Highly Tumor-reactive, Internalizing, Mouse Monoclonal Antibodies to Le\(^+\)-related Cell Surface Antigens\(^1\)

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ABSTRACT

Two monoclonal antibodies, designated BR64 (IgG1) and BR96 (IgG3), were generated that, according to immunohistology, bind selectively to carcinomas of the colon, breast, ovary, and lung. They have no or very low reactivity with normal human tissues, except for the fact that BR64 stains capillaries in the hearts from certain normal donors and that both monoclonal antibodies stain some epithelial cells from the gastrointestinal system, including stomach. Preliminary studies indicate that at least a portion of the epitope recognized by BR64 and BR96 is a \(\text{Le}^+\) carbohydrate chain. Both monoclonal antibodies can be "internalized" by antigen-positive tumor cells, since immunconjugates with ricin A-chain are cytotoxic. BR96 has the additional properties of being cytotoxic by itself, and it can mediate antibody-dependent cellular cytotoxicity and complement dependent cytotoxicity.

INTRODUCTION

MAb\(^1\) to human tumor-associated differentiation antigens offer promises for the "targeting" of various antitumor agents such as radioisotopes, chemotherapeutic drugs, and toxins (1-3). In addition, some MAb have the advantage of killing tumor cells via ADCC or CDC in the presence of human effector cells or serum (4), and there are even a few MAb that have a direct antitumor activity which does not depend on any host component (5).

Although hundreds of antitumor MAb have been described, there continues to be a need for MAb that have a high degree of reactivity with most common human neoplasms and low reactivity with normal cells. The need is particularly great for tumor-selective MAb that can "internalize" after they have bound to cell surface antigens, since such MAb are more effective carriers into the cytoplasm of toxins, chemotherapeutic drugs, and other agents directly linked to them. Although there are many MAb to lymphocyte antigens that internalize, only a few MAb to solid tumors have that property, with perhaps the best example being MAb to the transferrin receptor (6), which, unfortunately, has a low tumor specificity.

We report here on two mouse MAb, BR64 and BR96, that bind to a \(\text{Le}^+\) antigen, according to preliminary characterization with an ELISA assay. While several MAb described in the past show similar binding characteristics (7-11), both BR64 and BR96 have some special features. First, they have high tumor selectivity. Second, they both internalize. Third, BR96 has a direct cytotoxic effect on antigen-positive tumor cells, and it mediates ADCC and CDC. For these reasons, both BR64 and BR96 are of interest as candidates for clinical use.

MATERIALS AND METHODS

Control Antibodies. L6 is a MAb that binds to cells from most human carcinomas (10). It does not internalize.\(^4\) BR6 is an internalizing MAb to a protein antigen expressed at the surface of cells from many human carcinomas, including breast carcinoma;\(^3\) it was used as a control MAb in some experiments. P1.17 is a noninternalizing IgG2a mouse myeloma protein and does not bind to any human cells. It is available from American Type Culture Collection. MAb 24.1.1, which binds to the CALLA antigen (not expressed on carcinoma cells), was obtained from Dr. J. Ledbetter (ONCOGEN).

Tumor Lines. H3396 (referred to here as 3396) is a cultured line established from a metastasis of a human breast adenocarcinoma, which was explanted and maintained in culture at ONCOGEN. The 3396 line was used as an immunogen to obtain BR64 and BR96 and as a target for many of the tests described in this paper. Other cell lines, most of which were also established at ONCOGEN, are listed in the tables and figures. They were derived by culturing tissues from carcinomas of the lung, colon, or breast, from melanomas, or from lymphomas of B- or T-cell origin.

Establishment of Hybridomas. A previously described technique (12) was used. Briefly, 3-month-old BALB/c mice were immunized repeatedly with cells from carcinoma 3396, and their spleen cells were hybridized with NS-1 (for BR64) or P2x63-Ag 8.653 (for BR96) mouse myeloma cells. The initial selection of hybridomas was based on supernatant reactivity on 3396 cells but not on cultured human fibroblasts, when tested with an ELISA assay (13).

Hybridomas which produced MAb that could bind to 3396 cells but not to fibroblasts were cloned, expanded \textit{in vitro}, and further tested by immunohistology for MAb specificity. Those hybridomas that produced MAb that were preferentially reactive with cells from several different carcinomas without showing strong reactivity to normal cells were reclone, expanded, and again tested for specificity. Hybridoma cell lines BR64 and BR96 were both obtained by this procedure. They were injected into mice to develop ascites tumors. MAb secreted into the ascites were purified on Protein A-Sepharose (14) or by gel filtration on Sephadryl S-300. Purified MAb were used for all the experiments described in this paper.

Isotype Determination. Two different standard techniques were utilized to determine the class of immunoglobulin produced by the BR64 and BR96 hybridomas, \textit{i.e.}, Ouchterlony immunodiffusion with rabbit antibodies to various mouse immunoglobulin isotypes (Southern Biotechnology, Birmingham, AL) and ELISA isotyping using rabbit antisera raised to various mouse immunoglobulin isotypes and coupled to peroxidase (Zymed, South San Francisco, CA). Based on these procedures, it was determined that BR64 is an IgG1 and that BR96 is an IgG3.

Immunohistology. The PAP technique of Sternberger (15) was used, as modified by Garrigues \textit{et al.} (16). Frozen sections were prepared and treated with acetone. To decrease nonspecific background, sections were preincubated with normal human serum diluted 1/5 in PBS. The mouse MAb to be tested, rabbit anti-mouse IgG, and mouse PAP were diluted in medium containing 10% normal human serum and 3% rabbit serum. Rabbit anti-mouse IgG (Sternberger-Meyer Immunochemicals, Inc., Jarettsville, MD) was used at a dilution of 1/50. Mouse PAP (Sternberger-Meyer Immunochemicals) containing 2 mg/ml specifically purified PAP was used at a dilution of 1/80.

The slides were evaluated under code and regularly checked by an independent investigator, and typical slides were photographed by using differential interference contrast optics (Zeiss-Nomarski). The data are

\(^{1}\) I. Hellström et al., unpublished findings.

\(^{2}\) H. Jacques Garrigues, unpublished findings.

\(^{3}\) H. Jacques Garrigues, unpublished findings.

\(^{4}\) Hellström, unpublished findings.

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\(^3\) The abbreviations used are: MAb, monoclonal antibody(ies); ADCC, antibody-dependent cellular cytotoxicity; CDC, complement-dependent cytotoxicity; FACS, fluorescence-activated cell sorter; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; Fuc, fucose; Gc(Nac, N-acetylgalactosamine; Gal, galactose; RA, ricin A-chain; IMDM, Iscove's modified Dulbecco's medium.

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Lea-RELATED ANTIGEN MAb
expressed as positive, which means that at least one third of the cells
stained, or negative. No staining at all of normal cells was observed,
unless otherwise indicated. Both neoplastic cells and stroma cells were
observed in tumor samples and only the former were stained.

Binding Assays. Recognition of cell surface antigens by a MAb was
also identified by immunofluorescence, using a Coulter Epics C FACS
as previously described (10). The mean fluorescence intensity was
determined and the LFE was calculated from the mean fluorescence
intensity. The LFE of each test sample divided by the LFE of a negative
control gave a ratio of the brightness of cells stained by specific versus
control antibody.

Antigen Characterization. Coded samples of BR64 and BR96 were
sent to Dr. John Magnani at Biocarb (Gaithersburg, MD), where they
were screened against purified glycolipids having known carbohydrate
structures. The glycolipids were dried from methanol in microtiter wells
at 100 ng/well. Purified MAb were assayed at a concentration of 10
µg/ml in 0.01 M Tris-HCl, pH 7.4, containing 1% bovine serum
albumin. The binding of the MAb to immobilized glycolipids was tested
by an ELISA assay, and absorbance values were calculated as the
average of duplicate wells.

Internalization of BR64 and BR96. To determine whether BR64 or
BR96 could be internalized by carcinoma cells, immunoconjugates with
ricin A-chain were prepared. Uptake of the conjugate by the carcinoma
cells was assessed by determining to what extent the tumor cells were
killed by ricin A-chain.

Conjugation of a MAb to ricin A-chain was carried out as follows.
Deglycosylated ricin A-chain (Inland Labs, Austin, TX) (17) was treated
with dithiothreitol (5 IHM) prior to gel filtration on G-25 Sephadex,
cells was assessed by determining to what extent the tumor cells were
harvested (2:1 molar ratio to the MAb in PBS, the MAb having been previously modified with N-
succinimidyl-3-(2-pyridyldithio)propionate (Pierce, Rockford, IL) ac
according to the procedure of Lambert et al. (18). The reaction was
allowed to proceed for 12–24 h at room temperature, after which the
solution was diluted with 1 volume of H2O. Unconjugated MAb was
removed by using Blue Sepharose CL-6B (Pharmacia, Uppsala, Swe-
den) (19).

The conjugate and excess ricin A-chain were eluted with high salt
(10 × PBS) and subjected to further purification on Sephacryl-300
(Pharmacia) using PBS as eluant. The resulting conjugate was free of
unbound MAb or ricin A-chain and consisted mostly of 1:1 adducts.
The internalization of a MAb-RA conjugate by carcinoma cells was
measured using a thymidine uptake-inhibition assay. According to this
assay, the inhibition of [3H]thymidine incorporation into cellular DNA
is a measure of the cytotoxic effect of the given conjugate and reflects
the internalization of ricin A-chain by the cells.

Carcinoma cells were plated into a 96-well microtiter plate at 10⁴
cells/well, in 150 µl of IMDM medium containing 10% FBS. After the
cells had attached by overnight incubation, the BR64-RA or BR96-RA
immunotoxin (100 µl) was added in 10-fold dilutions to a final concen-
tration of 10 to 0.01 µg/ml. The reaction mixture was incubated for 6
h at 37°C in a 5% CO₂ incubator, after which [3H]thymidine were added
in 1 µCi/well and the plate was incubated for another 6 h at 37°C.
Subsequently, the plate was frozen at −70°C for at least 1 h
and thawed in a gel dryer for 15 min, and the cells were harvested onto
glass filters and counted in a beta-counter. The results were expressed
as the percentage of inhibition of [3H]-
thymidine incorporation into target cells treated with MAb BR96, as
compared to no antibody.

Tests for ADCC. Tests were carried out as previously described (4,
22), labeling target cells with 32Cr and exposing them for 4 h to human
lymphocytes and the MAb to be tested. The release of 32Cr from the
target cells was measured as evidence of tumor cell lysis (cytotoxicity).
Controls included the incubation of target cells alone or with either
lymphocytes or MAb separately. ADCC was calculated as the percent-
age of killing of target cells observed with MAb plus effector cells, as

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Number positive/number tested</th>
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<tr>
<td>BR64</td>
<td>BR96</td>
</tr>
<tr>
<td>Tumors</td>
<td></td>
</tr>
<tr>
<td>Lung carcinoma (non-small cell)</td>
<td>7/10</td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td>9/13</td>
</tr>
<tr>
<td>Colon carcinoma</td>
<td>12/14</td>
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<tr>
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<td>1/2</td>
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<tr>
<td>Endometrial carcinoma</td>
<td>3/3</td>
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<tr>
<td>Melanoma</td>
<td>0/3</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>0/5</td>
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<tr>
<td>Stomach carcinoma</td>
<td>3/3</td>
</tr>
<tr>
<td>Pancreatic carcinoma</td>
<td>3/2</td>
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<tr>
<td>Oesophagus carcinoma</td>
<td>3/2</td>
</tr>
<tr>
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<td>1/2</td>
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<tr>
<td>Normal tissues</td>
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<tr>
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</table>

* Independent testing by Dr. Willingham at NIH showed that capillaries in 3
  of 6 samples of heart stained with BR64 (they did not stain with BR96).
* Small population of the cells were positive.
* Acinar cells positive.
* Epithelial cells positive.
* Surface epithelial cells positive, basal cells negative.
compared to target cells being incubated alone. Several “criss-cross” experiments were done, in which carcinoma and melanoma cells were tested in parallel with MAb Br96 and the antimelanoma MAb MG-21, which does not bind to most carcinoma cells (22).

Tests for CDC. Our procedures, which have been published (4, 22), were identical to those used for the ADCC assays except that human serum (as a source of complement), diluted 1/3 to 1/6, was added in place of the effector cells.

RESULTS

Immunohistology

Table 1 summarizes data from immunohistological studies on frozen sections of malignant neoplasms (most of which were metastatic) and normal tissues. As shown in the table, both BR64 and BR96 reacted with the majority of carcinomas tested, including most carcinomas of the lung, breast, and colon, as well as carcinomas of the ovary, endometrium, stomach, pancreas, and esophagus. The staining was seen at the cell surface and was often, but not always, uniform. Sarcomas were not stained by either MAb and 0 of 8 melanomas were stained by BR64, as compared to 2 of 5 melanomas stained by BR96. Normal tissues, including blood, did not display any detectable staining except for the following. First, epithelial cells from the stomach and esophagus were stained by both MAb, as were acinar cells of the pancreas and scattered cells from the testes and tonsils. Second, cells from salivary glands and Paneth cells in the duodenum stained with both MAb when similar studies were performed by Dr. M. Willingham at the National Institutes of Health. Third, BR64 stained capillaries from 3 of 6 samples of normal heart when tested at the NIH, while no such staining was seen with 3 hearts studied at ONCOGEN. BR96 did not stain heart capillaries when studied either at ONCOGEN or at the NIH. Experiments are ongoing to verify whether the reactivity of BR64 with heart capillaries correlated with the expression of Lewis blood group antigens. There was no variation in the reactivity to various epithelial cells from organs such as stomach, esophagus, or pancreas when BR64 and BR96 were tested with normal tissues from different individuals.

Fig. 1 illustrates the staining of cells from a colon carcinoma by BR64 and the lack of staining of normal liver. Note that BR64 only stains the tumor cells, while the surrounding connective tissue is unstained, and that the staining of tumor cells is rather homogeneous. Formalin-fixed, paraffin-embedded sections of carcinomas could be stained equally well as frozen sections. The results with BR96 and BR64 were similar, with the exceptions shown in Table 1 and commented on above.

Benign tumors have not been studied with MAb BR64 or BR96 but will be included as part of an ongoing investigation of benign and malignant lesions of the esophagus. Likewise, further work is needed to establish whether primary and metastatic tumors vary in their reactivity to BR64 and BR96; the neoplasms studied so far have primarily been metastatic.

FACS Analysis of Live Cells

Fluorescein-conjugated MAb were incubated with viable cells from cultures of a variety of carcinomas, as well as T- and B-
UNRELATED ANTIGEN MAb

B »I t/rnchoim hn»P3HR-1

cell lymphoma

T c*ll lymphoma

MOLT-4

CM

Melanoma 2669

36?0

3806

Colon carcinomi

lin* C

HTC116

3600

3347

3619

kn* RCA

3630

MCF-7

Fig. 2. FACS analysis of the binding of BR64 and BR96 to cells grown in vitro. Various tumor cell lines were stained with MAb BR64 (•) and BR96 (G) (100 μg/ml) at a concentration of 10 μg/ml, followed by a fluorescein isothiocyanate-labeled secondary antibody. A ratio between the brightness (LFE) of cells stained by specific versus control antibody was determined on a FACS.

Preliminary Characterization of the Antigens to Which BR64 and BR96 Bind

Coded samples of BR64 and BR96 were sent to Dr. John Magnani (Biocarb, Gaithersburg, MD), who analyzed them with an ELISA assay for binding to known glycolipid antigens; this assay has been routinely used by Dr. Magnani for characterizing large numbers of antitumor MAb from different laboratories. According to this analysis (Fig. 3), BR64 bound specifically to plated Le' and H2 antigens (H2 has the same structure as Le' but lacks an internal fucose α1–3), while BR96 reacted with Le' and not with H2. Dr. Magnani concluded from these preliminary data that both MAb recognize a Le' antigen and that fucose α1–3 is part of the epitope recognized by BR96.

Killing of Carcinoma Cells by Immunotoxin as Evidence for MAb Internalization

Experiments were performed to determine if MAb BR64 or BR96 could be internalized by antigen-positive carcinoma cells. Different tumor lines were exposed to MAb-ricin A immunoconjugates, and internalization of the MAb was evaluated by determining whether the immunotoxins could inhibit the incorporation of [3H]thymidine into DNA. We have grouped the data presented in Fig. 4 into three categories. Group a contains tumors which, according to the data in Fig. 2, have high binding ratios for both BR64 and BR96. These tumors were killed by both BR64 and BR96 immunoconjugates but not by a BR6-RA conjugate, which did not bind to the tumor cells from group a. Group b tumors bind both BR64 and BR96 but the binding ratios for BR96 are substantially higher than those for BR64. BR96-RA was inhibitory, while the effect of BR64-RA was borderline and the BR6-RA conjugate, which did not bind to the cells, did not kill. Group c comprises tumors which bind very small amounts of BR64 or BR96 but bind BR6 well. These tumors were not significantly affected by BR64-RA or BR96-RA, but they were killed by the BR6-RA immunoconjugate. The immunotoxins were thus cytotoxic in an antigen-specific manner.
Three lines of evidence suggest that BR96 has a cytotoxic effect that does not depend on complement or host effector cells. They will be discussed separately. No such effects were seen with BR64 when similarly tested.

Morphology. Carcinoma cells (3396) were treated with various concentrations of BR96 or with the control MAb 24.1.1, and the effect of the MAb on cellular morphology was assessed after 72 h of incubation. As shown in Fig. 5, 3396 cells treated with 30 μg MAb 24.1.1 were well spread and formed intercellular contacts characteristic of epithelia. On the other hand, cells treated with the same concentration of BR96 were round and displayed few cell to cell contacts, and the number of cells remaining attached to the slide was reduced. The morphological effects of BR96 were dependent on antibody concentration and were apparent even at 1 μg/ml.

Killing Detected by FACS Analysis. Suspended cells from a variety of carcinoma lines were incubated on ice for 30 min with BR96 or control MAb at several different concentrations. After washing, they were stained by propidium iodide so that dead cells could be identified by their red fluorescence when the suspensions were analyzed with the FACS. Cells which could bind BR96 and cells not binding BR96 were studied in parallel. Fig. 6A demonstrates that incubation of cells from any of three antigen-positive carcinomas with BR96 rapidly killed them. Untreated or antigen-negative cells were not killed, i.e., they were not stained by propidium iodide (data not shown).

Inhibition of Thymidine Incorporation. We next determined whether treatment of antigen-positive cells with BR96 affected their ability to incorporate [3H]thymidine into DNA, as a measure of their viability. Target cells which had been allowed to adhere for 3 h were exposed to various concentrations of BR96 for 72 h. Six h prior to the completion of the 72-h treatment, they were pulsed with [3H]thymidine. As shown in Fig. 6B, BR96 caused an inhibition of [3H]thymidine incorporation into antigen-positive cell lines, and this effect was dose dependent. The antigen-negative cell line HCT116 was not affected by any concentration of BR96. We have, in addition, used a growth inhibition assay, as described by Linsley et al. (23), and obtained results in agreement with those presented in Fig. 6B (data not shown).

ADCC Mediated by BR96

Fig. 7A summarizes experiments in which BR96 was tested for ADCC in the presence of human effector cells, using a 4-h 51Cr-release assay. The cytotoxicity by effector cells alone (“natural killer effect”) varied for different preparations of human lymphocytes form 6 to 41%; it has been subtracted from the data given in the figure. Cells from five different carcinoma lines, which all bound BR96, were killed via ADCC at MAb concentrations down to 0.1 μg/ml, while cells from a sixth carcinoma, which did not bind BR96, were not. The requirement for antibody binding to obtain ADCC was further demonstrated by the fact that both of the two carcinomas which could bind a
different MAb, L6 (3619 and 2987), were killed by L6 via ADCC, while the others were not. Under the conditions of the 4-h \(^{51}\)Cr assay, BR96 alone caused the release of only 1% of the label, even when tested at a concentration of 10 \(\mu\)g/ml. BR64 gave a low level of ADCC in some experiments (data not shown).

**CDC Mediated by BR96**

Fig. 7B summarizes experiments similar to those performed to measure ADCC but, instead of effector cells, using complement in the form of human serum. As shown in the figure, CDC against cells binding BR96 was seen at a MAb concentration of 0.1–5.0 \(\mu\)g/ml, while there was no CDC against the BR96 antigen-negative lines HCT116 and 3347. The 3347 cells could, however, be killed when using MAb L6, which binds to these cells. Controls were always included in which BR96 was tested in the absence of complement. No killing by BR96 alone was detected by the 4-h \(^{51}\)Cr-release assay. BR64 did not give CDC.

**DISCUSSION**

We have obtained two mouse MAb, BR64 and BR96, which recognize cell surface antigens abundantly expressed (up to \(10^6\) molecules/cell) on the majority of human carcinomas. Preliminary data indicate that both MAb bind to a Le\(^+\) \(\text{[Fuc}_{\alpha\text{1-2}}\text{Gal}_{\beta1-4}\text{Fuc}_{\alpha\text{1-3}}\text{GlcNAc]}\) antigen when tested in an ELISA and that BR64 (but not BR96) also binds to an H2 glycolipid.
Le-related Antigen Mab

(Fucα1-2Galβ1-4GlcNAc). The latter observation suggests that fucose attached to GlcNAc may form a portion of the Le-related epitope recognized by BR96.

Several MAb recognizing Le' antigens have been described in the past (7–9, 11, 24–29), including one, L15, obtained by our group (10). There are, however, some interesting novel features of BR64 and BR96. First, while they, like other antitumor MAb, bind to some normal cells, their selectivity for tumor, as established by immunohistology on frozen sections, is higher than that of most other MAb that we have tested in the past (10). Second, BR64 and BR96 conjugated to ricin kill antigen-positive tumor cells, and recent studies by electron microscopy using gold-labeled BR64 have proven that this MAb is actually internalized by antigen-positive tumor cells1; our data thus suggest that BR64 and BR96 can be used to target various antitumor agents conjugated directly to them. Third, BR96 is toxic to antigen-positive tumor cells, and it mediates both ADCC and CDC.

It is unclear why the tumor specificity of BR64 and BR96 is higher than that observed for many other MAb reacting with Le' antigens. Indeed, Le' antigens have been found in ovaries (30), uterus (41), intestine (9, 24, 32–35), RBC (36), granulocytes (7), and gastric mucosa (7), while, on the other hand, the expression of Le' antigens in heart endothelium from some individuals was not previously reported. The high tumor specificity of BR64 and BR96, as well as their abilities to internalize (not previously described for MAb to Le'), suggests that the two MAb recognize some complex epitopes, a portion of which includes the Le' antigen. While the avidity of both BR64 and BR96 is rather low, a low avidity is not uncommon for MAb to glycolipid antigens.

The fact that BR96 mediates strong ADCC and CDC is not unexpected, since it binds to a cell surface antigen and is an IgG3, an isotype which often mediates ADCC and CDC (e.g., see Refs. 4 and 32). It was unexpected, however, that BR96 is cytotoxic by itself when tested under several different conditions, since very few MAb with such a cytotoxic activity have been described, the most notable exception being a MAb to an antigen encoded by the neu oncogene (5). One may speculate that the epitope to which BR96 binds is a receptor involved in some key cellular function or that it is associated with such a receptor. Further work is needed to understand why the toxic effect of BR96 did not result in the release of 3Cr during a 4-h assay, while it caused the rapid uptake of propidium iodide as detected by FACS analysis.

The tumor specificity of BR64 and BR96 and their ability to internalize make them suitable candidates for various therapeutic approaches; as usual, however, any initial clinical trials must be conducted carefully, with attention to toxicity caused by binding to normal cells. The fact that BR96 mediates ADCC and CDC and has a direct toxic effect on antigen-positive tumor cells should offer additional benefits.

ACKNOWLEDGMENTS

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REFERENCES


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