Inhibition of Growth of Colorectal Carcinoma in Nude Mice by Monoclonal Antibody

Dorothee M. Herlyn, Zenon Steplewski, Meenhard F. Herlyn, and Hilary Koprowski
The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104

ABSTRACT

Hybridoma-derived monoclonal anti-colorectal carcinoma antibodies suppressed the growth of colorectal carcinoma in nude mice as evidenced by a lower incidence of tumors, a longer latency period, and a smaller volume of tumors in antibody-treated than in control animals. The growth-inhibiting properties of monoclonal anti-colorectal antibodies seem to be specific for colorectal carcinoma cells. This is indicated by the lack of effect of the antibodies on the growth of melanomas or bronchogenic carcinomas and by the binding of the antibodies in vivo to colorectal carcinoma cells but not to lung or kidney cells from tumor-bearing animals or to other tumor cells implanted in other animals. Inhibition of tumor growth was most probably mediated by antibody-dependent cell-mediated cytotoxicity. The results of these studies could provide an approach to the study of immunotherapeutic possibilities for anti-colorectal carcinoma antibodies in humans.

INTRODUCTION

The paucity of recent attempts to use heterologous sera for immunotherapy of human cancer is due in part to the fact that it is difficult to obtain sera that are specific for tumor antigens (reviewed in Ref. 13). Attempts to produce tumor-specific antisera either by in vitro absorption of unwanted specificities present in polyclonal sera or by immunization with purified tumor antigen require an a priori knowledge of the antigenic composition of the compounds used for absorption or immunization. Although heterologous sera specific for tumor antigens have allegedly been raised (9, 10), it is extremely difficult to produce these sera in large enough volumes for use in human therapy.

Monoclonal antibodies produced by hybridoma cells (3) are directed against a single antigenic determinant and thus circumvent these problems. Furthermore, hybridoma cells can be injected into syngeneic hosts as solid or as ascitic tumors resulting in the production of sera or ascites with extremely high titers (in contrast to those of tissue culture supernatants) of hybridoma antibodies (4).

We demonstrated in a previous publication that anti-melanoma antibodies produced by hybridoma mass cultures suppressed the growth of human melanoma in nude mice (5). The same antibodies mediated tumor suppression in vitro through ADCC (1). In the present study, we investigated the effect of monoclonal anti-colorectal carcinoma antibodies secreted by hybridoma cells (2, 6) on the growth of human colorectal carcinoma tumors in nude mice. We thereby hoped to learn whether monoclonal antibodies which mediate ADCC against colorectal carcinoma cells in vitro (1) are also capable of inhibiting the growth of such tumors in vivo.

MATERIALS AND METHODS

Cell Lines

Human Cell Lines. The human cell lines used in this study are listed in Table 1. We included 6 colorectal carcinomas, 2 melanomas, one astrocytoma, one lung carcinoma, and one bronchogenic carcinoma. These cell lines have been described elsewhere (1, 2, 5, 6).

Mouse Cell Lines. BALB/c myeloma P3 × 63 Ag8 cells (hereafter called P3) (14) were obtained from C. Milstein.

Hybridoma Cell Lines. The hybridoma 1083-17-1A which produces colorectal carcinoma-specific antibodies and hybridoma 480-1-4 which produces antibody that cross-reacts with human cell lines of various origins are described elsewhere (2).

Antibody-dependent Cell-mediated Cytotoxicity

The ADCC test was performed as described elsewhere (1) except that lymphocytes from CBA/J mice were used as effector cells.

Production of Ascitic Fluid

BALB/c mice were inoculated i.p. with 10⁷ hybridoma 1083-17-1A cells or P3 mouse myeloma cells. Ascitic fluid from mice that developed tumors was collected 2 to 3 weeks after cell injection and centrifuged at 300 × g. Cell-free supernatant was stored at −20°C.

In Vivo Experiments

Thymus-deficient 6- to 8-week-old nude mice (nu/nu, BALB/c background) were inoculated under the skin of the neck with 10⁷ tumor cells in 0.3 ml Eagle's minimal essential medium containing 10% fetal calf serum. In the first and second experiments (Charts 1 and 2), control and experimental animals received i.p. injections of 0.3 ml ascitic fluid diluted 1:5 in PBS. Control animals received P3 ascitic fluid, and experimental animals received 1083-17-1A ascitic fluid (approximately 400 µg specific antibody). The first injection was given within 1 hr of tumor cell injection.

In the first experiment, antibody was administered once daily for 18 days. In the second experiment, animals were treated as above except that, after Day 18, antibody treatment was administered 4 times weekly throughout the observation period. In the third experiment (Chart 3), Alzet osmotic minipumps...
ADCC reactivity of monoclonal anti-colorectal carcinoma antibodies against cells of various human tumor lines

Hybridoma antibody of ADCC against cells of Colorectal carcinoma Melanoma Astrocytoma Lung carcinoma Bronchogenic carcinoma

<table>
<thead>
<tr>
<th>Hybridoma antibody</th>
<th>Colo. carcinoma</th>
<th>Melanoma</th>
<th>Astrocytoma</th>
<th>Lung carcinoma</th>
<th>Bronchogenic carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>1083-17-1A</td>
<td>7.1</td>
<td>17.6</td>
<td>26.8</td>
<td>8.9</td>
<td>15.7</td>
</tr>
<tr>
<td>480-1-4</td>
<td>22.1</td>
<td>12.0</td>
<td>21.2</td>
<td>8.9</td>
<td>12.0</td>
</tr>
</tbody>
</table>

* Based on hybridoma antibody dilutions showing highest ADCC values. ADCC data represent the average of 2 to 4 independently performed experiments. ADCC values of hybridoma antibodies are significantly different from P3-control values (p < 0.05).

Hybridoma Antibody 480-1-4 was included in order to demonstrate that cells of origin other than colorectal carcinoma are susceptible to lysis in ADCC assay.

**Table 1**

<table>
<thead>
<tr>
<th>% of ADCC* against cells of</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hybridoma antibody</td>
</tr>
<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td>SW 837</td>
</tr>
<tr>
<td>SW 948</td>
</tr>
<tr>
<td>SW 1116</td>
</tr>
<tr>
<td>SW 1222</td>
</tr>
<tr>
<td>SW 1345</td>
</tr>
<tr>
<td>SW 1463</td>
</tr>
<tr>
<td>SW 691</td>
</tr>
<tr>
<td>WM 9</td>
</tr>
<tr>
<td>SW 1088</td>
</tr>
<tr>
<td>SW 1271</td>
</tr>
<tr>
<td>MBA 9812</td>
</tr>
</tbody>
</table>

* Based on hybridoma antibody dilutions showing highest ADCC values. ADCC data represent the average of 2 to 4 independently performed experiments. ADCC values of hybridoma antibodies are significantly different from P3-control values (p < 0.05).

**Statistical Methods**

The method of least-squares regression (11) was used in all experiments for the estimation of tumor growth rates. Tests were carried out with the null hypothesis of no difference in tumor growth curves between control and experimental animals. The proportions of tumor-bearing mice in control and experimental groups were compared at each sampling point by the z test (12). Finally, the hypothesis of no difference in distribution of colorectal tumor size between experimental animals and controls was tested by the Mann-Whitney U statistic (8).

**Acid Extraction of Tumor, Kidney, and Lung Cells**

Nude mice were inoculated s.c. with $3 \times 10^7$ tumor cells. Starting 10 days later, they received one injection a day for 3 days of ascitic fluid containing monoclonal antibodies against colorectal carcinoma or containing P3 antibody. Tumor tissue, kidneys, and lungs were removed 24 hr after the last injection and were finely minced in PBS for production of a single-cell suspension. Cells were washed several times in PBS (until $E_{280nm} < 0.025$) for removal of nonspecifically attached serum proteins. Acid extraction of the cell contents was performed essentially as described by Witz et al. (15). Following extraction, the pH of the acid eluates was adjusted with 1 N NaOH to
cells, mice were treated for 14 days with anti-colorectal carcinoma (—) or P3 mouse myeloma antibody (— — -) released by osmotic minipumps. For an explanation of the tumor take ratio, see the legend to Chart 1.

7.2 to 7.5. The eluates were then dialyzed against 100 volumes of PBS and concentrated to one-tenth of the original cell volume. Finally, anti-colorectal carcinoma reactivity of the eluates was tested by RIA.

Evaluation of Antibody Activity in Sera, Ascitic Fluid, and Acid Eluates

The presence of monoclonal antibody either in the ascitic fluid or serum or in eluates of tumors and other tissues from mice treated with hybridoma antibody was determined by RIA as described (5).

RESULTS

ADCC Reactivity of Monoclonal Anti-Colorectal Carcinoma Antibody. The results of ADCC with Monoclonal Anti-Colorectal Carcinoma Antibody 1083-17-1A are shown in Table 1. The antibody mediated an ADCC reaction against 6 different colorectal carcinoma cell lines but not against melanomas, astrocytomas, lung carcinomas, or bronchogenic carcinomas. Normal human fibroblasts were also not lysed (results not shown). The susceptibility of the target cells to antibody-mediated cytotoxic reactions was demonstrated by their positive reaction to antibodies secreted by hybridoma 480-1-4. These antibodies nonspecifically lyse all cells of human origin.

In Vivo Tumor Growth Inhibition by Antibody 1083-17-1A.

The effectiveness of Antibody 1083-17-1A in suppressing the growth of colorectal carcinoma in nude mice is shown in Charts 1 to 3. In the first experiment (Chart 1), all mice inoculated with either melanoma (WM 9) or bronchogenic tumor (MBA 9812) cells and treated with Anti-Colorectal Antibody 1083-17-1A developed palpable masses by Day 10 after implantation. The masses grew rapidly, causing death in most of the animals from Day 35 on. The same large masses were observed in all animals inoculated with colorectal carcinoma (SW 948) cells and treated with the mouse myeloma antibody (P3). In contrast, none of the animals that received colorectal carcinoma cells and were treated for 18 consecutive days with anti-colorectal antibody showed palpable masses even by Day 35 of the experiment, i.e., 17 days after termination of the antibody treatment. From then on, it was possible to palpate small nodules first in 2 and then in all animals of this group. However, these nodules were much smaller than those observed in the control group, and their rate of growth was much slower (p < 0.001). The experiment had to be terminated on the 49th day because of the extremely poor health of the animals probably caused by daily handling (for 18 days) for injection of antibodies.

In the second experiment, 20 nude mice were inoculated with 1 x 10⁶ SW 948 colorectal carcinoma cells and were then treated with either Anti-Colorectal Antibody 1083-17-1A (10 mice) or P3 myeloma antibody (10 mice). Antibody treatment was performed essentially as described for the first experiment except that following the 18 daily injections, animals continued to be treated with antibody every second day. The results (shown in Chart 2) were that all mice treated with P3 antibody developed large palpable masses, 3 of the mice treated with the anti-colorectal hybridoma did not develop tumors at all, and the tumor growth in the remaining 6 was retarded in comparison to that in the control group (p < 0.05). Differences in tumor volume between the 2 groups were significant (p < 0.05 on each day of measurement). Again, excessive mortality of mice either because of massive tumor growth (in controls) or because of frequent handling (in both experimental mice and controls) led to termination of the experiment on the 45th day.

In the third experiment, 12 mice inoculated with colorectal carcinoma SW 948 received i.p. implants of osmotic pumps containing either anti-colorectal antibody (6 mice) or P3 mouse myeloma antibody (6 mice). Again, all control animals developed large tumors. Only 2 of the 6 mice in the experimental group developed small palpable nodules between Days 1 and 31 of the experiment. One additional mouse developed a nodule between then and Day 38 when the experiment had to be terminated. The differences between the 2 groups with respect to rate of tumor development and mean volume were statistically significant (p < 0.005 and < 0.05, respectively).

Antibody 1083-17-1A Activity in Nude Mouse Sera. Sera derived from tumor-bearing nude mice treated with Antibody 1083-17-1A showed binding activity in RIA against SW 948 cells approximately 3 times higher than the binding activity displayed by sera derived from control animals (Table 2).

Table 2

<table>
<thead>
<tr>
<th>Sera a,b from mice given injections of</th>
<th>Route of administration</th>
<th>Duration of treatment (days)</th>
<th>Hybridoma cpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW 948</td>
<td>i.p. c</td>
<td>10</td>
<td>1083-17-1A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P3</td>
</tr>
<tr>
<td>SW 948</td>
<td>Osmotic pumps d</td>
<td>2</td>
<td>1083-17-1A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>P3</td>
</tr>
<tr>
<td>MBA 9812</td>
<td>i.p. c</td>
<td>15</td>
<td>1083-17-1A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P3</td>
</tr>
</tbody>
</table>

a Tested by RIA.

b Serum pool from 5 mice/treatment group.

c Ascitic fluid (0.3 ml diluted 1:5) once daily starting 1 hr after tumor cell inoculation.

d First pump i.p. 1 hr after tumor cell inoculation. Second pump s.c. 7 days later. Pumping of antibody had been discontinued by Day 14.
that bind to tumor cells in vivo may inhibit tumor growth either in vitro (1). It is possible that monoclonal antibodies that bind to tumor cells in vivo may inhibit tumor growth either alone or in interaction with lymphocytes. Since the nude mice used in these experiments are athymic and deprived of mature T cells, it is probably the killer cells that participate in the ADCC reaction in vivo. A careful histological follow-up of tumors in antibody-treated and control mice may provide some information on the degree of host-derived cellular involvement at the tumor site.

Although nude mice are incapable of mounting a humoral antibody response to implanted colorectal carcinoma and although ascitic fluid produced by P3 cells does not inhibit tumor growth, it seems most probable that tumor growth suppression is mediated by monoclonal antibody.

These experiments do not provide specific information as to how late after tumor cell injection immunotherapy may be begun in order for suppression of tumor growth still to be successful. However, in one preliminary experiment which had to be terminated early because of high mortality of mice, hybridoma antibody did suppress tumor growth when first administered 7 days after tumor implantation (data not shown).

Tumor nodules were observed after tumor implantation in some of the hybridoma-treated mice. However, it is difficult based on the available experimental data to predict whether these nodules, which slowly increased in volume starting 25 days after tumor implantation, would have continued to grow progressively, as they did in animals treated with nonspecific antibodies, had the experiments not been terminated. In one experiment, cells obtained from a small mass were grown in tissue culture, and it was determined that these cells were still immunoreactive with Antibody 1083-17-1A.

**Antibody 1083-17-1A Activity in Acid Eluates from Tumors and Organs.** For mice treated with anti-colorectal carcinoma antibody, binding activity (as assayed in RIA on SW 948 cells) in eluates derived from colorectal carcinoma tumors was approximately 4 times higher than in eluates from lungs and kidneys. The binding activity of eluates obtained from control animals, i.e., those inoculated with melanoma cells and treated with anti-colorectal antibody and those inoculated with SW 948 cells and treated with P3 myeloma antibody, was also much lower than in eluates from colorectal tumors of mice treated with anti-colorectal carcinoma antibodies (Table 3).

**DISCUSSION**

Results of the experiments summarized in the preceding section indicate that the monoclonal antibody secreted by hybridomas obtained through fusion of mouse myeloma P3 cells with splenocytes of mice immunized with SW 1083 (7) colorectal carcinoma inhibits the growth of colorectal carcinoma SW 948 cells (7) implanted in nude mice. Hybridoma 1083-17-1A secretes IgG-1, which comes from the P3 parent, and IgG-2a, which is probably that antibody, produced by the splenocytes, that was found to bind in RIA and in the mixed hemadsorption assay to human colorectal carcinomas maintained in culture but not to 18 other human tumors or to 5 normal human fibroblasts tested (2, 6). Colorectal carcinoma cells exposed to Antibody 1083-17-1A were found to react positively in the immunofluorescence test (2). Furthermore, the antibody bound specifically to cells of 3 colorectal carcinomas removed from patients but not to adjacent intestinal mucosa removed from one of the same patients (2).

In the present study, Monoclonal Antibody 1083-17-1A mediated an ADCC reaction directed specifically against colorectal carcinoma cells (Table 1). It also seemed to bind specifically to colorectal carcinoma cells grown in nude mice but not to lung, kidney, or melanoma cells (Table 3). We were, therefore, not surprised to find that the growth of colorectal carcinoma tumors in nude mice is markedly affected by the presence of hybridoma antibody administered passively either by injection or through an osmotic pump.

The precise mechanism of this antitumor effect still remains unknown. Direct lysis in the presence of complement seems to be ruled out since Antibody 1083-17-1A does not show such an effect in vitro (1). It is possible that monoclonal antibodies that bind to tumor cells in vivo may inhibit tumor growth either alone or in interaction with lymphocytes. Since the nude mice used in these experiments are athymic and deprived of mature T cells, it is probably the killer cells that participate in the ADCC reaction in vivo. A careful histological follow-up of tumors in antibody-treated and control mice may provide some information on the degree of host-derived cellular involvement at the tumor site.

Although nude mice are incapable of mounting a humoral antibody response to implanted colorectal carcinoma and although ascitic fluid produced by P3 cells does not inhibit tumor growth, it seems most probable that tumor growth suppression is mediated by monoclonal antibody.

These experiments do not provide specific information as to how late after tumor cell injection immunotherapy may be begun in order for suppression of tumor growth still to be successful. However, in one preliminary experiment which had to be terminated early because of high mortality of mice, hybridoma antibody did suppress tumor growth when first administered 7 days after tumor implantation (data not shown).

Tumor nodules were observed after tumor implantation in some of the hybridoma-treated mice. However, it is difficult based on the available experimental data to predict whether these nodules, which slowly increased in volume starting 25 days after tumor implantation, would have continued to grow progressively, as they did in animals treated with nonspecific antibodies, had the experiments not been terminated. In one experiment, cells obtained from a small mass were grown in tissue culture, and it was determined that these cells were still immunoreactive with Antibody 1083-17-1A.

Although the results of this study may suggest an approach to the immunotherapy of human tumors, it should be emphasized that even if ADCC is responsible for the suppression of tumors in nude mice, it is not yet known whether mouse anti-colorectal antibody is capable of mediating ADCC with human rather than mouse killer cells. Also, the fact that the antibody adsorbs to a xenogeneic tumor graft in a nude mouse does not mean that it will also recognize a syngeneic tumor in a human host. Moreover, in contrast to the impaired immune response in nude mice, the immune response in a human host may counteract the effect of the tumor-specific antibody and prevent the antibody from reaching the target cells.

Although the use of nude mice has its obvious limitations, solutions to many of these problems may require investigations into the effect of hybridoma antibodies in a human host.

**ACKNOWLEDGMENTS**

The expert assistance of M. Prewett, R. Weiss-Wik, and L. Vitek is gratefully acknowledged. We thank Dr. Ron Copp, Pennsylvania Hospital, Philadelphia, for statistical analysis of the data.

**REFERENCES**

6. Koprowski, H., Steplewski, Z., Mitchell, K., Herlyn, M., Herlyn, D., and...


Inhibition of Growth of Colorectal Carcinoma in Nude Mice by Monoclonal Antibody

Dorothee M. Herlyn, Zenon Steplewski, Meenhard F. Herlyn, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/40/3/717

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.