Factors Affecting Passive Monoclonal Antibody Therapy of Moloney Sarcoma in BALB/c Mice¹

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ABSTRACT

We have developed a syngeneic monoclonal antibody (MoAb) (244-19A) which retards growth and contributes to cures of BALB/c mice bearing Moloney sarcoma cell (MSC) tumors (S. J. Kennel, T. Lankford, and K. M. Flynn, Cancer Res., 43: 2843–2847, 1983). The 244-19A epitope has not been detected in normal tissue or in any cultured cell (other than MSC) tested, including 15 different BALB/c sarcomas. MoAb 244-19A remains in circulation for a relatively long time in normal mice (t½ = 3.8 days), but it is cleared faster from tumor-bearing mice (t½ = 1.7 days), indicating a specific interaction of the antibody with the tumor. The 244-19A epitope is very labile. Osmotic lysis of cells or fixation with ethanol abolishes antibody binding. Trypsin treatment or fixation with gluteraldehyde reduces activity by 80 to 90%. Results from immunoprecipitation of radiiodinated MSC surface proteins indicate that the 244-19A epitope may reside on a M₄ 65,000 protein, distinct from the major C-type virus glycoprotein 70 of these cells. Several factors affecting passive MoAb therapy have been evaluated. Doses as low as 24 µg/mouse demonstrated a significant therapeutic effect; however, larger doses up to 1.5 mg/mouse produced progressively more cures. Since MoAb 244-19A is syngeneic in BALB/c mice, fractionated doses of antibody can be given over long periods of time without a host response to the MoAb. Fractionated doses showed a slight advantage over single dose therapy, but the difference was not statistically significant (P < 0.2).

Passive MoAb therapy has been effective in nu/nu mice, in BALB/c mice depleted of complement with cobra venom factor, and in BALB/c mice irradiated with 399 rads of X-rays; thus, therapy did not require complement, B-cells, or a cytotoxic T-cell response. Although tumor growth was retarded in nu/nu mice (T-cell-deficient), complete cures of tumor-bearing animals could not be accomplished even with large, multiple doses of antibody, indicating that cytotoxic T-cells eliminate residual tumor cells resulting in cures of BALB/c mice. Treatment of BALB/c mice with silica to deplete macrophage function did not affect therapy with MoAb 244-19A; however, treated animals still retained about 30% of their original phagocytic function, so macrophages cannot be eliminated as a possible host effector function.

INTRODUCTION

The immunological response of a host to a growing tumor is complex. When exogenous antibody is added (passive therapy), it may interact with cellular or humoral components of the ongoing host response. The efficacy of passive antibody therapy may be influenced by: (a) the chemical nature and membrane dynamics of the target antigen; (b) the circulation time and effective dosage of the antibody; and (c) interaction with the host effector mechanisms.

We have shown that passive administration of a syngeneic MoAb (244-19A) can inhibit the growth of transplanted Moloney sarcoma cells and result in complete cures in BALB/c mice, even when the antibody is given after establishment of significant tumor mass (1). While treatment in some cases (i.e., in nude mice) results only in tumor regression, all BALB/c mice tested which experience tumor regression and are tumor-free at Day 100 remained so for at least the ensuing year that they were held. These animals are termed "cured."

Since this model is one of the few in which passive MoAb therapy has been successful (2–6), we have examined several of the factors that may be responsible. This report documents that the target epitope for MoAb 244-19A is unique and may actually be tumor-specific and that therapy is effective in complement-depleted BALB/c mice, as well as in nu/nu mice.

MATERIALS AND METHODS

Cell Lines. MSC were isolated by Massicot et al. (7) were obtained from Dr. Yancy Gillespie (University of North Carolina, Chapel Hill, NC) at their 75th in vitro passage. The cells were used between passages 75 and 100 in McCoy’s medium containing 10% FCS and supplemented with penicillin and streptomycin. The other sarcomas used were kindly supplied by Dr. Albert DeLeo, Sloan Kettering Institute (New York, NY). References describing their origin are presented in Table 1. The origin of MoAb 244-19A, preparation of BALB/c ascites fluid, and purification of the MoAb are described elsewhere (1). IgG, from P3 X63 Ag8 myeloma and rat MoAb 133-13A reacting with P100 are described elsewhere (8). MoAb 280-4A is a mouse IgG subtype reacting with human fibrinogen (9).

Indirect Antibody Binding Assay. Cells, MSC or other sarcomas, were seeded at their normal split ratios into 96-well (Linbro) plates and were used for binding assays at 70 to 90% confluency. Spent medium was aspirated and primary antibodies (MoAb in ascites fluid or antisera) diluted in prewarmed medium were added (50 µl) to each well and incubated at 37°C on a platform rocker in humidified 5% CO₂ for 1 h. Medium was aspirated, and the wells were carefully washed twice with warm medium. Fifty ng of radioiodinated (¹²⁵I) secondary antibody (approximately 10,000 cpm/ng) was added in 50 µl of medium and allowed to incubate as before for 1 h. Purification and iodination of secondary antibodies (goat antibody to rat IgG and goat antibody to mouse IgG) are as described previously (8). After washing, individual wells were separated and analyzed for (¹²⁵I) in a Searle Model 1185 automatic gamma counter. Estimates of the amount of 244-19A activity (binding capacity) present were made by multiplying the dilution of ascites fluid necessary to give 50% maximal binding to MSC times the number of Ig representing 50% binding, times 20 to correct the values to 1-ml volumes. These

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MoAb THERAPY OF MSC IN MICE

Table 1

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Induced by (ref.)</th>
<th>Strain</th>
<th>MoAb 133-13A</th>
<th>MoAb P3</th>
<th>MoAb 244-19A</th>
<th>Ratio of 244-19A/P3</th>
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<tr>
<td>MSC</td>
<td>Virus (7)</td>
<td>BALB/c</td>
<td>27</td>
<td>1.6</td>
<td>13.0</td>
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<td>S160</td>
<td>MCA (6)</td>
<td>CFW</td>
<td>77</td>
<td>4.3</td>
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<td>CMS5</td>
<td>MCA (36)</td>
<td>BALB/c</td>
<td>24</td>
<td>0.7</td>
<td>1.2</td>
<td>1.7</td>
</tr>
<tr>
<td>11A(V)</td>
<td>Virus (39)</td>
<td>BALB/c</td>
<td>23</td>
<td>3.5</td>
<td>4.3</td>
<td>1.2</td>
</tr>
<tr>
<td>Meth A(a)</td>
<td>MCA (36)</td>
<td>BALB/c</td>
<td>47</td>
<td>2.4</td>
<td>1.5</td>
<td>0.6</td>
</tr>
<tr>
<td>CA2</td>
<td>MCA (36)</td>
<td>BALB/c</td>
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<td>1.8</td>
<td>2.0</td>
<td>1.1</td>
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<td>MCA (36, 47)</td>
<td>BALB/c</td>
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<td>BALB/c</td>
<td>33</td>
<td>1.8</td>
<td>1.6</td>
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<td>BALB/c</td>
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<td>1.5</td>
<td>1.2</td>
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<tr>
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<td>4.7</td>
<td>3.2</td>
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<tr>
<td>Meth A(s)</td>
<td>MCA (36)</td>
<td>BALB/c</td>
<td>32</td>
<td>1.9</td>
<td>2.0</td>
<td>1.1</td>
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</tbody>
</table>

values are reported as ABC-50.

Therapy Experiments. BALB/c mice, obtained from Cumberland View Farms (Clinton, TN) were used for therapy experiments at 6 to 12 weeks of age. BALB/c nu/nu mice were obtained from Life Sciences, Inc. (St. Petersburg, FL). Mice were given i.m. injections of 2 x 10^6 tumor cells suspended in 100 μl PBS after scraping from 100-mm dishes. Each antibody treatment consisted of an i.p. injection of syngeneic ascites fluid containing 24-4-19A MoAb or P3-lgG (5 mg/ml). Animals were monitored daily for tumor growth and mortality. Statistical significance of results was analyzed using a death time function described by Mantel (10). Significance values (P) were calculated using a 2-sided consideration.

To deplete complement, mice were treated with CVF (Cordis Laboratories, Miami, FL) according to the schedule of Cochrane et al. (11). Sera from representative animals were tested for complement activity by their ability to lyse sheep RBC activated with rabbit antibody and embedded in agarose.

To reduce phagocytic cells, mice were treated with 3.2 mg silica (12) in 100 μl PBS. Surviving animals (~70%) were tested for phagocytic function. Peritoneal exudate cells from peritoneal lavage with PBS were tested for their ability to engulf particles from India ink.

To compromise the B-cell function, animals were irradiated with 399 rads X-rays with a Noreco Model MG150, peak voltage of 150 kV at 12 ma filtered through 3 mm of aluminum. Animals were continuously rotated at 32 cm from over and under dual sources, receiving a dose of 150 rads/min.

Labeling Studies. Procedures for labeling of MoAb for distribution studies and the constructions of labeling indexes are published elsewhere (13). Cell surface iodination, immunoprecipitation, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and autoradiography were done for MSC exactly as described elsewhere (8) for Line 1 cells.

RESULTS

Antigen Characterization. Several different properties of the antigen molecule may prove to be important in passive antibody therapy. Among these are (a) the density and accessibility at the cell surface; (b) its function and membrane properties; and (c) whether the antigen is unique or found also on normal tissue.

We determined previously that MSC have about 10^6 binding sites per cell for MoAb 244-19A (1); however, we have not been able to quantitate the epitope in tumors, due to its lability. By

MoAb Therapy Experiments. BALB/c mice, obtained from Cumberland View Farms (Clinton, TN) were used for therapy experiments at 6 to 12 weeks of age. BALB/c nu/nu mice were obtained from Life Sciences, Inc. (St. Petersburg, FL). Mice were given i.m. injections of 2 x 10^6 tumor cells suspended in 100 μl PBS after scraping from 100-mm dishes. Each antibody treatment consisted of an i.p. injection of syngeneic ascites fluid containing 24-4-19A MoAb or P3-lgG (5 mg/ml). Animals were monitored daily for tumor growth and mortality. Statistical significance of results was analyzed using a death time function described by Mantel (10). Significance values (P) were calculated using a 2-sided consideration.

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MSC were iodinated with ^125I using lactoperoxidase, and the proteins were solubilized in Nonidet P-40 and subjected to double antibody immunoprecipitation with antibodies of several specificities. Immunoprecipitates were dissociated, and labeled proteins were separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and were detected by autoradiography (Fig. 1).

A long exposure time is necessary to allow visualization of a weak band at M, 65,000 precipitated by MoAb 244-19A (Lane 4). Control precipitates with control mouse MoAb 280-4A (Lane 5), normal goat IgG (Lane 3), or normal rat IgG (Lane 7) show little or no radioactivity banding at this molecular weight. Precipitates with goat antiserum to C-type virus gp70 precipitate a large amount of ^125I which bands at M, 70,000 characteristic of the gp70 from Moloney virus produced by these cells (14). Finally, we have used rat MoAb 133-13A to a surface glycoprotein of M, 100,000 from Line 1 lung tumor cells (8) as another positive control for the technique (Lane 6). This antibody precipitates a protein from MSC with a mobility corresponding to only about M, 90,000. The mobility of the band precipitated by MoAb 244-19A is only slightly different than that of the gp70 band. The amount of radioactivity, however, is much smaller, even though saturating amounts of MoAb were added, indicating that the protein recognized by 244-19A is not the major gp70 on MSC.

In order to rule out the possibility that MoAb 244-19A is binding to a unique MSC viral protein, binding assays were done on fibroblasts that had been infected productively with C-type virus harvested from MSC cell cultures. Both infected and uninfected cells were negative for binding of MoAb 244-19A, while a parallel experiment gave positive results on MSC cells (data not shown).

Finally, we have tested several other cell lines for binding of MoAb 244-19A in the indirect binding test. Positive controls were included (MoAb 133-13A) to document that cells were healthy and that the test gave reliable data. Ten sarcoma lines of independent origin from BALB/c mice all tested negative (Table I). This brings to 15 the number of sarcomas tested. Some of these are voice producers and some are not; some have been induced by chemicals, and some have not; and some are highly tumorigenic, while others (notably MSC) require 10^6 cells as a lethal dose. These results strengthen the claim (1) that the epitope recognized by MoAb 244-19A is unique and may qualify as a tumor-specific antigen.

Circulation Time and Distribution of MoAb. The amount of MoAb activity remaining in serum at various times after i.p. injection into normal or tumor-bearing mice was measured for the therapeutic MoAb 244-19A and a control MoAb 280-4A
which binds to human fibrinogen (9). Antibodies were mixed and injected, and the sera were collected when animals were sacrificed. Sera from different times postinjection were titered for activity of each of the MoAb. Data in Chart 1A show that MoAb 244-19A is cleared from the circulation of tumored animals faster than from normal animals ($t_\omega = 1.7$ versus 3.8 days). Control MoAb 280-4A (Chart 1B) has similar clearance times in both tumor-bearing and normal animals ($t_\omega = 4.9$ and 5.8 days, respectively). These data indicate that MoAb 244-19A is being preferentially bound and cleared from the circulation of tumor-bearing animals. In experiments in which rat MoAb are injected into mice (data not shown), the MoAb undergo rapid clearance between Days 5 and 8 due to host antibody to the rat MoAb. The fact that MoAbs 244-19A and 280-4A remain in the circulation of BALB/c mice is indicative that no significant anti-MoAb host response is triggered. This is expected, since both MoAb 244-19A and MoAb 280-4A are syngeneic in BALB/c mice.

The distribution of $^{125}$I MoAb in MSC tumor-bearing mice was determined at several times after injection i.v. For these experiments, MoAb 244-19A was radioiodinated with $^{125}$I, and control mouse monoclonal immunoglobulin from myeloma P3 was labeled with $^{131}$I. Data for a representative experiment (Table 2) indicate that no significant accumulation of MoAb 244-19A can be demonstrated in any organ or the tumor site. These data, coupled with the data for MoAb clearance (Chart 1), suggest that residence time of the MoAb in tumor tissue is short. We have been unable to detect "altered forms," either immune complexes or antibody fragments, of MoAb 244-19A in the serum of tumored animals (data not shown).

**Effective Therapeutic Dose of MoAb.** We have tested the effect of different doses of MoAb given i.p. at Days 1, 2, and 3 postinoculation with $2 \times 10^6$ MSC i.m. Antibody dosage was calculated as antibody activity in ascites fluid (ABC-50). Data in Chart 2 show that therapy is dose-dependent, with the best cure rates at the highest doses. There is some indication that the lowest dose used (24 $\mu$g) may have a significant effect. In several therapy experiments, parallel groups of animals were sacrificed, and antibody remaining in their serum was titered. We have found that circulating antibody, albeit at reduced levels, can easily be detected, even in tumor-bearing mice up to 5 days after the last injection. Significant antibody titers to MSC were not detected up to Day 15 in any animals given only control treatments, indicating that no humoral immune response to progres-

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**Chart 1.** Residual antibody activity remaining in serum of normal (C) or MSC tumor-bearing (B) BALB/c mice. Animals with 5-mm tumors were given injections i.p. with a mixture of ascites fluids containing MoAbs 244-19A and 280-4A. Sera collected at the times indicated were titered under standard conditions, and the ABC-50 calculated for duplicate animals at each point was normalized to the amount of each antibody injected. A, recovery of MoAb 244-19A activity; B, recovery of control MoAb 280-4A activity from the same sera.

**Chart 2.** Effect of total dose of MoAb-244-19A in producing long-term survivors of BALB/c mice given injections of $2 \times 10^6$ MSC cells. Animals were treated i.p. with MoAb 244-19A ascites fluid diluted in PBS or P3 ascites fluid (control) on Days 1, 2, and 3 post-tumor challenge to give the total dose indicated. A long-term survivor is an animal which is tumor-free at 100 days after challenge.
sively growing tumors could be detected.

In order to determine the most efficient treatment schedule, MoAb was administered as (a) a single dose on Day 1 after tumor inoculation; (b) the same total dose divided into 3 treatments on Days 1, 2, and 3; and (c) the total dose divided into 6 treatments at Days 1, 2, 3, 5, 7, and 9. In one experiment, high-titer ascites were used, and all animals in the different treatment groups were cured. Total doses had to be adjusted to achieve intermediate results. Two experiments with typical results were pooled, and the data are presented in Chart 3. All treated groups (single injection or split dose) showed statistically significantly longer survival times than did animals treated with P3 (control) ascites fluid (data for 1, 3, or 6 injections of control ascites are pooled). Although there is a trend for the split-dose regimen to be more effective than a single dose, the differences are not statistically significant (P < 0.2 between groups receiving the single treatment or 6 fractionated doses). Data on tumor size for these experiments give corresponding information (not shown).

Interaction with Host Effector Mechanisms. To assess whether a host T-cell function was essential to efficient MoAb therapy, BALB/c nu/nu mice bearing MSC tumors were treated with MoAb 244-19A. Survival data (Chart 4) show that animals given ascites fluid containing MoAb 244-19A on a 6-shot schedule survive longer than do animals given control ascites fluid containing P3 immunoglobulin. While survival is prolonged, all animals ultimately die of tumor burden, indicating that some host T-cell functions, reduced in these mice, is necessary to facilitate cures. This is consistent with the published T-cell-mediated mechanism of tumor rejection of low-dose cell challenge in the MSC tumor model (15). In another experiment, BALB/c nu/nu mice were treated with antibody on the 6-day schedule, and therapy continued every fifth day until death. Survival data (not shown) similar to those in Chart 4 were obtained, indicating that MoAb alone, even in large continuous doses, does not result in cures of nu/nu mice with MSC tumors.

To assess the role of complement in passive MoAb therapy, BALB/c mice were treated with CVF to deplete complement on the days when antibody was present (11). Tests on control animals indicated that complement was >90% depleted on Day 4 but had recovered by Day 10; hence, during the antibody treatment, complement was severely depleted. Data for tumor size (Chart 5) indicate that 244-19A significantly retards or prevents growth of MSC tumors, even in animals depleted of complement. MoAb 244-19A is an IgG2a, and has been used to kill MSC in vitro with complement (data not shown). However, this mechanism must not be a major factor to therapy in vivo.

One major theory of therapy mechanisms is that MoAb acts through Fc receptors of macrophage-like cells in an ADCC immune reaction (16–20). Mouse IgG2a subclass antibodies are most efficient in the mechanism, but IgG2b antibodies (i.e., 244-19A) may be active at some lower level (21, 22). To test the role of phagocytic cells in passive therapy, BALB/c mice were treated with silica to reduce macrophage function. The dose used was lethal for about one-third of the animals treated, while the remaining animals had lost only about 70% of the phagocytic function of peritoneal exudate cells (data not shown). Survival data for a therapy experiment done on these animals (Chart 6) show that MoAb 244-19A is still effective in the compromised animals; however, since macrophage function was only reduced by 70% with no loss in absolute numbers of exudate cells, the data do not rule out retention of a significant ADCC function.

We have not been able to produce active antibody fragments (either Fab or Fab') from MoAb 244-19A using several different enzyme treatments. While this is not unusual for IgG2a MoAbs (23), these fragments would be very useful in probing the mechanism of therapy.

Finally, we have conducted therapy experiments on BALB/c mice compromised with radiation. Chart 7 presents survival data for animals treated 1 day prior to cell injection with 250 rads of X-rays. Control antibody-treated animals die somewhat earlier than do unirradiated controls (data not shown), but MoAb 244-19A-treated animals all survived tumor-free. The immune status of these animals was not tested, but they must retain some T-cell function, or cures of MSC tumors would not be expected. One experiment was done on BALB/c mice receiving 750 rads of X-rays. Control antibody-treated animals bearing large tumors all died by Day 17. Four of 8 animals treated with MoAb 244-19A survived for more than 30 days, and 2 were free of tumors (data not shown). This level of X-rays is lethal in our colony and, hence, only tentative conclusions can be drawn from high-dose experiments.

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**Chart 3.** Effect of MoAb dose fractionation on survival of BALB/c mice challenged with 2 x 10⁵ MSC i.m. on Day 0. Animals were treated i.p. with the same total dose of 600 μg ABC-50 as a single injection on Day 1 (O), in 3 injections on Days 1, 2, and 3 (□), or in 6 injections on Days 1, 2, 3, 5, 7, and 9 (▲). Data for animals treated with the three different schedules of control (P3) ascites fluid were pooled (□). The results represent data for 2 separate experiments done at different times. Statistical analyses: control versus single injection, χ² = 4.5, P < 0.05; control versus 3 injections, χ² = 8.2, P < 0.01; control versus 6 injections, χ² = 13.4, P < 0.01; single injection versus 6 injections, χ² = 1.2, P < 0.2.
MoAb THERAPY OF MSC IN MICE

Chart 4. Therapy in BALB/c nu/nu mice. Survival of BALB/c nu/nu mice given injections (Day 0) i.m. with $2 \times 10^8$ MSC cells and treated with 360 µg ABC-50 of MoAb 244-19A (●) or P3 (control) (○) ascites fluids on Days 1, 2, and 3. Statistical differences in time to death, $\chi^2 = 13.7$, $P < 0.01$.

Chart 5. Effect of treatment of BALB/c mice with CVF on therapy with MoAb 244-19A. Animals were given injections of 5 units of CVF i.p. on Day 0, i.v. on Day 2, and i.p. on Day 3 after challenge with $2 \times 10^6$ MSC i.m. and were treated i.p. with 200 µg ABC-50 MoAb 244-19A or P3 (control) on Days 1, 2, and 3. Tumor diameter was measured for groups treated with CVF and 244-19A ascites fluid (●) or P3 ascites fluid (○) and for groups not treated with CVF and receiving 244-19A ascites fluid (●) or P3 ascites fluid (○) as a control group.

DISCUSSION

There have been some reports of passive antibody therapy of different types of experimental cancer (1–6, 22, 24–28). However, this approach has not been uniformly successful and so, many investigators have chosen to use antibodies as specific delivery systems for toxins and other chemotherapeutic drugs rather than rely on their direct effects. We have been successful in treating MSC tumors in BALB/c mice with MoAb (1). This system, while not the optimal model of human disease, still represents one of the few solid tumor models for which passive antibody therapy has worked. Furthermore, the fact that the MoAb used is syngeneic allows us to give MoAb over long periods without the complication of host response to the therapeutic MoAb. We have not been able to detect any host antibody to mouse immunoglobulin in general or to putative idiotypic determinants on MoAb 244-19A in any of our experiments. This fact, coupled with the observation that MoAb 244-19A binds to MSC cells with a relatively high binding constant, argues against involvement of the antibody in a "network effect" or as a direct-acting biological response modifier of the host immune system (29, 30).

Since so few antibody-tumor systems are available and since it is probable that human antibodies to human tumors will be generally available in the near future, we feel it is important to assess the factors which may predict success or failure of these antibodies in passive therapy (31). Three such factors have been evaluated: (a) the biochemistry of the target antigen; (b) the most effective treatment schedule; and (c) the effector mechanisms involved. For optimal assessment of each of these param-
MoAb THERAPY OF MSC IN MICE

etters, we would need a battery of antibodies of different sub-
classes and epitope specificities. To date, we have not been
able to produce such a panel of MoAbs.

We have made several attempts to characterize the target
antigen of MoAb 244-19A biochemically. Immunoprecipitation
data indicate that the epitope does not reside on any known cell
surface protein (including H-2 antigens or C-type viral gp70).
Still, the labile nature of the epitope recognized by this MoAb
makes absolute conclusions impossible. We have shown that
about 10^6 antibody binding sites are available on the surface of
each cultured MSC (1); however, we have been unable to quan-
titate antibody binding to tumor cells (in vivo) or to demonstrate
significant accumulation of injected MoAb at the tumor site.
These data would indicate that MoAb may be rapidly "turned
over" by tumor cells, as suggested by Scheinberg and Strand
(32). Another careful study in which imaging has been successful
makes use of a MoAb which turns over very slowly on the cell
surface (33). Studies in progress show that cultured MSC bind
and release immune complexed antibody at a high rate. Although
the membrane dynamics of the target antigen bound to antibody
may be important in some therapy systems, the fact that MoAb
244-19A is not toxic for MSC in vitro indicates that host factors
are essential. This fact also rules out the possibility that MoAb
244-19A blocks an essential cell function unless that function is
required only for growth in vivo.

The most intriguing property of the 244-19A epitope is its
exclusive expression in MSC. We have not detected the epitope
on any other sarcoma tested. Several mouse sarcoma antigens
defined by MoAbs (34–38) show similar specificities. They have
been associated with virus components (39) or epitopes on C-
type virus gp70s (36, 37). While gp70 variants are abundant (40),
our data indicate that the 244-19A epitope is probably not
associated with gp70 or H-2 antigens, but its residence on an
unusual or altered protein cannot be ruled out. Since MoAb 244-
19A circulates with near-normal half-life in normal animals, it
must not encounter significant concentrations of antigens. In
tumor-bearing animals in which antigen is present, the MoAb is
cleared rapidly. The fact that MoAb 244-19A does not react with
normal mouse tissue undoubtedly contributes to its efficient
therapeutic function. Most other systems in which MoAb has
proven effective in therapy have restricted epitope expressions.
For example, MoAb to human tumor antigen tested in nude mice
(26–28), MoAb to viral protein (5), or MoAb to immunoglobulin
idiotypes (24) have restricted specificities. However, MoAb to
differentiation antigens (2, 4) and to a specific glycolipid (6) have
also been effective, even though these antigens are present in
low amounts in the normal host.

Our experiments on the effective dose of antibody for therapy
show that amounts as low as 24 μg ABC-50 are effective but
that larger amounts result in more cures. Even at the lowest
effective dose tested, we calculate that animals have been
browned with about 100 times the amount of antibody necessary
to saturate all of the binding sites on the cell inoculum [2x 10^6
MSC cells (1)]. Results from dose fractionation experiments
indicate that split doses of MoAb over 3 to 9 days are slightly
more effective than a single dose; however, there is little statis-
tical significance (P < 0.2) between the different treatment sched-
ules. Since this antibody has a relatively long circulation time (3.7
days) in normal animals and 1.7 days in tumor-bearing animals,
we would predict the effect of dose fractionation to be minimal.

Systems using antibodies or antibody fragments with much
shorter residence time in the animals may show a more pronounced
effect.

Several experiments have been done to elucidate the mecha-
ism of action of MoAb therapy. Most IgM antibodies are not
effective (3, 19), although they are efficient fixers of comple-
ment. IgG1 (5, 28), IgG2a (3, 6, 28), IgG2b (1, 22), and IgG3
antibodies (2, 4, 17, 19) have been shown to be effective.
Complement involvement was indicated in early studies (2), but
the current theory is that MoAb interacts with an activated
macrophage population (16–20) to facilitate tumor rejection.

We attempted several experiments to test the mode of action
of MoAb 244-19A in animals. In the first of these, BALB/c nu/nu
mice, reduced in certain T-cell functions but containing a com-
potent ADCC system (17, 19, 41), were tested in the MSC
system. MSC tumors grew extremely fast in these animals,
reaching sizes of 2 cm within 2 weeks of cell injection. Treatment
with antibody retarded tumor growth, but it could not irradicate
the tumor, and the ultimate result was death. These tumor-
bearing mice were still capable of rapid clearance of antibody
from their circulation (t½ < 1 day) and, thus, failure of therapy
probably was not due to loss of antigen expression of a variant
cell population (42). When animals reject low doses of MSC, the
major definable immunological parameter is a cytokotoxic T-cell
response (15). While MoAb 244-19A treatment retards growth
of MSC tumors in nu/nu mice, none of the animals are cured. It
is probable that cures result only when residual tumor cells are
eliminated by cytokotoxic T-cells.

The role of an active complement system in therapy was
tested in BALB/c mice. Animals treated with CVF (11) were
deficient in complement for at least 4 days after the treatment.
This had no apparent effect on therapy with MoAb. MoAb-treated
animals had noticeably smaller tumors as early as 3 to 4 days
postimplant. Although it is possible that the complement system
recovered early enough to interact with residual antibody in the
circulation or on the tumor surface, the fact that a significant
tumor retardation was noted early after implant argues against
this interpretation.

Attempts were made to deplete BALB/c of phagocytic cells
by treatment with silice. This treatment did not alter the therape-
uttic efficiency of MoAb-244-19A. As noted in the results, only
about 70% of the phagocytic function was lost and, furthermore,
it is not clear that the subpopulation of phagocytic cells active in
ADCC (12) is impaired by silice treatment. It has been shown that
macrophages have receptors for IgG3 antibodies but at lower
levels than for IgG2a (21); therefore, it is possible that
macrophages may play a significant role in this therapeutic
model. The activity of MoAb 244-19A in ADCC in vitro is currently
being tested in collaboration with Dr. Dolf Adams, Duke Uni-
versity.

Therapy experiments have also been conducted in irradiated
mice. In mice given 399 rads of X-rays, it is clear that therapy
with MoAb 244-19A is not impaired. Irradiated BALB/c mice
show much less resistance to growth of MSC tumors, which is
consistent with the impaired immune function in these animals.
Many studies (43–45) have shown that both humoral (B-cell) and
cytotoxic lymphocyte (T-cells) responses are suppressed by at
least 90% at these doses. Fractionated doses of 200 rads are
effective in suppression of natural killer cell activity (45). The
effects of radiation on macrophages is difficult to assess, due to

CANCER RESEARCH Vol. 45 August 1985
3787
the heterogeneity of the populations, although it is clear that phagocytic function is quite radiation-resistant (46). Studies on ADCC function in irradiated mice have not been reported. Thus, it is possible that macrophages may represent a major effect on host function in the MSC passive therapy system.

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Fig. 1. Autoradiogram of sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of immunoprecipitates of MSC surface proteins radiiodinated with $^{125}$I and lactoperoxidase. Total label pattern. Lane 1, Lane 2, proteins precipitated from solubilized MSC by polyclonal goat antibody to C-type viral gp70; Lane 3, control normal goat serum; Lane 4, MoAb 244-19A; Lane 5, control MoAb 280-4A; Lane 6, rat MoAb 133-13A to P100; Lane 7, control normal rat serum. Molecular weight markers were run on each side of the gel, and their mobility, determined from gel staining, is indicated on the molecular weight scale.
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