ABSTRACT

In contrast to normal tissues, many malignant tumors contain a high proportion of dead and dying cells. The loss of membrane integrity that accompanies cellular degeneration permits macromolecules, including antibodies, to freely enter the cell cytoplasm. Based upon these observations, it was hypothesized that monoclonal antibodies to intracellular antigens, which are integral structural components and are retained by degenerating cells, may be used to target a wide range of human malignancies. Previous studies by our laboratory utilizing these principles have demonstrated the feasibility of imaging four different histological types of human cancer in a nude mouse model, using monoclonal antibodies directed against insoluble intranuclear antigens. The present study describes the application of this approach, designated tumor necrosis treatment, for the radioimmunotherapy of transplantable ME-180 human cervical carcinomas in the nude mouse. Groups of tumor-bearing nude mice received three weekly treatments of 150 or 300 μCi of 131I-labeled experimental (TNT-1) or control (Lym-1) monoclonal antibodies. Detailed biodistribution data, dosimetric evaluations, and therapeutic results are presented to demonstrate the effective and preferential targeting of 131I-labeled TNT-1 monoclonal antibody within the tumor. In the experimental groups, the dose delivered to the tumor was sufficient to induce clinical regressions in 88% of treated animals, without evidence of toxicity to normal tissues. Complete regressions were obtained in 25% of the mice treated with high dose TNT-1. Microscopic examination of the implantation sites of these mice demonstrated the presence of acute radiation damage and residual keratin-positive tumor cells showing marked evidence of degeneration. Dosimetric data obtained over the 3-week treatment period showed that, unlike control treated mice, which received approximately 500 cGy each week, the experimental animals received increasing doses of radiolabeled antibody with each treatment (averages for weeks 1, 2, and 3: 1066, 2046, and 2476 cGy, respectively). In accordance with these data, enhanced imaging and therapeutic responses were observed with each therapeutic dose in the TNT-1-treated groups, compared with controls. These results indicate that TNT-1 therapy produces an ever-expanding population of TNT-1-positive targets in the tumor as a result of the centrifugal killing of adjacent viable tumor cells. To help illustrate these results, a four-compartment model of the dose distribution kinetics of TNT-1 is presented for discussion with respect to the possible application of this method for the imaging and treatment of cancer in humans.

INTRODUCTION

A new method of cancer imaging and therapy which utilizes necrotic cells as a target for the selective binding of monoclonal antibodies to human cancers was recently reported by our laboratory (1). Designated tumor necrosis treatment, this approach is a radical departure from current methods which employ monoclonal antibodies to bind to tumor-associated cell surface antigens and require the use of different antibodies for each specific tumor cell type (2, 3). In contrast, TNT is based upon the hypothesis that monoclonal antibodies directed against abundant, insoluble, nuclear antigens show preferential localization in malignant tumors due to the presence of abnormally permeable degenerating cells not found in normal tissues. TNT is therefore applicable to a broad spectrum of human cancers and circumvents some of the limitations of monoclonal antibody therapy such as tumor heterogeneity and antigenic modulation (1).

It has long been recognized that rapidly growing tumors contain a proportion of degenerating or dead cells in addition to numerous proliferating cells (4–7) but, with attention focused upon attempts to kill dividing cells, the degenerating component has largely been ignored. Calculations of tumor cell loss have revealed that, in contrast to normal tissues, 50–90% of the progeny of tumor cell divisions shortly undergo degeneration and cell death (8, 9). In tumors, the imperfect vasculature and impaired phagocytic response appear to permit the accumulation of degenerating cells, often with the formation of large areas of necrosis, long recognized by pathologists to be a typical feature of malignant tumors (7). Thus, the accumulation within tumors of a high proportion of degenerating cells constitutes a major distinction between malignant tumors and normal tissues; in the latter, cell death occurs at a relatively low rate and is accompanied by a rapid and orderly removal of necrotic elements.

Degenerating and necrotic cells have distinctive properties, in particular an abnormal surface membrane permeability not found in viable cells (10, 11). The use of radiolabeled monoclonal antibodies against an intracellular antigen in melanoma (12) and myosin as a means of imaging myocardial infarcts (13) and, more recently, leiomyosarcomas and rhabdomyosarcomas (14) provides practical evidence of the feasibility of exploiting the loss of membrane integrity in degenerating cells in order to localize necrotic lesions. In our first report (1), imaging, biodistribution, and autoradiographic studies were performed in nude mice bearing four different human tumor cell lines, to demonstrate the binding of radiolabeled antinuclear monoclonal antibodies within tumors that contain necrotic cells. We now extend these studies by presenting data on the therapeutic potential of TNT in nude mice bearing transplantable tumors of the ME-180 human cervical carcinoma cell line. Dosimetric calculations are provided to demonstrate, in a quantitative manner, evidence of improved tumor uptake in animals receiving multiple radioimmunotherapeutic treatments.

MATERIALS AND METHODS

Antibody Purification and Radiolabeling. TNT-1 (IgG2a) monoclonal antibody, with specificity for nuclear histones,* was purified from mouse ascites using Protein-A affinity chromatography as described previously (1). Lym-1 (IgG2a), a monoclonal antibody reactive with the cell surface of B-lymphocytes but lacking reactivity with the ME-180 carcinoma cell line (15), was used as a control reagent and was obtained from

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* These data were supported by grants from Techniclon International, Inc. (Tustin, CA) and Lederle Laboratories, a Division of American Cyanamid, Inc. (Pearl River, NY).

1 A. L. Epstein, S. Gaffar, and G. Naeve, unpublished data.

The abbreviation used is: TNT, tumor necrosis treatment.

13II-Labeled TNT-1 Monoclonal Antibody1

1.5II-Labeled TNT-1 Monoclonal Antibody1

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The antibodies were radiolabeled with 125I (New England Nuclear Co., Boston, MA) or 131I (ICN Biomedicals, Inc., Irvine, CA) by the chloramine-T method (16). Unreacted radiiodine was reduced to iodide by adding sodium metabisulfite (Mallinckrodt, Inc., St. Louis, MO). The iodide was then absorbed by 100 μl of AG 1-X8 anion exchange resin (Bio-Rad Chemical Division, Richmond, CA) in phosphate-buffered saline, pH 7.4, that contained 1% bovine serum albumin (radioimmunoassay grade; Sigma Chemical Co.). After 1 min, the suspension was withdrawn and filtered in a Spin-X centrifuge filter unit (Costar, Cambridge, MA) to remove the resin. The radiolabeled antibodies were then diluted with phosphate-buffered saline to an appropriate volume for injection and used within 4 h after labeling. The specific activity of the radiolabeled antibodies was 1 mCi 125I or 131I/mg protein for the biodistribution studies and 6 mCi 131I/mg protein for the radioimmunoassay studies. The radiodinated IgG preparations were found to be aggregate-free by Superose-12 fast protein liquid chromatography. Approximately 75–80% of radiolabeled Lym-1 preparations were found to bind to Raji cells by live cell radioimmunoassay (15). The radiolabeled TNT-1 showed undiminished immunoreactivity when compared with the unlabelled antibody in a semiquantitative indirect immunofluorescence assay (17).

Tumor Model. The ME-180 human cervical carcinoma cell line was chosen as the tumor model on the basis of earlier experiments which showed good imaging of ME-180 tumors in nude mice using 131I-labeled TNT-1 antibody (1). Six-week-old female athymic nude mice (Simonsen Laboratories, Gilroy, CA) were inoculated in the thigh with 10⁷ ME-180 tumor cells in 0.2 ml RPMI 1640 medium (GIBCO Laboratories, Grand Island, NY) that contained 10% fetal calf serum (Hy clone Laboratories, Logan, UT), 100 units/ml penicillin G, and 100 μg/ml streptomycin sulfate. Tumors began to appear within 2 weeks after injection. The tumor volume was estimated by the formula

\[(\text{short dimension})^2 \times \text{(long dimension)} \times \frac{1}{2}\]

Animals were used for biodistribution or for therapeutic trials when the mean tumor volume reached approximately 0.5 cm³. Five days after the initiation of the clinical trials, the tumor-bearing mice were treated with Lugol’s solution in their drinking water to prevent thyroid uptake of dehalogenated radiiodine during the experiments.

Experimental Design of Radiotherapeutic Study. Five groups of nude mice (total of 31 animals) bearing ME-180 human cervical tumors in the thigh region received one of the following treatments: Group A (eight mice), 150 μCi 131I-conjugated TNT-1 antibody (experimental); Group B (eight mice), 300 μCi 131I-conjugated TNT-1 antibody (experimental); Group C (five mice), 50 μg unlabeled TNT-1 antibody (control); Group D (five mice), 150 μCi 131I-conjugated Lym-1 antibody (control); and Group E (five mice), 300 μCi 131I-conjugated Lym-1 antibody (control). The 131I-labeled antibodies were administered i.v. via the lateral tail vein on days 1, 8, and 15. For the first 3 weeks of treatment, animals were imaged at weekly intervals from their posterior side using a pinhole collimator and a Spectrum 91 gamma camera (Raythe on, Melrose Park, IL) for a total of 10,000 counts. In addition, tumor volumes were assessed by caliper measurements each week until the experiment was terminated at 9 weeks. Polaroid photographs of the mice were also taken at weekly intervals to record the visual appearance of the tumors in the control and experimental groups. Groups D and E were included as controls to demonstrate the effects of the radioactive dose when it was bound to an irrelevant monoclonal antibody of the same isotype as TNT-1.

Histological Studies of Tumor Tissue. At the completion of the therapeutic study, mice that showed major or complete regression were biopsied at the tumor site to document the absence or presence of residual tumor. Excised tissues were fixed overnight in 10% buffered formalin in preparation for paraffin embedding. Five-μm sections were stained with hematoxylin and eosin for histological examination. In addition, selected cases were stained for keratin by an immunoperoxidase method (19) to facilitate the recognition of scattered keratin-containing ME-180 carcinoma cells within scarred connective tissue.

Biodistribution Studies and Radiation Dosimetry. The biodistribution curves were plotted from the % dose/g for each organ and for each antibody at the various time intervals. The areas under the biodistribution curves were determined by the trapezoidal integration method (20). The initial concentration of radiolabeled antibodies in each organ was assumed to be 0%/g. Estimated cumulative radiation doses in tumor and other organs were then calculated for a 300-μCi 131I initial antibody dose by multiplying the integrated μCi/g by the γ-gy/μCi · h factor, which has been tabulated using the medical imaging radiation dose for '131I of 0.410 μGy/μCi · h (21). This value includes nonpenetrating particles and low energy X-rays but does not include the γ-radiation dose because of the low absorption of γ radiation in small tumors and organs of the mouse. Radiation doses of the first treatment were calculated using this method for an infusion of 300 μCi of 131I in TNT-1 and Lym-1 antibodies.

To estimate the doses of radiation that the mice received during more prolonged therapy, it was necessary to depart from the necropsy-biodistribution approach, which required sacrifice of an increasingly large number of tumor-bearing animals, in favor of a method utilizing ongoing sequential daily gamma camera imaging of individual tumor-bearing mice. Image analyses of these animals then provided an estimate of tumor/whole body antibody distribution during the three weekly injections of 131I-labeled TNT-1 or Lym-1. Nine ME-180 tumor-bearing nude mice were randomly divided into two groups and treated weekly for 3 weeks i.v. with either 300 μCi 131I-labeled TNT-1 (five mice) or Lym-1 control (four mice) as above. One to 6 days after each injection, the mice were imaged daily in a posterior position, as described above, for a total of 10,000 counts, giving a total of 162 images for both groups. Each image was recorded on 8 x 10 inch X-ray film (16 format) and stored in a 386 NEC computer using an imaging program designed by Cardiac Medical Systems Corp. (Springfield, WI). Whole body radiation doses for each mouse were measured daily using a CRC-7 dose calibrator (Capintec Inc., Pittsburgh, PA). Three areas in each image were defined using the Cardiac Medical Systems program: (a) region 1, whole body; (b) region 2, tumor; and (c) region 3, contralateral thigh for background subtraction (see Fig. 8). Total counts in each area were obtained after drawing each region on the screen image using a personal computer mouse. Based on the ratio of the counts in the tumor (region 2 minus region 3) and whole body, the net radiation doses delivered in the tumor (μCi) were estimated daily. For radiation dosimetry calculations, the data were corrected for the radiation decay of '131I from day 1 to day 6 postinjection. The results were expressed as the percentage of the original antibody injection dose/g of tumor. Gy were further estimated based on an extrapolation of 6–8 days and a standardized infusion of 300 μCi of TNT-1 antibody or radiolabeled control Lym-1, using the trapezoidal integration method described above.

RESULTS

Therapy Studies. In order to determine the maximum tolerated dose of 131I-labeled antibody in tumor-bearing nude mice, a preliminary experiment was performed in which escalating doses of radiolabeled antibody were administered to different groups of mice. By monitoring the WBC counts of the mice over several weeks, it was determined that a maximum cumulative...
TUMOR NECROSIS TREATMENT WITH MONOCLONAL ANTIBODY

lative dose of 900 μCi was safe to administer to the mice. Based upon these data, we chose two dose levels, namely 450 and 900 μCi, to be administered in three weekly fractionated doses as the experimental regimen. Of the 31 mice in this phase of the study, including 16 mice treated with 3 × 300 μCi 131I-labeled antibodies, none died within the experimental period of 9 weeks. As shown in Fig. 1, 131I-labeled TNT-1 produced a marked antitumor effect at both the low and high dose levels (Groups A and B). Tumor growth was inhibited in these groups and tumor volume did not significantly increase until 5 or 8 weeks after initiation of treatment for those mice receiving 150 or 300 μCi 131I-labeled TNT-1, respectively. In addition, 14 of 16 of the 131I-TNT-1-treated mice developed progressive cavitating necrosis within their s.c. tumors. Due to the extent of this necrosis, it was difficult to obtain accurate weekly measurements of tumor volume in the experimental groups. While most mice in these groups had tumors with extensive central necrosis and clearly showed a reduction in the amount of visible tumor, the overall tumor dimensions showed little change up to 5 weeks. Major regression was, therefore, defined to include those tumors with a greater than 30% shrinkage overall plus those that showed approximately the same dimensions but possessed a central cavity estimated to occupy one third or more of the total tumor volume. Minor regression was defined as those tumors showing a net decrease in size of less than 30% including an allowance for the presence of gross areas of necrosis. Complete regression was defined as total disappearance of macroscopic tumor.

Table 1 summarizes the results of the 131I-TNT-1 therapeutic trial in ME-180 cervical carcinoma-bearing nude mice. Two of the experimental mice in Group B (high dose) showed complete tumor ablation 5 weeks after initiation of 131I-TNT-1 therapy. In the high dose group, tumor growth was found to be inhibited substantially in all mice except one, which showed only minor inhibition compared to controls. Similarly, in the low dosage 131I-TNT-1-treated group (Group A), whereas one mouse was unresponsive to treatment, all of the other mice demonstrated various degrees of tumor regression. In contrast, most tumors in the control groups (Groups C, D, and E) continued to enlarge throughout the study, indicating that the tumors did not receive sufficient radiation to affect their growth or that the unlabeled TNT-1 monoclonal antibody had little or no cytotoxic effect on the tumor cells.

To illustrate the treatment results, Polaroid photographs of several mice and their gamma images obtained 7 days after the administration of 131I-antibody doses are shown in Fig. 2–4. Fig. 2, A, B, and C, are sequential images of an ME-180 human cervical carcinoma-bearing nude mouse 7 days after the first, second, and third treatments with 300 μCi 131I-TNT-1 antibody, respectively. Note the enhanced imaging of tumor in the second and third weeks of treatment, indicating that increased numbers of permeable degenerating cells were present at these times as a consequence of the therapy. Fig. 2D shows the same nude mouse at the time of the third image. The presence of cavitating necrosis in the central region corresponds to the therapeutic effect of the 131I-TNT-1 treatment. This tumor showed marked shrinkage over the next few weeks and was scored as a major regression.

Fig. 3 shows sequential images and photographs of a nude mouse that underwent complete regression of its tumor. The imaging pictures (Fig. 3, A, B, and C) were taken 7 days after the first, second, and third treatments with 300 μCi 131I-TNT-1 antibody, respectively. This tumor showed marked uptake of TNT-1 with the first dose and a more rapid therapeutic response when compared with the mouse shown in Fig. 2. The tumor in this nude mouse showed a cavitating necrotic area in the tumor by 3 weeks (Fig. 3D), with progressive shrinkage at 5 weeks (Fig. 3E) and total disappearance of the tumor at 7 weeks (Fig. 3F). Fig. 4A shows a nude mouse, bearing a human ME-180 cervical carcinoma in the left thigh, that was imaged 7 days after receiving an i.v. dose of 131I-Lym-1. Since Lym-1 is a B-cell-specific monoclonal antibody, the labeling observed at the tumor site was interpreted as indicative of the blood and tissue fluid pool of Lym-1 within the tumor. This interpretation was supported by the observation that, as the radioactivity decreased from the body blood pool, so it also cleared from the tumor, although at a somewhat slower rate. Consistent with this interpretation, there was no observable enhancement of tumor imaging after the second and third doses, a finding in marked contrast to the 131I-TNT-1-treated animals. Fig. 4B, C, and D, present the gross visual appearances of tumor-bearing mice from control groups C, D, and E at the end of the study period. All of these mice had progressively growing tumors at the sites of implantation and some showed the formation of satellite lesions.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. Tested</th>
<th>No. of complete regressions</th>
<th>No. of major regressions (&gt;50%)</th>
<th>No. of minor regressions (&lt;30%)</th>
<th>No. showing no effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (150 μCi 131I-TNT-1 × 3)</td>
<td>8</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>B (300 μCi 131I-TNT-1 × 3)</td>
<td>8</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C (unlabeled TNT-1 × 3)</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>D (150 μCi 131I-Lym-1 × 3)</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>E (300 μCi 131I-Lym-1 × 3)</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>
TUMOR NECROSIS TREATMENT WITH MONOCLONAL ANTIBODY

Fig. 2. Sequential images of ME-180 tumor-bearing nude mouse 7 days after the first (A), second (B), and third (C) treatments with 300 μCi of 131I-TNT-1 antibody. Note the improved imaging of tumor in the second and third weeks of therapy when increased amounts of necrotic cells were present as a consequence of the therapy. D, Appearance of nude mouse at the time of the third image. Left thigh tumor shows large central caviting necrosis with scab formation.

Fig. 3. Sequential images and photographs of a nude mouse that underwent a complete regression of its ME-180 tumor after three weekly treatments with 300 μCi of 131I-TNT-1 antibody. The scintigrams were taken 7 days after the first (A), second (B), and third (C) treatments. Note that, in this mouse, the first image is substantially greater than the one in Fig. 2A. Because of the larger initial therapeutic dose delivered in the first and second treatments, this mouse showed a less intense image of the tumor after the third dose, when the tumor had significantly shrunk in size. Photographic appearances of this mouse at 3 weeks (D), 5 weeks (E), and 7 weeks (F) show tumor regression.

Histological Examination. Histological examination of biopsy specimens from mice showing complete regressions by 5–7 weeks revealed the presence of small nests of tumor cells at the site of implantation, as shown in Fig. 5. Small numbers of residual keratin-positive tumor cells were identifiable. As evidence of acute radiation damage, all showed nuclear pyknosis, karyolysis, or karyorrhexis. In some sections, foci of granulocytes were observed clustered around fragments of degenerating tumor cells. The surrounding connective tissue showed dense hyaline fibrosis and endarteritis of small vessels, attesting to the delivery of significant amounts of radiation to the region.

Biodistribution and Dosimetry. The distribution of 131I-labeled TNT-1 and 125I-labeled Lym-1 was determined at 4 h and 1, 2, 4, 6, and 8 days after administration of the radiolabeled antibody mixture in cohorts of nude mice bearing ME-180 tumors. In this study, the simultaneous administration of TNT-1 and Lym-1 tagged with different labels permitted the quantitation of the percentage of injected dose/g of tumor and normal organs for both antibodies in the same mouse, thereby excluding errors due to individual differences between the animals in the experimental and control groups. As shown in Fig. 6, the tissue uptake of 131I-labeled TNT-1 was similar to that of 125I-labeled Lym-1 except in the spleen, which had significantly higher uptake of 125I-labeled Lym-1 than 131I-labeled TNT-1. The overall percentage of injected dose/g of tumor in mice given by 131I-labeled TNT-1 and 125I-labeled Lym-1 for days 1–8 was approximately the same.

The estimated radiation doses to the tumor and tissues for nude mice given 300 μCi 131I-labeled TNT-1 or Lym-1 antibodies are shown in Table 2. The doses represent the cumulative rad (cGy) doses to the tissues over 8 days and were calculated from the biodistribution data given in Fig. 6. As shown in this table, 300 μCi 131I-labeled TNT-1 delivered 875 rad doses to the tumor, compared to 799 rad from 300 μCi 131I-labeled Lym-1. These results are consistent with those of the tumor imaging studies since, in most mice, both antibodies showed approximately equivalent imaging of tumor after the first radiolabeled antibody dose treatment (Fig. 2A and 4A).

Since the above dosimetric data showed similar uptake by
Fig. 5. Histological appearance of the implantation site from a mouse with complete tumor regression. A, Low magnification (×40) reveals a relatively avascular scarred area with dense collagen formation. B, Intermediate magnification (×100) shows scattered bizarre tumor cells with degenerative changes, which under higher magnification (×250) (C) are characterized by nuclear pyknosis, karyorrhexis, or karyolysis. Viable tumor cells by histological criteria were not present.

Fig. 6. Biodistribution data for mice simultaneously treated with TNT-1 and Lym-1 monoclonal antibodies that were tagged with different radiolabels, showing %dose/g in normal organs and tumor over time. ▲, Lym-1; □, TNT-1.

Fig. 7. Dosimetric data collected over 3 weeks using a computerized imaging program for mice receiving three weekly doses of ¹³¹I-labeled TNT-1 (mice 1–5) or ¹³¹I-labeled Lym-1 (mice 6–9). Note the increased levels of doses in the second and third weeks of treatment for the experimental mice, compared with the controls. △, First week; □, second week; ○, third week; ---, extrapolation of data from 6–8 days.

Fig. 8. Example of computerized images used to calculate dosimetric data for experimental and control treatment groups shown in Fig. 7 and Table 3. Areas of interest are defined as: region 1, whole mouse; region 2, ME-180 tumor; and region 3, contralateral thigh. Images were obtained from mouse 5 on days 1 (A), 5 (B), 5 (C), and 7 (D) after the third treatment.
Table 2  Radiation dosimetry estimated from biodistribution data for 131I TNT-1 and 131I Lym-1 after first week of therapy.
Data are based on use of 300 μCi 131I-labeled antibodies.

<table>
<thead>
<tr>
<th>Organ</th>
<th>TNT-1 (cGy)</th>
<th>Lym-1 (cGy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>989</td>
<td>1186</td>
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<tr>
<td>Skin</td>
<td>509</td>
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</tr>
<tr>
<td>Muscle</td>
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<tr>
<td>Bone</td>
<td>254</td>
<td>267</td>
</tr>
<tr>
<td>Heart</td>
<td>411</td>
<td>411</td>
</tr>
<tr>
<td>Lung</td>
<td>699</td>
<td>476</td>
</tr>
<tr>
<td>Liver</td>
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<td>550</td>
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<tr>
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<td>1103</td>
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<td>179</td>
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<tr>
<td>Intestine</td>
<td>167</td>
<td>190</td>
</tr>
<tr>
<td>Kidney</td>
<td>358</td>
<td>330</td>
</tr>
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<td>ME-180 tumor</td>
<td>875</td>
<td>799</td>
</tr>
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</table>

Table 3  Radiation dosimetry estimated from imaging data for TNT-1 and Lym-1 after 1, 2, and 3 weeks of therapy.
Data are based on use of 300 μCi 131I-labeled antibodies. Each value represents the calculated dose for that week assuming that there is no accumulation from the previous dose.

<table>
<thead>
<tr>
<th>Mouse</th>
<th>1st wk (cGy)</th>
<th>2nd wk (cGy)</th>
<th>3rd wk (cGy)</th>
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<tr>
<td>TNT-1 therapy*</td>
<td>411</td>
<td>1018</td>
<td>1091</td>
</tr>
<tr>
<td>Lym-1 therapy*</td>
<td>406</td>
<td>1085</td>
<td>1856</td>
</tr>
<tr>
<td>3</td>
<td>1672</td>
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<td>8</td>
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</tr>
<tr>
<td>9</td>
<td>631</td>
<td>296</td>
<td>476</td>
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</table>

* Three 300-μCi doses weekly.

131I-TNT-1 and control Lym-1 for the first week, yet imaging and therapeutic data following repeated doses revealed marked differences between these treatments, further dosimetric studies were performed to quantitate tumor uptake in experimental and control mice after the second and third treatment doses. For this phase of the study, it was necessary to devise a new approach to substitute for the necropsy-biodistribution method, which would have required the commitment of a very large number of animals for a prolonged study and would have suffered from the disadvantage of animal-to-animal variations within small necropsy groups. Instead, an ongoing sequential study of individual mice using the gamma camera imaging system was performed to calculate the percentage of dose in the tumor and the whole animal. This method permitted us to generate dosimetric data using a smaller number of mice and minimized the problems associated with individual animal variation.

Table 3 and Fig. 7 show the radiation doses estimated from the imaging data for 131I-TNT-1 (mice 1–5) and Lym-1 (mice 6–9) after 1, 2 and 3 weeks of therapy. After the first week of treatment, the mean radiation dose delivered to the tumor was 1066 cGy (range, 411–2231) in the TNT-1 treatment group, compared to 580 cGy (range, 263–750) in the control Lym-1-treated group. In the second and third weeks of therapy, the mean tumor doses were 2046 cGy (range 1018–4008) and 2476 cGy (range, 1091–4275), respectively, in TNT-1-treated mice and were essentially unchanged (460 and 526 cGy) in the control Lym-1-treated mice, as illustrated in Fig. 7. Mice 3 and 4 of the TNT-1-treated group showed exceptionally high radiation doses in the tumor even in the first week of treatment (Table 3). Although the other mice (mice 1, 2, and 5) showed only moderate tumor uptake at this time period, all the mice in this group had progressively higher tumor localization in the following weeks of therapy. Even though mice 3 and 4 had the highest therapeutic doses delivered to the tumor (Table 3), mice 1, 2, and 5 also imaged well but they showed a lower overall delivered dose/g of tumor because of the relatively larger tumors in these animals. An example of the imaging data obtained in this study is shown in Fig. 8. In this figure, computerized imaging data are presented for mouse 5 during the third week of treatment with 131I-labeled TNT-1. As the unbound circulating antibody is cleared from the blood pool, the image of the tumor improved over time, demonstrating the therapeutic potential of TNT-1 in rapidly necrosing tumors.

**DISCUSSION**

In our previous study (1), we demonstrated that the numbers of abnormally permeable cells in four human tumors transplanted into nude mice were sufficient to permit the selective localization of two TNT antibodies, to the extent that successful imaging of tumors was achieved without evidence of normal tissue uptake. In the present study, we have extended these observations by testing the therapeutic potential of TNT-1.

The administration of 131I-labeled TNT-1, at either the 150 or the 300 μCi level for three successive weekly doses, produced significant inhibition of tumor growth within 7 days (Fig. 1). This inhibition was sustained for up to 6 weeks after therapy in those animals receiving high dose 131I-TNT-1. Two of these animals showed complete regression, with no gross evidence of tumor. Lesser degrees of regression were observed in 12 of the 14 remaining 131I-TNT-1-treated mice (Table 1). By contrast, only 50% of the 131I-Lym-1-treated controls showed evidence of regression and these were mostly minor. Yet, in spite of the obvious difference in therapeutic response, the biodistribution and dosimetry data (Table 2; Fig. 6) revealed little discernible difference for identical doses of radiolabeled TNT-1 and Lym-1 after 1 week of therapy. While not unexpected, this finding necessitated the design of a study to collect dosimetry data over a much longer time frame, covering a period of 3 weeks overall.

The experimental design was based upon the concept of a four-compartment model, shown in Fig. 9, for the distribution kinetics of radiolabeled TNT-1 and Lym-1 (control) monoclonal antibodies. In this four-compartment model, Lym-1 will behave in an identical fashion to TNT-1 within the first three compartments (blood, extravascular fluid, and intracellular fluid space of permeable cells). Both Lym-1 and TNT-1 peak in the blood (first compartment) immediately after injection and clear over a period of 6 or more days (Figs. 6 and 7). Both are IgG2a antibodies and, therefore, would be expected to enter and diffuse within the extracellular fluid space (second compartment) at a comparable rate. In normal tissues, diffusion from the blood to the extracellular space is slow but in the vicinity of the tumor, where vascular permeability is increased (22–26), the antibodies enter the extracellular space at an accelerated rate. Likewise, both antibodies would penetrate permeable cells (third compartment) in a similar fashion. However, once within the cells, TNT-1 would show progressive binding to the nuclear matrix in the cell ghost, whereas Lym-1 would remain free within the cytoplasm. With clearance of the antibody from the blood over several days, the concentration gradient would be expected to reverse and Lym-1, being unbound, would diffuse from within the cells to the extracellular fluid and to the blood, while TNT-1 would be retained in the tumor cell ghost. Thus, a differential accumulation of TNT-1 with reference to Lym-1 would be expected only after a sustained fall in blood levels, with a net diffusion of Lym-1 from...
the tumor intracellular and extracellular fluid compartments into the bloodstream.

This hypothesis is consistent with the dosimetry results (Fig. 6) and with the tumor regression observed after three therapeutic doses of radiolabeled antibody (Table 1). The passage of Lym-1 in the tumor intra- and extracellular fluid spaces is sufficient to deliver a radiation dose to the tumor on the order of 800 rad, accounting for the limited regression seen in half the Lym-1-treated groups (Table 1). Subsequently, as 131I-Lym-1 washes out, 131I-TNT-1 continues to irradiate viable tumor cells situated in proximity to the dead and dying cells in which TNT-1 is retained. With the passage of time, the dose of the radiation delivered to the tumor by TNT-1 will exceed and progressively diverge from that delivered by Lym-1. The initial biodistribution data (Fig. 6) contain a suggestion that this effect is becoming apparent, in the slightly higher levels of TNT-1 compared with Lym-1 at 8 days, a finding not seen in any of the normal tissues.

Furthermore, with additional doses of radiolabeled antibody, a more marked divergence of image intensity and therapeutic response for TNT-1 with reference to Lym-1 was observed. The radiation emitted by 131I-TNT-1 retained within the nuclei of dead or dying cells injures adjacent viable tumor cells, inducing cellular degeneration or death. These cells in turn become a target for subsequent doses of TNT. Indeed, with each successive dose of 131I-TNT-1, the tumor would be expected to contain a higher proportion of abnormally permeable cells and, therefore, show more intense imaging. These enlarging areas of degeneration and necrosis within the tumor provide an ever-expanding target for TNT-1 localization. Imaging and therapy would, therefore, be expected to become more effective as treatment proceeds, in marked contrast to other forms of monoclonal antibody therapy in which the observed response often decreases during treatment, due to antigenic modulation or the emergence of antigen-negative clones from a heterogeneous tumor population. In addition, there are data to suggest that radiation delivered at the tumor site serves to induce additional permeability increases in the microvasculature of tumors (27), thereby favoring further selective diffusion of radiolabeled antibody into the tumor.

The data from the extended 3-week dosimetry study (Table 3, Fig. 7) provided findings over the first week similar to those already described by the necropsy-biodistribution method (Table 2; Fig. 6). The TNT-1-treated animals, however, showed a substantial increase of approximately the same order of magnitude in the dose delivered in subsequent weeks (Fig. 7). The observation that two of the five 131I-TNT-1-treated mice received an initial tumor dose 3 to 5 times greater than the others (Table 3) is of interest and may be attributable to several factors that warrant further investigation. While tumor size alone may not be a factor, the tumor vascularity in relation to size is important in two respects. First, the degree to which the tumor vasculature provides adequate perfusion for all parts of the tumor is a major factor in determining the extent of cell degeneration and death (24, 28–30). This in turn determines the proportion of abnormally permeable cells and the extent to which nuclear antigens are accessible (i.e., the size of the third and fourth compartments). A poor blood flow would maximize cell death, providing a large target for TNT-1. Conversely, a poor blood flow would impede delivery of TNT-1 especially to sites of degeneration within the tumor, which by definition are likely to be furthest removed from functioning vessels (31). Other factors may play a role, including the extent to which these transplanted tumors develop neovascularization and the integrity of the small blood vessels around and within the tumor. The former may in part be an accident of location, i.e., whether or not the implantation site is in close proximity to vessels in the thigh. The latter issue is much more complex. Small vessels in the vicinity of tumors have been shown to be excessively permeable (22–25), while within the body of a malignant tumor blood frequently appears to pass through channels that partially lack endothelium. These factors determine the rate of passage between the first and second compartments shown in Fig. 9. The rate of diffusion of antibody within the extracellular fluid represents an additional variable (32) and the presence of inflammation, fibrin deposition, or fibrosis may influence access of the antibody to any permeable cells that may be present.

In conclusion, the results of our studies support the contention that TNT-1, an antibody directed against an intracellular component, can be used for the selective imaging of transplantable human tumors. Furthermore, the dosage of radiation delivered by the antibody can be escalated to the point that significant therapeutic responses are achievable in a squamous carcinoma with moderate radiosensitivity. The four-compartment model for the biodistribution kinetics of TNT-1 (Fig. 9) appears to be valid and provides an understanding of the mechanism by which an antibody without specificity to the tumor (represented by Lym-1) may deliver limited amounts of radiation to the tumor site. The concept of centrifugal killing of viable tumor cells around deposits of radiolabeled TNT-1 provides an explanation of the increased localization of 131I-TNT-1 after repeated dosages. Previous autoradiographic studies demonstrated localization of TNT-1 to the cell nuclei within areas of degeneration and necrosis, and the degree of retention of TNT-1 within the tumor appears to show a positive correlation with the extent of cell degeneration and death (i.e., the size of the third and fourth compartments (1). This last obser-
vation provides the rationale for a practical use of $^{131}$I-TNT in monitoring conventional forms of cancer therapy. The intensity of the image generated by a standardized dose of radiolabeled TNT-1 would be expected to correlate with the amount of cell death produced by therapy when following individual tumors over time. Furthermore, the demonstration that $^{131}$I-labeled TNT-1 can be used to deliver therapeutic doses of radiation to a tumor in an animal system opens the possibility that similar methods can be developed to deliver therapeutic doses to large bulky tumors in humans. In this regard, TNT-1 may be used both as an adjuvant to augment current therapies that themselves induce some degree of tumor necrosis and as a de novo therapy for those tumors in which the proportion of degenerating cells is high ab initio. Finally, malignant tumors of diverse histological type would be amenable to imaging and therapy by TNT-1, contingent only upon the presence of a sufficient number of degenerating cells within the tumor to serve as the target for initial antibody localization.

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Tumor Necrosis Treatment of ME-180 Human Cervical Carcinoma Model with $^{131}$I-Labeled TNT-1 Monoclonal Antibody

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