Antitumor Activity of Idarubicin-Monoclonal Antibody Conjugates in a Disseminated Thymic Lymphoma Model


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ABSTRACT

Many of the experimental approaches used in the search for new targeted drug delivery systems ignore the disseminated nature of metastatic disease; the development of more relevant tumor models is therefore a priority. A reproducible and tumor-specific model has been generated by inoculating (C57BL/6 × BALB/c) F1 (Ly-2.2*) mice i.v. with the Ly-2.1* murine ITT(1) 75NS E3 thymic lymphoma (E3). At a dose of 2x10⁶ cells, E3 tumors grew in a disseminated fashion, arising initially and predominantly in the lung and kidney, and later and less often in the thymus, spleen, and other tissues. In addition, histopathological examination and flow cytometry of blood did not detect E3 tumor cells in most organs or in the circulation throughout the course of disease. The mean survival time (MST) of untreated mice was both reproducible and proportional to the number of E3 tumor cells injected and was therefore used to demonstrate the suitability of this model for immunochemotherapeutic studies. When examining the antitumor efficacy of idarubicin monoclonal antibody conjugates, it was observed that the survival times of treated mice were consistent within groups and between experiments. The disseminated E3 (Ly-2.1*) tumor model, like the s.c. E3 tumor model, demonstrated the dose-dependent efficacy of idarubicin-anti-Ly-2.1 conjugate treatment and illustrated both the negligible antitumor activity and toxicity of idarubicin alone. Furthermore, lung and kidney weight measurements formally demonstrated that the increased MST of treated mice represented a reduction of E3 tumor burden in these organs. This model provides a useful tool for study of the immunochemotherapy of disseminated tumors in mice and further illustrates the antitumor activity of idarubicin-monoclonal antibody conjugates.

INTRODUCTION

Since MoAbs¹ can be used to localize a number of human tumors, many studies are focusing on the ability of these MoAbs to target chemotherapeutic drugs, toxins, radionuclides, and other antitumor agents in experimental murine tumor models. These models can be divided into those using leukemia or other nonsolid tumors usually confined to the peritoneal cavity (1-5) and those using s.c. solid tumors (6-11).

The systemic or i.p. treatment of s.c. solid tumors with immunoconjugates is more difficult, generally because the tumor is less accessible and penetration of the interior tumor mass is limited. Consequently, i.v. immunoconjugate therapy is often less effective than injection at or near the tumor site (12). However, like i.p. tumor models, transplanted tumors growing s.c. fail to provide a representative preclinical model for evaluating treatment of disseminated neoplastic disease. Total blood flow, rates of perfusion, and vessel permeability may vary significantly in tumors transplanted s.c. compared to those developing as metastatic deposits in organs and all of these vascular properties are of obvious importance in determining the uptake of circulating immunoconjugates by tumors (13). In addition, antigen expression, development of drug resistance, and metastatic and growth potential may also differ between transplanted s.c. tumors and tumor cell deposits growing in different organs. In this sense, few studies have considered the clinically observed disseminated nature of advanced cancer when determining the preclinical efficacy of immunoconjugate therapy (14, 15), yet it is probable that a heterogeneous response of tumors to immunochemotherapy will be observed (16).

Previously, E3, a clonal variant of the radiation-induced Ly-2.1* ITT(1) 75NS thymic lymphoma (17), has been used as both an i.p. and a s.c. tumor model in Ly-2.1~ (C57BL/6 × BALB/c) F1 (hereafter called B6CF1) mice to investigate the antitumor activity of anti-Ly-2.1 MoAbs covalently coupled to chlorambucil (18), methotrexate, aminopterin (19), N-acetyl-melphan (9), ricin (12), and Ida (11). Indeed Ida-MoAb conjugates were demonstrated to eradicate small (≤ 0.1 - cm²) s.c. E3 tumors at doses of at least 80 μg Ida, but were considerably less effective against larger (≥ 0.25 cm²) s.c. tumors at higher doses (LD₅₀ = 10.0 mg/kg Ida equivalent) (11).³ By contrast herein, following i.v. inoculation, E3 has been established, characterized, and utilized as a disseminated tumor model for evaluating the specific antitumor activity of Ida-MoAb conjugates. While the Ida-anti-Ly-2.1 conjugate demonstrated dose-dependent efficacy, prolonged treatment was the only schedule which completely prevented the development of disseminated E3 tumors. By contrast the control groups treated with PBS, Ida, Ida and anti-Ly-2.1, or an irrelevant Ida-MoAb could not increase the MST of mice. However, the administration of anti-Ly-2.1 at high doses prolonged the survival time of 20% of treated mice. This model appears both valid (results are reproducible, qualitatively and quantitatively) and selective (detectable difference between the specific antitumor activity and the toxicity of the preparations tested) and should aid future preclinical evaluation of other immunoconjugates.

MATERIALS AND METHODS

Mice

Normal female B6CF, mice were produced in the Department of Pathology, University of Melbourne; groups of 5-15 mice (8-12 weeks old) were used in each experiment.

MoAb

The MoAbs used were anti-Ly-2.1 (mouse IgG2a) reactive with the murine Ly-2.1 specificity (20) and 250-30.6 (mouse IgG2b) which recognizes an antigen present on normal and malignant human gastrointestinal epithelium (21). The MoAbs were isolated from ascites fluid by precipitation with 40% ammonium sulfate, dissolution in PBS, and dialysis with the same buffer. These crude preparations were either neutralized, MoAbs were then dialyzed against PBS, aliquoted, and stored old) were used in each experiment.

¹ Unpublished results.

References

1. This work was supported by Farmitalia Carlo Erba, Milan, Italy.
2. The abbreviations used are: MoAb(s), monoclonal antibody (ies); L₅₀, % lethal dose; FITC, fluorescein isothiocyanate.
3. Received 12/29/1988; accepted 10/22/90.
4. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

5. The abbreviations used are: MoAb(s), monoclonal antibody (ies); L₅₀, % lethal dose; FITC, fluorescein isothiocyanate.

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stored at −70°C. When tested by sodium dodecyl sulfate-polyacrylamide gel electrophoresis these MoAb preparations were estimated to be 90–95% pure.

Tumor Cells

*In Vitro.* The cell lines examined in this study included the Ly-2.1+ murine thymoma ITT(1) 75NS E3 (different only by this antigen in B6CF, mouse) (18) and the Ly-2.1+ murine T-cell leukemia BW5147 (allogeneic for B6CF) (22). Cells were maintained in vitro in Dulbecco’s modified Eagle’s medium (E3) or RPMI 1640 (BW5147) (Flow Laboratories, Sydney, Australia), supplemented with 10% heat-inactivated newborn calf serum (Flow), 2 mM glutamine (Commonwealth Serum Laboratories, Sydney, Australia), 100 μg/ml streptomycin (Glaxo, Melbourne, Australia), and 100 IU/ml penicillin (Commonwealth Serum Laboratories).

*In Vivo.* The E3 tumor was maintained in vivo by serial passage into B6CF, mice. Cells from the ascites fluid were washed and centrifuged (400 × g for 5 min) twice in PBS, resuspended in PBS, and injected i.v. (250 μl) into the lateral tail vein of mice. Mice were then subjected to a series of i.p. treatments and the survival recorded as the MST ± SD. In addition, at different time points the organs of control or treated mice were examined macroscopically for the presence of tumor, removed, and weighed prior to formalin fixation and staining by hematoxylin and eosin.

Biodistribution. [125I]-5-Iodo-2'-deoxyuridine, 32 μCi, with a specific activity of 956 μCi/ml (Amersham International Ltd., Amersham, Buckinghamshire, England), was used to label both E3 (from in vivo passage) and a negative control cell line BW5147 (from culture and does not grow in B6CF, mice) (8 × 10⁷ cells). The cells were incubated for 18–20 h at 37°C, washed three times in PBS, and resuspended to a final concentration of 1 × 10⁷ cells/ml (>95% cell viability as judged by the trypan blue exclusion test); a volume of 200 μl (2 × 10⁶ cells, 7–8 × 10⁶ cpm) was injected into the tail vein of each mouse. At various times after i.v. injection of radiolabeled cells, 4 mice from each experimental group were sacrificed, and blood and organs were removed, weighed, and thereafter counted in a gamma counter (LKB-Wallace 1260 Multigamma). Results were expressed as the percentage recovery of injected radioactivity (means ± SE) assuming a total blood volume of 7% of body weight.

Flow Cytometry Analysis. The presence of circulating viable E3 tumor cells in the blood of B6CF, mice were examined over a 28-day period after i.v. E3 tumor inoculation using FITC-labeled anti-Ly-2.1. Groups of 2–4 B6CF, mice given i.v. injections of 2 × 10³ E3 cells or control BW5147 cells were sacrificed and bled on days 1, 2, 4, 8, 14, and 28 after tumor inoculation. Blood (500 μl; some samples of normal B6CF, blood were “spiked” with (10⁻⁵–5 × 10⁷) E3 cells) was centrifuged (400 × g for 10 min) and the upper 100 μl buffy coat containing WBC and tumor cells were then removed and washed 3 times in PBS (1% bovine serum albumin) and 0.83% NH₄Cl to remove any RBC. These cells were added to 2-ml Eppendorf tubes and incubated with 100 μl (10 μg MoAb) FITC-anti-Ly-2.1 at 4°C for 1 h. Unbound antibody was removed by 3 washes with PBS (9000 × g, 5–10 s), before being finally resuspended in 500 μl of PBS (1% bovine serum albumin). Samples were run on a FACSscan (Becton Dickinson, Mountain View, CA) and data were recorded as the percentage of cells bound relative to a normal B6CF, blood negative control ± SE (quadruplicate determinations) as calculated by a HP310 system (Hewlett Packard Australia, Ltd., Melbourne, Australia).

Preparation of Ida-MoAb Conjugates

Intact MoAbs (2–3 mg/ml) in 0.05 M borate 0.075 M NaCl buffer (pH 7.8–8.0) were mixed with a 10–20 molar excess of 14-bromo-4-demethoxydaunomycin dissolved in N,N-dimethylformamide at 10 mg/ml. The reaction was performed at room temperature for 4 h, before centrifuging (9000 × g for 2 min) to remove any precipitate. Free 14-bromo-4-demethoxydaunomycin and other unreacted starting materials were removed by gel filtration chromatography using a Sephadex G-25 column (PD-10) and the conjugates were then passed through a column of Porapak Q to remove any adsorbed drug. The amount of Ida incorporated in the conjugate (2–4 molecules of Ida/molecule MoAb) was determined by absorbance spectrophotometry at 483 nm (ε₄₈₃ = 3.4 × 10⁶ M⁻¹ cm⁻¹) (11). Protein concentration was determined using the Bradford assay (11).

RESULTS

This study was conducted in two parts. Initially it was necessary to characterize the disseminated E3 tumor model; this was achieved by observing E3 tumor cell biodistribution and development and the survival time of mice inoculated with increasing doses of E3 tumor cells. Subsequently the model was used to test the ability of Ida-MoAb conjugates to reduce tumor burden and prolong the survival time of the mice.

Dose of E3 Cells Required for the Generation of Disseminated Tumors

Seven different doses of E3 tumor cells (2 × 10⁵ → 2 × 10⁸) were injected i.v. into groups of 10–15 B6CF, mice to determine whether a disseminated or metastatic tumor model could be produced. As shown in Fig. 1, a combination of macroscopic and light microscopic examination of the tissues of these mice at the time of death detected tumors in a number of different organs. Over the dose range tested, the predominant sites of tumor growth were lungs, thymus, kidney, and spleen (Fig. 1). However, at doses from 10³ to 2 × 10⁵ cells, lymph nodes and liver were also usually involved. It should be noted that a small percentage of mice that received the highest doses of E3 cells also developed tumors in capillary beds behind the eyes or s.c. but no tumors were detected in the brain, heart, muscle, gastrointestinal tract, or reproductive organs. Increasing the dose of E3 cells injected increased the likelihood of mice developing tumors in lymph node, lung, liver, and thymus (Fig. 1); this trend was not as obvious for the kidney and spleen which developed tumors in 30–80% of the mice irrespective of the dose of E3 cells injected.

Tumors did not arise in mice receiving doses ≤2 × 10⁴ cells. However, the MST of mice appeared to be proportional (coefficient of variation, 0.97) to the log number of E3 tumor cells injected over the dose range 2 × 10⁵–2 × 10⁸ cells (Fig. 2). In addition, the calculated MST (26.9 ± 1.0 days) of groups receiving 2 × 10⁶ E3 cells represents a value calculated from 4 experiments (total of 45 mice) therefore validating the quantitative reproducibility of survival as an end point in this model. Because the MST of mice receiving 2 × 10⁶ E3 cells was highly reproducible of survival as an end point in this model. Be-
ANTITUMOR ACTIVITY OF Ida-MoAb CONJUGATES IN THYMIC LYMPHOMA

Analysis of Tumor Development

Although the MST of mice receiving an i.v. inoculation of E3 tumor cells had been established, it was important for the purpose of scheduling treatment to determine the pathogenesis and development of E3 tumors in various organs.

Distribution of E3 Tumor Cells after i.v. Inoculation. Cells were injected i.v. into mice (4/group) and their distribution was examined within 3 days of inoculation (Tables 1 and 2). Initially almost all the E3 and BW5147 tumor (used as a negative or background control) cells were arrested in the lungs, that being the first capillary bed met by the tumor cells. From there the tumor cells were rapidly cleared with <1.5% of the injected dose left in the lungs 2 (BW5147) or 4 (E3) h after inoculation. Whether this difference between E3 and BW5147 lung clearance is important in tumor cell engraftment in the lung is not clear; however, it does partially account for the 30% lower total recovery of radioactivity observed in BW5147-inoculated mice after 2 h. Approximately 35% of the tumor cells (E3 and BW5147) passed through the lung capillaries, as an amount of radioactivity proportional to this number of cells was redistributed to other sites. One half of these viable tumor cells were found in the liver tissue, from which the cells were gradually cleared; the other half were found in the blood or remaining carcass (bone, skin, and muscle). Very few (<1.5%) E3 or BW5147 tumor cells were found in the spleen, kidney, or thymus suggesting that E3 tumors in these organs develop from a small number of tumor cells or arise later as metastases from other sites such as lung. The elevated levels (up to 7%) of radioactivity in the stomach and gastrointestinal tract several h after E3 or BW5147 tumor cell inoculation probably represents dehalogenated 125I of nonviable tumor cells cleared from the lung, liver, and blood inasmuch as no procedure was used to remove any radioactivity not associated with intact cells. Whole body counts revealed that <3% of the injected dose of radioactivity remained in E3- or BW5147-inoculated mice after 72 h, however, taking into account the rapid doubling time (~24 h) for E3 tumor cells, a significant number of the progeny will be unlabeled and therefore undetected in the organs of these mice. Therefore from these data it is not possible to estimate the number of E3 tumor cells present in the mouse 3 days after inoculation.

Histopathological and Flow Cytometric Analysis of E3 Tumor Development. A group of 28 B6CF1 mice were given i.v. injections of 2×10^6 E3 cells and sacrificed (4/time) on days 1, 2, 4, 6, 14, 21, and 25 after inoculation. The organs and blood of these mice were examined for the presence of E3 tumor cells by histopathology and flow cytometry, respectively. Light microscopic examination of hematoxylin and eosin-stained sections indicated considerable differences in the onset and growth rate of E3 tumor deposits in various organs. E3 tumor foci were detected in lung tissue within 6 days of inoculation (Fig. 3) and by day 21 had grown to involve 60–70% of the lung (Fig. 3). Histopathologically E3 tumor cells were not evident in the kidney until 14 days after tumor inoculation; however, within 3–4 weeks rapid tumor growth had destroyed 30–90% of the kidney (Fig. 3). Significant tumor deposits were not observed in the thymus until 14 days after inoculation and, when involved, in the spleen and liver (2 of 8 and 1 of 8, respectively) after 21 days. At the time of death (day 25), less than 15% of lung, thymus, and kidney tissue remained viable, the majority of mice dying as a result of respiratory or renal failure. As previously noted, no E3 tumor cells were found in the brain, heart, stomach, or gastrointestinal tract.

The possible initial presence and/or leukemic development of systemic E3 tumor was monitored by flow cytometry. At a detection limit minimum of 10^4 E3 tumor cells (as determined by normal B6CF1 blood samples spiked with increasing numbers of E3 cells), FACScan analysis could not detect FITC-anti-Ly-2.1-bound E3 cells in the blood of mice, 1, 2, 4, 8, 14, or 25 days after tumor inoculation (data not shown). As an

Table 1 Percentage of recovery of E3 tumor cells at various sites at different times after i.v. injection

<table>
<thead>
<tr>
<th>Site</th>
<th>0.0 h±</th>
<th>0.16 h</th>
<th>0.5 h</th>
<th>2.0 h</th>
<th>4.0 h</th>
<th>8.0 h</th>
<th>24.0 h</th>
<th>72.0 h</th>
</tr>
</thead>
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<td>Lungs</td>
<td>94.6 ± 1.3</td>
<td>67.1 ± 2.1</td>
<td>37.0 ± 1.5</td>
<td>14.5 ± 1.0</td>
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</tr>
<tr>
<td>Liver</td>
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<td>17.4 ± 0.7</td>
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<td>0.8 ± 0.1</td>
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</tr>
<tr>
<td>Kidney</td>
<td>0.0 ± 0.0</td>
<td>0.3 ± 0.0</td>
<td>1.5 ± 0.0</td>
<td>1.1 ± 0.1</td>
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<td>0.8 ± 0.0</td>
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<td>Thymus</td>
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<td>0.1 ± 0.0</td>
<td>0.2 ± 0.0</td>
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<td>Blood</td>
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<td>Stomach</td>
<td>0.0 ± 0.0</td>
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<td>Intestine</td>
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</tbody>
</table>

* Two × 10^6 [125I]-5-iodo-2′-deoxyuridine-labeled E3 tumor cells were injected i.v. into B6CF1 mice. Mean ± SE.

* Distribution of tumor at all time points was calculated from the injected dose (7–8 × 10^6 cpm).

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example, when normal B6CF, blood was used as a negative control (99% of the cells had fluorescence <70), and normal B6CF, blood spiked with increasing numbers of E3 cells (10^4, 5×10^4, 10^5, and 5×10^5) was used as a positive control (4, 20, 31, and 58% of cells respectively with fluorescence >70), no E3 tumor cells were detected in the blood of B6CF, mice that had been inoculated 2 days earlier. Thus due to their absence or small number (<10^4 cells), circulating E3 tumor cells could not be measured and utilized as a marker of immunoconjugate efficacy.

Because the majority of E3 tumor cells were initially entrapped in the lung (Table 1) and the development of tumor foci in this organ was rapid (Fig. 3) it appears that E3 tumors in the lung arise from the cells which lodge in this tissue immediately after inoculation. It is unclear from this analysis, however, whether E3 tumors in the kidney, spleen, liver, and thymus originate mechanically following inoculation or stem from single metastatic tumor cells or emboli of rapidly growing established E3 tumors in the lung.

Using the Disseminated E3 Tumor Model for Immunchemo-therapeutic Studies

Recently it has been demonstrated that the growth of day 4–5 (0.2 cm^3)-established s.c. murine and human tumors can be specifically and significantly inhibited by i.v. or i.p. administration of Ida-MoAb conjugates (11, 23). Indeed a number of small s.c. E3 tumors in B6CF, mice could be eradicated at dose of 80 μg of Ida-anti-Ly-2.1 conjugate, well below the single dose LD_{50} (10.0 mg/kg Ida equivalent) of this immunoconjugate (11). Herein, by i.v. injection of a dose of 2×10^6 E3 cells, a disseminated tumor model was established in which the MST of mice is highly reproducible. Using this end point, the efficacy of Ida-MoAb conjugates was examined (Table 3).

Groups of 10 B6CF, mice inoculated i.v. with 2×10^6 E3 cells were treated with i.v. injections of either (a) PBS, (b) anti-Ly-2.1, (c) Ida, (d) Ida and anti-Ly-2.1, (e) Ida-250–30.6 conjugate, or (f) Ida-anti-Ly-2.1 conjugate. As shown, a variety of doses of Ida-anti-Ly-2.1 conjugate and the control treatments were administered to establish their therapeutic efficacy and toxicity. The schedule of treatments were also varied to assess the efficacy of immediate (days 1 and 2 against minimal numbers of disseminated E3 cells), delayed (day 5–8 against established E3 tumors) or prolonged (days 1–5) therapy. As previously demonstrated B6CF, mice receiving 2×10^6 E3 cells i.v. had a MST of approximately 25 days (PBS; 24.1 ± 3.5 days) (Table 3). It was evident over the dose range tested that no therapeutic treatment (Ida, Ida and anti-Ly-2.1, or Ida-250–30.6) was able to increase the MST of mice. Indeed no dose of Ida alone was therapeutically effective (15–40 μg), the higher doses, 25 and 40 μg causing toxicity-related deaths (2 of 10 and 10 of 10, respectively) within 10 days of treatment. The lack of a synergistic effect with a combination of Ida and anti-Ly-2.1 was demonstrated by treating with a mixture of 15 μg of Ida and 2.7 mg anti-Ly-2.1, where all mice died with MST 27.3±3.2 days. By contrast, treatment with Ida-anti-Ly-2.1 conjugate prolonged the MST of mice in a dose-dependent manner (Table 3). At the highest dose of conjugate administered (110 μg), the MST was 24.2 ± 9.7 days; however, the smallest dose (40 μg) prolonged survival by only 4 days (MST 28.4 ± 7.2 days) (P < 0.001 and P < 0.05 compared to PBS controls). Surprisingly, over the dose range tested, there was no apparent difference in therapeutic efficacy between immediate (days 1–3) and delayed (days 5–8) treatment schedules. However, by prolonging the immediate treatment schedule (days 1–5; total, 90 μg), 75% (15 of 20) of the mice survived tumor free (Table 3). The specific antitumor activity of Ida-anti-Ly-2.1 conjugate was demonstrated by the ineffectiveness of 80 μg of Ida covalently bound to the nonspecific 250–30.6 MoAb (MST 25.7 ± 2.8). It is also apparent that opsonization of antibody coated tumor cells did not contribute significantly to the therapeutic effect of the Ida-anti-Ly-2.1 conjugate (80 μg Ida/4.6 mg anti-Ly-2.1) as anti-Ly-2.1 alone (4.6 mg) did not prolong the survival time of mice (MST 24.4 ± 2.7 days). However, when mice were treated with a total of 5.4 mg anti-Ly-2.1 on days 1–4 an antitumor effect was seen, where 20% of the mice survived. The survival times obtained were highly reproducible, within treated and control groups [small SD] and between experiments [Ida-anti-Ly-2.1 (80 μg) MST was calculated from 3 independent studies (10 mice/group)]. Toxic doses of Ida-anti-Ly-2.1 were not administered; however, the toxicity demonstrated by Ida alone was similar in both disseminated and s.c. E3 tumor models (LD_{50} ~ 1–2 mg/kg) (Table 3) (11).

Although the disseminated E3 tumor model primarily lends itself to survival experiments, macroscopic examination of the organs of treated mice at the time of death also revealed the different therapeutic effects obtained (Table 4). Even taking into account the unreliability of macroscopic examination and the possible variation between different experiments it appeared that a considerably lower proportion of Ida-anti-Ly-2.1-treated mice (20–30%) died with E3 tumor in their lungs than those

### Table 2 Percentage of recovery of BW5147 tumor cells at various sites at different times i.v. injection

<table>
<thead>
<tr>
<th>Site</th>
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<th>8.0 h</th>
<th>24.0 h</th>
<th>72.0 h</th>
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<tr>
<td>Lungs</td>
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<td>Liver</td>
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<td>0.5±</td>
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<td>Kidney</td>
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<td>0.2±</td>
<td>1.5±</td>
<td>4.2±</td>
<td>4.9±</td>
<td>2.0±</td>
<td>0.2±</td>
<td>0.1±</td>
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<td>0.4±</td>
<td>2.3±</td>
<td>3.5±</td>
<td>4.7±</td>
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<td>0.6±</td>
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<tr>
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<td>0.1±</td>
<td>0.2±</td>
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<td>0.8±</td>
<td>0.4±</td>
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</tr>
<tr>
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<td>12.0±</td>
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<td>11.9±</td>
<td>3.3±</td>
<td>3.3±</td>
<td>2.0±</td>
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</tbody>
</table>

**Table 2.** Percentage of recovery of BW5147 tumor cells at various sites at different times i.v. injection.

* Distribution of tumor at all time points was calculated from the initial dose (7–8×10^5 cpm).

* Two × 10^5 [^3]H-5-iodo-2'-deoxyuridine-labeled BW5147 tumor cells were injected i.v. into B6CF, mice. Mean ± SEM.
given control treatments (80–90%) (Table 4). By contrast, 70–90% of the mice inoculated i.v. with $2 \times 10^6$ E3 cells had tumors in the kidney at the time of death irrespective of the treatment administered (Table 4). These findings suggest that the increased MST of these mice is due to the prevention of respiratory failure. To examine this possibility and to formally demonstrate that Ida-anti-Ly-2.1 conjugate was reducing tumor burden, an experiment was designed wherein treated mice were sacrificed on day 21 or 30 and both their lungs and kidneys were weighed (Fig. 4). Groups of 10 B6CF1 mice inoculated i.v. with $2.0 \times 10^6$ E3 cells were treated (on days 1–2) with i.p. injections of either (a) PBS, (b) anti-Ly-2.1 (4.5 mg), (c) Ida (15 µg), (d) Ida-250–30.6 conjugate (80 µg), or (e) Ida-anti-Ly-2.1 conjugate (80 µg). Mice received equal doses of Ida and/or MoAb (anti-Ly-2.1 or 250–30.6), respectively, on both days 1 and 2 after tumor inoculation. A group of 5 normal B6CF1 mice not given injections of E3 tumor cells were sacrificed and their lungs and kidneys were weighed (mean weights: lung, 0.16 ± 0.01 g; kidney, 0.12 ± 0.01 g). PBS-, Ida-, Ida-240–30.6- or anti-Ly-2.1-treated mice all sacrificed 21 days after tumor inoculation had lung weights in the range 0.24–0.27 g and kidney weights of 0.19–0.26 g. By contrast, when sacrificed on day 21 the mean lung and kidney weights of Ida-anti-Ly-2.1-treated mice were 0.17±0.01 and 0.12±0.1 g, respectively (Fig. 4). Thirty days after tumor inoculation the surviving Ida-anti-Ly-2.1-treated mice (8 of 10) had mean lung and kidney weights of 0.17±0.02 g (data not shown). Thus the Ida-anti-Ly-2.1 conjugate treatment could more significantly inhibit E3 tumor growth in the lungs than in the kidney, and consequently fewer of these mice had macroscopically evident tumor in their lungs at the time of death (Table 4). Given the day 21 weight of control treated kidney, however, it is likely that a reduction of E3 tumor growth in the kidney was also responsible for the increased MST of Ida-anti-Ly-2.1 conjugated-treated mice.

DISCUSSION

The poor success rate of most immunotherapy and chemotherapy protocols in solid tumors, which are effective in experimental tumor systems, suggests that the models utilized inadequately represent the clinical disease (16). Most cancers are lethal because of their ability to metastasize from the primary tumor site and to proliferate at distant sites. It is therefore conceivable that therapies which are effective against disseminated tumor cells in experimental systems may be more applicable in the clinic. However, preclinical information on effective treatment of metastatic disease in animals is sparse, and even newer therapies such as immunotoxins and drug-MoAb conjugates have generally been tested against ascites tumors growing i.p. or well vascularized tumors implanted s.c. Indeed we have used the murine ITT(1) 75NS E3 thymoma (17) as a tumor specific i.p. (9, 18) or s.c. (9, 11, 12, 18, 19) tumor model to assess the antitumor efficacy of a number of different immunoconjugates in these two sites. Therefore a logical progression was to develop a disseminated tumor model in which the efficacy of immunoconjugates could be examined in tumors originating systemically in different organs like metastases observed in advanced cancer.

Initially it was demonstrated that tumors could grow in a number of different tissues when greater than $10^5$ E3 tumor cells were injected i.v. Lung, lymph node, thymus, liver, kidney, and spleen were all predominant sites of E3 tumor growth at the highest doses ($10^7–2 \times 10^8$) of cells injected (Fig. 1); however, only lung and kidney were consistently involved (>50% mice) when B6CF1 were inoculated with $2 \times 10^6$ cells. A linear relationship existed between the MST of mice and the log number of E3 tumor cells injected ($2 \times 10^5–2 \times 10^6$), while the validity of survival as an end point was demonstrated by reproducibility within and between experimental groups of B6CF1 mice (Fig. 2). Because the mice receiving $2 \times 10^6$ E3 cells i.v. had reproducible MST and large tumor burdens confined to a few organs, this dose was further characterized by analyzing tumor development and its suitability for immunoconjugate studies. Biodistribution studies (Tables 1 and 2) offered little evidence to suggest that E3 tumor cells have affinity for some organs, because most tumor cells, including allogeneic BWS147 cells

Fig. 3. Histological H & E sections of the disseminated E3 tumors of (A) lung (day 6), (B) lung (day 21), and (C) kidney (day 21). Note (A) small clusters of E3 tumor cells interspersed among surrounding lung tissue infiltrated by leukocytes (× 240), (B) invading mass of E3 tumor (left) bordering some remaining normal lung tissue (× 600), and (C) 50–80% of the normal kidney architecture is occupied by E3 tumor cells. Some remaining tubules and glomeruli are completely surrounded by the expanding tumor mass (× 240).
of the development of disseminated E3 tumors (Fig. 3) resulted in tumor deposition in the lung (data not shown) there therefore not possible to confirm whether E3 tumors arising in these organs. It is therefore not possible to confirm whether E3 tumors arising in the kidney, spleen, thymus, etc., were metastases from established lung E3 tumors resulting from cells lodging there immediately following i.v. inoculation. It was clear, however, that sequential i.v. passaging of kidney E3 tumors eventually resulted in tumor deposition in the lung (data not shown) therefore suggesting that E3 tumors of the kidney were not a unique population metastasizing only to the kidney.

Both biodistribution studies and histopathological examination of the development of disseminated E3 tumors (Fig. 3) indicated that E3 tumors in the lungs probably originated from cells entrapped immediately following tumor inoculation. Furthermore, the initial appearance of E3 tumors in the kidney (Fig. 4), thymus, spleen, and liver was considerably later than that observed in the lung (Fig. 4). The difference in development of E3 tumors between organs and the absence of detectable tumors in the brain (Table 1) are normally rapidly entrapped and destroyed in the lungs following i.v. administration (24, 25). In addition, very few E3 cells were detected in the kidney, spleen, or thymus (Table 2), are normally rapidly entrapped and destroyed in the lungs following i.v. administration (24, 25). In addition, very few E3 cells were detected in the kidney, spleen, or thymus (Table 2). It was clear, however, that sequential i.v. passaging of kidney E3 tumors eventually resulted in tumor deposition in the lung (data not shown) therefore suggesting that E3 tumors of the kidney were not a unique population metastasizing only to the kidney.

Both biodistribution studies and histopathological examination of the development of disseminated E3 tumors (Fig. 3) indicated that E3 tumors in the lungs probably originated from cells entrapped immediately following tumor inoculation. Furthermore, the initial appearance of E3 tumors in the kidney (Fig. 4), thymus, spleen, and liver was considerably later than that observed in the lung (Fig. 4). The difference in development of E3 tumors between organs and the absence of detectable circulating E3 tumor cells during the course of disease did not make histopathological or flow cytometric analysis of peripheral blood useful for immunochemotherapeutic studies. Unfortunately, the nature of the disseminated model does not enable any simple or reliable method to be used to measure the number of E3 tumor cells present in control or treated mice. To further investigate tumor morphology and Ly-2 antigen expression in these model systems. Such investigations may determine whether E3 tumor antigen expression, vasculature, and cell...
ANTITUMOR ACTIVITY OF Ida-MoAb CONJUGATES IN THYMIC LYMPHOMA

Fig. 4. Groups of 10 B6CF1 mice i.v. inoculated with 2×10⁶ E3 cells were treated i.p. on days 1 and 2 after tumor inoculation with PBS, Ida (total, 15 µg), anti-Ly-2.1 (total, 4.6 mg), Ida-anti-Ly-2.1 (total, 80 µg), or Ida-250–30.6 (total, 80 µg). On day 21 after tumor inoculation these mice were sacrificed and their lungs and kidneys (left and right) with any associated tumor mass were removed and weighed. A group of 5 normal B6CF1 mice was used to calculate normal lung and kidney weights (control) and results were expressed as the mean weight (g) ± SE. (bars).

REFERENCES

Antitumor Activity of Idarubicin-Monoclonal Antibody Conjugates in a Disseminated Thymic Lymphoma Model


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