Selective Cytotoxicity of the SM1 Monoclonal Antibody towards Small Cell Carcinoma of the Lung

Samuel D. Bernal, Mack Mabry, Rolf A. Stahel, James D. Griffin, and Jonathan A. Speak

ABSTRACT

A murine monoclonal antibody, SM1, is strongly reactive with the surface membrane of small cell carcinoma of the lung (1). SM1 antibody is selectively cytotoxic to small cell carcinoma (SCC) in vitro. The antibody is present in high titers in supernatant fluids or ascites obtained by i.p. injection of SM1 hybridoma cells into pristaned BALB/c mice. The cytotoxic effect of the antibody is reduced to one-half maximal activity only at dilutions greater than 1:40,000. The efficiency of tumor cell lysis is greatly enhanced by repeated treatments with antibody and complement. Using three treatments with antibody and complement, 99.9% of SCC cells are lysed, as determined by the chromium release assay. Similar efficiency of SCC cell kill was observed by clonogenic assays. SM1 antibody produces no significant antibody-dependent lysis of cell lines derived from non-SCC lung carcinomas and leukemia cells. The results from chromium release assay and clonogenic assays also indicate that the effect of SM1 antibody and complement on bone marrow cells is minimal and could be accounted for by the effect of complement alone.

INTRODUCTION

SCC3 of the lung has a propensity to metastasize to multiple distant sites including the bone marrow, adrenals, liver, and the brain (3). Although SCC is highly responsive to initial chemotherapy and radiotherapy, high relapse rates and resistance to further treatment remain the limiting factors in long-term survival from the disease (2, 6, 7, 8). Recently, intensive chemotherapy with autologous bone marrow transplantation was used for treatment of patients with SCC (4, 9, 10, 12, 13). The patients were generally selected for the treatment program if, presumably, their bone marrows were free of tumor cell metastasis. The histological stains used for diagnosis of metastasis to the bone marrow, however, have a limited ability to detect small numbers of SCC cells. We have recently found that close to 60% of bone marrow specimens from SCC patients diagnosed to be free of tumor contamination by Wright-Giemsa and hematoxylin-eosin stains, contain SCC cells by immunofluorescence staining with anti-SCC monoclonal antibodies (11). Thus, in future treatment programs, the criteria for patient selection, the consequences of reinfusing bone marrow contaminated with tumor cells, and the possibility of clearing SCC cells in contaminated marrow will need to be addressed.

In this paper, we report the selective toxicity of an anti-SCC monoclonal antibody in vitro. The optimum conditions for lysis and reduction of clonogenic ability of SCC cells are described.

MATERIALS AND METHODS

Cell Preparation

All the cell lines were grown in RPMI Tissue Culture Medium 1640 with 10% fetal calf serum and 1 mM glutamine. The SCC cell lines OH-1, SWC-2, and SLC-3 and their reactivity with SM1 antibody were described previously (1). Other lung cancer cell lines used were SL 164 and SL 170 (SCC cell lines), and SLC-6 (large cell carcinoma), which were developed in this laboratory. A549 is a human lung adenocarcinoma obtained from American Type Culture Collection. CEM is a human lymphoblastic leukemia cell line obtained from H. Lazarus at Dana-Farber Cancer Institute.

Fresh SCC tumor cells were prepared by mincing a tumor with scissors and scalpel. RPMI medium with 10% fetal calf serum was added to collect the tumor cells. The cells were washed, centrifuged at low speed to remove debris, and resuspended in fresh medium. The cell suspension was incubated for 5 hr at 37°C to allow fibroblast attachment to the culture dish. Separation of fibroblasts was confirmed by staining with anti-vimentin antibody. The cells remaining in suspension were then tested for reactivity with SM1 antibody.

Marrow cells from normal volunteers and from patients with SCC were prepared either by collection of the mononuclear cell layer over Ficoll-Hypaque or by centrifugation of the marrow sample after ammonium chloride lysis of RBC.

Preparation of Antibody

The preparation and propagation of the SM1 hybridoma culture was described previously (1). SM1 cells were injected into pristaned BALB/c mice for the production of ascites. The ascitic fluid was centrifuged at 1500 x g for 20 min. The clear supernatant was filtered on 0.2-μm Nalgene filters. The antibody preparations were incubated at 50°C for 20 min to inactivate complement. Aliquots were frozen at −80°C. Prior to use in cytotoxicity experiments, the antibodies were diluted with RPMI medium to obtain the appropriate concentration of antibody.

Purification of SM1 Antibody

Protamine-Sepharose was prepared by cyanogen bromide coupling of protamine to Sepharose 4B. SM1 ascites or supernatant was added to the gel and washed with dilute phosphate saline buffer (0.03 M phosphate-0.025 M NaCl). The washed gel was packed into a chromatography column and washed with dilute phosphate saline buffer to remove proteins other than IgM. The antibody was eluted with 1 M NaCl in 0.08 phosphate buffer, pH 7.4. The eluate was concentrated in a dialysis bag covered with Aqueadie (Calbiochem-Behring, La Jolla, CA). The antibody was further purified by passing through a 120-cm Sephacryl 300 column. The major antibody fraction was collected, and the individual tubes were tested for reactivity with SCC cells. The individual fractions with SCC reactivity were pooled and concentrated.
Immunofluorescence Staining

Suspension cells, cells attached to cover slips, or frozen sections were washed 3 times with RPMI without serum. Either 50 µl of purified antibody or 50 µl of ascites fluid diluted 1:1000 were applied to the test cells or tissues and incubated for 30 min at 37°C. The specimens were washed 3 times with PBS and incubated for 30 min with fluorescein-conjugated goat anti-mouse IgM (Meloy Laboratories, Springfield, VA) at 1:20 dilution with PBS. After 3 rinses with PBS, the cells were observed for fluorescence staining using a Zeiss epifluorescence microscope and by flow cytometric analysis using a EPICS V cell sorter. MPR, a mouse IgM monoclonal antibody (gift of Dr. E. Yunis, Dana Farber Cancer Institute) unreactive with OH1 and SW2 cells, was used as a negative control.

Cytotoxicity of SM1 Antibody and Complement toward Human Small-Cell Carcinoma

Chromium Labeling of Cells. SCC or marrow cells were labeled with ¹⁹Cr (sodium chromate) at 200 µCi/ml for 1 hr at 37°C. The cells were then washed with RPMI 1640 containing 1 mM glutamine and 4 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (wash buffer) and incubated in ice for 30 min. The cells were again washed with the wash buffer, and the concentration was adjusted to 2 x 10⁶ cells/ml. Each test sample contains 450 µl of cell suspension.

Treatment with Antibody and Complement. The appropriate dilution of purified antibody or ascites fluid in 50 µl of RPMI medium was added to the test cells, and the incubation was performed at 37°C for 30 min. Rabbit complement (final dilution, 1:20) was then added to the wells. The samples were incubated at 37°C for an additional 30 min. For multiple antibody treatments, the cells were washed with RPMI medium containing 1 mM glutamine and 4 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, and resuspended in the same medium. The antibody and complement treatments are then repeated. After the final treatment, the cells are washed twice with RPMI medium. The amount of chromium associated with the cell pellet was determined in a 

Chromium in control cells − chromium in antibody-treated cells

Chromium in control cells − chromium in detergent-lysed cells

Determinations were done in triplicate (S.D. = 2%).

Colony Formation Assays

For clonogenic assays with SCC cells, an exponentially growing culture of OH1 or SW2 cells was dispersed into small clumps and allowed to grow in suspension for 1 day. The cells were then disaggregated for a second time into single cells before treatment with antibody and/or complement. The cells were washed with growth medium and seeded into plates with a 1.2% agar base and 1.3% Methocel in medium with RPMI 1640 and 20% fetal calf serum. The seeding density included 10⁴, 10⁵, 10⁶, and 10⁷ cells/plate to increase accuracy in detecting a several log reduction in colony formation. Colony-forming assays for CFU (granulocytic/monocytic) and blast-forming units (erythroid) were performed as previously described by Griffin et al. (5).

RESULTS

Reactivity of SM1 Antibody with SCC Cell Lines and Tumors

We have previously reported that SM1 antibody is highly reactive with 3 of 3 SCC cell lines and 7 of 7 SCC tumors but is unreactive with a large number of other cell lines and tumors (1). We have extended these observations and found SM1 reactivity with 5 of 5 SCC cell lines and 25 of 25 SCC tumors tested so far. By immunoperoxidase staining of SCC tumor in the primary and in metastatic sites, we have found uniform reactivity with SM1 antibody. The SCC cell lines OH1 and SW2 used in the present study were found to be homogeneous with respect to their reactivity with SM1 antibody, although they were heterogeneous in their reactivity with SM7, another monoclonal antibody developed in this laboratory. When observed by fluorescence microscopy or flow cytometry, the different SCC cell lines were uniformly stained with SM1 antibody. We routinely counted several fields of at least 2000 cells after immunofluorescence staining and observed no or sometimes one SCC cell unreactive with SM1 antibody. Often, the unreactive cell looked damaged with irregular cell margins and granular cytoplasm. As shown by the flow cytometric pattern in Chart 1a, more than 99.9% of SCC-OH1 cells were strongly reactive with SM1 antibody. We have also examined the SM1 reactivity of fresh SCC cells by flow cytometry. Cells were isolated from a fresh sample of SCC tumor (disaggregated mechanically, washed three times to remove debris by low-speed centrifugation and resuspension in medium, and separated from fibroblasts as described in "Materials and Methods"). A very high proportion (more than 99.9%) of SCC cells was highly reactive with SM1 antibody (Chart 1c).

Lack of Reactivity of SM1 Antibody with Non-SCC Cells and Normal Marrow Cells

We have previously demonstrated the lack of reactivity of SM1 antibody by immunofluorescence and radioimmunoassay with a large number of non-SCC cell lines, non-SCC tumors, and normal cells (1). Further analysis of antibody reactivity was recently performed by flow cytometry. Chart 1a shows the flow cytometric pattern of OH1 cells. More than 99.9% of the cells are reactive with SM1 antibody compared to the lack of reactivity with CEM cells, a human lymphoblastic leukemia cell line. Normal bone marrow cells prepared either by Ficoll-Hypaque separation or by centrifugation after ammonium chloride lysis of RBC also show no reactivity with SM1 antibody (Chart 1b). Lack of SM1 reactivity was confirmed by immunofluorescence microscope scanning of large numbers of cells in bone marrow aspirates from 10 normal volunteers. However, we observed clumps of SM1-reactive cells in 25 of 36 bone marrow samples from SCC patients. Only 6 of 36 marrow samples were diagnosed to contain SCC cells by Wright-Giemsa or hematoxylin-eosin stains (11).

Lysis of SCC Cells by SM1 Antibody and Complement

Optimum Number of Treatments. Using SM1 antibody diluted to 1:1000 and complement concentration of 1:20, the effect of repeated treatments on the proportion of lysed SCC cells was determined by the chromium release assay. As shown in Chart 2, one treatment with SM1 antibody and complement lysed only 5% of SCC cells. Each additional treatment has a much greater effect; 50% of the cells are lysed after 2 treatments, and greater than 99.9% of the cells are lysed after 3 treatments. Whereas maximal cell lysis is obtained after 3 treatments with antibody and complement, the proportion of lysed cells continues to increase beyond 3 treatments with complement alone. In subsequent experiments, 3 treatments with antibody and complement were used for all the test cells.

Antibody Concentration. The effect of different concentra-
CYTOTOXIC ANTIBODIES TO LUNG CANCER

Chart 1. Flow cytometric pattern of cells labeled with SM1 antibody. The fluorescence intensity is expressed in arbitrary linear units. a, CEM, human lymphoblastic leukemia; OH-1, human small cell carcinoma cell line; b, normal human bone marrow nucleated cells; c, fresh small cell carcinoma.

fluorescence intensity

Percentage of lysis continues to be greater than 90% up to a dilution of 1:10,000. Further dilutions result in decreased cell lysis so that at a dilution of 1:40,000, approximately 60% of the cells are lysed (data not shown). SM1 antibody by itself resulted in less than 8% cell lysis even after 3 repeated treatments with high concentrations of antibody (dilution of 1:10).

Complement Concentration. The concentration of complement that yielded maximum synergism with antibody in lysis of OH-1 cells was at a dilution of 1:20 (Chart 4). Higher concentrations of complement, such as at dilutions of 1:10, resulted in significantly higher cell lysis in the absence of antibody. Dilutions of 1:50 or greater were much less effective in cell lysis even in the presence of high concentrations of antibody.

Effect of SM1 Antibody on SCC, Non-SCC Cells, and Normal Bone Marrow Cells. In Chart 6, different dilutions of antibody and complement were used to determine lysis of SCC cells compared to normal bone marrow cells. Incubation with SM1 antibody at different dilutions consistently resulted in much greater lysis of SCC cells than of normal bone marrow cells. Table 1 summarizes the cytotoxic effects of SM1 antibody and complement on SCC cells and normal bone marrow cells. Generally, 99.9% (3 logs) of small cell carcinoma cells are lysed after 3 treatments with SM1 antibody and complement. Approximately

Temperature Dependence. The effect of temperature used for incubation with antibody and complement on lysis of OH-1 cells was determined. As shown in Chart 5, the highest proportion of lysed cells was obtained at 37°, resulting in close to 4 logs of cell lysis. Although antibody and complement were less effective at 22°, more than 99.9% of the cells were lysed at this temperature. At 4°, however, fewer than 3 logs of cells were lysed, particularly at high dilutions of antibody.
CYTOTOXIC ANTIBODIES TO LUNG CANCER

Chart 2. Cytotoxicity of SM1 Antibody on SCC. Lysis of SCC cells by SM1 antibody. The amount of chromium released from SCC cells is determined after treatment with SM1 antibody and complement (●) or with complement alone (□).

Chart 3. OH-1 cell lysis mediated by SM1 antibody and complement. Effect of antibody dilution on the lysis of SCC cells in the presence or absence of complement.

Chart 4. Lysis of OH-1 (SCC) cells with SM1 antibody and complement. Effect of complement dilution on the lysis of SCC cells in the presence or absence of SM1 antibody.

Chart 5. Effect of temperature on lysis of SCC cells mediated by SM1 antibody and complement (1:10).

Chart 6. Lysis of SCC cells and normal bone marrow cells in the presence of SM1 antibody and complement.

10 to 15% of bone marrow cells from normal volunteers are lysed after treatment with SM1 antibody and complement. This is due to the effect of complement alone on normal bone marrow cells, which results in 10 to 17% lysis in the absence of antibody (Table 1). Rabbit complement preadsorbed with normal bone marrow cells and SCC cells produces 5 to 8% marrow cell lysis in the absence or presence of SM1 antibody. However, the combination of SM1 antibody and preadsorbed complement results in 98% lysis of SCC cells which is highly dependent upon the presence of SM1 antibody. The chromium release results are averages of triplicate experiments (S.D., approximately 2%).
Cytotoxic Antibodies to Lung Cancer

Table 1

<table>
<thead>
<tr>
<th>Cell target</th>
<th>SM1 + C'</th>
<th>C'</th>
<th>SM1</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCC cells-OH1</td>
<td>99.9</td>
<td>21</td>
<td>5</td>
</tr>
<tr>
<td>SCC cells-SW2</td>
<td>99.9</td>
<td>19</td>
<td>5</td>
</tr>
<tr>
<td>Marrow cells-1</td>
<td>15</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>Marrow cells-2</td>
<td>10</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Marrow cells-3</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>SM1 + C'</td>
<td>98.0</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>MPR + C'</td>
<td>99.9</td>
<td>6</td>
<td>8</td>
</tr>
</tbody>
</table>

* C', rabbit complement at dilution 1:20; C', rabbit complement preadsorbed with SCC and marrow cells used at dilution 1:20; MPR, monoclonal mouse IgM unreactive with OH1.

Table 2

<table>
<thead>
<tr>
<th>Incubation mixture</th>
<th>Reduction in SCC CFU (mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM1 (1:100) + complement (1:15)</td>
<td>99.98 ± 0.01*</td>
</tr>
<tr>
<td>SM1 (1:1000) + complement (1:15)</td>
<td>99.84 ± 0.04</td>
</tr>
<tr>
<td>SM1 (1:10,000) + complement (1:15)</td>
<td>99.79 ± 0.04</td>
</tr>
<tr>
<td>SM1 (1:100)</td>
<td>5.3 ± 0.8</td>
</tr>
<tr>
<td>Complement (1:15)</td>
<td>18.0 ± 1</td>
</tr>
</tbody>
</table>

* Mean ± S.D.

Effect of SM1 Antibody and Complement on Colony Formation by SCC Cells and Normal Bone Marrow Cells

Colony Formation by SCC Cells. Treatment with SM1 antibody and complement was found to be highly cytotoxic to SCC cells when assessed by the clonogenic assay. By varying the number of SCC cells plated on methocel from $10^2$ to $10^3$ cells/dish, we were able to detect reduction of cell growth by several orders of magnitude. The control cells were not treated with antibody but were washed, centrifuged, and incubated at 37°C, similar to test cells. After 3 treatments with SM1 antibody (1:100) and complement, there was 99.98% reduction of SCC CFU relative to control (Table 2). With higher dilutions of antibody (1:1000 and 1:10,000), the reduction in CFU was less but remained greater than 99%. Much lower reduction of CFU was observed with SM1 antibody alone or complement alone, similar to our results with the chromium release assay.

Colony Formation by Normal Bone Marrow Cells. We also tested the effect of SM1 antibody and complement on normal bone marrow precursors. In 3 separate experiments, treatment with the combination of SM1 antibody and complement did not result in significant reduction of CFU (granulocytic/monocytic) colonies relative to untreated controls or to cells treated with antibody or complement alone (Table 3). Similarly, there was no significant reduction in blast-forming units (erythroid) colonies after treatment with antibody and complement compared to untreated cells or cells treated with complement.

SM1 Antibody Purification and Effect on Other Non-SCC Cells

We have performed further purification of SM1 antibody from ascites and calculated the protein concentration. The total protein concentration in the ascites fluids used in these experiments was 40 mg/ml. An aliquot containing 200 mg of the protein in ascites fluid was precipitated by the addition of an equal volume of saturated ammonium sulfate. After dialysis in a sodium phosphate buffer, the antibody was purified by adsorption on protamine-Sepharose 4B column. Free serum proteins were removed by elution with sodium phosphate buffer, and the antibody was eluted with 1 M NaCl buffered solution. The antibody was further purified by passing through a 120-cm Sephacyl 300 column. The major antibody fraction was collected, and the individual tubes were tested for reactivity with SCC cells. The individual fractions with SCC reactivity were pooled and concentrated. From 200 mg of ascites protein, we obtained 120 mg of column-purified antibody. The IgM antibody protein fraction which is reactive with SCC cells is 60% of ascites protein or approximately 24 mg/ml. This purified antibody produced similar titers of complement-dependent cytotoxic antibody as ascites. Our results do indicate that in spite of the presence of other proteins in ascites fluid, we are able to demonstrate complement dependence, high level of cytotoxicity against SCC cells, and minimal cytotoxicity against normal marrow cells. Supernatant from SM1 hybridoma cultures, with dilutions of up to 1:500, also results in more than 99% lysis of SCC cells with no significant antibody-dependent lysis of normal bone marrow cells. In addition, no antibody-dependent lysis was observed with A549 (human lung adenocarcinoma), SL6 (large cell carcinoma of the lung), and CEM (human lymphoblastic leukemia). MPR antibody is also a mouse IgM antibody, but has no reactivity with SCC cells. After 3 treatments with MPR antibody and complement, only 5 to 8% of SCC cells were lysed, most of which was due to complement alone.

Discussion

We have previously described a murine monoclonal antibody, SM1, that is strongly reactive with the surface membrane of human SCC (1). This antibody is of particular interest since it is unreactive with many other cancers, including most non-small cell lung cancers and several cancers with neural characteristics such as neuroblastoma, melanoma, and bronchial carcinoid. The normal tissues examined thus far, including bronchial epithelium, lung parenchyma, liver, kidney, brain, erythrocytes, and bone marrow cells, are also unreactive. In immunoblots of membrane extracts from SCC cells, SM1 reactivity is observed with a major band of $M_\text{r}$ 50,000 and with a minor band of $M_\text{r}$ 25,000. SM1 was determined to be an IgM antibody by immunodiffusion and indirect immunofluorescence.

We now report that SM1 antibody is cytotoxic to SCC cells in the presence of complement. The conditions that result in opti-
maximum lysis of human SCC cells were defined using the chromium release assay. We found that as the number of treatments with antibody and complement was increased from one to 3 exposures, there was a dramatic increase in the proportion of lysed cells. Three treatments seemed to be optimal for antibody-dependent cell lysis, since greater than 99.9% of SCC cells were lysed in the presence of antibody and complement, whereas about 10 to 20% cell lysis was observed using complement alone. When 4 treatments were used, there was no further increase in cell lysis with antibody and complement, but more than 30% of the cells were lysed with complement alone. The requirement for repeated treatments with antibody and complement is likely to be due to exhaustion of complement during incubation. We have recently determined that one antibody incubation followed by 3 additions of complement 30 min apart is just as efficient in SCC cell lysis as 3 treatments with antibody and complement.4

SM1 antibody was found to be present in high titer in ascites fluids and hybridoma supernatants. Even dilutions of 1:10,000 of ascites fluid resulted in greater than 90% lysis of SCC cells, using the 3-treatment procedure. The high titer of cytotoxic antibody in these ascites fluids is of importance particularly if the antibody is to be used for treatment of large volumes of cells, such as bone marrow collected for autologous transplantation. However, we have also observed efficient antibody-dependent lysis of SCC cells using column-purified SM1 antibody or supernatant from SM1 hybridoma cultures. The cytotoxic effect of SM1 antibody was found to be strictly complement dependent, since even the highest concentration of antibody (dilution of 1:100) resulted in only 5 to 8% lysis of SCC cells in the absence of complement. The 1:20 dilution of the rabbit complement used in these studies was considered optimal for antibody-dependent SCC lysis. Lower dilutions of complement (1:10) resulted in greater cell lysis even in the absence of antibody. Higher dilutions of complement (1:50) reduced cell lysis to less than 90% even with high concentrations of SM1 antibody. Since antibodies may differ in the optimum temperature for complement-mediated cell lysis, we tested the cytotoxic effect of SM1 antibody at different incubation temperatures. At 4°C, the proportion of SCC cells lysed was clearly dependent upon the titer of antibody used but was generally less than 3 logs. At higher temperatures, the effect of varying antibody dilution between 1:100 to 1:1000 was less dramatic. The most efficient lysis was observed at 37°C, resulting in greater than 3 logs of cell lysis even at antibody dilution of 1:1000.

Thus, the SCC cell lines used in this study were efficiently lysed by the combination of SM1 antibody and complement. This is consistent with our observations that SCC cells in these cultures uniformly react with SM1 antibody. The SCC cell lines OH1 and SW2 were not selected for antigen density or lack of heterogeneity with respect to SM1 reactivity. Three other SCC cell lines tested showed very high proportions of SM1 reactivity. However, these cell lines were found to be heterogeneous with respect to reactivity with other anti-SCC monoclonal antibodies developed in this laboratory. We have also demonstrated by immunoperoxidase staining and fluorescence cell sorting that cells from fresh SCC tumors are highly reactive with SM1 antibody. Although we have found that 25 of 25 SCC tumors tested so far are reactive with SM1, more histological studies with SM1 antibody will need to be performed. Since heterogeneity is a common characteristic of tumor cells, it is possible that not all SCC cells within a tumor and not all SCC tumors will react with SM1 antibody. We are also characterizing additional anti-SCC monoclonal antibodies for reactivity and cytotoxic activity. The combination of several of these antibodies would then have a greater likelihood of alleviating the problem of tumor heterogeneity in diagnostic and therapeutic studies. Since SM1 antibody by itself is reactive with a very high proportion of SCC tumors, it is a good candidate for such a combination of anti-SCC reagents.

After treatment with SM1 antibody and rabbit complement, approximately 10 to 15% of normal bone marrow cells were lysed. This was due to the effect of complement, which by itself, lysed 10 to 17% of bone marrow cells. We have also found that preadsorption of complement with normal marrow and SCC cells reduced marrow cell lysis after treatment with SM1 antibody and complement. The low level of lysis (5 to 8%) was still due to the effect of complement alone. However, the combination of SM1 antibody and preadsorbed complement, retained the high level of antibody-dependent lysis of SCC cells. In addition to the chromium release results, our experiments indicate that after treatment with SM1 antibody and rabbit complement, there was marked reduction in tumor colony formation by SCC cells but no significant reduction in normal marrow cell colonies including CFU (granulocytic/monocytic) and blast-forming unit (erythroid). We have recently found that SM1 antibody is highly cytotoxic to SCC cells in the presence of human complement.4 Moreover, human complement appears to produce lower levels of nonspecific, antibody-independent lysis of normal bone marrow cells than rabbit complement. One incubation with SM1 antibody followed by 3 additions of human complement lysed more than 99% of SCC cells even in mixtures of 1 SCC:100 marrow cells.

Thus, SM1 antibody alone or in combination with other cytotoxic anti-SCC antibodies may be useful in eradicating SCC metastasis to bone marrow.

However, the clinical usefulness of monoclonal antibodies in the treatment of bone marrows contaminated with SCC cells remains to be established. Since our previous studies have shown that many patients with SCC metastasis to the bone marrow are not diagnosed by conventional histological stains (11), a significant proportion of patients is likely to have been entered into autologous bone marrow transplantation programs and reinfused with bone marrows contaminated with SCC. If pretransplant cryopreserved marrows are available for testing with more sensitive detection methods, it would be important to compare the times of relapse between patients who were infused with marrow containing SCC and those who received marrow free of metastasis as determined by monoclonal antibody techniques. If patients transplanted with antibody-negative, histology-negative marrows have a better prognosis that those who received antibody-positive, histology-negative marrows, we may be justified in using antibody-purging methods to try to improve the prognosis of transplanted SCC patients. In treatment programs that use marrow-lethal doses of chemotherapy and where the benefit of autologous bone marrow transplantation in reducing myelosuppression can be shown, the effects of antibody and

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complement exposure on marrow engraftment will need to be determined. Thus, numerous questions remain to be answered about the proper technique and benefit of autologous bone marrow transplantation on SCC patients. These are important questions, however, because in spite of the high response of this cancer to combination chemotherapy regimens, very few patients survive beyond 2 years (6).

REFERENCES

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