volume of PBS equal to 40% of the original serum volume. Normal gamma globulin was prepared by ammonium sulfate precipitation of (C57Bl/6 × Balb/c)F_1 serum; the final precipitate was dissolved in a volume of PBS equal to 27% of original serum volume.

Antibody purification and radiolabeling. The immune gamma globulin was enriched before labeling by affinity purification using a modified version of the procedure of Ehrlich and Witz (17). SVT2 cells were washed twice with PBS, and resuspended in serum-free RPMI 1640 medium at a concentration of 2.2×10^7 cells/ml. Aliquots containing 1.35 ml of the SVT2 cell suspension and 0.15 ml anti-SVT2 serum were combined in 15-ml conical test tubes (Falcon Plastics, Los Angeles, Calif.). These were incubated in an ice bath for 1 hr with occasional shaking. After incubation, the cells were pelleted and washed three times with PBS. Antibody remaining adsorbed to the packed cells was eluted by resuspending each pellet in 0.1 ml of 0.1 *M* citrate buffer, pH 3.5, for 15 min. The supernatants were pooled, neutralized with 1 *N* NaOH, and dialyzed against one liter of PBS overnight. The entire procedure—absorption, washes, elution, and dialysis—was carried out at 4°. The dialyzed eluate was added to 1.5 mg of anti-SVT2 Ig to produce "enriched" immune Ig.

Two preparations of enriched immune Ig were radiolabeled by the iodine monochloride method (16). Gamma globulin, 1.5 mg, was dialyzed against one liter of borate buffer (0.20 *M* boric acid, 0.16 *M* NaCl, 0.04 *M* NaOH, pH 8.0) overnight before iodination. ^{125}I (15 mCi) and 4 eq of ICl were used to label the immune gamma globulins to a specific activity of 4.5 and 5.3 mCi/mg. Normal (C57Bl/6 × Balb/c)F_1 serum was used as a protective protein for the labeled preparations at 20% of the final

volume. One milligram of normal Ig was labeled in a similar fashion with 1 mCi of ^{131}I and 4 eq of ICl.

The radiolabeled antibody preparations were once affinity-purified by the procedure described above. Aliquots containing 2.25 ml of the SVT2 cell suspension and 0.25 ml ^{125}I-labeled enriched anti-SVT2 Ig were combined and incubated in an ice bath 1 hr. Washes, elution, and dialysis have been detailed above. The ^{125}I-labeled once affinity-purified antibodies were further purified by *in vivo* screening (18). Approximately 400,000 and 700,000 cpm of Preparations I and II of once-absorbed and eluted antibody in 0.5 ml PBS were each injected ip into four normal mice. The mice were bled 24 hr later by cardiac puncture. Approximately 15% of the injected counts were recovered in the serum. ^{131}I-labeled normal Ig was also *in vivo* screened. Approximately 1.5×10^6 cpm of ^{131}I-labeled normal Ig was injected ip into five normal (C57Bl/6 × Balb/c)F_1 mice. Approximately 3% of the counts injected were recovered in the serum 24 hr later.

A portion of the enriched immune gamma globulin was passed over a protein A–sepharose column (19), and the individual IgG fractions were radiolabeled with ^{125}I. Each fraction was affinity-purified with SVT2 cells and *in vivo* screened in normal (C57Bl/6 × Balb/c)F_1 mice, and tested for binding to finely minced tumor pieces taken from immunosuppressed and nonimmunosuppresed mice.

In vivo distribution studies. The *in vivo* distribution of the radiolabeled purified anti-SVT2 antibodies was studied in nonimmunosuppressed and immunosuppressed mice. The immunosuppressed mice consisted of a group of 11 (C57Bl/6 × Balb/c)F_1 mice treated with 400 R of whole-body radiation from a General Electric 220-keV Maximar X-ray machine the day before tumor transplantation, and given a 0.25 mg sc dose of cortisone acetate on alternate days beginning with the day of tumor transplant. SVT2 tumors were started in the right flank in these mice with trocar transplants of SVT2 tumor maintained in irradiated (C57Bl/6 × Balb/c)F_1 mice. MOPC-315 tumors were started in the left flank in these mice with trocar transplants of MOPC-315 tumor maintained in Balb/c AnN (The Charles River Breeding Laboratories, Inc., Wilmington, Mass.) mice. The five nonimmunosuppressed mice received no cortisone or X-ray. The tumors were started in these mice at the same time they were transplanted into the immunosuppressed mice. Nineteen days after transplant, four tumor-bearing immunosuppressed mice received 0.3 ml of unlabeled (C57Bl/6 × Balb/c)F_1 anti-SVT2 serum ip. The next day, these four mice (Group III) and seven other immunosuppressed mice that were not given unlabeled antiserum (Group II) received ip a mixture of 0.01 μCi of Preparation I of ^{125}I-labeled enriched once affinity-purified/*in vivo*-screened antibody and 0.02 μCi ^{131}I-labeled, *in vivo*-screened normal (C57Bl/6 × Balb/c)F_1 gamma globulin. Group I containing five nonimmunosuppressed mice received 0.01 μCi of Preparation II of ^{125}I-labeled enriched once affinity-purified/*in vivo* screened antibody and 0.02 μCi ^{131}I-labeled, *in vivo* screened normal gamma globulin. Forty-eight hours after injection, mice were bled by cardiac puncture, dissected, and the tissues counted. For a week prior to injection of radiolabeled antibody and until dissection, the mice were given water containing 6×10^{-4} *M* KI to block thyroid uptake of iodine that could be freed by the catabolism of the labeled gamma globulins.

Results. High absorption ratios against SVT2 cells were obtained when testing serum from all groups of immunized mice. The sera were pooled, and the effect of concentration of antiserum on the absorption ratio obtained with SVT2 tumor cells indicated a titer of 1:8192. Table I demonstrates that the pooled anti-SVT antiserum reacted with SVT2 and SVA31 C14 cells, but did not bind to MOPC-315 myeloma cells of Balb/c origin, P-815-X2 mastocytoma cells of DBA/2 origin, RIF-1 radiation-induced fibrosarcoma cells of C3H/HeJ origin, B16-F1 melanoma cells of C57Bl/6 origin, or SCK spontaneous tumor cells of A/J origin.

The specificity of the ^{125}I-labeled anti-

TABLE I. ABSORPTION RATIOS OF (C57BL/6 × BALB/C)F_1 ANTI-SVT2 ANTISERUM WITH VARIOUS TUMOR CELLS

Serum[a]	Target cells	cpm of antiserum	cpm of normal serum[b]	AR[c]
Pooled progressor	SVT2	27,961	1157	24.2
	SVA31 C14	18,327	1230	14.9
	MOPC-315	5,113	4096	1.3
	P-815-X2	1,192	1268	0.9
	RIF-1	1,141	1018	1.1
	B16-F1	1,281	984	1.3
	SCK	1,680	1131	1.5

[a] A 1:50 dilution of mouse serum was incubated with 5×10^5 target cells for 30 min at room temperature. The cells were then washed four times, incubated with 10 μl of the ^{125}I-anti-globulin for 15 min at 0°, and then washed seven times. Each determination was performed in duplicate.

[b] Control serum was normal (C57Bl/6 × Balb/c)F_1 at a 1:50 dilution.

[c] Absorption ratio = ^{125}I cpm antiserum/^{125}I cpm normal serum (average of duplicate samples).

SVT2 antibody was tested at various stages of purification by an *in vitro* binding assay, as detailed in Table II legend. Table II shows the specific binding of enriched, affinity-purified, and affinity-purified/*in vivo*-screened antibody preparations. The affinity purification brought the specific binding to SVT2 cells from 1.2 to 42.0–43.7%. *In vivo* screening of affinity-purified antibodies did not increase the binding to SVT2 cells, but did reduce the nonspecific binding to P-815-X2 mastocytoma cells essentially to zero.

The dose–response effect of immunosuppression and change in titer of circulating host antibody to SVT2 is shown in Table III. We used techniques of analysis of variance and regression to determine the affect of radiation and cortisone on the AR. There is a significant slope (linear effect) of radiation on the absorption ratio for both the with- and without-cortisone groups of mice. However, the two slopes are significantly different with the steeper slope associated with the cortisone group.

The tissue to blood ratios of ^{125}I-labeled anti-SVT2 antibody and ^{131}I-labeled normal gamma globulin in the three groups of tumor-bearing mice are shown in Table IV. The SVT2 tumor load (mean ± SE) in

TABLE II. *IN VITRO* BINDING OF ^{125}I-LABELED (C57BL/6 × BALB/C)F_1 ANTI-SVT2 ANTIBODY AT VARIOUS STAGES OF PURIFICATION

Purification stage	Binding (%) to tumor cells[a] SVT2	P-815-X2
1. Enriched ^{125}I-gamma globulin		
Preparation I	1.2	—
Preparation II	0.8	0.8
2. Enriched, once absorbed and eluted		
Preparation I	42.0	6.7
Preparation II	43.7	3.5
3. Enriched, once absorbed and eluted/*in vivo* screened		
Preparation I	37.2	0.2
Preparation II	46.2	0.3
4. Enriched, twice absorbed and eluted		
Preparation I	54.3	3.3

[a] SVT2 tumor cells or P-815-X2 control cells were suspended in RPMI 1640 medium containing 5% calf serum at a concentration of 1.7×10^7 cells/ml. Six-tenths ml of the cell suspension (10^7 cells) was placed into 12 × 75-mm glass tubes, and a volume of antibody containing a minimum of 3000 cpm was added to duplicate tubes. The tubes were incubated at 37° for 1 hr with occasional shaking, and then counted in a Beckman Gamma 7000 counting system to determine the total cpm added. After three washes with 1 ml of RPMI 1640 medium containing 10% calf serum, the tubes were counted again. Mean values are shown. The variation between duplicate tubes did not exceed 10%.

TABLE III. EFFECT OF IMMUNOSUPPRESSION ON CHANGE IN TITER OF CIRCULATING HOST ANTIBODY IN MICE BEARING SVT2 TUMORS

Treatment	No. of mice in group	AR[a]
0 R	3	5.1 ± 0.4
0 R and cortisone	4	5.2 ± 0.4
200 R	4	4.0 ± 0.8
200 R and cortisone	3	3.6 ± 0.3
400 R	4	3.7 ± 0.5
400 R and cortisone	6	1.6 ± 0.4

[a] Mean ± SE obtained by dividing counts of ^{125}I-labeled sheep anti-mouse Ig bound to SVT2 cells incubated with serum obtained from individual mice in the different treatment groups, 3 weeks after sc transplantation of the SVT2 tumor, by the counts bound to SVT2 cells incubated with normal serum.

TABLE IV. TISSUE TO BLOOD RATIOS OF ^{125}I-LABELED ANTI-SVT2 ANTIBODY AND ^{131}I-LABELED NORMAL GAMMA GLOBULIN IN TUMOR-BEARING MICE INJECTED WITH ANTI-SVT2 SERUM[a]

	Group I (Nonimmunosuppressed)		Group II (Immunosuppressed)		Group III (Immunosuppressed + antiserum)	
Tissue	^{125}I-Antibody	^{131}I-Normal gamma globulin	^{125}I-Antibody	^{131}I-Normal gamma globulin	^{125}I-Antibody	^{131}I-Normal gamma globulin
SVT2 tumor	0.53 ± 0.12	0.30 ± 0.04	2.17 ± 0.23	0.55 ± 0.06	0.88 ± 0.11	0.55 ± 0.08
MOPC-315 tumor	0.43 ± 0.11	0.33 ± 0.07	0.66 ± 0.07	0.52 ± 0.03	0.32 ± 0.02	0.34 ± 0.01
Spleen	0.18 ± 0.02	0.17 ± 0.01	0.11 ± 0.04	0.19 ± 0.04	0.15 ± 0.03	0.18 ± 0.02
Liver	0.23 ± 0.01	0.19 ± 0.01	0.24 ± 0.01	0.21 ± 0.01	0.20 ± 0.02	0.16 ± 0.01
Heart	0.27 ± 0.02	0.26 ± 0.02	0.34 ± 0.07	0.23 ± 0.03	0.18 ± 0.04	0.25 ± 0.01
Lung	0.39 ± 0.02	0.40 ± 0.02	0.43 ± 0.10	0.45 ± 0.04	0.30 ± 0.03	0.42 ± 0.08
Kidney	0.28 ± 0.02	0.26 ± 0.01	0.31 ± 0.07	0.27 ± 0.02	0.24 ± 0.04	0.25 ± 0.03
Muscle	0.12 ± 0.02	0.12 ± 0.01	0.45 ± 0.13	0.52 ± 0.12	0.50 ± 0.15	0.42 ± 0.12
Skin	0.51 ± 0.09	0.41 ± 0.08	1.30 ± 0.24	1.17 ± 0.20	0.95 ± 0.12	0.87 ± 0.17
Small intestine	0.15 ± 0.04	0.14 ± 0.02	0.08 ± 0.02	0.11 ± 0.01	0.08 ± 0.03	0.08 ± 0.02

[a] Groups I–III consist of five, seven, and four mice, respectively. Groups II and III were immunosuppressed with radiation and cortisone and were inoculated with Preparation I of ^{125}I-labeled anti-SVT2 antibody and ^{131}I-labeled normal gamma globulin 20 days after tumor transplant. Group I was nonimmunosuppressed and received Preparation II of ^{125}I-labeled anti-SVT2 antibody and ^{131}I-labeled normal gamma globulin 25 days after tumor transplant. Mice were killed 48 hr after injection. Results are expressed as mean tissue to blood ratio (calculated on an individual animal basis and averaged for each group) ± SE.

Groups I–III was 0.31 ± 0.12, 0.44 ± 0.20, and 0.36 ± 0.11 g, respectively. The difference in SVT2 tumor load between any of the groups was statistically insignificant. There was significantly less ^{125}I-labeled antibody localized in the SVT2 tumor in Group I of nonimmunosuppressed mice compared with Group II of immunosuppressed mice, determined by two sample *t* test ($P < 0.01$). The localization of ^{131}I-labeled normal gamma globulin was lower in SVT2 tumor and skin in the nonimmunosuppressed mice (Group I, $P < 0.01$). The localization of ^{125}I-labeled antibody in SVT2 tumor in immunosuppressed mice (Group II) was reduced by passive administration of 0.3 ml anti-SVT2 serum (Group III, $P < 0.01$). The localization of ^{125}I-labeled antibody and ^{131}I-labeled normal gamma globulin was lower in the MOPC-315 tumor in mice injected with anti-SVT2 serum (Group III) compared with immunosuppressed mice (Group II, $P < 0.01$), but was similar to that seen in nonimmunosuppressed mice (Group I). The sera from the individual animals in the three groups were tested by the isotopic antiglobulin technique. The mean values for Groups I–III were 6.6, 1.8, and 5.6, respectively, indicating the presence of host-anti-SVT2 antibody in the nonimmunosuppressed mice. Table V shows the *in vitro* binding of the radiolabeled IgG antibody fractions to crude cellular preparations made by finely mincing SVT2 tumors taken from immunosuppressed and nonimmunosuppressed mice. There was lower binding to tumors taken from nonimmunosuppressed mice. Exposure of the cellular preparation made from tumor grown in nonimmunosuppressed mice to citrate buffer, pH 3.5, did not result in an eluate that showed antibody activity by the isotopic antiglobulin technique. Incubation of the cellular preparation with ^{125}I-labeled sheep anti-mouse IgG did not result in greater binding of the antiglobulin than that seen with a cellular preparation made from tumors grown in immunosuppressed mice.

Discussion. The possibility of using radiolabeled antibodies in syngeneic tumor models has been investigated in several other laboratories. Witz *et al.* (20) immunized syngeneic mice with Moloney virus-induced lymphomas. When labeled affinity-purified antibodies were injected into animals bearing the tumor, essentially the only organ which showed preferential uptake of the labeled antibody was the spleen. There was no such spleen fixation in normal mice, suggesting that the spleens from tumor-bearing mice contained tumor cells, antigen, or virus. De Vaux Saint Cyr (21) found no tumor localization when studying a radiolabeled syngeneic antibody against a hamster SV40 tumor. This lack of localization may have been the result of a disappearance of the virus-induced antigens

TABLE V. *IN VITRO* BINDING OF ANTI-SVT2 IMMUNOGLOBULIN FRACTIONS TO SVT2 CELLULAR PREPARATIONS

	Binding (%)[a]		
	SVT2 cells in culture	SVT2 tumor pieces from immunosuppressed mice	SVT2 tumor pieces from nonimmunosuppressed mice
^{125}I-IgG$_1$	29.5	26.9	11.7
^{131}I-Normal γ-globulin	—	3.2	1.9
^{125}I-IgG$_{2a}$	25.8	21.8	11.3
^{131}I-Normal γ-globulin	—	2.9	3.3
^{125}I-IgG$_{2b}$	15.8	22.9	5.5
^{131}I-Normal IgG$_{2b}$	—	4.8	3.8

[a] 1×10^7 SVT2 cells maintained in tissue culture or 0.1 g of a crude cellular preparation made by finely mincing tumors taken from immunosuppressed (400 R X-ray and cortisone) or nonimmunosuppressed mice, were incubated with a minimum of 2000 cpm of radiolabeled antibody or normal γ-globulin, and the cpm bound/cpm added initially (average of duplicate samples) determined.

from SV40 tumor cells *in vivo*. These antigens reappeared after the cells were cultured *in vitro*. In contrast, our results show that ^{125}I-labeled tumor-specific antibodies can be prepared from the sera of mice with progressively growing SVT2 tumors, and that when passively transferred these antibodies localize specifically in SVT2 tumors growing in immunosuppressed (X-ray and cortisone) mice. This localization was blocked by passive administration of anti-SVT2 serum or by nonimmunosuppression of the host. We used the F_1 hosts and SVT2 tumor to exactly duplicate the model and results reported by Ting (10, 11). The specificity of the antisera obtained from F_1 hosts was tested by absorption experiments. Their reactivity could only be abrogated by absorption with various SV40-transformed cells (10, 11). To assure that we were dealing with a tumor-specific antigen and not a histocompatibility antigen, we obtained the SVT2 tumor frozen from a tumor bank. In addition, the F_1 mice were from the same source at the NCI, where a new colony is started each year to prevent genetic drift. Finally, the antibody did not show any cross-reactivity with other tumors of C57Bl/6 or Balb/c background (Table I).

The results of the present study suggest that the presence of antibody, produced for example by an intact immune system, can block the localization of radiolabeled syngeneic tumor-specific antibodies to the SV40 tumor-specific cell surface antigen *in vivo*. The findings that immunosuppressed mice given anti-SVT2 serum had significant free antibody in the circulation and that the radiolabeled antibody fractions showed lower binding to tumors taken from nonimmunosuppressed mice, suggest that deposition of unlabeled antibody in tumors masks or modulates (21) antigenic sites on the SV40 tumor cell surface and prevents their recognition by radiolabeled tumor localizing antibodies. Witz *et al.* (22) reported that irradiation (400 rad) of the host prior to MDAY methylcholanthrene-induced sarcoma cell inoculation caused a decreased coating of tumor cells with immunoglobulin. Ran *et al.* (23) eluted cytotoxic antitumor antibodies from SEYF-a tumor cells using low pH buffers. However, acid elution gave no direct evidence of the presence of antibody on the SVT2 tumor *in situ*. Izzo *et al.* (24) reported that horse anti-rat lymphocyte serum blocked the localization in allogeneic skin grafts of radiolabeled histocompatibility antibody.

In Table IV, there was an increase of ^{131}I-labeled normal gamma globulin localization in SVT2 tumor in the immunosuppressed and immunosuppressed + antiserum groups relative to the nonimmunosuppressed group. This can probably be explained by the well-known increase in skin vascular permeability following X-ray (25). The localization of normal gamma globulin in the SVT2 tumor was not reduced in the immunosuppressed mice given antiserum (Group III) compared with immunosuppressed mice (Group II), but was reduced somewhat in muscle and skin. The reason for this reduction is not clear. However, it should be kept in mind that these differences involve tissue to blood ratios that are quite uniform and in most instances considerably less than 1, whereas the SVT2 tumor to blood ratio is greater than 2 at 48 hr in Group II. Ballou *et al.* (1, 26) reported that radiolabeled monoclonal antibody localized preferentially in a mouse teratinocarcinoma in syngeneic mice, with a tumor to blood ratio of 1.3 at 48 hr after injection. This ratio increased to 15 at 5 days after injection due to the rapid elimination of the IgM antibody from the blood.

Present studies using the SVT2 model have limitations in replicating what is presently being attempted clinically. First, to date, most radiolabeled antisera have been of heterologous derivation, goat and rabbit into man rather than comparable to the experimental situation of murine antibody into a murine host. In addition, most of the antigens studied in man have been tumor associated, i.e., in high concentration but not unique, nor immunogenic. HCG,[2] CEA, ferritin and AFP are generally not consid-

[2] Abbreviations used: HCG, human chorionic gonadotropin; CEA, carcinoembryonic antigen; AFP, α-fetoprotein.

ered immunogenic to the host nor have they been clearly demonstrated to produce host autoantibody.

The results suggest that a possible limitation in the use of radiolabeled antibodies for cancer diagnosis and therapy might be the production by the host of antibodies that bind to antigen sites on the tumor cell surface and block binding of labeled antibody. This would only be relevant to those antigens which induce autoantibody and only then if the heterologous-derived antibody would be similarly blocked. However, there is a high probability that monoclonal antibodies produced by hybridomas of human origin will eventually be used in cancer diagnosis and therapy, so that the results in mouse systems will be of importance in indicating the directions human studies should take.

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