Athymic Mouse Model of a Human T-Cell Tumor


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ABSTRACT

Because of the large number of different immunoconjugates which can be produced from monoclonal antibody-directed anticancer therapy, it would be useful to have in vivo tumor models to compare such preparations. Although historically human leukemias—lymphomas have been difficult to establish in athymic mice, we have succeeded in establishing human T-cell tumors from primary MOLT-4 cultures in 290 of 353 animals and have successfully transferred tumors in 42 of 45 animals during ten serial passages. The potential utility of this model for testing immunoconjugates of murine monoclonal antibody T101 has been confirmed by: (a) in all 148 tumors sampled including all passaged tumors the human T-cell antigen, T65, was expressed in a manner identical to that of cultured cells; (b) T101 was concentrated preferentially in the tumor; and (c) T101 injected by both the i.p. and i.v. routes bound to tumor and induced antigenic modulation to the same extent as that observed previously in vitro and in human studies.

INTRODUCTION

MoAbs3 may be a significant addition to the anticancer armamentarium because of their relative specificity for tumor-associated antigens and their ease of production (1, 2). In recent years there has been a substantial clinical experience with anhuman T-cell murine MoAb T101 in pilot and Phase I-II clinical trials in patients with cutaneous T-cell lymphoma (3, 4), T-cell leukemia (5), and chronic lymphocytic leukemia (6, 7). T101 and similar antibodies such as Leu-1 and OKT1 immunoprecipitate a M, 65,000–67,000 antigen (T65) that is restricted to thymocytes, malignant and normal T-cells, and chronic lymphocytic leukemia cells (8–10) and is not shed into the plasma in significant quantities. In the clinical studies with T101 over 50 patients have been treated at doses up to 500 mg during a single infusion. Transient decreases in circulating cell counts have been repeatedly reported but sustained antitumor effects have not been achieved. One reason for this may be the rapid modulation of the T65 antigen in the presence of T101 (11, 12), so that in vivo complement- or cell-mediated antitumor effects may not occur because of the limited antibody present on the cell surface. This may be why T101 is relatively ineffective in vitro in complement- and antibody-dependent cell-mediated cytotoxicity assays (13). However, the fact that the T101-T65 complex seems to be internalized in the course of modulation (12) suggests that toxic conjugates of T101 might have a pronounced cytotoxic effect on antigen bearing cells.

Technology exists for producing a variety of such immunoconjugates including attaching natural toxins such as ricin A chain, radioisotopes, or a variety of chemotherapeutic agents. Selection of any particular immunoconjugate for clinical use would be greatly facilitated if a suitable animal model existed for testing such T101 preparations. Unfortunately T65 is not expressed in animals other than higher primates (14) and T-cell tumors have been difficult to establish reproducibly in athymic mice (15, 16). In this paper we describe the successful establishment of a human T-cell leukemia line as a solid tumor in athymic mice. We also demonstrate T65 expression on growing tumor cells, T101 binding to the tumor in vivo, and modulation of the T65 antigen in the mouse following in vivo T101 infusions, thus confirming the potential utility of this animal model for studies of T101 and certain other anti-T-cell antibodies and their immunoconjugates.

MATERIALS AND METHODS

Tumor Establishment. This was done by a modification of the method described by Zeigler et al. (16). Four- to five-week-old female athymic BALB/c nu/nu mice were obtained from the Athymic Mouse Facility of the University of California San Diego Cancer Center. Animals received 200 cGy total body radiation via external beam weekly for 3 weeks and 1 week later were given s.c. injections of a mixture of 10–15 x 10e8 MOLT-4 (17) or CEM (18) human T-cell leukemia cells and 105 HT-1080 fibrosarcoma cells (19) in a 0.5-ml volume of RPMI 1640 10% fetal calf serum. Injected MOLT-4 cells had been growing in log phase in suspension culture in RPMI 1640 media with 10% fetal calf serum. HT-1080 cells were irradiated with 6000 cGy within 1 h of injection. Each animal was given s.c. injections in both anterolateral sides. As controls some animals were not irradiated, others were given injections only with 10–15 x 10e8 MOLT-4 or CEM cells, and others only with irradiated HT-1080 cells. Animals were then observed for the appearance of tumors.

Tumor Histology. At various time points after the appearance of tumor, animals were sacrificed and tissue samples were fixed in Bouin's solution, imbedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Monoclonal Antibody Immunofluorescence Studies. Cell suspensions of excised fresh tumor were incubated with various murine MoAbs to determine antigenic phenotype using indirect immunofluorescence and flow cytometry as described previously (4, 6, 11, 13). Briefly 25 μl containing 1.25 x 10e6 cells were incubated for 30 min at 4°C with 50 μl of a solution of T101 at a concentration of 10 μg/ml. Cells were washed twice and then incubated with a secondary fluorescein-conjugated, affinity-purified F(ab')1 goat anti-mouse antibody (Behring-Mannheim, Indianapolis, IN) for 30 min at 4°C. Cells were then washed twice, fixed in 1% formaldehyde, and analyzed cytofluometrically using a Cytofluorograf 50H (Ortho, Westwood, MA) with a 2100H computer. A nonspecific murine IgG2A, RPC5 was used as a control. Tumor cells were also tested for reactivity with L22, an antitransferrin receptor MoAb (20) and 3A1, another MoAb which detects an antigen on subsets of T-cells (21). T101 and 3A1 are two of the few monoclonal antibodies which react

1 This work was supported by the Veterans Administration, the Biological Response Modifiers Program of the National Cancer Institute per NCI-CM-37613-84, and the University of California San Diego Cancer Center.
2 Requests for reprints should be addressed, at VA Medical Center (V111E), 3350 La Jolla Village Drive, La Jolla, CA 92161.
3 The abbreviation used is: MoAb, monoclonal antibody.

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with antigens on T-cells and both are present on MOLT-4 cells sustained in culture.

In Vivo Binding of T101 and Antigenic Modulation. In 18 tumor-bearing animals 300–500 μg T101 was injected either i.p. or i.v. and animals were sacrificed at 2, 4, or 24 h. Tumors from three animals were analyzed at each time point for each route. Cell suspensions were obtained and assayed for binding of T101 by directly incubating with fluorescein-conjugated anti-mouse antibody as above and for saturation or residual T65 expression by indirect immunofluorescence by incubating with additional T101 in vitro, as described above. It was assumed that the mouse itself would serve as a negative control for any nonspecific binding of mouse immunoglobulins. In other animals we have performed serial evaluations in the same animal by aspirating with a 19- to 22-gauge needle to obtain a cell suspension and analyzed as above every 3–4 days for up to 3 weeks following an injection.

In Vivo Uptake of 111In-T101. 111In-T101 was kindly provided by Richard M. Bartholomew of Hybritech, Inc., La Jolla, CA. The indium labeling was performed using a modified bifunctional chelation technique detailed elsewhere (22, 23). In this technique 1.5 molecules of diethylene triamine pentaacetic acid was bound to the MoAb and 4.5–5 mCi of carrier-free indium chloride was linked to 1 mg T101 with an 85–99% labeling efficiency. The column-purified radiopharmaceutical has a 75–90% immunoreactivity based on competitive binding assays with unlabelled T101. For our studies 1 or 2 μg of 111In-T101 containing 5–10 μCi were injected i.v. into 15 tumor-bearing animals, the animals were sacrificed at 24 h and their organs were sampled for 111In as per a standard protocol detailed elsewhere (23). As a control 1 μg of another 111In-labeled murine monoclonal antibody which does not react with MOLT-4 was injected into another cohort of 15 MOLT-4 tumor-bearing animals and tissues were sampled in the same manner.

RESULTS

Table 1 summarizes the results of the various conditions for establishing the T-cell tumors. We were unable to establish tumors in nonirradiated athymic mice with either CEM or MOLT-4 cells whether or not HT-1080 cells were present. Even in irradiated mice we were unable to establish tumors using CEM cells alone and tumors were established in only 1 of 10 animals using MOLT-4 alone. In contrast in initial experiments in irradiated mice using HT-1080 cells tumors grew in 7 of 27 (26%) mice receiving CEM and HT-1080 compared to 30 of 40 (75%) receiving MOLT-4 and HT-1080. In addition when we examined these tumors for expression of the T65 antigen we found that it had markedly decreased in the CEM tumors compared to the primary cell culture, whereas in the MOLT-4 tumors T65 expression was the same as in the primary cells. For this reason MOLT-4 was selected for additional studies and subsequently tumors have been successfully grown in 290 of 353 (82%) irradiated athymic mice who received cultured cells of MOLT-4 and HT-1080.

Once tumors were established from primary culture we also attempted to passage the cells, initially with HT-1080 cells and subsequently without such cells. During the first three attempts 10–15 × 10⁶ tumor cells were injected with HT-1080 cells and tumors grew in 11 of 12 animals. Since that time tumor has been passaged without HT-1080 cells in 31 of 33 animals. Altogether a total of 398 irradiated athymic mice have been given injections of cultured or tumor-growing 8402 cells and tumors have grown in 332 or 84%. The tumors appeared within 10–14 days after injection and were extremely variable in size and growth rate. Fig. 1 illustrates the external appearance of the MOLT-4 tumors after variable growth periods. The median survival for 20 animals following establishment of tumor was over 8 weeks. The appearance, growth rate, and antigen expression has been similar for both the primary and the passaged tumors.

The tumors were white to beige in color and highly cellular in a liquid matrix when cut cross-sectionally. Necrosis was uncommon until tumors reached very large sizes, i.e., over a 4.0 cm² product of cross-sectional diameters. Even at this size necrosis was relatively uncommon. Histologically the cells were lymphoid in appearance with blue cytoplasm and large nuclei with fine granular chromatim and small nucleoli (Fig. 2). Residual radiated HT-1080 cells which presumably would have been large with eosinophilic cytoplasm (16) were not seen.

A total of 148 tumors have been examined for T65 expression over 9 months. In each and every case indirect immunofluorescence staining confirmed presence of the T65 antigen with over 95% of the cells positive for T101 with a median intensity of fluorescence similar to that of the cultured cell line (Chart 1). This degree of reactivity and intensity of antigen expression has been demonstrated on new tumors established from primary cultures and from the serially passaged tumors (Chart 1). Reactivity with L22, the antitransferrin receptor antibody, and 3A1, another T-cell marker, was also confirmed to be unaffected by establishment of the tumor in vivo.

In vivo distribution of 111In-T101 confirmed the selective uptake advantage in the tumor for T101 as opposed to the control IgG MoAb. Thirty tumor-bearing animals were divided into 2 groups with 15 receiving 111In-T101 and 15 the control MoAb. Data for

Table 1

<table>
<thead>
<tr>
<th>Conditions</th>
<th>CEM + HT-1080</th>
<th>MOLT-4 + HT-1080</th>
<th>HT-1080</th>
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</thead>
<tbody>
<tr>
<td>Non-irradiated and cultured cells</td>
<td>0/20</td>
<td>0/20</td>
<td>0/20</td>
</tr>
<tr>
<td>Irradiated mice and passaged tumor cells</td>
<td>0/20</td>
<td>7/27</td>
<td>1/10</td>
</tr>
<tr>
<td>Irradiated mice and passaged tumor cells</td>
<td>0/27</td>
<td>30/40</td>
<td>0/10</td>
</tr>
</tbody>
</table>

Fig. 1. Athymic mice bearing MOLT-4 tumors illustrating the extent and appearance of such tumors in this model.

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ATHYMIC MOUSE MODEL OF HUMAN T-CELL TUMOR

Chart 1. Histograms showing number of MOLT-4 cells on the ordinate and intensity of fluorescence of the secondary anti-mouse antibody following incubation with T101 on the abscissa. Cell suspensions were from primary MOLT-4 culture, from nude mouse tumor established from primary culture (first passage), and from tumor serially passaged from animal to animal (second to tenth passage).

<table>
<thead>
<tr>
<th>Passage</th>
<th>Relative Cell Number</th>
<th>Fluorescence Intensity</th>
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<tbody>
<tr>
<td>1st</td>
<td></td>
<td></td>
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<tr>
<td>2nd</td>
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<tr>
<td>3rd</td>
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<td>8th</td>
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<tr>
<td>9th</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10th</td>
<td></td>
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</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Cohort</th>
<th>No. of mice</th>
<th>Wt of tumor, range (g)</th>
<th>Wt of tumor, mean ± SE (g)</th>
<th>Blood</th>
<th>Bone</th>
<th>Heart</th>
<th>Kidney</th>
<th>Liver</th>
<th>Lung</th>
<th>Muscle</th>
<th>Skin</th>
<th>Spleen</th>
<th>Intestine</th>
<th>Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>0.058-10.2</td>
<td>1.72 ± 0.52</td>
<td>10.5 ± 0.9</td>
<td>1.3 ± 0.2</td>
<td>2.3 ± 0.3</td>
<td>4.9 ± 0.4</td>
<td>4.2 ± 0.4</td>
<td>3.8 ± 0.2</td>
<td>1.3 ± 0.2</td>
<td>3.3 ± 0.3</td>
<td>4.5 ± 0.6</td>
<td>1.5 ± 1.1</td>
<td>6.8 ± 0.9</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>0.96-9.8</td>
<td>1.18 ± 0.62</td>
<td>11.7 ± 0.8</td>
<td>1.9 ± 0.1</td>
<td>3.3 ± 0.2</td>
<td>6.0 ± 0.4</td>
<td>5.5 ± 0.3</td>
<td>5.7 ± 0.3</td>
<td>1.3 ± 0.2</td>
<td>4.8 ± 0.3</td>
<td>4.9 ± 0.3</td>
<td>1.5 ± 0.1</td>
<td>4.1 ± 0.8</td>
</tr>
</tbody>
</table>

*% dose/g tissue.

Table 2 Comparison of 111In-MoAb uptake by Molt-4 tumors in athymic mice

Cohorts of tumor-bearing animals received i.v. injections of 1-2 μg of 111In-T101 (5-10 μCi) or a control murine IgG MoAb labeled with 111In. Animals were sacrificed 24 h after being given injections and various organs and tissues were sampled, weighed, and the cpm was determined. The percentage of dose was determined by dividing the cpm in a given tissue by the total cpm injected into the animal.

<table>
<thead>
<tr>
<th>Cohorts</th>
<th>No. of mice</th>
<th>Wt of tumor, range (g)</th>
<th>Wt of tumor, mean ± SE (g)</th>
<th>Blood</th>
<th>Bone</th>
<th>Heart</th>
<th>Kidney</th>
<th>Liver</th>
<th>Lung</th>
<th>Muscle</th>
<th>Skin</th>
<th>Spleen</th>
<th>Intestine</th>
<th>Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-T101</td>
<td>15</td>
<td>0.058-10.2</td>
<td>1.72 ± 0.52</td>
<td>10.5 ± 0.9</td>
<td>1.3 ± 0.2</td>
<td>2.3 ± 0.3</td>
<td>4.9 ± 0.4</td>
<td>4.2 ± 0.4</td>
<td>3.8 ± 0.2</td>
<td>1.3 ± 0.2</td>
<td>3.3 ± 0.3</td>
<td>4.5 ± 0.6</td>
<td>1.5 ± 1.1</td>
<td>6.8 ± 0.9</td>
</tr>
<tr>
<td>In-IgG</td>
<td>15</td>
<td>0.96-9.8</td>
<td>1.18 ± 0.62</td>
<td>11.7 ± 0.8</td>
<td>1.9 ± 0.1</td>
<td>3.3 ± 0.2</td>
<td>6.0 ± 0.4</td>
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</tbody>
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0.19 and 0.24. Thus binding throughout the 24-h period was confirmed by the failure of additional T101 to substantially increase T101 binding in vitro. There was evidence of in vivo antigenic modulation in that the proportion of cells directly staining with T101 in vivo decreased during the 24-h time period but cultures of those cells which were allowed to incubate in vitro reexpressed the antigen to pretreatment levels in the absence of T101. Hence this study confirmed that a 300- to 500-μg dose produced saturating conditions in the tumor and that T101 serum levels were sustained for over 24 h.

In a subsequent experiment the tumor from one animal who received 500 μg T101 was aspirated every 3 days for 9 days and then weekly for 2 weeks and cells were assayed for T101 binding in residual binding sites. Evidence of in vivo binding and antigenic modulation persisted up to 16 days but the T65 antigen had been completely reexpressed by 23 days.

DISCUSSION

Based on our work it appears that the MOLT-4 tumor is readily established in athymic mice and the in vitro antigen characteristics are such that it should be a good model for testing immunonjugates of T101. This study confirms the report of Zeigler et al. (16) who were able to produce such tumors in 19 of 25 irradiated mice with inoculations bilaterally of 2 × 10⁶ MOLT-4 cells. Using 10–15 × 10⁶ cells from primary cultures we have established tumors in 82% of animals, and using cells from s.c. MOLT-4 tumors we have established tumors in 93% of animals. Using this larger number of cells we have been able to produce tumors within 10 days, which is somewhat more rapid than the 2–3 weeks required to establish tumors using the smaller cell inoculum (16). The appearance of tumors within 1–2 weeks and the subsequent survival of animals with growing tumors suggests this model would be useful for an animal tumor response study.

It is important to emphasize the negative attempts to establish human T-cell tumors using related methods. The necessity of
radiating animals was illustrated by our inability to establish tumors in the absence of radiation with CEM or MOLT-4 cells, whether or not HT-1080 cells were present. The importance of adding HT-1080 as a "feeder cell" was demonstrated by our inability to establish CEM without HT-1080 cells and finding only a 10% tumor rate with MOLT-4 cells alone. Presumably HT-1080 provided some growth factor which may have been required initially but which could be obtained from the animal or by autocrine mechanisms once the tumor had been established for a few days. The significance of individual cell lines was shown by the high tumor rate for MOLT-4 in the presence of HT-1080 (82%) compared to the low success rate with CEM (26%). In addition and crucial for our purposes in developing this model is that once established CEM had a decreased expression of the T65 target antigen while MOLT-4 expressed this antigen at the same level as did cultured cells. Thus in terms of both the probability of tumor establishment and the persistence of the T65 target antigen the MOLT-4 tumor was superior to CEM.

Of particular significance is the suitability of this model for T101 testing. We have demonstrated the persistent expression of T65 and the ability of T101 and radiolabeled T101 to bind to this tumor in the in vivo model. Although tissue sampling was performed at only 24 h after injection there was already a statistically significant increased specific uptake of $^{111}$In in the tumor. There is a larger and broader clinical experience with this similar cell line than with probably any other antibody-antigen system (3-7). Phase I trials have laid a foundation for in vivo and in vitro antigenic modulation by the anti-human T-cell monoclonal antibody T101. Cancer Res., 44: 5921-5927, 1984.


Fig. 2. Fixed section of MOLT-4 tumor growing in athymic mouse. H & E. × 400.
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