GYNECOLOGIC ONCOLOGY 47, 93–101 (1992)

Localization and Therapy of Human Cervical Tumor Xenografts with Radiolabeled Monoclonal Antibody 1H10

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Received November 25, 1991

Murine IgG_3 monoclonal antibody (Mab) 1H10, which recognizes a tumor-associated antigen expressed on the surface of more than 40% of human cervical carcinoma tissues, was used for *in vivo* localization and therapy of cervical tumor xenografts. A human cervical carcinoma cell line, CaSki, was used as our experimental tumor system. Mab 1H10 antigen expression on the surface of CaSki cells was found to be cell-cycle independent. The ability of Mab 1H10 $F(ab')_2$ to bind to CaSki tumor xenografts was verified by direct immunohistochemical staining of thin tumor sections with a Mab 1H10-peroxidase conjugate. Radioimmunoscintigraphy of nude mice bearing CaSki tumors after iv administration of [^{131}I]1H10 $F(ab')_2$ showed clear tumor images 48 hr after Mab injection. Radiolabeled Mab 1H10 $F(ab')_2$ was found to specifically localize in solid CaSki tumors 96 hr after antibody injection. Radioactivity in tumor tissue was 4 times higher than that in kidney tissue and over 6 times higher than that in liver tissue. Mab 1H10 $F(ab')_2$ binding to xenografted CaSki tumors was 17 times greater than a control IgG_3 $F(ab')_2$ after 96 hr. Therapy of athymic mice bearing established CaSki tumors with three iv injections of 100 μCi [^{131}I]1H10 $F(ab')_2$ resulted in extensive tumor necrosis and significant suppression ($p < 0.05$) of tumor growth compared to that in control mice. These results indicate that Mab 1H10 $F(ab')_2$ may be clinically useful for detection or treatment of cervical cancer.

INTRODUCTION

The concept of targeting a tumor with antibodies is usually attributed to Paul Ehrlich, who at the turn of the century discussed the *in vivo* potential of "magic bullets." However, it was not until the classic studies of Pressman and Korngold [1] that research on tumor targeting using antibodies was documented. In recent years the production of monoclonal antibodies (Mabs) specific to tumor markers and tumor-associated antigens has greatly advanced the field. Antibody-mediated diagnosis and therapy of several cancers are being tested [2,3].

Despite the widespread use of cervical cytologic screening programs, cervical carcinoma is still a serious problem [4–6]. The ineffectiveness of current management methods for advanced or metastatic cervical carcinoma argues that research related to improving staging, detection of early recurrence, and treatment of cervical carcinoma is needed. For these reasons, clinical benefits should result from developing monoclonals that aid in staging and determining the extent of recurrent disease and offer opportunities for improved treatment of advanced and metastatic cancer.

Monoclonal antibodies that recognize tumor-associated antigens can be labeled with radioisotopes and used to localize or treat tumors. We have previously shown that human bladder tumor xenografts can be imaged and treated with ^{131}I-labeled antibody [7,8]. In this study, Mab 1H10, generated using CaSki cells, a human cervical carcinoma cell line, was examined for cervical carcinoma localization and therapy. Frozen tissue immunohistochemical studies have demonstrated that Mab 1H10 reacts with more than 40% of cervical carcinoma tissues obtained at surgery with no apparent cross-reaction to normal human tissues and cells [9]. We have previously shown that Mab 1H10 $F(ab')_2$ fragments conjugated with *Pseudomonas* exotoxin [10] or doxorubicin [11] can suppress the growth of established cervical carcinoma xenografts in nude mice. In this study, the ability of radio-

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0090-8258/92 $4.00

labeled Mab 1H10 $F(ab')_2$ to localize in xenografted cervical tumors was quantified. In addition, we show that ^{131}I-labeled Mab 1H10 $F(ab')_2$ may be useful for the detection and treatment of cervical carcinoma.

MATERIALS AND METHODS

Cell lines. All cells used for immunization or establishment of xenografted tumors were maintained in RPMI medium supplemented with 5% heat-inactivated fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin. CaSki cervical carcinoma cell line was provided by Dr. R. A. Pattillo (Medical College of Wisconsin, Milwaukee). H2669 cell line was provided by Dr. I. Hellström (University of Washington, Seattle).

Reagents. Propidium iodide, ribonuclease A, pepsin, 2-iminothiolane, and horseradish peroxidase were purchased from Sigma Chemical Company (St. Louis, MO). Sepharose CL-4B protein A, Con A Sepharose, and S-300 gels as well as 3-(2-pyridyldithio)propionic acid *N*-hydroxy succinimide ester were from Pharmacia LKB Biotechnology (Uppsala, Sweden). ACA 34 gel was obtained from IBF Biotechnics (Villeneuve-la-Garenee, France).

Animals. BALB/c mice were obtained from the animal room of the Institute of Biomedical Sciences, Academia Sinica, Taipei, R.O.C. BALB/c *nu/nu* mice were obtained from the Cancer Research Laboratory, Tri-Service General Hospital (Taipei, R.O.C.).

Antibody preparation. Mab 1H10 was generated against CaSki human cervical carcinoma cell-surface antigen as previously described [9]. Antibody was purified [12] from ascites produced in BALB/c mice or from culture supernatant obtained from hybridomas grown in serum-free medium using an Opticell 5200R cell-culture system. Hybridoma cells producing anti-influenza virus IgG_3, Mab HK-PEG-1, used as a control antibody, were obtained from the American Type Culture Collection (Rockville, MD).

Staining for DNA content of tumor cells. A previously described technique for DNA staining [13] was used. Briefly, propidium iodide was added at a working concentration of 4 μg/ml to the cell suspension, 1–5 min before flow cytometric analysis. Since propidium iodide stains all double-stranded nucleic acids, ribonuclease A at a concentration of 0.5 mg/ml was added to ensure DNA-specific staining.

Flow cytometric analysis. Analysis was performed using an EPICS flow cytometer (Coulter Corporation). Fluorochrome labels were excited at 488 nm with an air-cooled argon laser and emitted light was detected by multiplier tubes after passing through appropriate filters. Optical alignment of the instrument was monitored using alignment fluorospheres (EPICS). CaSki cells saturated with Mab 1H10 and labeled with FITC-goat anti-mouse IgG were used for normalization of the green fluorescence signal, with the peak signal placed in channel 150 of a 256-channel scale by adjusting the photomultiplier voltage and amplifier settings. At least 10,000 cells were analyzed in each experiment.

Preparation of $F(ab')_2$ immunoperoxidase. $F(ab')_2$ fragments of Mab 1H10 or Mab HK-PEG-1 were prepared by dialyzing pure antibody against 0.1 *M* sodium acetate, pH 4.0, and then digesting each milligram of antibody with 10 μg pepsin for 12 min at 37°C. Pepsin was deactivated by raising the pH to 8.0 and undigested IgG was removed by affinity chromatography on Sepharose CL-4B protein A while free pepsin and small peptides were removed by size-exclusion chromatography on AcA 34 or S300 gel. $F(ab')_2$ fragments were assayed for purity and activity and then stored at −70°C. Purity of $F(ab')_2$ fragments was greater than 95% as determined by sodium dodecyl sulfate–polyacryamide gel electrophoresis under nonreducing conditions. $F(ab')_2$ fragments retained over 90% of the antigen-binding activity of the original IgG molecules as assayed by enzyme-linked immunosorbent assay [11]. Peroxidase was linked to $F(ab')_2$ fragments as described [14]. An average of 4.0 3-(2-pyridyl-dithio)propionyl groups were introduced into each $F(ab')_2$, as measured by the method of Carlsson *et al.* [15] while an average of 0.7 to 0.9 SH groups were introduced into each peroxidase when measured as described by FitzGerald [16]. $F(ab')_2$ peroxidase conjugates were purified through Con A Sepharose and S-300 columns. Optimal conjugate concentration was determined to minimize nonspecific binding by enzyme-linked immunosorbent assay.

Tumor models. Female BALB/c *nu/nu* mice (4–6 weeks old) were injected sc in the right thigh with 1×10^7 CaSki cells or H2669 cells (human melanoma cell line) in 0.2 ml of PBS to establish solid tumors. Tumors were subjected to nuclear imaging and immunohistochemical studies after 3 to 4 weeks when they grew to a size of approximately 10 mm in diameter.

Immunohistochemical staining. Xenografted cervical carcinoma and adjacent normal tissues were obtained immediately after autopsies and stored at −70°C. Cryostat sections (5 μm) of tumor tissue were treated with 3% H_2O_2 in methanol to deactivate endogenous peroxidase activity and antibody reactivity was observed by direct immunoperoxidase assay. Briefly, an optimal dilution of Mab 1H10 or Mab HK-PEG-1 $F(ab')_2$ peroxidase conjugate was incubated with tissues followed by reaction with 3,3′-diaminobenzidine and counterstaining with hematoxylin. Staining above the negative control back-

ground (control Mab peroxidase conjugate staining) was considered to be a positive result.

Radiolabeling of Mabs and nuclear imaging. Mabs 1H10 and HK-PEG-1 F(ab')$_2$ were radiolabeled with ^{131}I using the Chloramine T procedure [17] to a specific activity between 5.0 and 6.0 μCi/μg. Iodinated Mabs were tested by direct binding to CaSki cells and other cell lines to ensure maintenance of specificity and immunoreactivity. No change in F(ab')$_2$ immunoreactivity was found. For nuclear imaging studies, mice were given Lugol's solution in their drinking water 2 days before injection to prevent thyroid uptake of dehalogenated radioiodine. ^{131}I-labeled 1H10 F(ab')$_2$, 100 μCi, was injected intravenously via the tail vein and each nude mouse was then imaged from the back with a γ-camera (Elscint Model Apex 400). Images were obtained at 24, 48, 72, and 96 hr. All data were recorded on a computer system and stored on floppy disks.

Biodistribution of radiolabeled Mab. Groups of three mice were sacrificed and dissected at 24, 48, and 96 hr after iv injection of 100 μCi [^{131}I]1H10 F(ab')$_2$ or [^{131}I]HK-PEG-1 F(ab')$_2$. Tumors, blood, brain, heart, lung, liver, spleen, gut, kidney, bladder, uterus, and muscle were weighed on an analytical balance and assayed for radioactivity using a multichannel γ-counter. Results are expressed as follows: (a) Specific uptake of antibody in tumor or tissue (cpm/mg); (b) ratio of specific activity in normal mouse tissues compared to tumor tissue ((cpm/mg normal tissue)/(cpm/mg tumor)); and (c) localization index, defined as the ratio of specific (Mab 1H10) to nonspecific (Mab HK-PEG-1) antibody uptake in tissue or tumor, normalized to the radioactivity in the blood of different mice. Localization index is calculated by

$$\text{Localization index} = \left(\frac{\text{cpm/mg tissue}}{\text{cpm/mg blood}}\right)_{\text{Mab 1H10}} \Bigg/ \left(\frac{\text{cpm/mg tissue}}{\text{cpm/mg blood}}\right)_{\text{Mab HK-PEG-1}}.$$

A localization index of 1 indicates that Mab 1H10 and HK-PEG-1 binding to a particular tissue were equivalent while a high index value indicates that Mab 1H10 binding was relatively higher than control Mab binding.

In vivo therapy with radiolabeled Mab 1H10. Twenty female BALB/c *nu/nu* mice were injected sc in the right flank with 10^7 exponentially growing CaSki cells. Three days later, mice were fed Lugol's solution in their drinking water. On Days 5, 12, and 19, groups of five mice were treated by iv injection of 100 μCi [^{131}I]1H10 F(ab')$_2$ (27 μg protein), [^{131}I]HK-PEG-1 F(ab')$_2$ or free ^{131}I. Tumor size and body weight were measured twice a week. Tumor volumes were estimated by weight × height × length/2 [18]. Statistical analysis was performed by the Student t test.

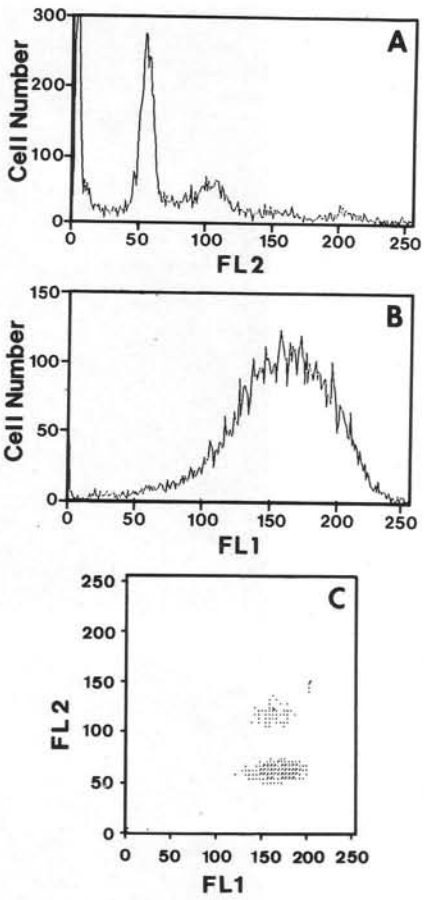

FIG. 1. Simultaneous flow cytometric analysis of Mab 1H10 antigen expression and cell cycle of CaSki cells. (A) DNA histogram of cells stained with propidium iodide, (B) surface antigen expression shown by membrane immunofluorescence of cells incubated with Mab 1H10 and anti-mouse IgG FITC, and (C) dot plot of DNA staining (FL2) versus antigen expression (FL1). There was no significant difference in surface antigen expression during different phases of the cell cycle.

RESULTS

Mab 1H10 expression. Figure 1A shows that resting cells in the G_0 and G_1 phases of the cell cycle have a normal diploid quantity of DNA. During the G_2/M phase, the DNA content doubled, while in the synthesis phase there was an intermediate quantity. Thus, a characteristic double-peak histogram for replicating cells was depicted graphically by the flow cytometer (Fig. 1A). Mab 1H10 binding to CaSki cells was measured by indirect FITC immunofluorescence (Fig. 1B). FITC green fluorescence (FL1) was detected simultaneously with propidium iodide's red fluorescence (FL2) and there was no significant difference in surface antigen expression during different phases of the cell cycles (Fig. 1C).

Mab 1H10 binding to xenografted tumor sections. CaSki tumor xenografts were removed from athymic mice, sectioned, and stained with Mab 1H10

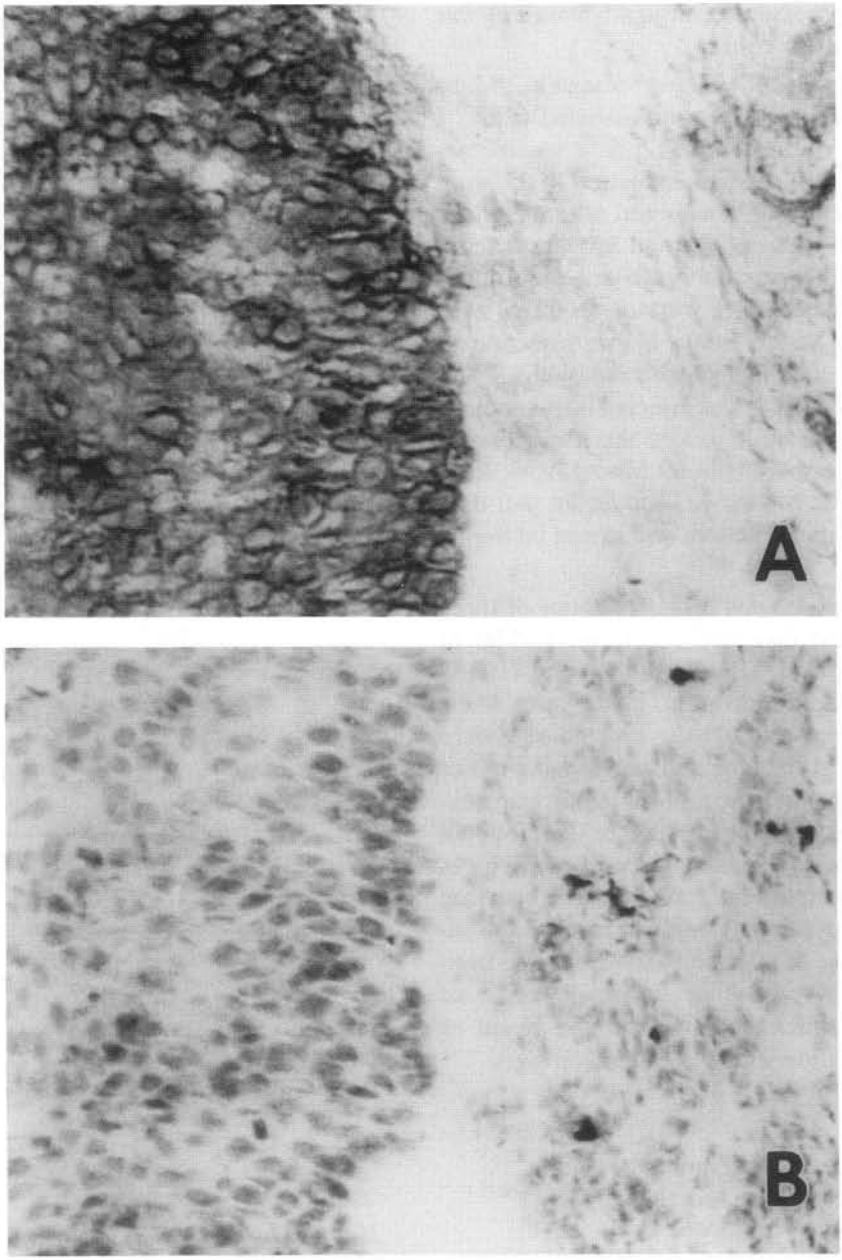

FIG. 2. Direct immunoperoxidase staining of tissues obtained from xenografted CaSki tumors by (A) Mab 1H10 F(ab′)$_2$-peroxidase conjugate and (B) Mab HK-PEG-1 (control) F(ab′)$_2$-peroxidase. Hematoxylin counterstaining of nuclei was observed on normal adjacent tissue (A and B, right) and tumor tissue incubated with control immunoconjugate (B, left), but only tumor tissue incubated with Mab 1H10 F(ab′)$_2$-peroxidase showed a positive membrane reaction (A, left).

F(ab′)$_2$-peroxidase or control Mab HK-PEG-1 F(ab′)$_2$-peroxidase conjugates. Specific binding of Mab 1H10 F(ab′)$_2$-peroxidase was observed on CaSki tumor cells but not on surrounding normal tissue (Fig. 2A). No staining of CaSki tumor xenografts or normal tissue with control IgG_3 antibody–peroxidase conjugate was observed (Fig. 2B).

In vivo detection of cervical carcinoma. Figure 3 shows that iv injected [^{131}I]1H10 F(ab′)$_2$ specifically accumulated in CaSki xenografts, especially 48–96 hr postinjection at which time radiolabeled Mab 1H10 F(ab′)$_2$ allowed clear visualization of human cervical tumors. Blood pool images of lung, heart, or liver were not prominent compared with high-intensity imaging of tumors. In contrast, [^{131}I]1H10 F(ab′)$_2$ was unable to image H2669 melanoma xenografts, which do not express Mab 1H10 antigen, suggesting that Mab 1H10 accumulation in CaSki tumors was mediated by specific antibody–antigen interactions rather than by nonspecific uptake due to changes in blood flow at the tumor implantation site.

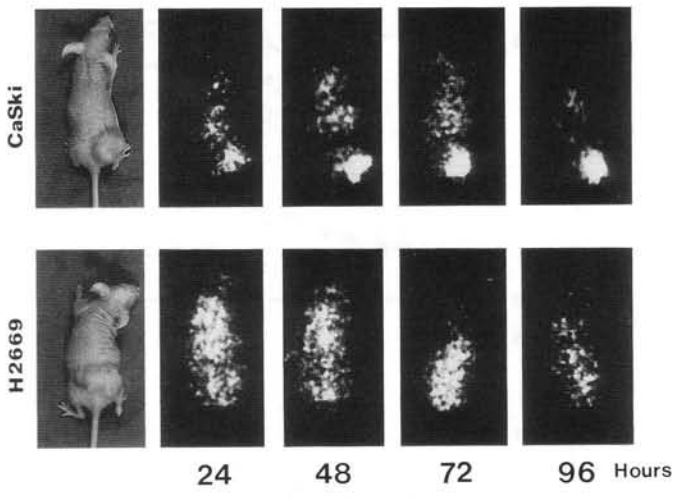

FIG. 3. Nuclear imaging by [^{131}I]1H10 F(ab')$_2$ of nude mice bearing human tumor xenografts. Left photographs illustrate the location of tumors. Subsequent images are of mice bearing CaSki cervical (top) or H2669 melanoma tumors (bottom) at 24, 48, 72, and 96 hr after iv administration of [^{131}I]1H10 F(ab')$_2$.

In vivo distribution of Mab 1H10 F(ab')$_2$. Cervical carcinoma tumors showed a higher uptake of radioactivity than normal tissues at all times (Fig. 4). The ratio of radioactivity in normal tissue compared to tumor tissue decreased throughout the experiment, indicating that Mab 1H10 F(ab')$_2$ cleared from normal tissue more rapidly than from tumors (Table 1). Normal tissue uptake appeared to be related to blood circulation and urine pooling. For example, at 48 hr, the highest tissue/tumor ratios were found in the lung, liver, kidney, and bladder. Tissues not associated with the blood and urine pools, such as muscle and uterus, took up relatively little Mab. The very low antibody uptake in the brain is likely due to the blood–brain barrier. Table 2, listing the localization index, provides further evidence of specific localization. Analysis of localization indices shows that there was an increasingly selective uptake of Mab 1H10 F(ab')$_2$ by CaSki tumors with increasing time; after 96 hr, there was 17 times more Mab 1H10 F(ab')$_2$ than HK-PEG-1 F(ab')$_2$ taken up by CaSki tumors. Twenty-four hours after injection of antibody, the localization index was greater than one for several organs including liver, gut, kidney, and bladder. This may have been due to a greater nonspecific uptake of Mab 1H10 by these organs. Alternatively, faster clearance of Mab 1H10 from the blood pool compared to Mab HK-PEG-1 would also result in elevated localization indices. The localization indices for normal tissue, however, approached unity at 96 hr, indicating that normal tissue uptake of both Mab 1H10 and HK-PEG-1 were equivalent at this time.

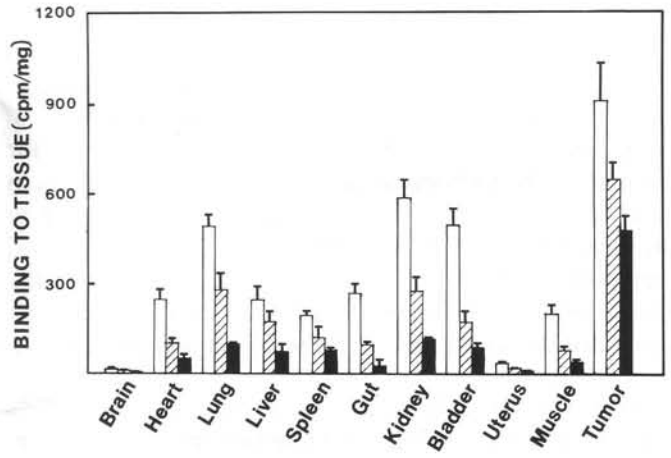

FIG. 4. Biodistribution of [^{131}I]1H10 F(ab')$_2$ in nude mice bearing CaSki cervical tumors (□) 24 hr; (▨) 48 hr; (■) 96 hr. Results represent the mean of three mice and error bars show the SD.

TABLE 1
Tissue Specificity of Radiolabeled Mab 1H10 F(ab')$_2$ Localization in Tumor-Bearing Nude Mice

	[^{131}I]Mab 1H10 F(ab')$_2$ (Tissue/tumor)[a]		
Tissue	24 hr	48 hr	96 hr
Tumor[b]	1.00	1.00	1.00
Brain	0.016 ± 0.004	0.015 ± 0.001	0.008 ± 0.007
Heart	0.269 ± 0.004	0.157 ± 0.014	0.108 ± 0.023
Lung	0.539 ± 0.035	0.427 ± 0.055	0.208 ± 0.013
Liver	0.267 ± 0.015	0.265 ± 0.033	0.149 ± 0.045
Spleen	0.211 ± 0.010	0.183 ± 0.042	0.168 ± 0.008
Gut	0.292 ± 0.012	0.150 ± 0.010	0.052 ± 0.002
Kidney	0.640 ± 0.015	0.416 ± 0.046	0.244 ± 0.015
Bladder	0.540 ± 0.034	0.260 ± 0.038	0.183 ± 0.019
Uterus	0.036 ± 0.005	0.026 ± 0.003	0.022 ± 0.002
Muscle	0.217 ± 0.005	0.119 ± 0.015	0.078 ± 0.016

Note. Groups of three mice bearing established CaSki tumors received 100 μCi [^{131}I]Mab 1H10 F(ab')$_2$ by iv injection at 0 hr. Mice were sacrificed at 24, 48, and 96 hr and specific radioactivity of tissues was determined.

[a] Values are means of three mice at each time point ± SD and were calculated from (cpm/mg tissue)/(cpm/mg tumor).

[b] Mean values of [^{131}I]Mab 1H10 F(ab')$_2$ uptake in tumors at 24, 48, and 96 hr were 902 ± 125, 637 ± 61, and 467 ± 51 cpm/mg, respectively.

Cervical carcinoma therapy with [^{131}I]1H10 F(ab')$_2$. Figure 5A shows that three iv injections of radiolabeled Mab 1H10 F(ab')$_2$ effectively suppressed CaSki tumor growth while tumors continued to grow in mice that received radiolabled Mab HK-PEG-1 F(ab')$_2$, free ^{131}I or human serum albumin. Tumors in mice treated with [^{131}I]1H10 F(ab')$_2$ were significantly smaller ($P < 0.05$) than tumors in mice treated with [^{131}I]HK-PEG-1 F(ab')$_2$; on Day 47, mean tumor size in [^{131}]1H10 F(ab')$_2$-treated mice was 66 mm^3 compared to 770 and 1330 mm^3 in [^{131}I]HK-PEG-1 F(ab')$_2$ and human serum albumin treated mice, respectively. Radiolabeled Mab 1H10

TABLE 2
In Vivo Distribution of [^{131}I]1H10 F(ab′)$_2$ Compared to [^{131}I]HK-PEG-1 F(ab′)$_2$

Tissue	Localization index[a]		
	24 hr	48 hr	96 hr
Blood	1.00	1.00	1.00
Brain	0.92 ± 0.18	0.91 ± 0.19	0.52 ± 0.42
Heart	1.43 ± 0.19	1.01 ± 0.15	1.02 ± 0.17
Lung	1.90 ± 0.16	1.50 ± 0.09	1.53 ± 0.22
Liver	2.96 ± 0.09	1.51 ± 0.20	1.35 ± 0.21
Spleen	2.80 ± 0.70	1.78 ± 0.31	2.73 ± 0.17
Gut	4.94 ± 0.87	2.18 ± 0.40	1.17 ± 0.25
Kidney	2.26 ± 0.30	1.41 ± 0.05	1.05 ± 0.08
Bladder	4.67 ± 0.58	1.42 ± 0.12	1.33 ± 0.16
Uterus	0.90 ± 0.24	0.93 ± 0.57	1.49 ± 0.60
Muscle	1.34 ± 0.03	1.46 ± 0.26	1.63 ± 0.13
Tumor	10.83[b] ± 1.23	12.64[b] ± 4.17	17.53[b] ± 8.42

Note. Groups of three athymic mice bearing CaSki tumor xenografts were injected iv with 100 μCi [^{131}I]1H10 F(ab′)$_2$ or control IgG$_3$ F(ab′)$_2$ and radioactivity was compared at different times.

[a] Localization index represents a normalized ratio of Mab 1H10 binding compared to control Mab binding in tissues and was calculated as described under Materials and Methods. Results represent the mean ratios ± SD obtained using three mice for each Mab.

[b] Significantly ($P < 0.05$) different from normal tissues using the Student *t* test.

F(ab′)$_2$ appeared to be nontoxic to treated mice; average body weight of Mab 1H10-treated mice was not significantly different from that of control mice (Fig. 5B).

Nontreated CaSki tumors appeared as nonkeratinized, large cell, squamous cell carcinoma (Fig. 6A). After [^{131}I]Mab 1H10 F(ab′)$_2$ treatment (Week 3), tumor nuclei exhibited pyknosis and karyolysis. Extensive tumor necrosis was also evident (Fig. 6B).

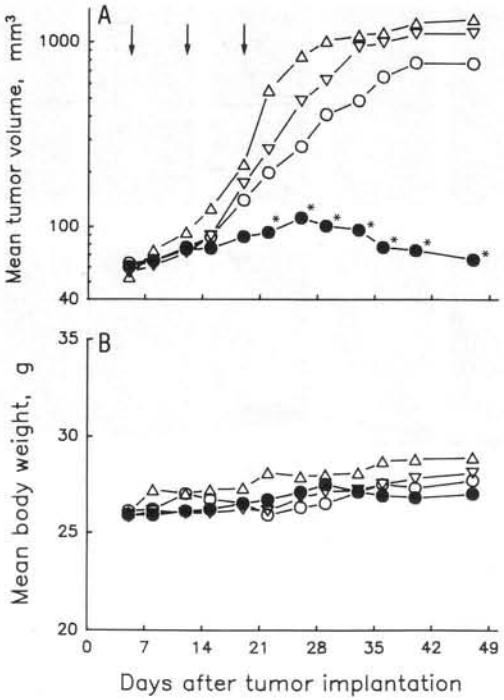

FIG. 5. Radioimmunotherapy of athymic mice bearing CaSki cervical tumors. Mice were injected sc in the right flank with 10^7 CaSki cells on Day 1. On Days 5, 12, and 19, groups of five mice received iv injections of human serum albumin (△) or 100 μCi [^{131}I]1H10 F(ab′)$_2$ (●), [^{131}I]HK-PEG-1 F(ab′)$_2$ (○), or free ^{131}I (▽). (A) Mean tumor volumes and (B) mean body weight of each group are shown. Significant differences in tumor size between [^{131}I]HK-PEG-1 F(ab′)$_2$ (control) and [^{131}I]1H10 F(ab′)$_2$ treated mice are indicated (*$P < 0.05$).

DISCUSSION

In this study, we have reported results of the reaction of Mab 1H10 F(ab′)$_2$ with a surface antigen expressed on human cervical tumors which may be clinically useful for localization or therapy of human cervical carcinoma. We found that the expression of Mab 1H10 antigen on CaSki cervical carcinoma cells is independent of the cell cycle. We also showed that Mab 1H10 binding to tumor antigen can be determined by direct tissue immunoperoxidase staining. In addition, radiolabeled Mab 1H10 F(ab′)$_2$ proved to be an excellent antibody for cervical carcinoma localization and *in vivo* therapy using a nude-mouse model.

Tumor heterogeneity is a serious impediment to the successful application of Mabs for diagnosis and therapy. Heterogeneity may exist not only within the primary tumor, but within metastasis, which presumably arose from a single cell from the primary tumor. However, some antigenic heterogeneity is related to the cell cycle [19]. These observations suggest that an ideal tumor antigen would be cell-cycle independent. Mab 1H10 antigen is strongly expressed on the tumor cell surface during all phases of the cell cycle. Thus Mab 1H10 might be expected to give maximum binding to cervical carcinoma cells independent of time.

A conjugate formed by linking horseradish peroxidase to Mab 1H10 F(ab′)$_2$ was found to specifically bind to CaSki tumor tissue but not to adjacent normal tissue *in situ*. Although still in the early stages of cervical carcinoma Mab research, various Mabs have been used for the immunohistochemical detection of tumor markers in cervical smears and cervical tissues [20–22] in order to improve the specificity and sensitivity of the conventional diagnostic technique. The specificity and reactivity of Mab 1H10 with human cervical carcinoma tissues has been described elsewhere [9]. In this study, immunohistochemistry studies were used to confirm expression of Mab 1H10 antigen on CaSki cells *in vitro* and *in vivo* because conflicts have been reported [23]. Direct immunoperoxidase staining is rapid and simple and may be a convenient technique for testing cervical smears or for tissue diagnosis.

Mab 1H10 F(ab′)$_2$ was shown in a nude-mouse model

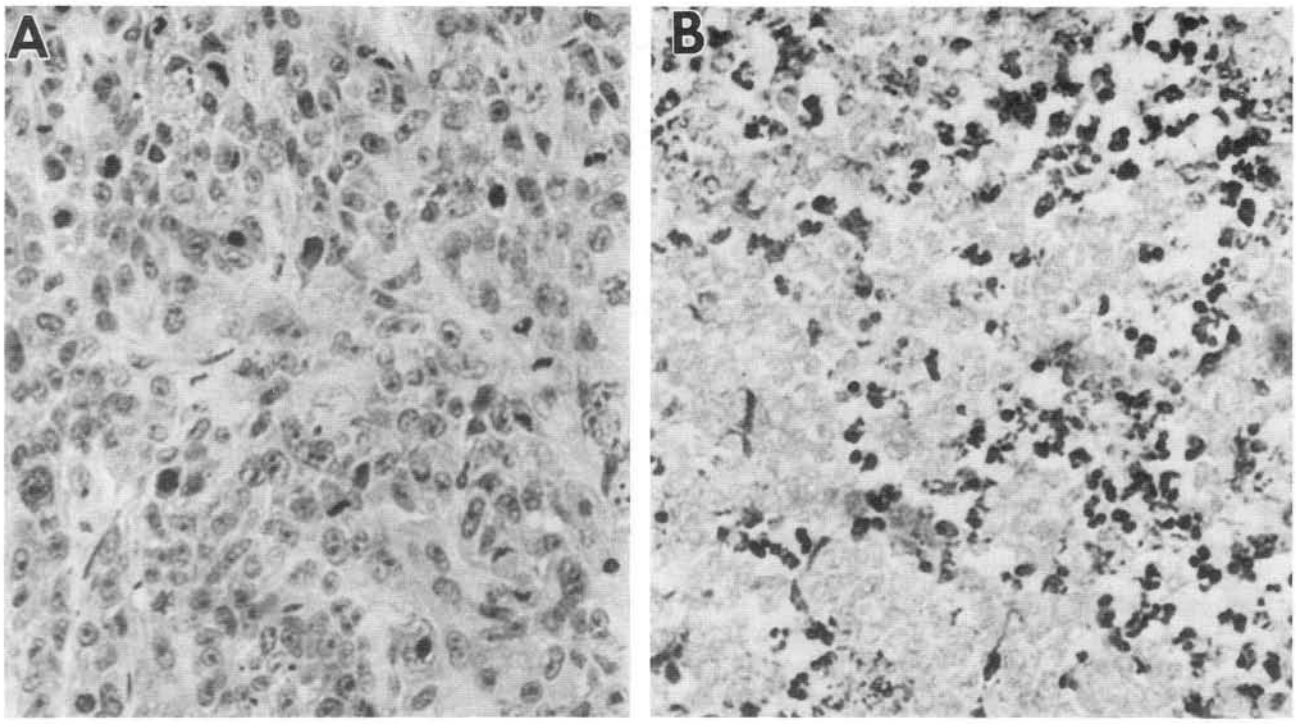

FIG. 6. Morphology of CaSki tumors before and after radioimmunotherapy. CaSki tumors were removed from athymic mice on Day 1 (A) or Day 30 (B) after treatment with three fractions of 100 μCi [^{131}I]1H10 F(ab')$_2$ (B). Sections were stained with hematoxylin and examined at 400× magnification.

to be a promising vehicle for *in vivo* cervical carcinoma localization and imaging. The F(ab')$_2$ fragment of Mab 1H10 was used rather than whole IgG for several reasons. First, the purpose of radiolabeled antibody is to quickly reach the target and react with the tumor cells. F(ab')$_2$ fragments should be superior to intact antibody, since the half life of the F(ab')$_2$ in the blood circulation is shorter than intact immunoglobulin [24]. Second, murine antibodies are known to elicit anti-mouse antibody responses in humans [25]. The Fc portion of IgG is believed to be the most immunopotent region of the immunoglobulin and its removal may reduce some of its immunogenecity in humans. Finally, removal of the Fc portion should also prevent nonspecific Fc-receptor binding on normal cells and toxic effects induced by activation of complement. In our imaging studies, CaSki tumors were clearly visualized by Day 3 with [^{131}I]1H10 F(ab')$_2$. Melanoma tumors which do not express the Mab 1H10 antigen were not imaged.

The differential uptake of [^{131}I]1H10 F(ab')$_2$ between tumor and normal tissues became more pronounced over time, indicating that normal tissue binding decreased faster than tumor binding. Greatest changes were seen in the lung, kidney, and bladder. Radiolabeled antibody reached the tumor via blood circulation and most antibody appeared to be excreted through the urinary system. Thus, blood-enriched organs such as the lung and liver and urine pooling organs such as the kidney and bladder showed elevated background radioactivity during the first 48 hr after antibody injection. Mab 1H10 F(ab')$_2$ accumulation in CaSki tumors was specific as shown by the significantly higher localization index for CaSki tumors compared to that of normal tissue ($P < 0.05$). The difference in indices between tumor and blood also suggest that 1H10-defined antigen is not shed into serum. This is important for radioimmunoscintigraphy where circulating antibody can elevate blood pool activity and interfere with imaging.

The specific localization of radiolabeled Mab 1H10 F(ab')$_2$ in cervical carcinoma xenografts encouraged us to attempt tumor radioimmunotherapy with [^{131}I]1H10 F(ab')$_2$. Three iv injections of 100 μCi [^{131}I]1H10 F(ab')$_2$ suppressed the growth of established CaSki cervical tumors. Tumor suppression by radiolabeled Mab 1H10 F(ab')$_2$ was specific as tumors continued to grow in mice treated with [^{131}I]HK-PEG-1 F(ab')$_2$, free ^{131}I, or human serum albumin. Conjugates formed by linking Mab 1H10 F(ab')$_2$ with *Pseudomonas* exotoxin [10] or doxorubicin [11] have also been shown to suppress the growth of established CaSki tumors in nude mice. Radiolabeled Mab 1H10, however, may possess advantages over these conjugates for therapy of cervical carcinoma in humans.

Treatment with *Pseudomonas* exotoxin or doxorubicin conjugates required eight doses to obtain the same tumor suppression afforded with three fractions of [^{131}I]1H10 F(ab′)$_2$. [^{131}I]Mab 1H10 toxicity was also minimal, in contrast to treatment with *Pseudomonas* exotoxin conjugates in which several mice died during therapy [10]. In addition, while toxin or drug conjugates should only be effective against tumor cells that express tumor-associated antigen, radiolabeled Mab should also be able to kill surrounding cells present in heterogeneous tumor-cell populations. [^{131}I]1H10 F(ab′)$_2$ (110 kDa) is also much smaller than Mab 1H10 F(ab′)$_2$ linked to *Pseudomonas* exotoxin (176 kDa) or doxorubicin (170 kDa) which should allow more extensive tumor penetration [26]. Anti-conjugate immune responses should also be minimal for radiolabeled antibody compared with toxin or drug conjugates. Targeting isotopes directly to the cancer site may also reduce the possibility of developing second cancer after conventional radiation therapy [27].

In summary, [^{131}I]1H10 F(ab′)$_2$ has been shown to localize in human cervical tumor xenografts in nude mice and cause tumor cell necrosis and suppression of tumor growth. Mab 1H10 appears to possess sufficient specificity to be applicable to the cytodiagnosis and histodiagnosis of cervical cancer. Studies aimed at staging and early detection of recurrent cervical cancer are now in progress. In order to use radioimmunotherapy for human cancer treatment, however, there are many questions that remain to be answered and are the focus of ongoing research in many laboratories around the world. The concept of targeted delivery of diagnostic or therapeutic agents is so promising that clinically beneficial immunoconjugates will likely be developed in the near future.

ACKNOWLEDGMENTS

We thank J. Co Ng for monoclonal antibody preparation, Y. H. Chen for antibody labeling. M. C. Wu for nude mice preparation, and S. I. Guan for nuclear imaging. This work was supported in part by National Science Council, Taipei, Taiwan, R.O.C. Grants NSC 80-0418-B016-03, 81-0418-B016-02 and Academia Sinica, R.O.C.

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