The Antitumor Activity of an Anti-CD22 Immunotoxin in SCID Mice With Disseminated Daudi Lymphoma Is Enhanced by Either an Anti-CD19 Antibody or an Anti-CD19 Immunotoxin

By Maria-Ana Ghetie, Karsten Tucker, James Richardson, Jonathan W. Uhr, and Ellen S. Vitetta

The antitumor activities of immunotoxins (ITs) constructed with deglycosylated ricin A chain (dgA) and either anti-CD19 (HD37) or anti-CD22 (RFB4) monoclonal antibodies were compared in SCID mice with disseminated human Daudi lymphoma (SCID/Daudi). As reported previously, after intravenous injection with Daudi cells, SCID mice develop disseminated lymphoma, which infiltrates the vertebral column and causes paralysis of the hind legs before death. The mean paralysis time (MPT) has been taken as an end point in this tumor model. We have previously reported that early treatment of SCID/Daudi mice with RFB4 coupled to dgA prolongs the MPT in a manner consistent with the killing of 4 logs of tumor cells. In the present study, we show that HD37-dgA kills 2 logs of tumor cells. The lower potency of the HD37-dgA is consistent with its lower IC_{50} on Daudi cells in vitro. We further show that the antitumor activity of a mixture of HD37-dgA and RFB4-dgA is significantly enhanced in SCID/Daudi mice and is consistent with the killing in excess of 5 logs of tumor cells. However, identical enhancement was observed when a mixture of the RFB4-dgA and the HD37 antibody was administered. In contrast, enhancement was not observed when mice were injected with a mixture of the RFB4 antibody and the HD37-dgA. The results indicate that a “cocktail” of HD37 antibody and RFB4-dgA immunotoxin can have significant antitumor activity in this mouse model of lymphoma and suggest that combinations of particular antibodies and ITs may have cooperative antitumor activity.

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MATERIALS AND METHODS

Cells. The in vitro adapted surface IgM+ Burkitt’s lymphoma cell line, Daudi, was maintained by serial passages in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin, and 100 mmol/L L-glutamine (complete medium). The cells were grown in a humidified atmosphere of 5% CO_{2} and air. Cells were washed with sterile phosphate-buffered saline (PBS), and the cell suspension was adjusted to an appropriate concentration for inoculation into SCID mice (5 × 10^{7}/mL). The cell viability was determined by trypan blue exclusion.

Animals. Female SCID mice (C.B-17 SCID/SCID) were obtained from our colony at the University of Texas. They were housed and maintained in a specific pathogen-free (SPF) facility. Animals were given autoclaved food and sterile water ad libitum, and all manipulations were performed in a laminar flow hood.

SCID/Daudi mice. Six- to ten-week-old SCID mice were injected intravenously (IV) with 5 × 10^{7} Daudi cells in 0.1 mL PBS. Mice were observed daily and were killed at the onset of paralysis, which routinely occurred 10 to 15 days before death.

Antibodies. Mouse IgG1 monoclonal antibodies (MoAbs) specific for CD22 (RFB4) or CD19 (HD37) and the purified isotypematched (IgG1) myeloma protein (MOPC-21) (control) were used. Both RFB4 and HD37 were prepared by Abbott Biotech (Needham Heights, MA). The MOPC-21 myeloma protein was obtained from Cappel (West Chester, PA).

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Preparation of (Fab')2 fragments of HD37. (Fab')2 was obtained by digesting HD37 with pepsin as described previously.11 (Fab')2 fragments were purified by gel permeation high performance liquid chromatography (HPLC) using a preparative 21.5 × 600 mm TSK 3000SWG column (Ultrospec; Pharmacia, Uppsala, Sweden).

Ricin A chain. dgA was prepared as described previously12 and was purchased from Inland Biologicals (Austin, TX).

ITs. HD37-dgA, RFB4-dgA, and MOPC-21-dgA were prepared as described previously.13 The purity of the ITs was determined by electrophoresis under nonreducing and reducing conditions on 7.0% sodium dodecyl sulphate-polyacrylamide gels13 and by gel permeation HPLC on an analytical 7.5 × 600 mm TSK 3000SW column (Ultrospec; Pharmacia).

Characterization of the ITs. The HD37-dgA and RFB4-dgA ITs used in these studies are described in Table 1. The data show that they are of the LD50 dose of each IT in a single course of four equal doses on days 1, 2, 3, and 4 after tumor inoculation. Other mice were injected with antibody alone in amounts equivalent to those used in the respective ITs. Mice were injected with the following cocktails: (1) RFB4-dgA (20% LD50) + HD37-dgA (20% LD50); (2) RFB4-dgA (20% LD50) + 20% LD50 equivalent amounts (RFB4, HD37, or MOPC-21); (3) HD37-dgA (20% LD50) + 20% LD50 equivalent amounts of antibodies (HD37, RFB4, or MOPC-21); and (4) MOPC-21-dgA (dose equivalent to the average of RFB4-dgA [20% LD50] and HD37-dgA [20% LD50]) + 20% LD50 equivalent amounts of antibodies (MOPC-21 or RFB4). Three groups of mice were treated with HD37 antibody (40% LD50 equivalent) at different stages of tumor growth. Two other groups of five mice were treated with either HD37 antibody or with its F(ab')2 fragment. Mice were observed daily for the onset of posteriors paralysis or paralysis. Gross and microscopic examination were performed on organs from groups of killed mice. Excess tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin-eosin.

Pharmacokinetics of ITs in SCID/Daudi mice. The ITs were labeled with Na125I by the IODO-GEN (Pierce, Rockville, IL) technique, mixed with cold IT, and injected into the tail vein of mice challenged with 5 × 106 Daudi cells 1 day earlier. Two mice were injected with 125I-RFB4-dgA + (cold) RFB4-dgA (40% of the LD50 dose) as 4 equal doses starting 1 day after tumor inoculation. The total radioactivity injected was 9.3 × 106 cpm/120 μg IT/mouse. Sera were collected 0, 1, 2, 4, 8, 24, 48, 72, 96, and 128 hours after the fourth injection.

One mouse was injected with 125I-HD37-dgA (20% of the LD50 dose) administered in 4 equal doses on days 1 through 4 after tumor inoculation. Total radioactivity injected was 1.0 × 107 cpm/60 μg/mouse. The levels of 125I-ITs in heparinized blood samples (44.7 μL) were determined at 0, 1, 2, 4, 8, 24, 48, 72, 96, and 128 hours after the last dose.

The pharmacokinetics were calculated using the noncompartmental determination as recommended by Perrier and Mayerson15 because after 4 inoculations the distribution of the immunotoxins between the intravascular and extravascular compartments had already taken place. A computer program kindly provided by Dr K. Vyas from Merck Sharp and Dohme Research Laboratory (West Point, PA) was used for the calculation of all pharmacokinetic parameters.

RESULTS

The pharmacokinetics of HD37-dgA and RFB4-dgA ITs used in these studies are described in Table 1. The data show that they differ only in their ability to kill Daudi cells in vitro. Thus, HD37-dgA is 10-fold less cytotoxic to Daudi cells than RFB4-dgA, as assessed by their IC50.

The pharmacokinetics of HD37-dgA and RFB4-dgA in SCID/Daudi mice. As shown in Table 2, there was no significant difference between the pharmacokinetics of the two ITs. The area under the curve (AUC) for HD37-dgA was lower than that for RFB4-dgA, reflecting the smaller amount injected. The volumes of distribution (around 5 mL) were higher than the blood volumes, indicating that the ITs were distributed in the tissues. Most significantly, the half-lives (T\(\frac{1}{2}\)) were extremely long, as has been reported for antibodies in SCID mice.16

With regard to the peak concentrations and T\(\frac{1}{2}\), it should be noted that in B lymphoma patients receiving the maximum tolerated dose (MTD) of RFB4-dgA, the peak serum levels averaged 3.5 μg/mL,14 which is 5- to 10-fold lower than the values observed in SCID/Daudi mice. Furthermore, the T\(\frac{1}{2}\) in patients averaged 10.1 hours,14 as compared with 60 hours in SCID/Daudi mice. Nevertheless, objective responses were observed in greater than 30% of the patients. Hence, it is possible that, in the presence of large tumor burdens, the tumor cells act as an antigenic sink, and that the serum levels of the IT are therefore lower and the T\(\frac{1}{2}\) are shorter. Further studies in both patients and SCID/Daudi mice with different tumor burdens will be required to explore this possibility.

The effect of ITs and antibodies on the mean paralysis time (MPT). As described previously,12 the MPT represents an accurate measurement of the antitumor activity of ITs in

Table 1. Characterization of ITs Used in SCID/Daudi Mice

<table>
<thead>
<tr>
<th>Assay</th>
<th>HD37</th>
<th>RFB4</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS-PAGE/HPLC on TSK3000</td>
<td>69</td>
<td>70</td>
</tr>
<tr>
<td>(% of major peak)</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>76% of dissociation in vitro (h)</td>
<td>45</td>
<td>ND*</td>
</tr>
<tr>
<td>% of dissociation in vivo (h)</td>
<td>801</td>
<td>971</td>
</tr>
<tr>
<td>Antibody activity (% of initial)</td>
<td>7.8</td>
<td>8.0</td>
</tr>
<tr>
<td>dgA activity in reticulocyte assay (kIC50, mol/L × 10^{-19})</td>
<td>4.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Daudi killing assay (kIC50, mol/L × 10^{-11})</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>LD50 (mg/kg)</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Endotoxin by LAL-assay (EU/mg)</td>
<td>801</td>
<td>971</td>
</tr>
</tbody>
</table>

**Abbreviation:** SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; LAL, limulus amebocyte lysate.

*Eighty-four percent nondissociated IT at 12 hours.77

†kIC50 = 3.6 ± 0.4 × 10^{-16} mol/L.

‡kIC50 = 9.1 ± 1.8 × 10^{-16} mol/L.

§in BALB/c mice by IV bolus inoculation; survival recorded at 14 days.
ANTI-CD19/ANTI-CD22 ITS IN SCID/DAUDI MICE

The SCID/Daudi model. From the dose-dependent curve of MPT we can estimate the number of tumor cells that are killed in vivo after treatment with ITS if we assume that the only direct effect of the therapeutic agent is rapid cell killing. Table 3 summarizes the MPT and the estimated number of cells killed by the three ITS and their corresponding antibody. The experiments show that at a 40% LD50 dose of either the RFB4-dgA or the HD37-dgA, the MPT is extended as compared with either PBS or with MOPC-21-dgA (P < .001). Treatment with a 40% LD50 dose of RFB4-dgA killed 99.99% (4 logs) of Daudi cells, whereas a similar dose of the HD37-dgA killed 99.45% (2 logs) of tumor cells. HD37-dgA was less effective than RFB4-dgA, as shown by the statistically significant difference between the MPTs of the treated mice (73.2 v. 54.9 days, P < .001). The lower potency of the HD37-dgA is consistent with its inferior in vitro activity (Table 1). The effect of the two ITS became comparable at 20% of the LD50 dose, as shown by the MPTs of the treated mice (50.0 v. 53.8 days, P > .05). Furthermore, when administered at either 40% or 20% of the LD50 dose, the antitumor activity of RFB4-dgA was dose-dependent, whereas that of the HD37-dgA was not. Thus, the MPT of mice injected with RFB4-dgA at 40% of the LD50 dose was significantly higher than that at 20% of the LD50 dose (73.2 v. 50.0 days, P < .001), whereas the effects of HD37-dgA at both 20% and 40% of the LD50 dose were the same (54.9 v. 53.8 days, P > .05).

The two antibodies, RFB4 and HD37, had modest but statistically significant antitumor activities of their own, corresponding to 1 to 2 logs of tumor cell killing. An irrelevant isotype-matched murine plasmacytoma IgG (MOPC-21) had no effect at either dose. The HD37 antibody was more effective than the RFB4 antibody at both doses (MPTs of 57.8 v. 46.2 days, P < .001; 43.6 v. 38.4 days, P < .05). Hence, when the antitumor activities of the two ITS are compared with those of their corresponding antibodies, the MPT of mice injected with RFB4-dgA as compared with RFB4 was significantly longer at both doses. In contrast, HD37-dgA and HD37 showed identical antitumor activities at 40% of the LD50 dose and modest differences at 20% of the LD50 dose (or its equivalent in milligrams for antibody).

Further characterization of the antitumor activity of HD37 antibody. Because the HD37 antibody was as effective as its IT in extending the MPT of SCID/Daudi mice, we further determined whether this particular antibody could prolong survival when administered at later stages of tumor growth. As shown in Table 4, the antitumor effect of HD37 was achieved only by injecting the antibody at a dose equivalent to that contained in 40% of the LD50 dose of the IT early after tumor inoculation (days 1 through 4). To determine whether treatment with HD37 was dependent on its Fc fragment, we compared the antitumor activity of intact antibody with that of its F(ab')2 fragment. As shown in Table 5, both the F(ab')2 and IgG had antitumor activity of HD37 Antibody During Different Stages of Tumor Growth

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Treatment Regimen</th>
<th>MPT ± SD (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MOPC-21-dgA</td>
<td>Days 1-4</td>
<td>26.0 ± 1.7 (5)</td>
</tr>
<tr>
<td>MOPC-21</td>
<td>Days 1-4</td>
<td>52.4 ± 2.6 (5)</td>
</tr>
<tr>
<td>MOPC-21-dgA</td>
<td>Days 10-13</td>
<td>62.0 ± 4.2 (5)</td>
</tr>
<tr>
<td>HD37</td>
<td>Days 21-24</td>
<td>32.8 ± 4.1 (5)</td>
</tr>
</tbody>
</table>

The SCID mice were treated with a dose of antibody equivalent to that present in 40% LD50 dose of the IT. A single course of treatment was administered in 4 equal doses. Numbers in parentheses represent the number of mice in each group. The difference between HD37 (days 1 through 4) and MOPC-21 is significant (P < .001), but the others are not (P > .05).

Table 3. The Effect of Different Doses of ITS or Antibodies on the Survival of Mice and the Extrapolated Killing of Tumor Cells in Vivo

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Dose (% of LD50 or its equivalent in mg of antibody)</th>
<th>MPT ± SD (d)</th>
<th>Killing of Daudi Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>0.0 ± 0.0 (5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MOPC-21-dgA</td>
<td>20.0 ± 3.2 (5)</td>
<td>23.0 ± 4.1 (5)</td>
<td>0</td>
</tr>
<tr>
<td>MOPC-21</td>
<td>10.0 ± 3.2 (5)</td>
<td>23.0 ± 4.1 (5)</td>
<td>0</td>
</tr>
<tr>
<td>RFB4-dgA</td>
<td>30.0 ± 3.2 (5)</td>
<td>23.0 ± 4.1 (5)</td>
<td>0</td>
</tr>
<tr>
<td>RFB4</td>
<td>30.0 ± 3.2 (5)</td>
<td>23.0 ± 4.1 (5)</td>
<td>0</td>
</tr>
<tr>
<td>HD37-dgA</td>
<td>40.0 ± 3.2 (5)</td>
<td>23.0 ± 4.1 (5)</td>
<td>0</td>
</tr>
<tr>
<td>HD37</td>
<td>40.0 ± 3.2 (5)</td>
<td>23.0 ± 4.1 (5)</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: Vd, volume of distribution; MRT, mean residence time; AUC, area under the curve.

*The results represent the average of two mice.
†The results were obtained with one mouse.

Table 2. The Pharmacokinetics of RFB4-dgA and HD37-dgA in SCID/Daudi Mice

<table>
<thead>
<tr>
<th>Total dose (µg)</th>
<th>RFB4-dgA</th>
<th>HD37-dgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 × 10^6 Daudi cells</td>
<td>310.0 ± 16.3 (5)</td>
<td>18.3 ± 1.13 (5)</td>
</tr>
<tr>
<td>4 × 15 = 60 (µg/mL)</td>
<td>57.9 ± 61.3 (5)</td>
<td>0.065 ± 0.053 (5)</td>
</tr>
<tr>
<td>5.6 ± 4.8 (mg/mL)</td>
<td>85.1 ± 89.6 (5)</td>
<td>..........</td>
</tr>
</tbody>
</table>
activities when administered as four equal injections, even though the former would be predicted to have a 10-fold shorter T1/2 in vivo (unpublished results). The amount of F(ab')2 injected was not enough (on a molar basis) to offset the difference in T1/2s.

The effect of combination therapy. We next determined whether the two ITs had a cooperative antitumor effect when administered together. As shown in Table 6, the administration of combinations of ITs or IT and antibody extended the MPT. For example, a mixture of a 20% LD50 dose of RFB4-dgA and a 20% LD50 dose of HD37-dgA showed cooperative antitumor activity, resulting in an MPT significantly longer (103.1 days) than that obtained with either individual IT at either a total of 20% of the LD50 dose (50.0 or 53.8 days, P < .0001) or at 40% of the LD50 dose (73.2 or 54.9 days, P < .0001) (Tables 3 and 6). The cocktail therefore prolonged survival in a manner consistent with the killing of greater than 5 logs of tumor cells as compared with 2 to 4 logs for a 20% to 40% LD50 dose of RFB4-dgA or 2 logs for either a 40% or 20% LD50 dose of HD37-dgA. Enhanced antitumor activity was also observed when RFB4-dgA was administered with HD37 antibody instead of HD37-dgA. In contrast, when a mixture of RFBCdgA and RFBC antibody was injected, the MPT of the IT was decreased (37.0 v 50.4 days) and was even shorter than that observed using the combination of RFBCdgA and MOPC-21 IgG (51.5 days). This indicates that the RFBC antibody exerted an expected inhibitory effect on the antitumor activity of its IT, probably by competing for binding sites on the tumor cells. In contrast, the antitumor activity of HD37-dgA was not decreased when it was administered with HD37 antibody (53.8 v 50.2 days), suggesting that the antitumor activity of HD37-dgA is not due to its toxin moiety, i.e., the antibody and the IT had similar antitumor activity and there was no competition or cooperativity between the two agents in vivo. Finally, HD37-dgA administered in combination with RFBC or MOPC-21 had the same antitumor activity as HD37-dgA alone, indicating that the antitumor activity of this IT was not potentiated by RFBC.

Macroscopic and microscopic evaluation of organs from treated mice. All mice developed prominent tumor infiltrations in the vertebral column. The tumor cells replaced marrow, produced osteolysis of vertebral bone, and extended into the spinal canal to compress the cord. Compression of the cord resulted in posterior paralysis. Neoplastic infiltrates were common in the bones of the skull and mandible and commonly produced meningeal infiltrates, but clinical signs relating to disfunction of the brain or cranial nerves were not evident.

Lymphoma was common in the lungs (19 of 22) and often was associated with a bacterial pneumonia (12 of 22), but the pneumonic lesions were not responsible for death.

No major differences were observed between the tumor burden or sites of tumor growth in mice treated with either one of the two ITs or with cocktails with the possible exception of a reduced tumor burden in the ovaries of the mice treated with RFB4-dgA as compared with HD37-dgA. Microscopic examination showed that the sites of tumor growth in the spinal canal were the same in mice treated with combination therapy as in untreated mice even though tumor developed much more slowly.

**DISCUSSION**

In previous studies1,2 we have shown that SCID mice inoculated IV with CD19+ CD22+ Burkitt’s lymphoma cells (Daudi) develop extranodal disease in ovaries, kidneys, and bone marrow and that these mice showed hind legs paralysis before death. When SCID/Daudi mice were treated with a 40% LD50 dose of RFB4-dgA, there was a prolongation in survival consistent with a 4-log killing of tumor cells.2 Antitumor activity of this IT, either as a Fab'-dgA or an IgG-dgA has also been observed in phase 1 clinical trials in patients with advanced NHL.3,4 In the case of the IgG-dgA, at MTD peak levels of 3.5 μg/mL, IT could be achieved in blood.

The present work was undertaken with two goals: (1) to study the antitumor activity of an IT directed against the
CD19 antigen (HD37-dgA) and to compare its activity with that of RFB4-dgA; and (2) to study the antitumor effect of combination therapy with these two ITs and their corresponding antibodies. Our studies show that (1) at 40% of the LD50 dose, at which serum levels of RFB4-dgA could be maintained at 31 μg/mL, and at which the T½ was 57.9 hours, RFB4-dgA kills 4 logs of tumor cells in vivo, whereas HD37-dgA kills 2 logs; (2) at 40% of the LD50 dose or an equivalent amount of antibody, HD37-dgA is as effective as HD37 in prolonging the survival of mice; (3) a mixture of a 20% LD50 dose of RFB4-dgA and either a 20% LD50 dose of HD37-dgA or an equivalent amount of HD37 antibody results in enhanced antitumor activity as compared with that of either agent alone; and (4) a mixture of HD37-dgA and either RFB4 or HD37 does not show enhanced antitumor activity.

The effect of combination therapy with ITs or ITs and antibodies has not been investigated in SCID mice with human lymphoma. However, it has been reported that cocktails of ITs are more effective in preventing the growth of Hodgkin’s tumors in triple beige nude mice than single ITs (A. Engert and P.E. Thorpe, personal communication, 1990). Studies in nude mice have also shown that combinations of two ITs directed against non-T-cell acute lymphocytic leukemia (non-T-ALL) cells are more effective than single ITs. It has also been shown that the delivery of saporin to murine lymphoma in vitro and in vivo by a pair of bispecific antibodies is significantly better than either one individually. Finally, studies performed in vitro have shown that a cocktail of three anti-T-cell ITs was significantly better than single ITs.

Our previous studies have shown that both RFB4 and HD37 antibodies recognize only B cells in a panel of more than 40 normal human tissues. However, RFB4 reacts with 15% to 100% of tumor cells in 60% to 70% of patients with B-cell lymphoma, whereas HD37 recognizes 50% to 100% of the tumor cells from greater than 90% of B lymphomas. Hence, HD37-dgA should be effective on a wider range of tumors than RFB4-dgA, despite the fact that HD37-dgA is 10-fold less cytotoxic in vitro to Daudi cells. As described in this study, at 40% of the LD50 dose, HD37-dgA was less effective than RFB4-dgA in extending the survival of mice. This result is consistent with the lower cytotoxic activity of HD37-dgA in vitro. Because the two ITs had different effects at higher doses (40% of the LD50 dose), but not at lower doses, (20% of the LD50 dose) the optimal dose of a given IT may vary depending on the nature and density of the antigen that it recognizes on tumor cells and the mechanisms involved in cytotoxicity or cytostasis. In this regard, RFB4-dgA is more effective than HD37-dgA at killing Daudi cells in vitro as well, even though these cells express similar densities of CD19 and CD22.

We have also shown that at 40% of the LD50 dose of HD37-dgA or its equivalent in antibody, survival was prolonged only when the agents were administered early. The antitumor activity of the HD37 antibody is consistent with results of earlier work of Vuist et al showing that a mouse IgG2a MoAb directed against CD19 inhibited the tumor growth of Daudi lymphoma xenografts in nude mice. Treatment was effective only if the antibody was administered early after tumor inoculation. Interestingly, later treatment was also effective if the antibody was administered along with interleukin-2 (IL-2). The antitumor effect might be a result of natural killer (NK)-mediated antibody-dependent cell-mediated cytotoxicity (ADCC). Thus, peritoneal exudate cells were able to inhibit the proliferation of Daudi cells in vitro in the presence of anti-CD19 antibody. Similarly, spleen cells preincubated with IL-2 induced ADCC against Daudi cells sensitized with anti-CD19. The same anti-CD19 antibody was administered to six patients with NHL and one patient showed a partial remission. In contrast to the above studies, another anti-CD19 (B43) antibody did not show any antitumor effect in SCID mice with human t(4;11) cell leukemia. This indicates that the HD37 antibody might not have the same antitumor effect on all CD19+ tumors or that the antitumor effect is dependent on the epitope that the anti-CD19 antibody recognizes. In our study, it is unlikely that ADCC or complement were involved in the antitumor effect of HD37, because F(ab’)2 fragments were also effective. We have also ruled out an apoptotic mechanism because the in vitro incubation of Daudi cells with various concentrations of HD37 does not induce characteristic DNA fragmentation (data not shown). Therefore, the antitumor effect of the HD37 antibody may be due to a novel type of death similar to that described for an anti-IgM antibody on a human B-lymphoma cell line.

The cytostatic effect of HD37 might also be related to the reported inhibitory activity of several anti-CD19 antibodies, including HD37 (IgG or as Fab’ fragment), on B-cell proliferation induced by anti-Ig antibody alone or in combination with B-cell growth factor. It has been shown that anti-CD19 can arrest the entry of stimulated tonsilar B cells into S-phase by inhibiting DNA, but not RNA, synthesis. If anti-CD19 antibody can induce the same downregulatory signal in Daudi cells, then it is possible that it blocks or impairs the proliferation of the tumor cell in vivo in the absence of effector cells in the host. It should be emphasized that Daudi cells respond to treatment with anti-CD19 by a rapid increase in intracellular Ca2+ even though this effect has not been reported in normal B cells.

In conclusion, our results indicate that the antitumor activity of cocktails of anti-CD22-dgA and anti-CD19-dgA or anti-CD22-dgA and anti-CD19 antibody is better than that of any of these three agents alone. The finding that anti-CD19 and anti-CD22-dgA have enhanced antitumor activity suggests that combinations of antibodies and immunotoxins directed against different target antigens should be considered in designing future clinical trials with these reagents.

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The antitumor activity of an anti-CD22 immunotoxin in SCID mice with disseminated Daudi lymphoma is enhanced by either an anti-CD19 antibody or an anti-CD19 immunotoxin

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