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 have 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) 111In-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 antihuman 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 antigen bearing 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 fluorocytometry as described previously (4, 6, 11, 13). Briefly 25 μl containing 1.25 x 10⁶ 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')₂ 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 cytfluorometrically using a Cytotuffograf 50H (Ortho, Westwood, MA) with a 2100H computer. A nonspecific murine IgG2A, RCCP 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...
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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 pentacetic acid was bound to the MoAb and 4.5–5.5 mCi of carrier-free indium chloride was linked to 1 mg T101 with an 85–95% labeling efficiency. The column-purified radiopharmaceutical has a 75–90% immunoreactivity based on competitive binding assays with unlabeled 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 chromatin 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

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**Table 1**

Human T-cell tumor establishment in athymic mice

<table>
<thead>
<tr>
<th>Conditions</th>
<th>CEM + HT-1080</th>
<th>MOLT-4 + HT-1080</th>
<th>HT-1080</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-irradiated</td>
<td>0/20</td>
<td>0/20</td>
<td>0/20</td>
</tr>
<tr>
<td>and cultured cells</td>
<td>0/20</td>
<td>7/27</td>
<td>10/10, 30/40 *</td>
</tr>
<tr>
<td>Irradiated mice and</td>
<td>31/33</td>
<td></td>
<td>11/12</td>
</tr>
<tr>
<td>cultured cells</td>
<td></td>
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</tbody>
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* Subsequent total of 290 of 353 (82%).

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**Fig. 1.** Athymic mice bearing MOLT-4 tumors illustrating the extent and appearance of such tumors in this model.
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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 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. |
|---|---|---|
| No. of mice | 15 | 15 |
| Wt of tumor, range (g) | 0.058-10.2 | 0.96-9.8 |
| Wt of tumor, mean ± SE (g) | 1.72 ± 0.52 | 1.18 ± 0.62 |
| Blood | 10.5 ± 0.9 | 11.7 ± 0.8 |
| Bone | 1.3 ± 0.2 | 1.9 ± 0.1 |
| Heart | 2.3 ± 0.3 | 3.3 ± 0.2 |
| Kidney | 4.9 ± 0.4 | 6.0 ± 0.4 |
| Liver | 4.2 ± 0.4 | 5.5 ± 0.3 |
| Lung | 3.8 ± 0.2 | 5.7 ± 0.3 |
| Muscle | 1.3 ± 0.2 | 1.3 ± 0.2 |
| Skin | 3.3 ± 0.3 | 4.8 ± 0.3 |
| Spleen | 4.5 ± 0.6 | 4.8 ± 0.3 |
| Intestine | 1.5 ± 1.1 | 1.5 ± 0.1 |
| Tumor | 6.8 ± 0.9 | 4.1 ± 0.8 |

* % dose/g tissue.

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 immunconjugates 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 × 10⁵ 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 and similar natural antibodies than with probably any other antibody-antigen system (3–7). Phase I trials have laid a foundation for T101-immunoconjugate studies but there is a variety of potential T101 immunoconjugates which might be suitable for in vivo study in humans including various radioisotopes, derivatives of natural toxins such as the ricin A chain, and chemotherapeutic agents such as doxorubicin, methotrexate, and vinblastine. The persistence of T65 antigen in vivo and its modulation in the presence of T101 in vivo suggest that this athymic mouse model should be extremely useful in the screening of various T101 immunoconjugates for toxicity, stability, and antitumor efficacy. Small T101 immunoconjugates could be given i.p. which would be an advantage if multiple treatments are to be used in such trials because of the fragility of athymic mice tail veins. This animal model may be relevant for clinical testing of other agents which could be useful in T-cell cancers and perhaps also for other agents which may be useful in transplantation or immunosuppression.

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REFERENCES


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