A Monoclonal Antibody against a Human B Lymphoblastoid Cell Line Induces Tumor Regression in Mice

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Abstract

We have developed a monoclonal antibody (BAT) to Daudi B lymphoblastoid cell line membranes. The antibody was selected for its ability to stimulate lymphocyte proliferation. Splenocytes of BALB/c or C57BL/6 mice given i.v. injections of 10 μg/mouse of BAT exhibited increased proliferation and cytotoxic activity. A single i.v. administration of BAT monoclonal antibody 2 weeks after B16 melanoma cell inoculation resulted in a striking antitumor effect as manifested by the elimination of lung metastases and prolonged survival of the treated mice. BAT monoclonal antibody was also effective in the regression of tumors in mice bearing 3LL (Lewis lung carcinoma) and MCA-105 (fibrosarcoma). Transfer of $10^9-10^8$ splenocytes from mice that had been given injections of BAT to B16- or 3LL-inoculated recipients led to a reduction of lung metastases. Splenocytes from B16-inoculated mice that were cured by BAT were more effective than those from mice treated with BAT alone against recipients bearing either B16 or 3LL tumors. The antitumor activity of BAT is related to its immunostimulatory properties.

Introduction

Agonistic mAbs to a number of T cell determinants were previously found to induce proliferation, activation, and differentiation of T cells (1). The best known of such mAbs is the one directed against the CD3 antigen. mAb to the CD3 determinant was able to induce clonal proliferation, elicit mitogenic activity, and also trigger the cytolytic process in T lymphocytes (2-4). Additional T lymphocyte agonistic mAbs were also reported. They include mAbs against CD2 (5), CD5 (6), CD69 (7), and CD28 (8, 9), an antigen that interacts with its ligand B7 which is present on antigen-presenting cells (10). In vivo antitumor activity of anti-CD3 was described (11, 12).

Anti-CD28 was also reported to slow the growth of murine melanoma, although complete rejection did not occur. These results suggest that a provision of costimulation to T cells can enhance an antitumor immune response (13).

We have previously shown that a monoclonal antibody to Daudi human B lymphoblastoid cell line was stimulatory for murine thymocytes and human peripheral T cells (14). We produced a new series of monoclonal antibodies which were selected by their ability to bind Daudi cells and to induce human PBL proliferation. Such a mAb, designated BAT, induced cytolytic activity in human PBL. ΔBAT also exhibited immunostimulatory properties in the mouse, expressed by induction of proliferation and cytolytic activity in splenocytes. Since stimulation of the immune system can induce antitumor activity, we studied the effect of BAT in several mouse tumor models and found that it manifested a remarkable antitumor effect. This report described the antitumor properties of this mAb.

Materials and Methods

Generation and Purification of BAT mAb. BALB/c mice were immunized by membranes from Daudi cells. Membranes were emulsified with complete Freund's adjuvant and administered i.m., i.p., and s.c. Three weeks later mice were boosted i.v. with the membrane preparation in saline. Spleen cells harvested 3 days later were fused with myeloma NS-O cells using polyethylene glycol. Spleen cells harvested 3 days later were fused with myeloma NS-O cells using polyethylene glycol. The hybridomas were grown in selective media according to the method of Kohler and Milstein (15). BAT was selected by its ability to bind the Daudi cell line in a cell-bound enzyme-linked immunosorbent assay (16) and by its ability to induce proliferation of human PBL, using $[^3H]$hy- midine uptake assay. Supernatant of BAT clone was precipitated by 50% ammonium sulfate, dissolved, and dialyzed against PBS. Further purification was done on CNBr-activated Sepharose (Pharmacia) to which the F(ab')$_2$ fragment of goat anti-mouse IgG was bound. The purified antibody was elicted from the column with glycine buffer (0.2 M, pH 2.7) and dialyzed against PBS.

Cell Cultures. Splenocytes of mice given i.v. injections of BAT or controls given injections of mouse IgG3 isotype control (Sigma) were washed in PBS and suspended and cultured at $2 \times 10^6$ to $10^7$ cells/ml in RPMI media supplemented with 5% fetal calf serum, glutamine, pyruvate, and antibiotics. Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO$_2$.

Thymidine Incorporation. Incorporation for each mouse was done by suspending 200 μl of splenocytes in culture medium into 96-well flat bottomed plates. $[^3H]$Thymidine (1 μCi/well) was added for 8–12 h. Cultures were harvested into glass filter paper and radioactivity was counted using a liquid scintillation counter.

Cytotoxicity Assay. Cytotoxicity assays were done as follows. Target cells (10$^5$) were mixed with 50 μCi of $[^51Cr]$chromate for 1 h in serum-free medium. They were washed three times with complete medium, resuspended, and plated at 10$^4$ cells/well in 96-well round bottom plates. Effector cells were splenocytes removed from mice 10 days after i.v. injection with BAT mAb or from control mice. The cells were washed three times and mixed with target cells at various effector:target ratios. The plates were incubated overnight at 37°C in 5% CO$_2$. The culture supernatants were harvested and counted in a gamma scintillation counter. Maximum isotope release (MR) was produced by incubation of the targets with Triton X-100. Spontaneous release (SR) was measured by incubation of the targets with medium alone (spontaneous release did not exceed 20% of maximum isotope release). The percentage of lysis was calculated by

$$EX - SR \times 100 \quad MR - SR$$

where EX is the experimental effector release.

Mouse Tumor Models. Three mouse tumor models were used: B16 melanoma; 3LL Lewis lung carcinoma; and MCA-105 (methylcholanthrene-induced fibrosarcoma). The tumor cells were grown in culture and obtained from a large stock of frozen cells, prepared ahead. The stock was prepared from in vivo s.c. growth of B16 and 3LL tumors and i.p. growth of MCA-105 cells. Tumors removed in sterile conditions were dispersed in cold PBS, centrifuged, and cultured in large flasks. The cells were removed and kept frozen in 20%
serum, 70% medium, and 10% dimethyl sulfoxide. Stock cell cultures were cultured for 2–3 times in vitro and used for in vivo injections. B16 was injected i.v. at 5 × 10⁶ cells/mouse. 3LL and MCA were injected i.v. at 2 × 10⁶ cells/mouse. These tumor cell concentrations were optimal for the induction of lung metastases in the lungs.

**B16 in Vivo Treatment.** Purified BAT mAb was injected i.v. into C57BL/6 or BALB/c mice at various concentrations. Ten days later spleens were removed and studied. BAT mAb at 10 μg/mouse was injected i.v. 2–3 times in vitro and used for in vivo injections. B16 was injected i.v. at 5 × 10⁶ cells/mouse. 3LL and MCA were injected i.v. at 2 × 10⁶ cells/mouse. These tumor cell concentrations were optimal for the induction of tumor lesions in the lungs.

**Results**

A mAb (BAT) was raised against membranes of B lymphoblastoid cells (Daudi) and selected for binding to Daudi cells and for inducing in vitro proliferation of human PBL. Western blot analysis revealed that this IgG3 mAb reacted with a 47–50-kDa protein from Daudi cell membranes. Fluorescence-activated cell sorter analysis, using an anti-CD3 and BAT mAb double staining technique, indicated that BAT mAb binds to human T cells. In vitro studies indicated that BAT mAb binds to mouse splenocytes and induced proliferation and cytoxicity in these cells. C57BL murine splenocytes were incubated for 5 days in vitro in the presence of none, 1, 10, and 100 ng/ml of BAT mAb. CYTOTOXICITY (as measured by 51Cr release) against B16 melanoma target cells at a 50:1 effector:target ratio was 7.4, 12.3, 17.4, and 24.9%, respectively.

BAT mAb also exhibited immunostimulatory properties in vivo (Table 1). Injection of BAT mAb to BALB/c or C57BL mice induced a moderate but significant increase in proliferation and cytoxicity. Maximal stimulation by BAT was attained at doses of 1–10 μg/mouse. BAT at doses less than 1 μg/mouse were less stimulatory (data not shown). Target cells were syngeneic to the effector cells. YAC is a natural killer cell-sensitive target cell and MR-29 and B16 are natural killer cell resistant. Results depicted in Table 1 are from animals that had been tested 10 days post-BAT treatment. Splenocytes from mice that had been treated with BAT for time intervals of less than 10 days had less stimulatory response. Maximal stimulatory response was maintained up to 19 days post-BAT administration (data not shown).

Following the observation that splenocytes of mice given injections of BAT mAb were cytotoxic to a variety of tumor cells in vitro, we investigated the antitumor properties of BAT against established tumor models in mice. They include B16 melanoma, 3LL Lewis lung carcinoma, and MCA-105 fibrosarcoma. In all these tumor models, mice given i.v. injections of the tumor cells developed visible lung lesion and died between 25 and 35 days post-tumor inoculation. A striking activity of BAT mAb injected into the tumor-inoculated mice resulted in a marked decrease or complete elimination of the tumor lesions (Table 2A; Fig. 1A).

Lung weights correlated with the tumor burden and were also markedly decreased in BAT-treated mice. Lung weights of tumor-

### Table 1. Proliferation and cytotoxicity of splenocytes from mice given injections of BAT mAb

<table>
<thead>
<tr>
<th>BAT mAb concentration (μg/mouse)</th>
<th>Specific 51Cr release (% ± SEM) at specified cell target</th>
<th>[3H]Thymidine incorporation (cpm × 10⁶ ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B16</td>
<td>MR-29</td>
</tr>
<tr>
<td>0</td>
<td>3.9 ± 0.5</td>
<td>16.0 ± 2.7</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>(n = 8)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>1</td>
<td>13.2 ± 1.4</td>
<td>31.7 ± 2.8</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>(n = 8)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>10</td>
<td>14.0 ± 1.2</td>
<td>25.2 ± 2.1</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>(n = 8)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>P &lt; 0.001</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

### Table 2. Antitumor effect of BAT mAb

**A. BAT-induced reduction in lung metastases**

<table>
<thead>
<tr>
<th>Tumors</th>
<th>B16</th>
<th>3LL</th>
<th>MCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAT treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of mets</td>
<td>(n = 24)</td>
<td>+</td>
<td>(n = 11)</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>1–10</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>11–50</td>
<td>8</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>&gt;250</td>
<td>16</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Lung wt (g)</td>
<td>0.803 ± 0.26</td>
<td>0.315 ± 0.66</td>
<td>1.014 ± 0.21</td>
</tr>
</tbody>
</table>

**B. BAT administration at different times in relation to B16 melanoma inoculation**

<table>
<thead>
<tr>
<th>Day</th>
<th>No. of mice</th>
<th>No. of mets.</th>
<th>Lung wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>10</td>
<td>155.0 ± 28</td>
<td>0.892 ± 0.19</td>
</tr>
<tr>
<td>–6</td>
<td>3</td>
<td>61.0 ± 0.08</td>
<td>0.983 ± 0.01</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>148.0 ± 36</td>
<td>0.425 ± 0.01</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>0.0 ± 0.0</td>
<td>0.243 ± 0.07</td>
</tr>
<tr>
<td>14</td>
<td>7</td>
<td>0.0 ± 0.0</td>
<td>0.240 ± 0.08</td>
</tr>
<tr>
<td>19</td>
<td>4</td>
<td>2.2 ± 1.3</td>
<td>0.338 ± 0.01</td>
</tr>
<tr>
<td>23</td>
<td>23</td>
<td>75.4 ± 44</td>
<td>0.840 ± 0.24</td>
</tr>
</tbody>
</table>

* Lung metastases (mets.) were scored 24 days post-tumor inoculation using a Zeiss stereomicroscope.
* a Tumor was inoculated at day 0.
* b BAT mAb (10 μg/mouse) was injected i.v. at day 14.
* c n, number of mice.
* Mean ± SD of lung weights from n mice.
* d Day of BAT mAb administration relative to tumor inoculation at day 0.
MONOCLONAL ANTIBODY WITH ANTITUMOR PROPERTIES

Fig. 1. Effect of BAT mAb on mouse tumors. A, photomicrograph of lungs from mice that were inoculated with B16 melanoma (a), 3LL-Lewis lung carcinoma (b), or MCA-105 fibrosarcoma (c). Upper row, lungs from mice that were inoculated with the tumor alone; Lower row, same as above, followed 14 days later by i.v. injection of BAT mAb (10 μg/mouse) for each tumor. B, survival time of BAT treated animals 2 weeks after B16 melanoma inoculation. C, survival time of BAT treated animals 2 weeks after 3LL inoculation.

Inoculated BAT-treated mice was similar or close to the lung weights of control animals. BAT induced complete regression of B16 melanoma tumor in 25 of 27 animals, 6 of 11 in 3LL, and 4 of 9 in MCA-105 inoculated mice (Table 2A). A photomicrograph of lungs from animals that were inoculated with the three tumor models tested and treated with BAT by a single injection 2 weeks later is depicted in Fig. 1A. No visible metastases can be seen in BAT-treated B16- and MCA-inoculated mice.

The ability of BAT to fully regress lung lesions was also reflected by the prolonged survival time of the BAT-treated animals. The majority of the animals were followed up for 5 months, showed no ill effects, and were free of metastases upon pathological examination. We then investigated the antitumor effect of BAT mAb injected at different times in relation to B16 tumor inoculation. As was shown above (Fig. 1A; Table 2A) and as seen in Table 2B, mice given injections of the melanoma cells and treated 10–14 days later by BAT mAb administration were found to be free of metastases and had normal lung weights. A marked decrease, although not complete, of number of metastases was noticed 5 days after tumor inoculation and as late as 19 days post-tumor administration. Injection of BAT on the same day as injection of tumor cells had no therapeutic effect.

The mechanisms of the antitumor activity of BAT could be related to its immunostimulatory property (Table 1). On the other hand the possibility that the antibody has a direct effect against the tumor was also considered, particularly in view of the observation that BAT binds to B16 cells. We could not demonstrate, however, a direct cytotoxic effect of BAT mAb on B16 cells by treating the tumor cells, in vitro, in the absence and presence of complement.

Based on adoptive transfer experiments our data strongly suggest that immune mechanisms are involved in the BAT antitumor effect (Table 3). Splenocytes from mice that had been treated with BAT mAb alone or first given injections of B16 melanoma cells and then treated with BAT mAb, were transferred to recipient mice. These mice were inoculated either with B16 melanoma or with 3LL tumor cells. The results in Table 3 demonstrate that adoptive transfer induced their tumor regression. Splenocytes (10^6) of B16 melanoma-BAT-treated mice resulted in complete elimination of melanoma tumor cells in B16 recipients and pronounced tumor regression in 3LL
T cells, and induces cytotoxicity and antitumor effects, it is possible that BAT recognizes a costimulatory ligand. This is based on the following observations: (a) BAT antitumor activity was less effective with a molecular size of 47—50 kDa. Data indicated that this protein has a monomeric structure and that the epitope recognized by BAT resides on the protein moiety. Costimulatory cell ligands were described in interactions between B and T cells which provide agonistic stimuli for the T cell to proliferate and generate cytotoxic activity (10, 17—19).

Since BAT was prepared against B lymphoblastoid cells, binds to T cells, and induces cytotoxicity and antitumor effects, it is possible that BAT recognizes a costimulatory ligand.

Our experimental data support the hypothesis that BAT mediates its antitumor activity via immune mechanisms. This is based on the following observations: (a) BAT antitumor activity was less effective in sublethally irradiated animals; (b) antitumor activity could be mediated by an adoptive transfer of splenocytes from BAT-treated mice; (c) mice that were cured of B16 melanoma by BAT mAb were partially resistant to further challenge with the tumor.

The effector cells that mediate the antitumor effect include T cells. Since BAT was prepared against B lymphoblastoid cells, binds to T cells, and induces cytotoxicity and antitumor effects, it is possible that BAT recognizes a costimulatory ligand.

We have reported here on the antitumor properties of a mAb that was raised against a human B lymphoblastoid cell line. To our knowledge, no similar activity has been reported previously.

The stimulatory effect of BAT on human lymphocytes, in addition to its antitumor effect in mice, provides a basis for the potential use of this antibody in humans.

**Discussion**

We have reported here on the antitumor properties of a mAb that was raised against a human B lymphoblastoid cell line. To our knowledge, no similar activity has been reported previously.

The ligand recognized by BAT mAb has been characterized thus far only by its molecular size. Using Western blot analysis we have detected in Daudi cells and Jurkat T cell line a BAT-binding protein with a molecular size of 47—50 kDa. Data indicated that this protein has a monomeric structure and that the epitope recognized by BAT resides on the protein moiety. Costimulatory cell ligands were described in interactions between B and T cells which provide agonistic stimuli for the T cell to proliferate and generate cytotoxic activity (10, 17—19).

Since BAT was prepared against B lymphoblastoid cells, binds to T cells, and induces cytotoxicity and antitumor effects, it is possible that BAT recognizes a costimulatory ligand.

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The effector cells that mediate the antitumor effect include T cells. This conclusion is based on our recent finding that in vivo depletion of Thy-1.2 positive cells by an anti-Thy-1.2 reduced the antitumor effect of BAT. In addition, in adoptive transfer experiments splenocytes from BAT-treated mice that were injected with anti-Thy-1.2 antibody 48 h prior to transfer showed reduced antitumor activity as compared to controls. These results are in accord with our findings that BAT mAb binds and activates human T cells.4

The adoptive transfer experiments indicated that splenocytes alone from B16-inoculated and BAT-treated mice exhibited antitumor effect against both B16 and 3LL. This indicates that BAT enhances non-specific cellular effector mechanisms. The adoptive transfer experiments also revealed that the presence of tumors potentiated the enhancement of BAT-induced generation of effector cells. This could result from the costimulatory effect of the tumor on T cells by ligands that normally reside primarily on accessory cells. Such a ligand, B7, was recently demonstrated on tumor cells (13).

Anti-CD3 was reported to elicit an antitumor effect in a few mouse tumor models (11, 12). Our unpublished results indicate that under similar experimental conditions BAT had a curing effect whereas anti-CD3 had only a partial antitumor effect in B16-bearing mice.

### Table 3 Adaptive transfer-induced tumor regression

<table>
<thead>
<tr>
<th>Donor group</th>
<th>B16 (10^7 splenocytes transferred)</th>
<th>3LL (10^8 splenocytes transferred)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Untreated</td>
<td>&gt;250</td>
<td>1.09 ± 0.09</td>
</tr>
<tr>
<td>B. BAT-treated</td>
<td>89 ± 10^6</td>
<td>0.67 ± 0.04</td>
</tr>
<tr>
<td>C. B16-injected and BAT-treated</td>
<td>4 ± 4</td>
<td>0.32 ± 0.01</td>
</tr>
</tbody>
</table>

**References**

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