Elimination of Clonogenic Tumor Cells from Human Bone Marrow Using a Combination of Monoclonal Antibody:Ricin A Chain Conjugates

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ABSTRACT

Effective autologous bone marrow transplantation for leukemia and lymphoma is likely to depend upon the selective removal in vitro of malignant cells from normal human bone marrow precursors. Highly specific cytotoxic conjugates formed by coupling ricin A chain to monoclonal antibodies might prove useful for the selective elimination of malignant cells. Consequently, ricin A chain conjugates have been prepared with several different murine monoclonal antibodies and tested for their ability to eliminate clonogenic Burkitt’s lymphoma cells from an excess of human bone marrow. The most active reagents included an antibody:A chain conjugate which bound to the nonpolymorphic chain of the la molecule and another which reacted with the μ heavy chain of cell surface immunoglobulin. Conjugates formed with anti-common acute lymphoblastic leukemia antigen, anti-M₂, 26,000 glycoprotein, and anti-B₁ were much less active on these Burkitt’s cells, contrasting with results of complement-dependent tumor cell lysis. Tumor cell kill was partially inhibited by the addition of greater than 2 × 10⁶ human bone marrow cells/ml but could be potentiated by increasing the concentration of conjugate or by the addition of 10 mM ammonium chloride. In the presence of ammonium chloride, at least 4 logs of clonogenic tumor cells could be eliminated within 24 h from a 20-fold excess of bone marrow using 10⁻⁷ M ricin A chain linked to one or two different antibodies. Similar treatment of normal human bone marrow temporarily inhibited granulocyte-macrophage colony-forming units (cell) formation but did not compromise establishment of continuous bone marrow cultures. The degree of selective elimination of tumor cells with A chain antibody conjugates was comparable to that achieved with 4-hydroperoxycyclophosphamide or with multiple monoclonal antibodies and complement.

INTRODUCTION

One requirement for autologous bone marrow transplantation is the complete removal of malignant cells from human bone marrow while sparing normal progenitors. Selective elimination of malignant cells has been achieved through the use of cytotoxic drugs (1) and of antibodies (2), in both animal systems (3, 4) and clinical studies (5, 6). In recent reports, monoclonal antibodies have been used in combination with heterologous complement (2) or have been conjugated with intact ricin (7) or ricin A chain (8–10). Given antibodies of appropriate specificity, selective destruction of tumor cells has been achieved using some systems in vitro. It remains to be determined, however, which antigenic determinants will prove to be optimal targets for conjugates containing ricin A chain and monoclonal antibodies.

Recently, we have conjugated ricin A chain to several different monoclonal antibodies which react with well-defined antigenic determinants expressed on Burkitt’s lymphoma tumor cell lines. As these lines exhibit high clonogenic efficiency, it has been possible to study elimination of several logs of tumor cells in the presence of a 20-fold excess of irradiated human bone marrow. Optimal conditions for the selective elimination of Burkitt’s tumor cells have been defined, and the effect of treatment on normal human bone marrow has been evaluated in short-term and continuous bone marrow cultures.

MATERIALS AND METHODS

Burkitt’s Lymphoma Tumor Cell Lines. Namalwa, Bjab, Bjab 113, CA 46, and JD 38 were used in this study (11). Cells were grown in RPMI 1640 medium (Microbiological Associates, Walkersville, MD) containing 10% heat-inactivated FBS² (Gibco Labs., Grand Island, NY), 2 mM L-glutamine, 1 mM sodium pyruvate, 10 mM hydroxyethylpiperazineethanesulfonic acid, penicillin (100 units/ml), and streptomycin (100 μg/ml). Cells were cultured at 37°C in 5% CO₂-95% humidified air. Each of the Burkitt’s lymphoma cell lines bore multiple antigenic determinants including la, CALLA, gp26, B₁, B₂, and cell surface immunoglobulin.

Monoclonal Antibodies. An IgG₁ monoclonal antibody, designated 287, reacts with the μ heavy chain expressed on normal and malignant B-cells.⁶ The J₂ antibody is an IgG₂a, murine monoclonal reagent that reacts with the nonpolymorphic portion of the M₂, 32,000 A chain of the la complex that is expressed on a majority of B-cell cancers (12). J₅ is an IgG₂a murine monoclonal antibody (13) directed against the CALLA; J₂ and J₃₀ are, respectively, an IgM and an IgG₂a reactive with the gp26 cell surface glycoprotein previously described (14). B₁ is an IgG₂a antibody that recognizes a M₂, 35,000 B-cell surface antigen (15). Monoclonal antibodies directed against human immunoglobulin light and heavy chain determinants have been developed (16).

To evaluate reactivity of these reagents with the cell lines used in the study, indirect immunofluorescence assays were performed. Burkitt’s lymphoma cells were incubated for 30 min at 4°C in medium containing a 1:100 dilution of each antibody in PBS with 5% FBS. Cells were washed twice in the same medium without antibody and incubated for 30 min at 4°C with a 1:20 dilution of fluorescein-conjugated goat anti-mouse immunoglobulin (Meloy Lab., Inc., Springfield, VA). Fluorescence intensity was evaluated using an Ortho Cytofluorograph 30-L (Ortho Diagnostics, Westwood, MA).

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¹ The abbreviations used are: FBS, fetal bovine serum; CALLA, common acute lymphoblastic leukemia antigen; GM-CFU-C, granulocyte-macrophage colony-forming units (cell); MEM, minimal essential medium; PBS, phosphate-buffered saline; gp26, M₂, 26,000 glycoprotein.
² L. Nadler, personal communication.
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Bone Marrow. After obtaining informed consent under protocols approved by the human studies committee of the Dana-Farber Cancer Institute, bone marrow was obtained from healthy volunteers and anti-coagulated with preservative-free heparin (Pan Heparin; Abbott Laboratories, N. Chicago, IL). Mononuclear cells were separated on discontinuous gradients of Ficoll-diatrizoate (lymphocyte separation medium; Litton Bionetics, Kensington, MD) using methods described previously (2). Cells at the interface were collected, washed twice, and resuspended in Eagle's MEM containing 1% hydroxyethylpiperazineethanesulfonic acid and 5% FBS. Prior to studies of tumor cell elimination, bone marrow cells were irradiated to 5000 rads. Studies with normal clonogenic precursors were performed on marrow that had not been irradiated. For incubation with different conjugates, normal marrow was diluted to a concentration of 2 × 10^6 nucleated cells/ml.

Ricin A Chain Conjugates. The procedure used for preparation of conjugates has been described (10). Briefly, affinity-purified ricin (E-Y Lab., San Mateo, CA) was reduced with 5% 2-mercaptoethanol, and the A chain was isolated on a column of acid-treated Sepharose (10, 16) (Pierce Chemical Co., Rockford, IL). Monoclonal antibodies were precipitated from ascites with 50% saturated ammonium sulfate and purified on a sephacryl S-300 column (Pharmacia Fine Chemicals, Piscataway, NJ). Each monoclonal was reacted with a 6-fold molar excess of the heterobifunctional SPDP reagent for 30 min and passed through a G-25 column. A 2-fold molar excess of reduced ricin A chain was then reacted with the SPDP-substituted monoclonals for 16 h at 37°C. The reaction mixture was applied to a Sephacryl S-300 column, and the fractions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Only fractions with an A chain:antibody ratio of 1:1 were used.

Treatment of Cells. Suspensions were prepared with 1 × 10^6 Burkitt's lymphoma cells/ml with or without the addition of 2 × 10^7 irradiated nucleated bone marrow cells/ml in Eagle's MEM with 5% FBS. The ricin A chain monoclonal antibody conjugate was added at 5 × 10^{-8} or 1 × 10^{-7} final molar concentration. Similar concentrations of nonconjugated monoclonal antibodies were used as a control. Where indicated, cells were treated in the presence of 10 mM ammonium chloride (Fisher Scientific Co., Fairlawn, NJ) as previously described (17). All incubations were carried out at 37°C in 5% CO2-95% humidified air. At the end of 12–72 h of treatment, cells were washed twice and resuspended in RPMI 1640 medium with 10% heat-inactivated FBS. Intervals were chosen based on earlier studies (10) which indicated increasing effect of ricin A chain conjugates with increasing time in culture. Incubation at 37°C for longer than 72 h was considered inconsistent with adequate bone marrow survival.

Treatment of Cells with Monoclonal Antibodies and Complement. For complement-mediated cytotoxicity assays, similar suspensions of Burkitt's lymphoma cells were prepared with or without the addition of a 20-fold excess of human bone marrow. Cells were incubated for 15 min at 0°C with a 1:100 dilution of ascites containing nonconjugated antibody. Rabbit complement (Pel Freeze, Inc., Glen Rock, NJ) was absorbed with an equal volume of human AB erythrocytes for 1 h on ice. Absorbed complement was then added to Burkitt's tumor cells at a final dilution of 1:10. After 30-min incubation at 37°C, cells were sedimented, and the treatment was repeated twice for a total of three cycles. After the third treatment, cells were washed twice and resuspended in RPMI 1640 medium with 10% FBS.

Clonogenic Assay. To measure the number of clonogenic units surviving treatment with conjugates or with antibody and complement, a limiting dilution was used as previously described (18). Briefly, cells were resuspended at 1 × 10^6 per ml, and ten serial 5-fold dilutions were made in RPMI 1640 medium with 10% FBS. From 6–10 aliquots (100 μl) of each dilution were plated in 96-well flat-bottomed microtiter plates that had already been seeded with 10^5 irradiated bone marrow cells per well in 100 μl of clonogenic medium. Plates were incubated for 14 days at 37°C in 5% CO2-95% humidified air. Wells with progressively growing tumor colonies were scored with the unaided eye, and their morphology was confirmed by examination under an inverted phase microscope. In previous studies, excellent correlation has been obtained between visual scoring and uptake of [3H]thymidine by tumor colonies. The number of clonogenic units remaining in 10^6 tumor cells was calculated using a Spearman-Karber estimator (19). With this assay a 10-fold difference in clonogenic survival generally attains statistical significance.

Continuous Bone Marrow Cultures. The entire procedure has been published elsewhere (20). Briefly, 4 × 10^7 mononuclear cells from human bone marrow were resuspended in 9 ml of McCoy's Medium 5A containing 12.5% horse serum, 12.5% FBS, 5 mM NaHCO₃, 0.8% essential amino acid mixture (Gibco Lab., Grand Island, NY), 0.4% nonessential amino acid mixture, 1 mM sodium pyruvate, 2 mM l-glutamine, 0.8 mM serine, 0.15 mM l-asparagine, 0.4% MEM-Vitamin solution (Gibco), penicillin (100 units/ml), streptomycin (100 μg/ml), and 10^{-4} M hydrocortisone. Cells were grown at 33°C in 2% CO2-98% humidified air. For each medium change, all nonadherent cells were removed and centrifuged at 200 × g for 5 min. One-half of the total cells was retained for study, and the other half of the cells was returned to the flask in 9 ml of fresh medium. Nonadherent cells were counted weekly. Aliquots of cells at 0, 7, and 21 days were used for colony-forming assays as described (20, 21).

RESULTS

Reactivity of Monoclonal Antibodies with Burkitt's Lymphoma Cell Lines. Analysis of the cell lines by indirect immunofluorescence after incubation with each of the nonconjugated monoclonal antibodies indicated that they were heterogeneous with regard to the expression of cell surface determinants (Table 1). CALLA, gp26, and B1 are expressed on all of the cell lines to varying degrees. Ia is uniformly present at high levels on all cell lines. The μ chain is present on all cell lines with the exception of JD-38. Among the cell lines which bear surface membrane immunoglobulin, all are reactive with monoclonals against the κ-light chain with the exception of the Namalwa, which bears the κ-light chain and is not reactive with the 4F7 reagent against the κ-light chain. When anti-μ and anti-κ were used in combination, staining with anti-κ was sufficiently intense that no additional increment in indirect immunofluorescence could be detected by adding anti-μ when measured on a logarithmic scale (data not shown).

Treatment with Whole Ricin. Each of the cell lines was tested for the susceptibility to whole ricin (Fig. 1). Portions of medium containing 10^4 cells were incubated with increasing concentrations of ricin ranging from 10^{-12}-10^{-4} M. Some heterogeneity was observed among the different cell lines, but 50% inhibition of clonogenic growth was observed with ricin at concentrations between 10^{-10} and 10^{-9} M. Maximum tumor cell lysis was observed at 10^{-10} M in the case of CA 46 and Bjab, whereas Bjab 113, JD 38, and Namalwa required higher concentrations of whole ricin for complete inhibition.

Treatment with Monoclonal Antibody:Ricin A Chain Conjugates. Each of the five cell lines was tested with each of six different conjugates. As indicated in Fig. 2, conjugates reactive with CALLA, gp26, and B1 were relatively ineffective. By contrast, conjugates reactive with the α-chain of Ia were consistently effective against each of the five cell lines. Immunological specificity of the anti-λ:ricin A chain conjugate was tested in two ways. Addition of a 50-fold excess of unconjugated antibody to Namalwa cells 10 min before incubation with the conjugate at 5 × 10^{-9} mol blocked cytotoxicity. Similarly, the conjugate was tested against CEM cells which are Ia negative. Incubation of the samples at 37°C for 72 h failed to demonstrate cytotoxic activity (Table 2).

Variable results were obtained with conjugates that reacted with cell surface membrane immunoglobulin. Of particular inter-
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Table 1

Reactivity of monoclonal antibodies with Burkitt’s lymphoma cell lines

<table>
<thead>
<tr>
<th>Cells</th>
<th>Control</th>
<th>anti-CALLA</th>
<th>anti-gp26</th>
<th>Bjb</th>
<th>anti-B1</th>
<th>I-2</th>
<th>289 anti-µ</th>
<th>4F7 anti-K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Namalwa</td>
<td>3.04 (7)</td>
<td>98.0 (191)</td>
<td>97.4 (170)</td>
<td>4.3 (6)</td>
<td>93.2 (283)</td>
<td>3.9 (39)</td>
<td>2.6 (5)</td>
<td></td>
</tr>
<tr>
<td>Bjab</td>
<td>20.3 (18)</td>
<td>83.0 (74)</td>
<td>88.2 (204)</td>
<td>64.3 (105)</td>
<td>90.6 (310)</td>
<td>56.4 (119)</td>
<td>55.3 (260)</td>
<td></td>
</tr>
<tr>
<td>Bjab 113</td>
<td>8.3 (14)</td>
<td>26.9 (33)</td>
<td>90.0 (184)</td>
<td>78.6 (126)</td>
<td>91.9 (277)</td>
<td>81.6 (320)</td>
<td>77.8 (380)</td>
<td></td>
</tr>
<tr>
<td>CA 46</td>
<td>1.6 (6)</td>
<td>67.1 (63)</td>
<td>25.2 (24)</td>
<td>31.8 (31)</td>
<td>95.8 (411)</td>
<td>89.5 (209)</td>
<td>90.4 (301)</td>
<td></td>
</tr>
<tr>
<td>JD 38</td>
<td>4.6 (5)</td>
<td>87.6 (113)</td>
<td>55.9 (80)</td>
<td>26.4 (27)</td>
<td>90.9 (275)</td>
<td>6.9 (7)</td>
<td>4.3 (12)</td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses, median channel for fluorescence intensity.

Fig. 1. Clonogenic Burkitt’s lymphoma cells surviving treatment with whole ricin. Samples containing 10⁶ tumor cells were incubated with increasing molar concentrations of ricin for 24 h at 37°C in MEM with 5% FBS. Cells were washed 3 times with incubation medium and plated in a limiting dilution clonogenic assay. Namalwa (C), JD38 (B), CA46 (C), Bjab (D), and Bjab 113 (E) were studied.

Fig. 2. Percentage of clonogenic Burkitt’s lymphoma cells surviving treatment with different monoclonal antibody:ricin A chain conjugates. Samples containing 10⁶ cells from each of five cell lines were incubated with different monoclonal antibody:ricin A chain conjugates (5 × 10⁻⁸ M) for 72 h at 37°C. Cells were washed 3 times and plated in a clonogenic assay. The percentage of clonogenic cells surviving treatment was calculated relative to the nontreated controls. Anti-µ: A chain inhibited clonogenic growth of Namalwa, BJAB, BJAB 113, and CA46 (P < 0.01). Anti-KA chain inhibited all cell lines (P < 0.01). Anti-CALLA:A chain inhibited all cell lines (P < 0.01) with the exception of Bjab 113.

Table 2

Specificity of anti-la:ricin A chain conjugate: clonogenic units surviving treatment of Namalwa and cell lines with 5 × 10⁻⁸ anti-la:A chain for 72 h

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Control</th>
<th>Anti-la:A chain</th>
<th>Anti-la:A chain + anti-la</th>
</tr>
</thead>
<tbody>
<tr>
<td>Namalwa (la)</td>
<td>5.7 × 10⁶</td>
<td>1.6 × 10⁶⁺</td>
<td>5.7 × 10⁶⁺</td>
</tr>
<tr>
<td>CEM (la)</td>
<td>4.3 × 10⁶</td>
<td>3.3 × 10⁶⁺</td>
<td>ND³</td>
</tr>
</tbody>
</table>

* Differences from control and from anti-la:A chain plus anti-la (P < 0.01).
* Incubated with 50-fold of molar excess of unconjugated antibody 10 min at 4°C before treatment.
* ND, not done.

Fig. 3. Comparison of Treatment with Different Monoclonal Antibodies plus Complement to Treatment with Different Conjugates. In an earlier paper (18), multiple treatments of clonogenic malignant cells with multiple monoclonal antibodies and complement proved more effective than treatment with each individual antibody. More than 3 logs of tumor cells could be eliminated with a combination of J5, J2, and B1 in the presence of absorbed rabbit complement. It was not known whether the same antibodies conjugated with ricin A chain would prove equally effective in the absence of rabbit complement. One difficulty in performing a direct comparison of the two materials for eliminating tumor cells was the fact that J2 was an IgM antibody, and the degree of conjugation with ricin A chain was difficult to quantitate. Consequently, an IgG2a antibody, designated J30, which recognized the same epitope as J2 was conjugated with ricin A chain. When a combination of J5, J30, and B1 was used against the Namalwa cell line in the presence of rabbit complement, more than 3 logs of Burkitt’s cells could be eliminated (Fig. 3). By contrast, the ricin A chain conjugates individually and in combination could eliminate no more than a single log of tumor cells in this test system.

Elimination of Tumor Cells with Conjugates in the Presence or Absence of Bone Marrow. la and cell surface membrane immunoglobulin are useful targets for conjugate-mediated tumor cell killing. The Namalwa and Bjab 113 cell lines were incubated with the anti-la or anti-µ:A chain conjugates in the presence or absence of marrow for 24 h prior to assay of clonogenic cells by limiting dilution. When the two conjugates were tested individ-

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CALLA (P < 0.01). Antibody plus complement anti-B1 (P < 0.01) were used as carrier. Significant inhibition of clonogenic growth of Namalwa when J5 anti-CALLA (P < 0.01), J2 anti-gp26 (P < 0.05), anti-CALLA plus anti-gp26 (P < 0.01), and anti-CALLA plus anti-gp26 plus anti-B1 (P < 0.01) were used as carrier. Significant inhibition of clonogenic growth was obtained with all complement groups. Antibody plus complement was significantly more effective than antibody:A chain for all groups except anti-CALLA (P < 0.01).

Table 3
Clonogenic Namalwa cells surviving treatment with monoclonal antibody:ricin A chain conjugates

Namalwa cells (1 x 10⁶) were incubated for 24 h at 37°C in the presence of 5 x 10⁻⁹ M anti-μ:A chain and/or anti-la:A chain conjugates. Where indicated, 2 x 10⁶ irradiated human bone marrow cells were added prior to incubation. Some incubations were carried out in the presence of 10 mM ammonium chloride. After treatment, cells were washed 3 times, and clonogenic units were assayed.

<table>
<thead>
<tr>
<th>NH₄Cl (10 mm)</th>
<th>Anti-μ chain A chain</th>
<th>Anti-la chain A chain</th>
<th>Anti-μ:ricin A + anti-la:ricin A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absence of bone marrow</td>
<td>3.3 x 10²</td>
<td>1.4 x 10²</td>
<td>2.0 x 10²</td>
</tr>
<tr>
<td>+</td>
<td>5.0 x 10⁻⁹</td>
<td>1.2 x 10⁻⁹</td>
<td>1.2 x 10⁻⁹</td>
</tr>
<tr>
<td>20-Fold excess of bone marrow</td>
<td>3.3 x 10²</td>
<td>1.9 x 10²</td>
<td>1.5 x 10²</td>
</tr>
<tr>
<td>+</td>
<td>6.6 x 10⁻⁶</td>
<td>3.6 x 10⁻⁶</td>
<td>1.2 x 10⁻⁹</td>
</tr>
</tbody>
</table>

a Differs from control (P < 0.01).
b Differs from anti-la:ricin A chain (P < 0.01).
c Differs from control (P < 0.01) and from cells incubated with ammonium chloride (P < 0.01).
d Differs from anti-la:A chain (P < 0.05).

Fig. 3. Clonogenic Namalwa cells surviving treatment with either monoclonal antibodies and complement or monoclonal antibody:ricin A chain conjugates. Namalwa cells (10⁶) were treated 3 times for 30 min with monoclonal antibodies and complement (C') or once for 24 h with ricin A chain conjugated at 5 x 10⁻⁹ M in the presence of 10 mM ammonium chloride. Ricin A chain conjugates inhibited clonogenic growth of Namalwa when J5 anti-CALLA (P < 0.01), J2 anti-gp26 (P < 0.05), anti-CALLA plus anti-gp26 (P < 0.01), and anti-CALLA plus anti-gp26 plus anti-B1 (P < 0.01) were used as carrier. Significant inhibition of clonogenic growth (P < 0.01) was obtained with all complement groups. Antibody plus complement was significantly more effective than antibody:A chain for all groups except anti-CALLA (P < 0.01).

Effect of Increased Molar Concentration of the Conjugates.

The maximum effect of the anti-la:A chain conjugate was obtained at a concentration between 1 x 10⁻⁸ and 1 x 10⁻⁷ M (Fig. 4), with complete elimination of clonogenic growth occurring when the concentration of conjugates was between 5 x 10⁻⁸ and 1 x 10⁻⁷ M. In initial experiments the concentration of conjugates was kept at 5 x 10⁻⁸ M because of possible nonspecific toxicity. In an attempt to shorten the incubation time, Bjab 113 cells were incubated with a standard concentration of conjugates (5 x 10⁻⁸ M) or twice the standard concentration (1 x 10⁻⁷ M) for 12 h in the presence of 10 mM ammonium chloride. Under these conditions the higher concentration of each conjugate produced tumor cell elimination comparable to that which could be achieved when the individual conjugates were used at a lower concentration in combination (Table 5).

Effect of Conjugates on Normal Human Bone Marrow in Continuous Culture.

For effective autologous bone marrow transplantation, tumor cells must be eliminated without destroying pluripotent stem cells required for reconstitution of normal hematopoietic elements. Consequently, the toxicity of different ricin A chain:monoclonal antibody conjugates was tested with normal human bone marrow. Ricin A chain conjugates with anti-μ and with anti-la were evaluated alone and in combination. Bone marrow cells (3-4 x 10⁷) were incubated with two different molar concentrations of the two conjugates (either 5 x 10⁻⁸ or 1 x 10⁻⁷ M) for 24 h in the presence of 10 mM NH₄Cl. Nonadherent cell
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**Table 5**

<table>
<thead>
<tr>
<th>Clonogenic Bjab 113 cells surviving treatment with a combination of different monoclonal antibody:ricin A chain conjugates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bjab cells (1 x 10⁶) were incubated for 12 h in the presence of either 5 x 10⁻⁸ M or 1 x 10⁻⁷ M ricin A chain:anti-µ and/or anti-la conjugates. All experiments were carried out in the presence of 10 mM ammonium chloride.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Control</th>
<th>Anti-la: ricin A</th>
<th>Anti-µ: ricin A</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 x 10⁻⁸ M</td>
<td>2.5 x 10⁶</td>
<td>1.4 x 10⁶</td>
</tr>
<tr>
<td>1 x 10⁻⁷ M</td>
<td>1.2 x 10⁶</td>
<td>1.2 x 10⁵</td>
</tr>
</tbody>
</table>

* Indicates a difference from control (P < 0.01).

**Table 6**

<table>
<thead>
<tr>
<th>Cumulative nonadherent cell production from continuous bone marrow cultures after treatment of bone marrow cells with anti-µ: A chain and/or anti-la conjugates at 5 x 10⁻⁷ M concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonies/clusters.</td>
</tr>
</tbody>
</table>

**Table 7**

<table>
<thead>
<tr>
<th>Cumulative nonadherent cell production from continuous bone marrow cultures after treatment with ricin A chain:anti-la and/or anti-la conjugates at 1 x 10⁻⁷ M concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonies/clusters.</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Given the defined reactivity and extraordinary titer of monoclonal antibodies, there has been increasing interest in the use of these reagents for serotherapy. Antibodies may be either used alone or may be conjugated with drugs or toxins. Although the use of immunotoxins in vivo presents certain problems (23), conjugates might be used to remove malignant cells from human bone marrow prior to autologous transplantation (24). In our present study, a combination of different monoclonal antibody:A chain conjugates with appropriate specificity could eliminate more than 4 logs of Burkitt’s lymphoma cells, without compromising the ability of normal marrow to grow in continuous culture. Our results confirm earlier studies that suggest the GM-CFU-C bears la determinants (25, 26). Nevertheless, marrow treated with the anti-la:A chain conjugate was capable of generating new GM-CFU-C after 14-21 days in culture, demonstrating that a more primitive hematopoietic cell may be harmed by the anti-la:A chain conjugate.

The clonogenic assay for Burkitt’s tumor cells not only measures a cell population capable of progressive growth but is also capable of detecting up to 5 logs of tumor cell killing. Conventional assays of ⁵¹Cr release can at best detect only 2 logs of tumor cell killing. This, of course, a model system, and it remains to be seen whether tumor cells isolated directly from patients will prove as susceptible to killing by monoclonal antibody:ricin A chain conjugates.

Comparable degrees of tumor cell elimination could be ob-
tained by multiple treatments with different monoclonal antibodies and complement or by treatment with monoclonal antibody:ricin A chain conjugates. The optimal antigenic targets differed, however, for the two techniques. Monoclonal antibodies reactive with CALLA, gp26, and B1 proved quite effective for complement-dependent lysis, but they were ineffective when conjugated with ricin A chain. As the anti-μ antibody was not complement fixing, a direct comparison between complement-dependent lysis and conjugate-mediated lysis was not possible. The lack of efficacy of anti-CALLA was somewhat surprising, as this antigen is thought to be partially internalized during modulation (27). In earlier studies, Burkitt’s lymphoma cells were not optimally susceptible to monoclonal antibody:A chain conjugates (10), and more recent reports suggest that potentiating agents other than NH4Cl may greatly influence the potency of A chain conjugates (22, 28). The choice of two different conjugates with different specificity could be particularly important, given antigenic heterogeneity of cells within a given tumor population. In addition, heterogeneity is likely to be encountered in tumors from different patients. In the present study, however, different conjugates exerted an additive effect, and comparable elimination of tumor cells could be achieved by increasing the concentration of the more potent conjugates. Interestingly, the additive effects of anti-la and anti-μ conjugates could not be explained simply on the basis of additive binding, as much more of the anti-la conjugates bound to Namalwa cells than did anti-μ. These data suggest that translocation of the anti-μ conjugate might be more efficient than that of the anti-la conjugate.

Our data indicate that treatment with ricin A chain conjugates can be comparable in efficacy to other in vitro treatments which utilize multiple antibodies plus complement or 4-hydroperoxycyclophosphamide (18, 29). In each case, these other techniques have eliminated up to 4 logs of malignant lymphoma cells without compromising the ability of marrow to populate continuous cultures (29). In our assays, marrow has been treated at concentrations of 2 × 10^6 nucleated cells/ml. For these experiments a 5% contamination with malignant cells has been evaluated. If remission marrow actually contained 0.5% clonogenic malignant cells, currently available techniques should eliminate tumor cells quantitatively. The more difficult problem in the clinic will be the elimination of tumor cells from the remainder of the patient.

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