Monoclonal Antibody Therapy of Spontaneous AKR T-Cell Leukemia

Christopher C. Badger, Howard Shulman, Arthur V. Peterson, and Irwin D. Bernstein

ABSTRACT

We have previously shown that monoclonal antibodies against the Thy 1.1 differentiation antigen can inhibit the outgrowth of a lethal inoculum of transplanted AKR T-leukemic cells. In the present report we have extended these studies to examine antibody therapy of aged AKR/J mice with spontaneous leukemia. Infusion of anti-Thy 1.1 antibody in frankly leukemic mice led to uniform early mortality from cell lysis and agglutination. In contrast, anti-Thy 1.1 antibody therapy of mice in remission following treatment with cyclophosphamide prolonged remission duration (P < 0.001) and modestly prolonged survival (P < 0.01) compared to treatment with irrelevant antibody or chemotherapy alone. The major cause of failure was relapse of leukemia. In 85% (47 of 55) of cases relapse was due to cells that continued to express Thy 1.1, but in 15% of these relapsing animals all leukemic cells failed to express the target antigen. Our results suggest that monoclonal antibody against a normal T-cell antigen can add to the antileukemic effects obtained with chemotherapy alone. Nevertheless, the clinical benefit of unmodified antibody was modest, and antibodies conjugated to cytotoxic agents may be needed to overcome the limitations of unmodified antibodies.

INTRODUCTION

Monoclonal antibodies have been successfully used to prevent the growth of a number of transplanted tumors (1-11). While the results of antibody therapy in these experimental models suggest that antibodies can have an antitumor effect, the potential usefulness of antibody for treating spontaneous malignancies remains uncertain. There may be important differences between transplanted and naturally occurring disease in total body tumor burden and access of antibody or effector cell populations to the tumor cells. Furthermore, the results of early clinical trials in patients with advanced disease have been disappointing (12).

In previous studies, we have shown that monoclonal antibodies against the Thy 1.1 differentiation antigen can successfully prevent the growth of a lethal inoculum of transplanted syngeneic AKR/J, SL2, T-cell leukemia cells (1-5). In those studies, anti-Thy 1.1 antibody could eliminate a maximum of 3 × 10^6 cells from a single implantation site. The therapeutic effects of antibody were limited by the inability of the host to eliminate antibody-coated tumor cells at the inoculation site and by the metastatic growth of variant tumor cells lacking the target antigen (1).

The occurrence of spontaneous T-cell leukemia in aged AKR mice has allowed us to extend our studies of the monoclonal antibody therapy of leukemia to a model analogous to human lymphoid malignancies (13, 14). Approximately 80% of AKR mice between 6 and 12 mo of age develop T-cell malignancies which arise in the thymus, metastasize widely, and progress to overt leukemia. In the present report we evaluated the treatment of these spontaneous leukemias with monoclonal antibodies against the Thy 1.1 antigen alone and in combination with chemotherapy.

MATERIALS AND METHODS

Animals. Retired AKR/J breeders (6-8 mo old) were obtained from Jackson Laboratories (Bar Harbor, ME). Animals were examined twice weekly for the presence of leukemia as indicated by enlarged spleen, thymus, and lymph nodes (13). Spleen, lymph node, thymus size and initial WBC count were scored semiquantitatively at the time of entry into treatment according to the following criteria: thymus: 0, normal; 1, enlarged; lymph node: 0, normal; 1, one node <3 mm in diameter; 2, >one node or one node >3 mm in diameter; spleen: 0, normal; 1, one node palpable; 2, significantly enlarged; WBC: 0, <10,000; 1, WBC, 10,000-40,000; 2, WBC >40,000.

Each of these semiquantitative indices (I) was combined in an overall tumor burden index according to the formula

\[ I = (I_{\text{thymus}}) + (I_{\text{lymph node}}) + (I_{\text{spleen}}) + (I_{\text{WBC}}) \]

Once treatment was initiated, all animals were housed singly. Food, water, and bedding were sterilized, and drinking water contained neomycin (1 mg/ml) and oxytetracycline (0.2 mg/ml) alternating every 2 wk with acidification (HCl, pH 2.5). Following treatment, animals were examined twice weekly until death when an autopsy was obtained to determine the presence of leukemia histologically and the cause of death. Clinical relapse was determined by the same criteria as for diagnosis.

Antibodies. The preparation of the IgG2a anti-Thy 1.1 monoclonal antibody 19E12 for therapy has been previously described (1). Control antibody G3G6 is an IgG2a monoclonal antibody reactive with human platelets. Hybridoma cells were inoculated i.p. in syngeneic mice for production of ascites. The pooled 19E12 ascites had a complement-dependent cytotoxic titer of 5 × 10^{-7} (50% lysis end point) and contained approximately 4 mg of antibody per ml. G3G6 ascites were not cytotoxic against mouse lymphoid cells. Ascites were sterilized by filtration through a 0.22-μm filter. Antibody therapy was administered as unpurified ascites.

Immunofluorescence Studies. Tissue samples (lymph node, spleen, or thymus) were examined for expression of Thy 1.1 and surface immunoglobulin by immunofluorescence as previously described (1). Briefly, tissue samples were minced in RPMI 1640 and 2% bovine serum albumin plus 0.02% sodium azide, and viable cells were isolated on a Ficoll-Hypaque density gradient. Cells were then incubated with (a) FITC-labeled goat anti-mouse IgG (Bionetics Laboratory Products, Lithton Bionetics, Inc., Kensington, MD) to determine SIg+, (b) 19E12 antibody plus FITC-labeled anti-IgG to determine Thy 1.1 expression, or (c) FITC-labeled goat anti-human IgM to determine nonspecific binding. Binding to the cell surfaces was analyzed by flow microfluorimetry using the fluorescence-activated cell sorter (FACS-systems; Becton Dickinson & Co., Sunnyvale, CA). Leukemic cells were identified based on size (forward light scatter).

Statistical Methods. For the analysis of remission duration and survival times, standard methods for the analysis of time-to-event data were used: Kaplan-Meier estimates of relapse and survival curves (15, 16); the log rank test for two-sample tests of significance (16); and the
RESULTS

Thy 1.1 Expression by Spontaneous Leukemias. Ninety-five% of animals (270 of 285) thought to have spontaneous leukemia on the basis or organomegaly had lymph node cells at diagnosis with a clearly defined leukemic cell population in flow microfluorimetry studies. The 5% (15 of 285) of animals with lymphadenopathy that had cytfluorograms similar to those of lymph node cells from normal animals were presumed to have lymphadenopathy on the basis of infection or trauma and were excluded from further analysis. The results of the antigenic analysis of the 270 leukemic mice are shown in Table 1. Two hundred nine of 270 (77%) of the tumors expressed the Thy 1.1 antigenruen but not surface immunoglobulin. Seventeen of 270 (6%) of the animals had leukemias that had high levels of surface immunoglobulin (fluorescence equivalent to normal B-cells, without increase following incubation with anti-Thy 1.1 antibody). These leukemias were presumably B-cell tumors, although the possibility that these cells were antibody-coated T-cells was not rigorously excluded. Eighteen of 270 (7%) of the leukemias had neither Thy 1.1 nor Slg (null cell), and 26 of 270 (10%) of the tumors had mixed T- and null cell populations. The 270 animals with leukemia were subsequently entered into trials of antibody therapy.

Treatment with Anti-Thy 1.1 Alone. We initially treated a group of mice with spontaneous leukemia with antibody immediately after diagnosis. Infusion of either 0.4 or 3.2 mg of anti-Thy 1.1 antibody in these frankly leukemic mice resulted in death of all animals (10 of 10) within 48 h after antibody infusion. Autopsy examination of these animals revealed striking intravascular aggregates of fibrin, cell aggregates, and cellular debris in the pulmonary (Fig. 1) and, to a lesser extent, renal vessels. There was no histologically apparent reduction in leukemic cells present in spleen and other organs. Because this therapy was prohibitively toxic to overtly leukemic animals, in subsequent studies mice were first treated with cyclophosphamide to induce remission prior to antibody infusion (see below).

Antibody Treatment in Combination with Chemotherapy. To evaluate the effect of antibody in combination with chemotherapy in spontaneous leukemia, we treated mice with an initial dose of cyclophosphamide (150 mg/kg) i.p. to induce remission. Following the first dose of cyclophosphamide, 80% of animals achieved remission (resolution of previously enlarged lymphoid organs) by Day 4. These animals received a second dose of cyclophosphamide (150 mg/kg) and were randomized to subsequent therapy. Five treatment regimens were examined. A prolonged antibody regimen (0.4 mg i.v., Day 4, followed by 0.2 mg i.p. twice weekly) was compared to a single low dose of 0.4 mg i.v., Day 4, as well as a single high dose of 3.2 mg i.v., Day 4. An irrelevant control antibody given in the same volume of ascites as the continuous anti-Thy 1.1 regimen (100 μl, Day 4, followed by 50 μl, twice weekly) was included as well as chemotherapy-alone controls. A preliminary analysis showed no evidence of an influence of irrelevant antibody treatment on relapse or survival. Therefore, data from the two control groups were pooled in subsequent analyses.

Anti-Thy 1.1 antibody treatment significantly ($P < 0.001$, log rank test) prolonged remission duration when anti-Thy 1.1-treated animals were compared to the combined controls. Furthermore, each of the three anti-Thy 1.1 antibody treatment regimens significantly prolonged remission duration when individually compared to the combined control groups ($P < 0.001$; Fig. 2). The 3.2-mg dose appeared slightly more effective compared to the 0.4-mg single dose or the 0.4-mg continuous regimen in prolonging remission at early time points (<40 days), although this difference was not significant ($P > 0.1$).

Similarly, animals treated with anti-Thy 1.1 antibody survived significantly longer than animals treated with control antibody or chemotherapy alone ($P < 0.01$, log rank test). However, treatment with anti-Thy 1.1 antibody had less of an effect on absolute survival than on remission duration, and when each individual anti-Thy 1.1 treatment group was compared to the combined controls, the prolongation of survival approached significance only for the 3.2-mg single dose (Fig. 3; $P = 0.1$, log rank test). The 0.4-mg single dose or 0.4 mg/kg followed by continuous treatment did not significantly prolong survival when these groups were individually compared to combined controls ($P > 0.1$).

Influence of Leukemic Cell Burden. In studies of transplanted leukemia the primary factor determining the success of antibody therapy was the number of cells implanted in the tumor inoculum (1). To determine whether the total number of leukemic cells present at diagnosis, and thus presumably the number of leukemic cells remaining after chemotherapy, influenced the success of antibody therapy, a number of indicators of leukemic cell burden were examined. Semiquantitative measures of initial thymus, spleen, and lymph node size were combined with WBC count into an overall tumor burden index (see “Materials and Methods”). The influence of this tumor burden index on relapse and survival rates was then examined using a Cox regression analysis (Table 2). In animals treated with cyclophosphamide alone or cyclophosphamide plus control antibody, tumor burden had a major influence on relapse rate: animals with large tumor burdens relapsed at a higher rate than those with small burdens ($P = 0.03$). The effect of tumor burden was substantial with the relapse rate increasing an estimated 70% for each increase of 1 unit of the tumor burden index. A similar but less pronounced effect of tumor burden on survival was suggested in these animals ($P = 0.10$). In the anti-Thy 1.1 antibody-treated animals the influence of tumor burden on relapse and survival rates depended on the dose of antibody infused. Animals treated with a single low dose of antibody (0.4 mg) behaved similarly to control animals. Those animals with a larger tumor burden had higher rates of relapse ($P = 0.04$) and of death ($P = 0.03$) than those with smaller tumor burdens. In contrast, following treatment with either the continuous or single large dose (3.2 mg) anti-Thy 1.1 antibody regimens, animals with larger tumor burdens did not relapse or die at a greater rate than those with smaller tumor burdens ($P > 0.1$). These observations suggested that the high dose (3.2 mg) or continuous anti-Thy 1.1 antibody treatments were slightly more effective than a single low dose (0.4 mg) in eliminating leukemic cells.

Thy 1.1 Expression in Relapsing Leukemia. Although anti-

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**Table 1 Antigenic expression at diagnosis**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thy 1.1+, Slg−</td>
<td>209 (77)*</td>
</tr>
<tr>
<td>Thy 1.1−, Slg+</td>
<td>17 (6)</td>
</tr>
<tr>
<td>Thy 1.1−, Slg−</td>
<td>18 (7)</td>
</tr>
<tr>
<td>Mixed Thy 1.1+, Thy 1.1−</td>
<td>26 (10)</td>
</tr>
<tr>
<td>Total</td>
<td>270 (100)</td>
</tr>
</tbody>
</table>

* Expression of Thy 1.1 and Slg by lymph node cells obtained prior to treatment as determined by indirect immunofluorescence and flow microfluorimetry (see “Materials and Methods”). Animals without identifiable leukemic cell populations have been excluded.

* Numbers in parentheses, percentage.
Thy 1.1 antibody was able to prolong remission duration, the majority of both anti-Thy 1.1-treated and control animals eventually relapsed. Relapse in antibody-treated animals could have been a result of failure to eliminate all cells expressing Thy 1.1. Alternatively, it could have resulted from the emergence of variant cells lacking the Thy 1.1 antigen, as had been observed in studies of transplanted leukemia (1). For this reason expression of Thy 1.1 by leukemic cells in lymph nodes obtained at relapse from animals treated with each of the antibody regimens was determined (Table 3). All leukemias uniformly expressed Thy 1.1 prior to treatment. A proportion of animals treated with each of the anti-Thy 1.1 regimens had some leukemic cells which failed to express Thy 1.1. The proportion of relapses containing Thy 1.1- cells appeared slightly higher in animals treated with the continuous antibody regimen (9 of 10, P < 0.01, Fisher's exact test) or the 3.2-mg single dose (4 of 8, 0.05 < P < 0.10) than in animals treated with the 0.4-mg single dose (2 of 13). In contrast, all (17 of 17) control animals relapsed with leukemic cells that uniformly expressed Thy 1.1. These results again suggested the continuous and single large (3.2 mg) dose of antibody had a slightly greater antileukemic effect.

### Table 2 Influence of leukemic cell burden

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Survival (RR)</th>
<th>Relapse (RR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Thy 1.1 Ab*</td>
<td>86</td>
<td>1.13 (P = 0.16)</td>
<td>1.10 (P = 0.38)</td>
</tr>
<tr>
<td>Control (cyclophosphamide alone + control Ab)</td>
<td>20</td>
<td>1.48 (P = 0.10)</td>
<td>1.70 (P = 0.03)</td>
</tr>
<tr>
<td>Anti-Thy 1.1 Ab (0.4 mg, then 0.2 mg)</td>
<td>35</td>
<td>1.10 (P = 0.42)</td>
<td>1.04 (P = 0.80)</td>
</tr>
<tr>
<td>Anti-Thy 1.1 Ab (0.4 mg)</td>
<td>25</td>
<td>1.47 (P = 0.03)</td>
<td>1.50 (P = 0.04)</td>
</tr>
<tr>
<td>Anti-Thy 1.1 Ab (3.2 mg)</td>
<td>26</td>
<td>0.88 (P = 0.54)</td>
<td>0.92 (P = 0.75)</td>
</tr>
</tbody>
</table>

* RR, relative risk, denotes the relative increase in rate of death or relapse per unit increase of the tumor burden index. The significance levels are for a test that the relative risk equals 1.0; i.e., tumor burden was not related to the rate of death or relapse. A relative risk greater than 1.0 indicates that animals with larger tumor burdens relapsed or died at a greater rate than those with a smaller tumor burden. For example, in the control group the rate of relapse increased an estimated 70% with each 1 unit increase in the tumor burden index (RR = 1.70). In accordance with this estimate, animals in the control group with the maximum possible tumor burden index (I = 8) had a relapse rate 24 times [i.e., (1.70)^8] that of animals with the minimum tumor burden (I = 2) required for diagnosis.

* Ab, all anti-Thy 1.1-treated animals.
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Table 3 Thy 1.1 expression in lymph node at relapse

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Thy 1.1+ (n)</th>
<th>Mixed (n)</th>
<th>Thy 1.1− (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Thy 1.1 antibody (0.4 mg, then 0.2 mg)</td>
<td>10</td>
<td>1 (10)</td>
<td>4 (40)</td>
<td>5 (50)</td>
</tr>
<tr>
<td>Anti-Thy 1.1 antibody (0.4 mg)</td>
<td>13</td>
<td>11 (85)</td>
<td>2 (15)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Anti-Thy 1.1 antibody (3.2 mg)</td>
<td>8</td>
<td>4 (50)</td>
<td>3 (38)</td>
<td>1 (13)</td>
</tr>
<tr>
<td>Control antibody (100 μl, then 50 μl)</td>
<td>9</td>
<td>9 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cyclophosphamide alone</td>
<td>8</td>
<td>8 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

* All animals were treated with cyclophosphamide (150 mg/kg), Days 0 and 4, prior to antibody therapy.
* Expression of Thy 1.1 as determined by flow microfluorimetry on lymph node cells obtained at time of clinical relapse.
* Numbers in parentheses, percentage.

Table 4 Thy 1.1 expression in lymph node, spleen, and thymus at relapse

<table>
<thead>
<tr>
<th>Organ</th>
<th>n</th>
<th>Thy 1.1+ (n)</th>
<th>Mixed (n)</th>
<th>Thy 1.1− (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph node</td>
<td>24</td>
<td>17 (71)</td>
<td>5 (21)</td>
<td>2 (12)</td>
</tr>
<tr>
<td>Spleen</td>
<td>22</td>
<td>11 (50)</td>
<td>8 (36)</td>
<td>3 (14)</td>
</tr>
<tr>
<td>Thymus</td>
<td>20</td>
<td>16 (80)</td>
<td>3 (15)</td>
<td>1 (5)</td>
</tr>
</tbody>
</table>

* Number of animals with identifiable leukemic cells in organ.
* Expression of Thy 1.1 by spleen, lymph node, and thymus cells at clinical relapse as determined by indirect immunofluorescence.
* Numbers in parentheses, percentage.

In a subsequent experiment we evaluated the possibility that such antigen-negative cells were present only in lymph nodes. Thy 1.1 expression by cells from spleen, thymus, and lymph node was determined in animals relapsing after treatment with continuous anti-Thy 1.1 therapy, since this regimen had resulted in the greatest proportion of animals with antigen-negative relapses (Table 4). Antigen-negative cells were present in spleen (11 of 22 mice) and thymus (4 of 20 mice) as well as lymph nodes (7 of 24 mice). They were, however, the sole cause of relapse only in a minority of animals (3 of 24 mice). Thus, antibody appeared to be able to prevent relapse with cells expressing Thy 1.1 in all lymphoid organs in a few instances and to eliminate a major fraction of leukemic cells expressing Thy 1.1 in many animals. However, most animals [47 of 55 (85%) in both studies] had relapses in which some cells continued to express the target antigen.

Toxicity. In contrast to the therapy of frankly leukemic mice, infusion of anti-Thy 1.1 antibody following remission induction did not result in immediate death with 85% of animals living greater than 14 days. Anti-Thy 1.1 antibody treatment resulted in a higher rate of non-leukemic death with 31% of animals treated with anti-Thy 1.1 antibody dying in remission as compared to 12% of control animals. Autopsy examination revealed pneumonia or other infection as the predominant (78%) non-leukemic cause of death. Serial creatinine and bilirubin levels obtained twice weekly after initiation of therapy with anti-Thy 1.1 in a subgroup of 20 animals were uniformly normal. Thus the toxicity of anti-Thy 1.1 antibody in mice in remission appeared limited to an increase in infection.

Influence of Antibody on Thy 1.1-negative Leukemias. Anti-Thy 1.1 antibody had no apparent effect on remission duration or survival in the small number of animals with either Slg+ or Thy 1.1−, Slg−, cell leukemias (data not shown). Antigenic analysis of relapsing leukemias in these animals showed the same pattern as on diagnosis. Interestingly, there was also no evidence for a prolongation of remission duration or survival in animals whose leukemias initially contained a mixture of Thy 1.1+ and Thy 1.1− leukemic cells (not shown).

DISCUSSION

Although the passive serotherapy of malignant disease with monoclonal antibodies has shown promise in a number of experimental models (1-11), the results of clinical studies have been, for the most part, disappointing. In the majority of cases, treatment of patients with far-advanced disease has resulted in, at most, transient partial remissions (12). In addition, significant toxicity has been encountered following rapid infusion of antibodies reactive with circulating cells (18, 19). In contrast to these early clinical trials, studies in experimental models have focused on the treatment of relatively small amounts of transplanted tumor, and there may be important differences in the therapy of transplanted tumors as compared to naturally occurring disease.

In the present study we have extended our previous examination of the therapeutic effects of anti-Thy 1.1 antibody against murine T-cell lymphoma (1-5) to aged AKR mice with spontaneous leukemia, a model analogous to human lymphoid malignancies. Infusion of anti-Thy 1.1 antibody in frankly leukemic mice led to early mortality from rapid cell lysis and agglutination. The observation of cell aggregates and cellular debris in pulmonary vessels suggests that, if infusion of antibody results in significant cell lysis, it may be toxic when large numbers of circulating cells are present. A similar phenomenon may account for the pulmonary toxicity observed in clinical studies following rapid infusion of large doses of anti-T-cell antibodies (18), or antibodies reactive with granulocytes (19).

At the same time, the lack of effect of anti-Thy 1.1 antibody on overall cell burden in spontaneous AKR leukemia and the failure to achieve more than transient reductions in circulating leukemic cells in most clinical studies (12) suggest that antibody alone will not have a significant impact in situations of bulk disease.

Antibody used as an adjunct following initial cytoreductive chemotherapy, however, can have a significant therapeutic effect. Anti-Thy 1.1 antibody significantly prolonged chemotherapy-induced remission in mice with spontaneous leukemia compared to chemotherapy alone or chemotherapy combined with control antibody. In spite of a significant prolongation of remission duration, anti-Thy 1.1 antibody treatment only modestly prolonged survival. The failure to observe a greater influence of antibody treatment on survival was presumably a result of an increase in infectious deaths in anti-Thy 1.1 antibody-treated mice, probably from severe immune suppression related to elimination of normal T-cells (20). In the clinical setting it should be possible to overcome this increase in infectious deaths through the use of aggressive supportive measures. Nevertheless, the majority of the anti-Thy 1.1 antibody-treated animals not dying of infection eventually relapsed. Relapse in these animals resulted from a variety of causes.

A proportion of anti-Thy 1.1 antibody-treated animals may have been cured of their original disease. The high frequency of leukemic induction (17%/mo) in aged AKR mice, and thus presumably the rate of reinduction of new leukemias in mice cured of their original disease, combined with the rapid doubling time [mean, 24 h (13, 14)] makes it difficult to determine what proportion of these relapses represented reinduction of...
new leukemias. However, the prolonged duration of remission in some anti-Thy 1.1 antibody-treated animals (30% greater than 50 days) suggests that, in at least some cases, antibody therapy resulted in cure of the initial leukemia (see Refs. 13 and 14). The effectiveness of antibody when used after remission induction with chemotherapy was presumably a result of reducing leukemic cell numbers into the range where host effector mechanisms could eliminate a major portion of remaining cells expressing the target antigen. Studies of transplanted tumors have suggested that a maximum of $10^5$-$10^6$ cells at a single site can be eliminated by antibody (1, 8, 11, 21).

Although it seems likely that some animals were cured of their original leukemia, a major portion of relapses was probably a result of a failure to eliminate all leukemic cells. In addition, even continuous anti-Thy 1.1 antibody treatment was unable to prevent reemergence of leukemia in animals that were potentially cured. In 85% of cases these leukemias contained some cells that continued to express the Thy 1.1 antigen. The failure to eliminate these cells was presumably a result of limitations in the ability of host effector mechanisms to eliminate antibody-coated tumor cells. Evidence from a number of studies suggests that the antitumor effect of antibodies is mediated primarily through a cellular mechanism (6, 10, 22, 23). In addition, AKR mice are complement deficient (24), and antibodies which mediate antibody-dependent cellular cytotoxicity are more effective than those that do not (25). Effector cell function has been shown to be reduced in AKR mice with spontaneous leukemia (26), and effector cell function may have been further reduced by the induction chemotherapy. However, previous studies have failed to show an influence of pretreatment with cyclophosphamide on the effectiveness of antibody therapy (Ref. 23; Footnote 4). Whether a more effective cytoreductive regimen could be given while maintaining effector cell populations and result in an improvement in the results of antibody therapy is uncertain.

Even if all cells that expressed the target antigen were eliminated, relapse would have still been a major cause of failure. Forty-nine of anti-Thy 1.1 antibody-treated animals had relapses that contained cells that failed to express the Thy 1.1 antigen, and in a minor proportion (10–15%) of animals, relapse appeared to be solely due to these antigen-negative cells. A similar emergence of cells lacking the target antigen has been shown to limit therapy of B-cell lymphoma with anti-idiotypic antibodies in clinical trials (27). It seems likely that such antigen-negative cells may ultimately limit the effectiveness of a single antibody and that multiple antibodies against multiple antigens may be required to eliminate all malignant cells.

The influence of antibody dose on the effectiveness of therapy was less clear in mice with spontaneous leukemia than in mice with transplanted disease. In our studies of transplanted leukemia a high dose (3.2–3.6 mg) of antibody was clearly more effective than lower doses (1). In contrast, in mice with spontaneous leukemia in remission, our results were only suggestive that a high dose was more effective than a single lower dose, and there was no evidence that it was superior to multiple lower doses. The differences in the influence of antibody dose between these two models were possibly a result of differences in access of either antibody or effector cells to the s.c. inoculation site in the studies of transplanted leukemia as compared to the more diffuse organ involvement present in spontaneous leukemia. Alternatively, a greater antileukemic effect of the 3.2-mg dose may have been obscured by a high rate of leukemia reinduction. Nevertheless, both models suggested that there is little, or no, advantage for multiple doses of antibody, provided that a sufficiently high dose is infused initially.

Our results suggest that the treatment with antibody directed against a normal differentiation antigen on tumor cells can significantly add to the therapeutic benefits achieved from chemotherapy alone. Nevertheless, the clinical improvement seen was of a modest order which presumably resulted from two major factors limiting antibody therapy. The first was a limit in the host effector mechanism(s) required to eliminate antibody-coated tumor cells that expressed the target antigen. One promising approach to overcoming this limitation is to render the antibody itself directly cytotoxic by the use of antibody conjugates. Antibody conjugates with cytotoxic drugs or toxins have the potential for increasing the therapeutic efficacy against cells that express the target antigen (28, 29), while maintaining antibody specificity. Nevertheless, since a second major factor limiting antibody therapy is the presence of cells lacking the target antigen, it may be advantageous to use antibody-radionuclide conjugates which, when bound to antigen-positive cells, can kill adjacent antigen-negative cells as a result of emitted radiation. The use of $^{111}$In-labeled anti-Thy 1.1 antibody has already proven significantly more effective than antibody alone against transplanted leukemia (30) and will now be tested in mice with spontaneous leukemia. Successful results would suggest the clinical applicability of this approach.

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REFERENCES


* Unpublished observations.
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