Identification of a Monoclonal Antibody, TV-1, Directed against the Basement Membrane of Tumor Vessels, and Its Use to Enhance the Delivery of Macromolecules to Tumors after Conjugation with Interleukin 2

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ABSTRACT

mAbs reactive with epitopes expressed on tumor vessels were evaluated as universal delivery agents of peptides with vasoactive properties to enhance the uptake of macromolecules in tumors. Unlike other reported approaches to target tumor vessels, a mAb designated TV-1 targets a basement membrane antigen that is found in all tissues but that is accessible only in tumor vessels, making it an alternative vehicle for the delivery of biologically active peptides to tumors. A panel of 30 monoclonal and polyclonal antibodies was screened by immunohistochecmy on sections of human tumors, normal vascular endothelium, and connective tissues. Five antibodies were chosen for in vivo evaluation, including two anti-fibronecctin antibodies (TV-1, HFN 7.1), one anti-basic fibroblast growth factor antibody (anti-BFGF), and two antibodies reactive with a mesenchymal cell antigen (TP-1, TP-3). Three nude mouse tumor models characterized by varying degrees of vascularization (low to high) were used. After chemical conjugation to interleukin 2 (IL-2), these antibodies were used to pretreat tumor-bearing nude mice 3 h before injection with a radiolabeled mAb directed to the transplanted tumors. Pretreatment with TV-1/IL-2 or HFN 7.1/IL-2 produced a 3-fold higher tumor uptake of radiolabeled compared to control mice pretreated with mAb alone. The other three vasoactive immunoconjugates failed to show significant increases in these tumor models. When TV-1/IL-2 was compared with the specific vasoconjugate (Lym-1/IL-2) as a pretreatment in the Raji lymphoma model, which has low vascularization, TV-1/IL-2 yielded approximately 60% of the tumor uptake seen with Lym-1/IL-2. In comparison, pretreatment with TV-1/IL-2 in the LS174T colon carcinoma model, which has high vascularization, yielded approximately the same tumor uptake seen with the B72.3/IL-2 vasoconjugate, which directly targets the tumor cells. These studies demonstrate that a mAb directed against fibronecctin in the endothelial subcellular matrix can be used to deliver vasoactive agents to tumors.

INTRODUCTION

Radioimaging and biodistribution studies in animal tumor models have revealed that an extremely small percentage of the injected dose of radiolabeled mAbs (1–4%) accumulates in the tumor mass, despite the relatively large size of the tumor and the marked antigenic differences between the tumor and the host (1, 2). Likewise, patient studies have revealed that an even lower fraction of the injected dose (0.01–0.1%) can be shown to localize at the tumor site (3–5). Thus, only a small amount of radiolabeled antibody actually contributes to therapy, and the bulk of the radionuclide preparation is dispersed throughout the body where it is capable of producing dose-limiting toxicity.

Recognizing that the key factor for successful therapy is the amount of radiolabel delivered to the tumor with respect to normal tissues, our laboratory has attempted to generate new approaches to enhance antibody uptake in experimental tumor models. As schematically shown in Fig. 1, our initial focus was to develop methods that selectively alter the blood flow and/or vascular permeability of tumors because these important physiological factors control the egress of macromolecules from the vasculature into the tumor mass. Previous studies from our laboratory (2, 6) have shown that antibodies directed against tumor-associated cell surface antigens or intracellular antigens residing in degenerating tumor cells (Fig. 1, target B) can be used to deliver peptides with vasoactive properties such as IL-2, tumor necrosis factor α, and IL-1β to tumor xenografts in nude mice to alter the physiologic characteristics of tumor vessels without concomitant changes in normal tissues. These studies resulted in a 3–4-fold increase in the amount of radiolabel accumulating at the tumor site, with no noticeable changes observed in normal tissues and organs. Mechanism of action studies revealed that this increased localization to the tumor was due to changes seen in the blood volume, vasopermeability, or both, depending on which vasoactive agent was used. After these experiments, it was hypothesized that if vasoactive peptides could be delivered directly to tumor vessels (Fig. 1, target A) instead of tumor cells, this approach might be even more universally applicable, eliminating the need to target different tumor-associated antigens.

The challenge, therefore, was to identify an antigen present in tumor vessels that was not identified in the vasculature of normal tissues. Earlier observations that blood vessels in the vicinity of neoplasms are structurally abnormal (7–9) and show marked endothelial fenestration, revealing the underlying basement membrane and perivascular connective tissue, led to the hypothesis that an antibody against these normal vascular structures might localize preferentially to tumors, while sparing blood vessels in the rest of the body, where the intact endothelium would preclude access of injected antibody to the underlying vascular structures. A search, therefore, was initiated to identify a mAb that could preferentially target tumor vessels by this approach. As a universal carrier of potent vasoactive agents, this mAb could be used as a pretreatment to alter the physiology of tumor vessels and enhance the preferential accumulation of diagnostic and therapeutic radionuclides at the tumor site.

MATERIALS AND METHODS

Sources of Reagents

mAbs Lym-1 (IgG2a), recognizing a polymorphic variant of the HLA-Dr antigen on B cells and related malignancies, and TNT-1 (IgG2a), recognizing nuclear histone H1, were obtained from Techniclonne International, Inc. (Tustin, California). mAb B72.3 (IgG1), recognizing the tumor-associated glycoprotein TAG-72, was a gift from Celltech, Ltd. (Slough, United Kingdom). Recombinant IL-2 was provided by Amgen Corporation (Thousand Oaks, CA). Radioactive iodine (125I) was obtained as sodium iodide in 0.05 N sodium hydroxide solution (ICN Biomedicals, Irvine, CA). Chloramine-T and all other immunochromes were purchased from Sigma Chemical Co. (St. Louis, MO).

Antibodies

A panel of 30 monoclonal and polyclonal antibodies was assembled based on their reported reactivity against antigens known to exist in the walls or...
obtained from commercial sources or other investigators (Table 1); the remain

vasoactive immunoconjugates.

Immunohistological Studies

tumors. Immunohistological Studies were used. Tumors included large cell carcinoma of the liver, primary hepatocellular carcinoma of the liver, breast carcinoma, and adenocarcinoma of the colon. Tissues were snap frozen in isopentane for storage in liquid nitrogen as described elsewhere (17). Before use, sections were cut at 5 μm on a cryostat and fixed briefly (30 s) in acetone. Immunohistochemical stains were performed with the use of an immunoperoxidase ABC (avidin-biotin conjugate) system, with diaminobenzidine/hydrogen peroxide as the substrate and hematoxylin as the counterstain (18). Slides were mounted in buffered glycerol for examination. The secondary or link antibody was a biotinylated horse anti-mouse immunoglobulin (Vector Laboratories, Inc., Burlingame, CA) for use with primary murine mAbs, or biotinylated goat anti-rabbit immunoglobulins (Vector Laboratories) for use with rabbit polyclonal primary antibodies. All primary antibodies were titrated by doubling dilution to obtain optimal intensity of specific staining with minimal nonspecific background reactivity (19).

Western Blotting

To confirm the identity of the antigen recognized by TV-1, Western blotting was performed with the use of fibronectin purified from the supernatant of SV40-transformed human lung fibroblasts (WI-38VA13, American Type Culture Collection, Rockville, MD) as described previously (20, 21). Briefly, the transformed WI-38 cells were grown in RPMI 1640 (Life Technologies, Gaithersburg, MD) supplemented with 10 μM dexamethasone (Sigma) and 10% FCS (HyClone Laboratories, Logan, UT) depleted of bovine fibronectin. Fibronectin was then purified from the cellular supernatant by affinity chromatography on gelatin-Sepharose columns (Pharmacia, Uppsala, Sweden). The purified fibronectin (500 ng) and biotinylated broad-range molecular weight standards (Bio-Rad, Hercules, CA) were loaded onto a 7.5% SDS-polyacrylamide gel, electrophoresed under reducing conditions, and transferred to a Hybond-C nitrocellulose membrane (Amersham, Arlington Heights, IL). The blot was blocked with 2% nonfat milk in PBS-0.1% Tween 20 and incubated at room temperature for 1 h with TV-1 (1:5000 v/v in PBS-Tween). After three washes, the blot was incubated for 1 h with horseradish peroxidase-conjugated goat anti-mouse IgG (1:30,000; Caltag Laboratories, South San Francisco, CA) and horseradish peroxidase-conjugated streptavidin (1:50,000; Caltag). After three washes, binding was detected with the use of the QuickDraw chemiluminescent visualization system (National Diagnostics, Atlanta, GA), and the blot was exposed to X-ray film.

Radioliodination of mAbs

The mAbs used in these studies were radiolabeled with 125I by a modification of the chloramine-T method (13). Briefly, 400 μCi 125I and 10 μl of a 43 mM aqueous solution of chloramine-T were added to a 5-ml test tube containing 100 μl of mAb (10 mg/ml) in 100 μl PBS. The reaction was quenched after 3 min with 20 μl of 5% sodium metabisulfite. The radioliodinated antibody was purified by adding 100 μl of anion exchange resin (AG 1-X8; Bio-Rad) in 0.1 M PBS, pH 7.4, containing 1% BSA. After 1 min the suspension was withdrawn and filtered in a Spin-X centrifuge filter unit (Corning Costar, Cambridge, MA) to remove the resin. The radiolabeled mAb was diluted with PBS for injection, stored at 4°C, and administered within 4 h after radiolabeling.

Preparation of Antibody/IL-2 Immunoconjugates

Conjugation of IL-2 to mAbs Lym-1, B72.3, and TNT-1 was performed as described previously (2). Coupling reactions were initiated by adding IL-2 to antibody, 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-p-toluene-sulfonate, and N-hydroxysulfosuccinimide in a 1:2.5:50 ratio by weight to give a total volume of 0.3 ml in 0.1 M phosphate buffer, pH 7.4. The reactions were incubated overnight at 4°C. After centrifugation, the soluble, coupled antibody was chromatographed on a Sephadex G-100 column calibrated with blue dextran. From the antibody concentration and radioactivity, one or two molecules of IL-2 were calculated to be bound to each antibody molecule. Immunoconjugates retained a minimum of 75% of the antibody-binding reactivity compared with the respective native antibody, determined as described previously (2).

In Vivo Studies

Tumor Models. Raji lymphomas were grown in the left thigh of female, six-week-old, BALB/c athymic nude mice (Harlan-Sprague-Dawley, Indianapolis, IN) by s.c. injection of a 0.2-ml inoculum consisting of 4 × 107 Raji cells and 4 × 106 human fetal fibroblast feeder cells (2). Three days before injection, the mice were irradiated with 400 rads with the use of a cobalt irradiator to ensure a high take rate of implanted cells. The tumors were grown for 15–18 days to a diameter of approximately 1 cm. The LS174T human colon carcinoma cell line was used to produce tumors in female, six-week-old, BALB/c nude mice by s.c. injection of 2 × 107 tumor cells into the left thigh (2). The tumors reached 1 cm in diameter by approximately 12–15 days.

The ME-180 cervical carcinoma cell line produced comparably sized tumors 4 weeks after s.c. injection of 107 cells into the thigh of female, six-week-old, BALB/c nude mice (13).

Dosimetric Studies. Studies were established to document localization, expressed as the percentage of injected dose for each antibody. Baseline dosimetric studies were performed in all three model systems, utilizing in each case 125I-labeled doses of the relevant antibody (Lym-1 for the Raji tumor, B72.3 for the LS174T tumor, and TNT-1 for the ME-180 model). For all in vivo studies, vasoconjugates and antibodies were administered to mice via the i.v. route. Lym-1 and TNT-1 were used as F(ab')2 fragments, whereas B72.3 was used as whole antibody. Three or 4 days after injection of radiolabel (3 days for Lym-1 and TNT-1; 4 days for B72.3), groups of mice were sacrificed, and the major organs, including blood, were weighed and counted in a γ counter. In each case the uptake of injected dose by tumor was determined by dosimetry as described below. This phase of the experiments established the basal levels of tumor uptake for each system, expressed as percentage of total injected dose/g of tumor. All experiments were then repeated in additional groups of 5 mice each, after priming with antivessel antibody/IL-2 immunoconjugate, with the use of one of the antibodies selected on the basis of the immunohistological studies described previously. Five different antivessel antibodies were evaluated, each conjugated with an identical final dose of IL-2. An equivalent dose of TNT-1/IL-2 conjugate was used for comparison in each study.

Biodistribution Studies. Biodistribution studies were performed as above with the use of identical doses of the relevant antibodies for each model system but included pretreatment with one of the IL-2-conjugated antivessel antibodies. Percentage uptake of injected dose of radiolabeled Lym-1, B72.3, or TNT-1 was then calculated as a fraction of the total administered dose and compared with the values obtained in the baseline study as a means of measuring the effect of prior treatment with the antivessel antibody/IL-2 immunoconjugate. In addition, tumor:organ ratios (cpm/g tumor:cpm/g organ) were determined as a measure of normal tissue uptake. On the basis of previous data (2, 6), a 3-h interval was allowed to elapse between injection of the antivessel/IL-2 immunoconjugate and injection of the radiolabeled Lym-1, B72.3, or TNT-1.

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**Immunohistological Studies**

Patterns of immunohistochromatic staining were evaluated with reference to the presence and intensity of a positive reaction in the connective tissue or basement membrane of the vessel wall, or in perivascular connective tissue (Table 1). The localization of stain within the connective tissue of the tumor stroma or in necrotic areas within tumor was also recorded. Finally, evidence of positive reactivity on the surface of endothelial cells or in serum trapped within vessels was noted as the basis for excluding antibodies from *in vivo* studies. The results recorded in Table 1 are based on a semiquantitative score of the intensity of staining reaction: − (no evidence of staining) to ++ (intense positivity).

Of several antifibrin antibodies evaluated, only one gave evidence of reactivity with vessel wall components. This antibody also gave some staining of perivascular connective tissue, stromal cells, and necrotic zones within the tumor. Antifibrin 5D8 stained only necrotic zones within the tumor. Of the three antifibronectin antibodies, two (HFN 7.1 and TV-1) reacted strongly with vessel walls and surrounding connective tissue. Because TV-1 was produced originally by our laboratory, it was necessary to demonstrate its immunoreactivity to human cellular fibronectin. As shown in Fig. 2, Western blot studies using affinity-purified cellular fibronectin confirmed its immunoreactivity to this glycoprotein, which had a *M*₅ of approximately 250,000 after reduction. Both HFN 7.1 and TV-1 localized to necrotic areas within the tumor. Fig. 3 demonstrates the reactivity pattern of TV-1 in a section of human small cell lung carcinoma and illustrates its specificity for vessel wall components. Antibodies to laminin, collagen type IV, and vitronectin also showed positive staining in vessel walls and perivascular connective tissue but had little staining of tumor stroma or necrotic zones within tumor. Antibody LN-3 and an antibody to fibrinogen gave some activity within vessel walls but were disqualified from further consideration because of staining of endothelial cells or serum.

**Vascular Volume Studies.** Assessment of the vascular volume of the three s.c. transplanted human tumor models was determined as described previously (2, 22). Briefly, groups of mice were administered 1.2 μg of glucoheptanoic γ-lactone i.v., followed 30 min later with 50 μCi of ⁹⁹mTcO₄⁻. Within 30 min, more than 95% of the ⁹⁹mTc in the blood was bound to RBC. At the time of sacrifice, the organs were removed and weighed, and the samples were counted in a γ counter to determine ⁹⁹mTc activity. The data were expressed as ml of blood/g of organ. From these data, the mean ± SD was calculated for each group.

**RESULTS**

**Immunohistological Studies**

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![Western blot analysis of TV-1 on purified human cellular fibronectin. Numbers, molecular weights in thousands.](#)
Fig. 3. Immunohistochemical staining pattern of TV-1 on a section of human small cell lung carcinoma demonstrating reactivity to tumor vasculature. X240.

Table 2. Effects of antivessel mAb/IL-2 vasoconjugate pretreatment on TNT-1 uptake

<table>
<thead>
<tr>
<th>Vasoconjugate</th>
<th>Tumor uptake&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% injected dose/g (SE); n = 3.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNT-1 alone (control)</td>
<td>1.28 (0.30)</td>
<td></td>
</tr>
<tr>
<td>TV-1/IL-2</td>
<td>4.35 (0.63)</td>
<td></td>
</tr>
<tr>
<td>HFN 7.1/IL-2</td>
<td>4.09 (0.28)</td>
<td></td>
</tr>
<tr>
<td>anti-BFGF/IL-2</td>
<td>1.68 (0.10)</td>
<td></td>
</tr>
<tr>
<td>TP-1/IL-2</td>
<td>1.55 (0.12)</td>
<td></td>
</tr>
<tr>
<td>TP-3/IL-2</td>
<td>1.79 (0.13)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Nude mice bearing ME-180 human cervical carcinoma xenografts were injected with mAb/IL-2 vasoconjugate 3 h before injection with 125I-labeled TNT-1. Three days later the mice were sacrificed, and the percentage of injected dose in the tumor was determined.

In vivo studies

In consideration of these findings, five antibodies met the requirements for further in vivo studies, namely, positive reactivity with vessel wall components (+ or – perivascular connective tissue) but complete absence of reactivity with endothelium and serum. Antibodies that localized within the connective tissue stroma of tumors or in necrotic zones of tumors, but not immediately in the vicinity of vessels, were not examined further in this study, because immunoconjugates composed of these antibodies would not be likely to have a strong effect on distant tumor vessels.

In Vivo Studies

Preliminary Screening Studies for Permeability Enhancement. The five antibodies selected for in vivo studies were TV-1 and HFN 7.1 (both antifibronectin antibodies), anti-BFGF (antibody to basic fibroblast growth factor), and TP-1 and TP-3 [antibodies to a Mr 80,000 mesenchymal cell antigen (16, 23)]. The ability of each of these antibodies, after conjugation with IL-2, to increase the uptake of radiolabeled TNT-1 into ME-180 human cervical carcinoma transplants in nude mice was evaluated. Results are displayed in Table 2, with reference to a primary dose of unconjugated TNT-1 as the baseline control. Both of the antifibronectin antibodies increased the amount of a subsequent dose of radiolabeled TNT-1 localizing to the tumor site by more than 3-fold (3.4- and 3.2-fold, respectively). The remaining 3 antibodies (anti-BFGF, TP-1, and TP-3) gave less than a 40% increase under identical conditions.

The similar results obtained with antibodies TV-1 and HFN 7.1 reflect their almost identical pattern of reactivity as observed by immunohistochemistry. TV-1 was selected for further studies in three different tumor models.

Testing of Three Tumor Models with TV-1/IL-2. Three tumor model systems were chosen to test the effectiveness of TV-1 as a delivery agent for IL-2. Vascular volume studies shown in Fig. 4 demonstrated that these three tumor models varied greatly in the amount of vascularization, despite their common site of implantation. As shown in Table 3, TV-1/IL-2 pretreatment was tested in each of these tumor models and compared to groups of mice receiving no pretreatment or tumor-specific antibody/IL-2 immunoconjugate administration. In all cases, prior treatment with TV-1/IL-2 provoked a marked increase in localization of the subsequently administered radiolabeled specific antitumor antibody, compared with mice receiving no prior treatment. The TV-1/IL-2 conjugate gave enhanced delivery of the appropriate specific antitumor antibody that compared favorably with results obtained when the specific antibody itself was used as the pretreatment conjugate. Interestingly, the effectiveness of TV-1/IL-2 was dependent on the level of intrinsic blood volume or vascularization of the model tumor. The LS174T human colon carcinoma model, which contains approximately a 10-fold higher vascular volume than the Raji human Burkitt’s lymphoma model in nude mice (Fig. 4), showed similar uptake after TV-1/IL-2 pretreatment or tumor-specific B72.3/IL-2 pretreatment. As shown in Fig. 5, 4-day...
biodistribution studies comparing no pretreatment with B72.3/IL-2 and TV-1/IL-2 pretreatments demonstrate the marked improvement in localization of radiolabeled antibody to the tumor after either pretreatment (Fig. 5A). Normal tissues remained unaffected by these pretreatments, and tumor:organ ratios were therefore extremely high, ranging from 25:1 to 500:1 (Fig. 5B). Contrarily, Lym-1/IL-2 was more effective than TV-1/IL-2 in the Raji model, which was found to have a low amount of vascularization. In the ME-180 model, which has a vascularization level somewhat greater than the Raji model, pretreatment with TV-1/IL-2 and TNT-1/IL-2 had almost an equal effect. Hence, TV-1/IL-2 successfully altered the vascular permeability of three diverse tumor models but was maximally effective in the system with the highest degree of vascularization.

**DISCUSSION**

In this study, we identified a mAb, designated TV-1, that targets an antigen that is common to endothelial basement membranes and connective tissue stroma but that is normally inaccessible to large plasma proteins due to the tight junctions that exist between endothelial cells in normal tissues and organs. In solid tumors, where the endothelium is structurally abnormal (7-9) and the vasculature exhibits a heightened permeability (24, 25), the antigen is exposed, allowing i.v. administered mAb to penetrate the vessel walls and target the tumor vessel endothelial subcellular matrix. We demonstrated that TV-1 may be used as a universal carrier of potent vasoactive agents, such as IL-2, to enhance the uptake of tumor-specific mAbs. From a panel of 30 monoclonal and polyclonal antibodies with specificities for antigens expressed in connective tissue and blood vessels (Table 3), 5 antibodies that immunohistologically demonstrated binding to vessel wall and perivascular connective tissue antigens were selected for subsequent *in vivo* studies. Antibodies that bound endothelial cells or serum components were excluded from further consideration. Of the five selected antibodies, two mAbs with specificity for fibronectin produced significantly enhanced tumor uptake when chemically conjugated to IL-2 and used as a pretreatment in the TNT-1/ME-180 human cervical carcinoma model in nude mice (Table 2). Finally, in each of three different tumor models, TV-1/IL-2 as a pretreatment was shown to enhance the uptake of radiolabeled tumor-specific mAbs (Table 3). TV-1/IL-2 was maximally effective in the tumor model demonstrating the highest degree of vascularization.

Numerous mAbs with specificities for human endothelial cells have been produced. Whereas the majority of these mAbs recognizes antigens on the luminal aspect of most normal blood vessels or a subset of normal vessels (26-34), several groups have developed mAbs with varying degrees of specificity for tumor vessels (35-39). It has been suggested that the differences between tumor and normal vessels can be exploited to treat solid tumors by the destruction of their vasculature (40). Identification of an antigen specific to the endothelial cells of tumor vessels would facilitate the development of mAb-based therapies directed against such antigens. A model system in which to test vascular targeting was developed by Burrows et al. (41). To test the efficacy of this novel approach, these investigators chose to induce tumor vessel endothelial cells to produce an antigen that could be targeted with a mAb. For these studies, a neuroblastoma cell line was transfected with the gene for IFN-γ, the secretion of which induces the expression of MHC class II antigens on tumor vascular endothelium. In a nude mouse model, the targeting of such transfected tumors with a mAb that recognizes a MHC class II antigen was shown to be specific for tumor vessels. In a subsequent study, treatment of the tumors with an immunotoxin conjugated to the mAb resulted in marked tumor regression following hemorrhagic necrosis after endothelial injury (42). These experiments demonstrate the utility of destroying tumor vessels to treat solid tumors. The necessity to identify a definitive tumor vessel-specific antigen remains, although a recently generated mAb against endoglin shows promise as a tumor-

**Table 3 Comparison of tumor- and vessel-specific mAb vasoconjugates on mAb uptake in human tumor xenografts**

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Radiolabeled antibody</th>
<th>Tumor uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAJI model (low vascularization)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>B72.3/B72.3</td>
<td>125I-Lym-1 (Fab')2</td>
</tr>
<tr>
<td>Lym-1/IL-2</td>
<td>B72.3/B72.3</td>
<td>125I-Lym-1 (Fab')2</td>
</tr>
<tr>
<td>TNT-1/IL-2</td>
<td>B72.3/B72.3</td>
<td>125I-Lym-1 (Fab')2</td>
</tr>
<tr>
<td>TV-1/IL-2</td>
<td>B72.3/B72.3</td>
<td>125I-Lym-1 (Fab')2</td>
</tr>
<tr>
<td>ME-180 model (low to moderate vascularization)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>B72.3/B72.3</td>
<td>125I-TNT-1 (Fab')2</td>
</tr>
<tr>
<td>TNT-1/IL-2</td>
<td>B72.3/B72.3</td>
<td>125I-TNT-1 (Fab')2</td>
</tr>
<tr>
<td>TV-1/IL-2</td>
<td>B72.3/B72.3</td>
<td>125I-TNT-1 (Fab')2</td>
</tr>
<tr>
<td>LS174T model (high vascularization)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>B72.3/B72.3</td>
<td>125I-Lym-1 (Fab')2</td>
</tr>
<tr>
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</table>

* Nude mice bearing human tumor xenografts were injected with mAb/IL-2 vasoconjugate 3 h before injection of 125I-labeled mAb. Three or 4 days later the mice were sacrificed, and the percentage of injected dose in the tumor was determined. In each tumor model, pretreatments with TV-1, relevant, and TNT-1 mAb/IL-2 vasoconjugates were compared.

* % injected dose/g (SD); n = 3.

* Three-day biodistribution.

* Four-day biodistribution.
Fig. 6. Schematic diagram of tumor cross section showing possible mechanism of TV-1 specificity for tumor vessels. A, diagram of normal tissue vessel with tightly junctioned endothelium. B, diagram of tumor vessel showing fenestrated endothelium and binding of TV-1 to underlying subcellular matrix.

A NORMAL TISSUE VESSEL

Parenchymal cells

Endothelium

MAb (TV-1)

Blood

Basement membrane

B TUMOR VESSEL

Tumor cells

Endothelium

MAb (TV-1)

Blood

Basement membrane

Accessible antigenic sites

specific vascular marker, though several normal tissues are also targeted by this antibody.4

Instead of targeting an antigen specific to the endothelial cells of tumor vessels, Dvorak et al. (43) suggested exploiting the persistently hyperpermeable state of tumor vessels by targeting an antigen located in the connective tissue stroma. Such an approach would obviate the identification of a tumor endothelial cell-specific antigen. The specificity dependent on the heightened permeability of tumor vessels, however, would be lost if the patient had healing wounds, which would also be targeted by a mAb against an antigen within connective tissue stroma. It should be noted, however, that wounds heal in a short period of time (1-3 days), but tumor lesions do not undergo similar resolution. The authors proposed conjugating the mAb with a cytotoxin that acts at a distance, such as a therapeutic radionuclide, necessary to effect tumor cell death at varying distances from the targeted stromal antigens (43).

As illustrated schematically in Fig. 6, we have developed an alternative approach that also takes advantage of the structural differences between tumor-associated and normal vessels. Initially, our focus was to enhance antibody uptake in experimental tumor models by generating chemical conjugates composed of tumor-specific antibodies and peptides with vasoactive properties, such as IL-2, tumor necrosis factor α, and IL-1β. These immunoconjugates, when used as pretreatments for radiolabeled tumor-specific antibodies in nude mouse tumor models, resulted in a 3-4-fold increase in the accumulation of radiolabel in the tumor without affecting normal tissues (2, 6). We hypothesized that delivering biologically active peptides directly to tumor vessels, instead of to tumor cells, might be more successful in enhancing the uptake of tumor-specific mAbs, because the vasoactive agents were found to act through increasing vascular permeability and/or blood flow. Cognizant of the hyperpermeable state of tumor vessels, we chose to search for an antigenic target located within the vascular endothelial basement membrane or connective tissue stroma and now report that certain epitopes of fibronectin appear to be suitable for this approach. When conjugated to IL-2, the antifibronectin mAb TV-1 produces enhanced tumor uptake of radiolabeled tumor-specific antibodies, while sparing normal tissues. By inducing a rapid and reversible vasopermeability at the tumor site, TV-1/IL-2 as a pretreatment is most successful in tumor models that show the highest degree of vascularization.

Fibronectin is a high molecular weight glycoprotein that has been implicated in such diverse processes as embryogenesis, wound healing, coagulation, and oncogenesis (see reviews in Ref. 44). Composed of three types of repeating subunits, fibronectin consists of multiple domains that have been shown to mediate interactions with cells, heparin, fibrin, and collagen (45). Several isoforms of fibronectin are generated through alternative splicing of fibronectin mRNA precursors, resulting in the exclusion or inclusion of three subunits, ED-A, ED-B, and IIIIC (46, 47). It has been shown that an ED-B-containing fibronectin isoform has limited distribution in normal adult tissues, though it shows relatively high levels of expression in fetal and tumor tissues (48). In these studies, immunohistochemical analyses of tumor tissue sections using a mAb specific for the ED-B-containing isoform demonstrated its expression in the interstitium of many tumor types. Earlier, a mAb was generated that recognized a different fibronectin domain found in fetal and tumor tissues that was absent in the

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majority of normal adult tissues (49). It is conceivable, therefore, that mAb TV-1 targets one of the "oncofetal" domains characterized in these studies. Presently, we are mapping the epitope recognized by TV-1. Epitopes of fibronectin other than the one targeted by TV-1 may also turn out to be useful for tumor targeting approaches.

In conclusion, mAb TV-1 represents a universally applicable reagent for the delivery of vasoactive agents to tumor vessels to enhance tumor uptake of radiolabeled mAbs and presumably other macromolecules in the diagnosis and treatment of human malignancies. In addition, tumors with high vascular content, such as Kaposi's sarcoma, hemangiosarcomas, and renal carcinomas, would be especially good targets for the delivery of cytotoxic reagents (drugs, prodrugs, toxins, and radionuclides) that can destroy the tumor by its vasculature. Finally, a targeting agent such as TV-1 may be used to prevent further tumor vascularization and spread by delivering potent antiangiogenic factors that are currently being used in experimental investigations (50–56).

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