Production and Characterization of a Bispecific IgG Capable of Inducing T-Cell–Mediated Lysis of Malignant B Cells

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Bispecific monoclonal antibodies (bsabs) recognizing both CD3 and a tumor antigen can redirect T-cell–mediated cytoxicity toward cells bearing that antigen. Such bsabs have been shown to be more effective than monospecific monoclonal antibodies (MoAbs) at preventing tumor growth in animal models of B-cell malignancy. The current studies describe the production and preliminary evaluation of a bsab designed to induce the lysis of malignant human B cells by human T cells. The bsab was obtained from a hybrid-hybridoma cell line produced by fusing OKT3-secreting hybridoma cells with hybridoma cells that secrete 1D10. 1D10 is a MoAb that recognizes an antigen found on a majority of malignant human B cells that has not been detected to a significant degree on normal resting or activated lymphocytes. High performance liquid chromatography (HPLC) was used to separate bsab from monospecific antibodies that were also present in the hybrid-hybridoma antibody product. The bsab was then evaluated in vitro for its ability to induce lysis of malignant B cells by activated T cells. The bsab consistently induced extensive lysis in vitro of 1D10 (+) cells, including both cell lines and cells obtained from patients with a variety of B-cell malignancies. No such effect was seen with activated T cells alone or activated T cells with monospecific antibody. No increased lysis was seen with 1D10 (−) cell lines. The bsab also mediated lysis of malignant B cells by autologous T cells. We conclude bsab containing an OKT3 arm and a 1D10 arm can induce T-cell–mediated lysis in a manner that is both potent and specific. This supports further evaluation of this bsab as a potential immunotherapy of B-cell malignancy.

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The antigen recognized by the MoAb designated 1D10 has not been as extensively evaluated as antigens recognized by other anti-B-cell antibodies. Nevertheless, 1D10 appears to be well suited for bsab-directed therapy. The 1D10 antigen is present on the large majority of B- and pre-B-cell malignancies, including those that are relatively differentiated (chronic lymphocytic leukemia [CLL]) and those that are relatively undifferentiated (pre-B acute lymphoblastic leukemia [ALL] and Burkitt’s lymphoma). It also reacts with lymphocytes from individuals with acute Epstein-Barr virus (EBV) infection, but not to a detectable degree with normal resting or activated B cells or other normal cell populations.\textsuperscript{34} Immunoprecipitation of the antigen recognized by 1D10 indicates it is a heterodimer of 32 Kd and 28 Kd that is not HLA-DR, HLA-DP, or HLA-DQ, but may represent an HLA D-region gene that is not normally expressed. It is expressed on 1D10 (+) cell lines at a density that is generally greater than HLA class I but less than HLA class II. Preliminary studies also indicate that 1D10 is not shed to a significant degree into the circulation of animals bearing large tumor burdens with 1D10 (+) tumors. Further, antigenic modulation of the 1D10 antigen does not occur after addition of 1D10 antibody (unpublished data).

In this report we describe the production and in vitro evaluation of a bsab that recognizes CD3 on human T cells and the antigen recognized by 1D10. These studies show that a bsab produced from anti-CD3 and 1D10 can induce T-cell–mediated lysis of malignant B cells bearing the 1D10 antigen, and that this effect is both specific and potent.

**MATERIALS AND METHODS**

**Monospecific antibody production.** The OKT3 secreting hybridoma\textsuperscript{32} (American Type Culture Collection, [ATCC], Rockville, MD) was grown in RPMI 1640 (Irvine Scientific, Santa Ana, CA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (J.R. Scientific, Inc, Woodland, CA), 100 \( \mu \)g/mL L-glutamine, 100 \( \mu \)g/mL penicillin-streptomycin (K.C. Biological, Lenora, KS), and 5 \( \times \) 10\( ^{-5} \) \( \mu \)moles/L 2-mercaptoethanol (complete medium). Ig product was purified from supernatant by precipitation with 45% saturated ammonium sulfate, followed by Protein A immunoaffinity chromatography to yield a preparation that was greater than 95% Ig by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Immunoreactivity was confirmed by indirect immunofluorescence staining of mononuclear cells obtained from normal donors. The 1D10-secreting hybridoma (supplied by Dr Roger Gingrich, University of Iowa) was grown in complete medium. Cells, 2 \( \times 10^5 \), were injected into a mini-flx path hollow fiber bioreactor (Amicon Div, Beverly, MA). Cells were continuously bathed with complete medium containing 2.5% fetal calf serum (FCS). After 1 week, daily harvests of antibody-containing supernatant were collected. Supernatant represented IgG as indicated by SDS-PAGE. Immunoreactivity was reconfirmed by indirect immunofluorescent staining of Raji cells.

**Bsab production.** A bsab-secreting hybrid-hybridoma was produced by fusing OKT3-secreting hybridoma cells with 1D10-secreting hybridoma cells. An ouabain-resistant, aminopterin-sensitive OKT3 hybridoma cell line was selected by growing 10\( ^6 \) OKT3 hybridoma cells sequentially in media containing increasing concentrations of 8 azaguanine (up to 0.13 mmol/L), then increasing concentrations of ouabain (up to 1.0 mmol/L). The resulting cells were subcloned by limiting dilution. Hybrid-hybridomas were then produced by the fusion of, using 38% polyethylene glycol, of 10\( ^6 \) hypoxanthine-aminopterin-thymidine (HAT)-sensitive, ouabain-resistant OKT3-secreting hybridoma cells with 10\( ^6 \) HAT-resistant, ouabain-sensitive 1D10-secreting hybridomas. Fused cells were placed in 50-mL HAT-ouabain media consisting of complete media supplemented with 1 mmol/L ouabain and HAT (Sigma, St Louis, MO) with the aminopterina at a final concentration of 0.4 \( \times 10^{-2} \) mmol/L, and were plated in two 24-well plates. The parental OKT3 line did not survive in the presence of aminopterina, and the parental 1D10 line did not survive in the presence of ouabain, thus selecting for growth of hybrid-hybridomas displaying both ouabain resistance (from OKT3) and aminopterina resistance (from 1D10). Two weeks after the fusion, supernatant from wells containing growth was tested for the secretion of functional bsab by enzyme-linked immunosorbent assay (ELISA) and flow cytometry (see below). The selected hybrid-hybridoma was subcloned twice, then expanded in serum-free media consisting of HB101 (Irvine Scientific, Santa Ana, CA) supplemented with 100 \( \mu \)g/mL L-glutamine and 100 \( \mu \)g/mL penicillin-streptomycin. A mini-flx path hollow fiber bioreactor was then inoculated with 2 \( \times 10^8 \) hybrid-hybridoma cells. After 1 week, daily harvests of bsab-containing supernatant were collected for a period of up to 8 weeks. The desired bispecific Ig fraction was purified from the supernatant by cation-exchange high performance liquid chromatography (HPLC). (Beckman Model 342; Berkeley, CA). This was done by dialyzing the hybrid-hybridoma supernatant twice against a 50-mmol/L malonate buffer (pH 4.8) and loading it onto a TSK SP-SPW 150 \( \times 2.5 \) mm Ultra-Pac column (TOSO-HAAS, Philadelphia, PA). Antibody was then eluted with a 0.18- to 0.50-mol/L NaCl gradient at a flow rate of 4.0 mL/min. Protein-containing fractions, as defined by UV light absorbance at 280 nm, were analyzed for their immunoreactivity and isotype composition using the ELISA and flow cytometric assays outlined below.

**Evaluation of antibody reactivity.** ELISA and flow cytometric assays were used to evaluate for the presence of bsab in hybrid-hybridoma supernatant, and to assess the reactivity of various antibody fractions. In the ELISA, serial dilutions of supernatant or antibody fractions were added to microtiter plates coated with 10 \( \mu \)g/mL goat antiguinea IgG antibody (Southern Biotech, Birmingham, AL). After a 30-minute incubation at room temperature, plates were washed and alkaline phosphatase-labeled goat antiguinea IgG2a or IgG1 was added. After repeat incubation, plates were washed and developed with p-nitrophenyl phosphate substrate. A positive signal indicated the presence of IgG1 or IgG2a isotypes. When the assay was performed using plates coated with anti-IgG2a and later incubated with anti-IgG1-alkaline phosphatase, a positive signal indicated the presence of single Ig molecules composed of both IgG2a and IgG1 heavy chains. In the indirect immunofluorescent assay, supernatant or antibody was added to 1D10 (+) cells (Raji or HO-85) or cells that were mostly CD3 (+) (peripheral blood lymphocytes [PBLs]) for 30 minutes on ice. After washing, fluorescein isothiocyanate (FITC)-labeled goat antiguinea IgG was added. Cells were evaluated by flow cytometry for fluorescence to evaluate for the presence of bound antibody. To further characterize the nature of the bound Ig, isotype specific (anti-IgG2a and anti-IgG1) FITC-conjugated reagents were used.

**Effector cells.** PBLs were isolated from theuffy-coat fraction of donor blood by density gradient centrifugation. Monocytes and macrophages were depleted by adherence to plastic (1 hour at 37°C) followed by nylon wool adsorption. Lymphocytes were cultured 5 to 7 days in complete medium that was supplemented with recombiant human interleukin-2 (rhl-2) (Cetus Oncology, Emeryville, CA) at 300 IU/mL, OKT3 at 10 \( \mu \)g/mL, or both IL-2 and OKT3. Effector cells were washed twice in complete media before use.
Target cells. Malignant human B cell lines included 697-ALL (a pre-B-ALL), HO-85 (a large cell lymphoma), Raji (a Burkitt's-like lymphoma), and RPMI 8866 and BURKHART (EBV-transformed human B-cell lines). All cell lines were grown in complete medium and used at a time of exponential growth. Malignant B-cells were obtained from patients by density centrifugation of the PB or pleural fluid.

Cytotoxicity assay. Target cells were labeled with $^{51}$Cr by adding 100 μCi of $^{51}$Cr (Amer sham Corp., Arlington Heights, IL) to 10^6 target cells in 1 mL complete media. After 1-hour incubation at 37°C, the cells were washed twice. CLL cells required a modified labeling consisting of a 3-hour incubation in a 50-mmol/L Tris buffer containing 300 mmol/L NaCl, 1 mmol/L Na$_2$PO$_4$, and 10 mmol/L KCl (pH 7.4). Labeled target cells (50 μL) and effector cells (50 μL) at the desired E:T ratio were plated in v-bottom microtiter plates (Corning Glassworks, Corning NY). MoAb (100 μL) in complete media at the desired concentration was then added. All samples were plated in triplicate. Plates were centrifuged, incubated for 4 hours at 37°C, re centrifuged, and tumor cell lysis was measured by determining the amount of $^{51}$Cr released into 100 μl of cell-free supernatant from each sample. Counting was done in a Beckman model 5500 gamma counter. The percent specific lysis was determined by the following equation: (E - S/M - S) × 100, where E is the average of triplicate sample counts per minute for sample. S is the spontaneous release in sample with target cells only, and M is the maximal release for samples with target cells and 100 μg of 1% NP-40 detergent. Spontaneous $^{51}$Cr release was always less than 20% of maximum release.

RESULTS

Production and evaluation of the 1DT3-D bsab. Twelve hybrid-hybridoma colonies grew in HAT-ouabain medium after the fusion. All produced bsab as indicated by both ELISA and flow cytometric evaluation of supernatant. The colony that produced the most bsab as indicated by these assays was subcloned by limiting dilution two times. This hybrid-hybridoma was designated 1DT3D when ELISA and flow cytometry confirmed it continued to produce bsab. Because hybrid-hybridomas contain two productive heavy-chain alleles and two productive light-chain alleles, the hybrid-hybridoma supernatant would be expected to contain IgG molecules composed of several combinations of heavy chains and light chains in addition to the desired bsab, including IgGs that are monospecific-monovalent, monospecific-bivalent, and "nonsense" with no functional variable region.

Purification of the bsab from most of the unwanted IgGs was accomplished by cation-exchange HPLC. Ionic differences between both the 1D10 and OKT3 heavy chains and their corresponding light chains allowed for separation of bsab from monospecific parental antibodies. Fractionation of supernatant resulted in eight reproducible protein fractions, six of which represented IgG as seen in Fig 1. The immunoreactivity of each fraction was determined by repeating the screening process described above that uses the isotype mismatch between 1D10 (IgGl) and OKT3 (IgG2a), and reactivity of antibody with T cells or malignant B cells. For example, an antibody fraction added to Raji cells only adhered if there was an intact 1D10 arm, and only stained with goat antimouse IgG2a-FITC if the OKT3 heavy chain was also present; thus, fluorescence in this assay indicated individual IgG molecules are present that contained both an intact 1D10 arm and the heavy chain from OKT3, with or without an OKT3 light chain. In a similar manner, T cells incubated with IgG followed by goat antimouse IgG1 FITC were fluorescent only if the IgG were composed of an intact OKT3 arm and the 1D10 heavy chain, with or without the 1D10 light chain.

The monospecific 1D10 and OKT3 fractions, as determined by these assays, had elution properties similar to the MoAbs produced by the parent hybridomas. Only one fraction (fraction 4) was identified that was positive on all assays, indicating it contained the bsab. Other fractions that did not contain bsab (fractions 3 and 6) displayed complex patterns of reactivity that could not be attributed to a single antibody type based on heavy- and light-chain composition. Some individual antibody molecules in fraction 3 did, however, contain one IgGl and one IgG2a heavy chain as indicated by ELISA and flow cytometry. Therefore, light-chain mismatches were responsible for the inability of these antibodies to bind to CD3' or 1D10' cells. Separation of these populations from bsab must have been based in part on ionic differences between the 1D10 and OKT3 κ light chains. Similarly, IgG in fraction 6 contained only IgG2a heavy chains and so most likely separated from parental OKT3 because of the presence of at least one mismatched 1D10 light chain. It is likely the fraction designated bsab also contained light chain mismatches, particularly when considering the fact that nonsense antibodies with reciprocal switching of light chains are likely to have ionic properties that are indistinguishable from bsab. Nevertheless, the fraction that contained bsab was designated 1DT3D bsab and used in subsequent studies.

When the 1DT3-D hybrid-hybridoma was grown in a hollow fiber mini-flo path bioreactor inside a standard bench top CO2 incubator, total IgG was produced at a rate of approximately 2.0 mg/d, from which approximately 0.15 mg of purified bsab was obtained after fractionation. Production from a single unit, which remained stable over a period of 6 to 8 weeks, could therefore result in the isolation of approximately 6 to 8 mg of purified bsab.

Cytotoxicity. The ability of 1DT3D bsab to direct T-cell-mediated lysis was tested in a series of chromium-release assays. Effector cells were obtained from normal donor PB mononuclear cells by culturing them in OKT3 and IL-2 for 5 to 7 days. 1DT3-D was able to direct T-cell-mediated lysis of three 1D10 (+) cell lines derived from patients with a variety of B-cell malignancies (Table 1). This lysis was dependent on the presence of both bsab and activated T cells, and was not mediated by T cells in the presence of monospecific MoAb alone. For example, T cells at an effector:target (E:T) ratio of 10:1 in the presence of 10 μg/mL bsab induced 81% specific lysis of labeled Raji cells, while OKT3 and 1D10 alone (data not shown) or in combination (Table 1) at the same concentration resulted in less than 5% specific lysis. No significant lysis was seen when other Ig fractions, representing mismatched IgG populations or monospecific antibody, were substituted for bsab.
(data not shown). The results were essentially the same when HO-85 and 697-ALL cells were used as targets. Absolute values of specific lysis between cell lines is not applicable because the assays are separate experiments involving separate preparations of effector cells. The roles of the bsab and effector cells were further evaluated by varying the concentration of 1DT3D bsab, with increased lysis compared with monospecific antibody seen at concentrations as low as 5 ng/mL (Fig 2A). Similarly, in a separate experiment using effector cells from a different donor, bsab-mediated lysis is affected by the E:T ratio with activity seen at ratios as low as 0.3:1 (Fig 2B). The specificity of 1DT3-D bsab was evaluated by using 1D10 (−) transformed human B-cell lines (Burkhart and RPMI 8866) as target cells. These were chosen because they share many of the other antigens found on other malignant B cells. Minimal specific lysis was seen with high concentrations of 1DT3D bsab and high E:T ratios, confirming the need for target antigen to be present on the target cells to have effective lysis.

Effector cell source and preparation also had an impact on bsab-mediated lysis. Lysis mediated by bsab was seen with T cells obtained from all normal donors; however, the potency of this lysis varied. The variability of lysis was not caused solely by conditions used for T cell activation, as T cells activated in parallel varied in their bsab-directed lytic potential, and the relative potency of T cells obtained from the same donor was consistent from donation to donation. Those T cells that were less potent at inducing bsab-directed lysis of Raji cell lines were also less potent at inducing the lysis of HO-85 and 697-ALL cells. Effector cells activated with IL-2 alone showed bsab-directed lysis, but also induced a higher degree of nonspecific lysis, presumably because of the presence of natural killer (NK) activity. T cells activated with OKT3 were not capable of inducing bsab-directed lysis (data not shown).

The ability of 1DT3-D bsab to direct T-cell-mediated cytolysis of fresh malignant cells was tested using target cells from patients with various B-cell malignancies and peripherally accessible malignant cells. These included cells designated PC-ALL (leukemic blasts from a patient with pre-B acute lymphoblastic leukemia), KH-CLL (lymphocytes from the PB of a patient with CLL), BJ-LCL (mononuclear cells from a malignant pleural effusion in a patient with diffuse large cell lymphoma), and WAS-TCL (mononuclear cells from the PB of a patient with a stage IV T-cell lymphoma and a high percentage of circulating malignant cells). PC-ALL, KH-CLL, and BJ-LCL cells were greater than 90% 1D10 (+), while WS-TCL cells were 1D10 (−). All cells labeled well with 51Cr and showed less than 30% nonspecific release in a 4-hour assay. Specific lysis of all 1D10 (+) target cells was mediated by bsab, whereas bsab did not

Table 1. T-Cell-Mediated Lysis of Malignant Lymphoid Cells

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>1D10 Expression</th>
<th>% Specific Release (1DT3D induced)</th>
<th>% Specific Release (monospecific 1D10 and OKT3 induced)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raji*†</td>
<td>+</td>
<td>81.34 ± 2.7</td>
<td>3.7 ± 0.9</td>
</tr>
<tr>
<td>HO-85†‡</td>
<td>+</td>
<td>72.12 ± 3.8</td>
<td>3.8 ± 1.2</td>
</tr>
<tr>
<td>697-ALL†‡</td>
<td>+</td>
<td>76.95 ± 2.9</td>
<td>9.2 ± 3.0</td>
</tr>
<tr>
<td>Burkhart‡§</td>
<td>–</td>
<td>1.9 ± 0.6</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>RPMI 8866‡</td>
<td>–</td>
<td>5.1 ± 0.6</td>
<td>4.9 ± 1.3</td>
</tr>
</tbody>
</table>

Patient samples

<table>
<thead>
<tr>
<th>Allogenic effectors</th>
<th>% Specific Release (1DT3D induced)</th>
<th>% Specific Release (monospecific 1D10 and OKT3 induced)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH-CLL††</td>
<td>+</td>
<td>42.9 ± 2.0</td>
</tr>
<tr>
<td>PC-ALL§</td>
<td>+</td>
<td>53.0 ± 2.2</td>
</tr>
<tr>
<td>BJ-LCL††</td>
<td>+</td>
<td>27.2 ± 2.4</td>
</tr>
<tr>
<td>WS-TCL††</td>
<td>–</td>
<td>2.0 ± 3.0</td>
</tr>
</tbody>
</table>

Autologous effectors

<table>
<thead>
<tr>
<th>% Specific Release (1DT3D induced)</th>
<th>% Specific Release (monospecific 1D10 and OKT3 induced)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC-ALL§</td>
<td>+</td>
</tr>
<tr>
<td>BJ-LCL††</td>
<td>+</td>
</tr>
</tbody>
</table>

Data are shown as mean ± standard error of mean (n = 3). Background lysis was determined from samples containing target cells but no antibody or effector cells.

* Antibody concentration 10 μg/mL.
† E:T ratio 10:1.
‡ Antibody concentration 5 μg/mL.
§ E:T ratio 5:1.

Fig 1. Elution profile of 1DT3D bsab on cation exchange HPLC. Hybrid-hybridoma supernatant was dialyzed against 50 mmol/L, malonate buffer at pH 4.8, and loaded onto 21.5 × 150 mm TSK SP-5PW Ultra-Pac column (TSK-HAAS). Protein was eluted over an increasing NaCl gradient at a flow rate of 4.0 mL/min and detected by UV light absorbance. Protein fractions were collected and immunologically evaluated for IgG content, isotype, and reactivity with target cells.
DISCUSSION

The ability of bsabs to target effector cell-mediated lysis of malignant cells in a non-major histocompatibility complex (MHC)-restricted fashion has been shown in vitro in a variety of malignancies, and more recently in vivo in animal tumor models. These studies suggest bsab-based immunotherapy might be more effective than unlabeled MoAbs. The functional specificity of bsabs, ie, their ability to activate T cells only in the presence of cells expressing the target antigen, could help avoid some of the toxicities that can be seen with other forms of immunoconjugates.

Despite this promise, systemic therapy with bsab has yet to be evaluated in clinical trials. The major reason for this is the difficulty involved in producing adequate amounts of purified bsab in a consistent fashion. Most in vitro studies of bsab have relied on bsab produced by chemical conjugation. Production of such chemical heteroconjugates on a small scale is not difficult; however, producing and purifying well-defined heteroconjugate bsabs from either intact antibodies or antibody fragments in a reproducible manner is quite complex, particularly when trying to produce the large quantities of bsab that would be needed for a clinical trial.

Recently, two techniques have been described that may help overcome some of the problems associated with the heterogeneity of chemical heteroconjugates. Glennie et al have had success with disulfide exchange using F(ab'), fragments of both antibodies. This technique is limited by the need to have both antibodies susceptible to enzymatic cleavage, and by the tendency for homodimers to form with the resulting decrease in yield and need for extensive purification procedures. Kostelny et al have produced a high yield of bsab in a reproducible manner by using genetic techniques. Their approach involves the production of F(ab'), connected via the Fc to the leucine zipper region of the transcription factors Fos and Jun. This takes advantage of preferential heterodimer formation displayed by these proteins (so-called "leucine zippers"), and is attractive in that it is applicable to all antibodies and results in a high yield of bsab. However, it is not yet known whether the presence, on the bsab, of peptide sequences that correspond to oncogene products will impact on the in vivo behavior of bsab produced in this manner.

The bsabs obtained from hybrid-hybridomas, such as those described in the current studies, have the advantage of being true IgG molecules that are obtainable in a reproducible manner from a well-described clone of cells. We have found the composition of the antibodies produced by hybrid-hybridomas is consistent during the period of time required for antibody production if the cells are first extensively subcloned. However, problems do exist with this approach. Hybrid-hybridoma supernatants contain up to 10 different IgG molecules consisting of various combinations of heavy and light chains. The isolation of the relevant bspecific component can be challenging. It is often particularly difficult to separate antibodies based on light-chain composition. Affinity chromatography can be used to obtain the purified bsab; however, this requires large amounts of immobilized antigen or anti-idiotype that are
often not available. We were fortunate that both the heavy chains and the light chains of OKT3 and 1D10 displayed different ionic properties as observed with cation-exchange chromatography. This allowed for separation of bsab from parental OKT3, parental 1D10, and some of the mismatched IGs. Our fractionation and testing procedures did not exclude coelution of bsab with some light-chain mismatches. Nevertheless, the majority of unwanted IgG populations were removed.

The other major limitation to the use of bsabs produced by hybrid-hybridomas is the relatively low yield of bsab that results after removal of unwanted antibody populations. Our final recovered bsab contained only 7.5% of total IgG secreted by the hybrid-hybridoma. The true importance of this low yield is not clear, as we do not know how much bsab will be required to obtain a significant effect in vivo. Indeed, the in vitro data presented here indicate that bsab is effective at inducing a high degree of lysis of antigen-positive cells at very low bsab concentrations and at low E:T ratios under conditions where lysis is not observed with monospecific MoAbs. These results are similar to those observed in other studies, including our evaluation of bsab therapy in the 38C13 murine lymphoma model. In this immunocompetent, syngeneic system, bsabs were produced from hybrid-hybridomas in a manner similar to that described here (although fractionation of the hybrid-hybridoma antibody product was not as effective as that described above). The bsabs were composed of an anti-Id arm (tumor specific) and an anti-CD3 arm. In vitro, bsab in this model was capable of inducing specific lysis of tumor cells by syngeneic, activated T cells in a manner similar to that described above. In vivo, bsab at a dose of 10 μg/mouse was more effective than was monospecific antitumor or monospecific anti-CD3 alone or in combination. Although IL-2 alone had no effect on tumor growth, bsab and IL-2 were markedly synergistic. This combination was able to eliminate a tumor load 100- to 1,000-fold greater than was monospecific antitumor antibody. Therapy was not effective in animals bearing large tumor burdens. This failure of therapy apparently was caused by the emergence of idiotypic variants and the presence of circulating idiotype, which are problems that have been documented after other forms of anti-idiotype therapy.

In summary, anti-CD3–based bsabs can redirect T-cell cytotoxicity, and inhibit neoplastic cell growth both in vitro and in vivo. The 1D10 antigen is well suited as the target of bsab therapy in the 38C13 model, in combination with the in vitro studies of 1DT3-D bsab, suggest this approach to immunotherapy of B-cell malignancy deserves further attention.

ACKNOWLEDGMENT

The authors thank David Woods, Jan Kintzle, James Hillstrom, and Justin Fishbaugh for their excellent technical assistance.

REFERENCES


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