Inhibition of Friend Leukemia Cell Visceral Metastases by a New Monoclonal Antibody and Role of the Immune System of the Host in Its Action

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ABSTRACT

We developed a syngeneic mouse IgG2a monoclonal antibody (MAb) A9D41 directed against the Friend leukemia virus envelope gp70 antigen present on the cell surface membranes of virus producer 3C18 Friend leukemia cells (FLC). A9D41 showed a marked antitumor activity in mice given injections of gp70 positive 3C18 FLC, but it was ineffective in mice given injections of gp70 negative 745 FLC or unrelated tumor cells. A9D41 was particularly effective in inhibiting the development of 3C18 FLC liver and spleen metastases. MAb was also effective as adjuvant therapy in inhibiting visceral metastases after excision of an established s.c. FLC tumor, and combined therapy of A9D41 with mouse interferon α/β was more effective than MAb or interferon α/β alone. The immune system of the host played a decisive role in the antitumor action of A9D41. Thus, although MAb was cytotoxic for 3C18 FLC in vitro in the presence of rabbit complement, the F(ab')2 fragment was ineffective in vitro, and the antitumor effect of MAb was abolished in mice treated with an antibody to CD4 and diminished in natural killer cell-deficient beige and athymic nude mice. MAb-treated mice surviving injection of FLC developed an immune response to 3C18 FLC.

INTRODUCTION

In the course of our studies on the tumorigenicity and metastatic capacity of different lines of FLC (1-3) we observed that antibodies to FLC could be detected in the sera of mice given injections of low tumorigenic FLC, whereas antibody was not detected in the sera of mice given injections of highly metastatic FLC. Furthermore, we showed that the development of antibodies to FLC surface antigens was an important part of the IFN α/β-induced suppression of FLC visceral metastases (4). To pursue these studies on the therapeutic usefulness and the mechanisms of action of antibody to FLC, we isolated and characterized a mouse MAb A9D41 capable of recognizing Friend viral gp70 antigens expressed on the cell membranes of Friend virus producer FLC. We show, herein, that this MAb exerts a target cell specific antitumor effect in syngeneic DBA/2 mice, and is particularly effective in inhibiting the development of FLC metastases in the liver and spleen, even after i.v. injection of large numbers of FLC. The results suggest that several mechanisms of action may be important. Thus, although A9D41 MAb was directly cytotoxic for virus producer FLC in the presence of complement in vitro, an intact immune system also proved essential in achieving an optimal therapeutic effect, and MAb-protected, FLC-injected mice developed an immune response to FLC. These results may be germane to the therapeutic use of monoclonal antibodies in patients.

MATERIALS AND METHODS

Mice

DBA/2 mice were obtained from the pathogen-free breeding colonies of either Charles River Italia (Milan) or the Institut de Recherches Scientifiques sur le Cancer (Villejuif). DBA/2 athymic nu/nu mice were obtained from a colony maintained at the Institut Curie (Orsay, France). Breeding pairs of DBA/2 J-CO bg+/bg beige (bg/bg) and heterozygote +/bg mice were obtained through the courtesy of Dr. G. Carlson (The Jackson Laboratory, Bar Harbor, ME). A colony of bg/bg and +/bg was then raised and maintained at Villejuif, France. These mice were shown in our laboratory to be deficient in natural killer cell activity.

Tumor Cells

The origin of IFN α/β-sensitive Friend leukemia virus-negative 745 FLC and IFN-resistant Friend leukemia virus-positive 3C18 FLC has been described (5, 6). The ESb lymphoma, a spontaneous metastatic variant of a methylchloranthrene-induced T-cell lymphoma (7), was obtained from V. Schirrmacher (Heidelberg, Germany). Sp2/0.Ag8 mouse myeloma and hybridoma clones (8), as well as all cell lines described herein, were cultured in RPMI 1640 medium supplemented with antibiotics, l-glutamine, and 10% fetal calf serum, using standard conditions for cells in suspension.

Quantitative Estimation of Number of FLC

Peritoneum. Mice were killed and the peritoneal cavity was washed with 3 ml of cold RPMI medium containing 10% fetal calf serum. The total number of cells recovered from each mouse was estimated by colony formation in agarose (9).

Liver. The techniques for estimating the number of FLC in the liver by colony formation have been previously described in detail (10).

IFN and Control Preparations

Mouse IFN α/β was prepared from suspension cultures of mouse sarcoma C243 cells inoculated with Newcastle disease virus. The methods of production, partial purification, and assay have been described (11). IFN was assayed by inhibition of cytopathic effect of vesicular stomatitis virus on L-cells in monolayer cultures in Falcon microplates. Units are expressed in mouse reference units. The specific activity of partially purified IFN was approximately 2 × 10^5 units/mg protein.

Immunization Protocol and Hybridoma Production

Murine splenocytes were obtained by immunizing 8-week-old male DBA/2 mice with in vitro-passaged low tumorigenic 3C18 FLC. Mice were inoculated i.p. once weekly for 3 weeks with 10^5 FLC. In the course of the immunization experiments we observed that daily treatment of FLC-injected DBA/2 mice for 2 weeks with mouse IFN α/β (10^4 units/mouse/day) resulted in a 3- to 4-fold greater antibody response to FLC than was observed in untreated FLC-injected mice. For hybridoma production we therefore used spleens from IFN α/β-treated FLC-immunized mice. Murine splenic B-lymphocytes were fused with
Sp2/01-Ag8 (a nonsecreting myeloma cell line) as previously described were screened for the presence of anti-FLC antibodies by radioimmunoassay and immunoblotting techniques. Ten-twiced-cloned cell lines exhibiting similar levels of anti-FLC antibody production were obtained after selective screening for anti-FLC-positive cultures. The immunoglobulin class was determined by double immunodiffusion in agarose.

Purification and 125I-labeling of A9D41 MAb

Hybridoma cells were injected i.p. into BALB/c nude mice. Twenty days after cell injection, the ascitic fluids were harvested and the MAb was partially purified by ammonium sulfate precipitation. The protein concentration of this MAb preparation was 10 mg/ml. In most of the experiments to be described, we have used this partially purified MAb preparation. Some experiments were performed with the use of a highly purified MAb preparation. Briefly, the partially purified material was first passed through a G-25 Sephadex column in a 20 mM Tris-Cl buffer (pH 7.7). Two ml of the G-25 eluate containing the protein fraction was run on a Mono-Q anion exchange column by fast protein liquid chromatography (Pharmacia). The immunoglobulin fraction was eluted with a NaCl gradient (0–0.5 M) and the IgG peak was recovered at approximately 0.2 M NaCl. The purified MAb was 125I-labeled by the chloramine-T method (12) with a 20-s reaction time. The labeled antibody was separated from the reaction products by purification on a PD-10 prepacked column (Pharmacia).

Monoclonal Antibodies

Besides the A9D41 MAb, 3 control immunoglobulins were used: a highly purified BALB/c myeloma IgG2a MOPC (obtained from Dr. G. Bordenave, Institut Pasteur, Paris, France); a partially purified MOPC, IgG2a (obtained from Dr. M. Stanislawsky, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France); and a rat MAb R-FLC. This MAb was produced by a cloned hybridoma cell line obtained after fusion of spleen cells from a rat immunized with in vivo-passaged FLC with a rat myeloma cell line. This MAb recognized a M, 60,000 protein expressed on the cell membrane of in vivo-passaged 3C18 and 745 FLC. The rat hybridoma cells [MAb GK1-5 (13)] producing MAb to CD4 were provided by Professor G. Forni (Institute of Microbiology, University of Turin, Italy). The immunoglobulin fraction of ascitic fluids was partially purified by ammonium sulfate precipitation. DBA/2 mice given injections i.v. of 500 µg of the anti-CD4 immunoglobulin resulted in an apparently complete depletion of CD4-positive spleen cells, 6–9 days after inoculation of antibody, as determined by flow cytometric analysis with the use of an FITC-labeled anti-CD4 MAb. The immunoglobulin fraction of ascitic fluids of the rat hybridoma cells [MAb 53–6.7 (14)] producing MAb to CD8 was separated by ammonium sulfate precipitation and further purified on a protein G column (Pharmacia, Upsala, Sweden). Injection i.p. of 1 mg of anti-CD8 immunoglobulin into DBA/2 mice resulted in an apparently complete depletion of CD8-positive spleen cells when tested 8 days thereafter as determined by flow cytometric analysis, using an FITC-conjugated anti-CD8 MAb.

Preparation of F(ab')2

The F(ab')2 fragment from highly purified A9D41 was prepared by papain digestion (15) with subsequent separation on a protein A Sepharose column, followed by separation on an anionic exchange column by Dr. C. Gugliemetti (Biosys, Compiègne, France). The purity of the F(ab')2 was determined by sodium dodecyl-sulfate-polyacrylamide gel electrophoresis.

Titration of Antibodies to FLC in Sera of FLC-injected DBA/2 Mice

Titration of antibodies to 3C18 FLC was performed by a radioimmunoassay, using a 125I-labeled anti-mouse immunoglobulin, as described in detail elsewhere (4).

Complement-mediated Cytotoxicity

Complement-mediated cytotoxicity was assessed in a radioactive chromium release assay. FLC from cell cultures were radioiodelabeled by incubation of 5 x 10⁶ FLC with 200 µCi of sodium [125I]chromate (Amersham) for 2 h at 37°C in 0.8 ml RPMI medium plus 5% fetal calf serum and then washed extensively. Labeled target cells (1 x 10⁶) were incubated in round-bottomed microplates (Nunclon, Roskilde, Denmark) with serial 2-fold dilutions of MAb in 0.15 ml total volume at 4°C for 1 h and subsequently with a 1:30 dilution of rabbit complement (Low Tox-M, Cederlane Laboratories, Ontario, Canada) for 30 min at 37°C; 0.075 ml of supernatant was collected at the end of the incubation period and counted for 3γ-radioactivity. Base-line release determined in the presence of complement was always less than 15%. The antibody titer was determined as the last serum dilution which gave at least 10% specific cytotoxicity.

Fluorescence-activated Cell Sorter Analysis

Cell pellets (1 x 10⁶ cells) were treated with 50 µl of partially purified A9D41 MAb (20 µg/ml in PBS), incubated at 4°C for 30 min, and washed 3 times. These cells were then incubated with a FITC-labeled goat anti-mouse immunoglobulin antibody (20 µg/ml) (GAM-F, Cappel, Westchester, PA) at 4°C for 30 min. After appropriate washings, the cells were incubated with propidium iodide (Calbiochem, San Diego, CA). The cell suspension (5 x 10⁶ cells/ml) was analyzed on a bench-top flow cytometer (FACScan, Becton Dickinson, Mountain View, CA). Fluorescence measurement was determined and compared in homogeneously defined (per cell size and/or DNA content) cell populations. Dead cells stained with propidium iodide were excluded from the analysis. Fluorescence signals were collected in logarithmic mode and relative cell number per channel in linear mode. For each cell sample, the fluorescence histogram obtained with an irrelevant IgG2a monoclonal antibody was used as a control to discriminate between negative and positive cells.

Western Blot Analysis of FLC Membrane Proteins Recognized by Anti-FLC Antibodies

Cell plasma membrane fractions were obtained by using previously described techniques (2, 16). Aliquots of Nonidet P-40 membrane fractions corresponding to 10⁶ cells were treated according to the method of Laemmli (17) and loaded onto a 10% polyacrylamide sodium dodecyl sulfate slab gel. After electrophoresis, proteins were transferred onto nitrocellulose membranes (Bio-Rad) for 4 h at 250 mA. Nitrocellulose membranes were saturated by incubation with 2% BSA in PBS for 1 h at room temperature. Strips were incubated overnight at 4°C with different sera diluted 1:100 in PBS containing 2% BSA. After washing with PBS containing 0.2% BSA and 0.1% Nonidet P-40, the strips were incubated with 1 µCi/ml 125I-labeled anti-mouse immunoglobulin F(ab')2, for 2–4 h at room temperature. After washing, the nitrocellulose strips were wrapped in Saran wrap and exposed at −80°C for 2 to 12 h with Fuji-ray film, using an intensifying screen.

Statistical Analysis

Within each experimental group, the one-way variance analysis test was used after verification of homogeneity of the variances by Bartlett's test, and subsequently the means were compared by using Duncan's and/or Tukey's test. When necessary, an inverse transformation of the survival times was performed to homogenize the variances.

RESULTS

Importance of Antibody Response to Tumor-associated Antigens in Suppression of Friend Leukemia Cell Growth in Mice

DBA/2 mice given injections i.p. of in vitro-passaged low tumorigenic 3C18 FLC were resistant to an i.p. challenge with highly tumorigenic in vivo-passaged 3C18 FLC (Fig. 1), but not
to unrelated L1210 lymphoma cells (data not shown). Kinetic experiments showed that it was necessary to preinject in vivo-passaged FLC into DBA/2 mice at least 7 days before challenge to obtain a protective effect (data not shown). Low levels of antibodies to FLC detected by radioimmune assay were present in the sera of mice given injections of in vitro-passaged FLC as early as 7 to 10 days after FLC injection (anti-FLC titer 1:40) and higher titers (i.e., 1:320–1:640) were found at 14 and 21 days.

To ascertain the relevance of these anti-FLC antibodies in the protection of FLC-immunized mice to FLC challenge, we determined the antitumor activity of sera from FLC-immunized mice in standard Winn assays. As shown in Fig. 2, sera from 3 different FLC-immunized mice exhibited a clear-cut inhibitory effect on the development of s.c. 3C18 FLC tumors as compared with control FLC-injected mice. These sera did not exert any effect when injected together with L1210 or RBL5 tumor cells (data not shown). Sera from normal DBA/2 mice did not exert any anti-FLC activity (Fig. 2).

Characterization of the anti-FLC antibodies by Western blot analysis revealed a strong reactivity to 3C18 FLC membrane proteins in the M, 65,000–85,000 region of the gel (Fig. 3). No specific reactivity was found with membrane proteins from mouse RBL5 lymphoma cells, human erythroleukemia K562 cells, or normal DBA/2 splenocytes (Fig. 3).

Isolation and Characterization of Monoclonal Antibody A9D41 against FLC gp70 Surface Antigen

The data cited in the preceding paragraphs suggested that antibodies to specific FLC surface antigens were important in the resistance of immunized DBA/2 mice to challenge with highly tumorigenic 3C18 FLC. We attempted, therefore, to obtain monoclonal antibodies to specific tumor-associated FLC antigens in order to test their antitumor activity. From 10 twice-cloned cell lines secreting anti-FLC antibody, a hybridoma cell line A9D41 (IgG2a) was isolated and characterized.

We investigated the reactivity of the A9D41 MAb for different FLC lines by fluorescence-activated cell sorter analysis. Fig. 4 shows that the A9D41 MAb exhibited a clear-cut reactivity on both in vitro-passaged virus producer 3C18 and 745 FLC (Fig. 4, A and C), as well as on Friend virus producer in vivo-passaged 3C18 FLC (Fig. 4B). However, the A9D41 MAb did not react with a 745 FLC line (Fig. 4D) previously selected and characterized as a Friend virus nonproducer FLC variant (6). The A9D41 MAb did not react with other tumor cell lines (mouse ESB, RBL-5, L1210, and L929 cells, or human K562 cells) or with normal mouse cells (i.e., peritoneal macrophages, thymocytes, or spleen cells) (data not shown). These results suggested that the MAb reacted with a Friend viral antigen on virus producer FLC.

We next compared by Western blot the reactivity of a polyclonal goat antibody to gp70, the sera from DBA/2 mice immunized with 3C18 FLC, and A9D41 MAb, with membrane proteins from virus producer and virus nonproducer FLC. As can be seen in Fig. 5, all 3 immunoglobulins reacted with membrane proteins from gp70-positive 3C18 FLC (Fig. 5, Lanes 1–3). The A9D41 reacted with 2 bands in the M, 65,000–85,000 region of the gel (Fig. 5, Lane 3), which were not clearly separated in the Western blot patterns with the other sera. It has previously been shown that the 2 bands in the M, 65,000–85,000 region constitute the gp70 antigen and the precursor protein with a molecular weight of 85,000 (6). In contrast, both the goat antibody to gp70 antigen and A9D41 MAb were negative when tested on the gp70-negative 745 FLC (Fig. 5, Lanes 4 and 6). These results indicated that both the goat anti-gp70 antibody and the A9D41 MAb recognized the gp70 Friend virus antigen. In contrast, sera from mice immunized with 3C18 FLC reacted with M, 65,000 protein(s) from cell membranes of gp70-negative FLC (Fig. 5, Lane 5), suggesting that these sera recognized both the gp70 antigen (Fig. 5, Lane 2) as well as a cell membrane antigen present on virus nonproducer FLC (Fig. 5, Lane 5).

As shown in Fig. 6 the reactivity of the purified 125I-labeled A9D41 MAb to 3C18 FLC plasma membranes was abrogated by an excess of polyclonal antibody to gp70, whereas a control goat serum did not inhibit this reactivity.

Antitumor Effects of A9D41 MAb in Mice Given Injections of FLC

Inhibition of Development of FLC Tumors Implanted s.c. and i.p.

Injection (s.c.) of gp70-positive 3C18 FLC resulted in rapid tumor growth, visceral métastases, and death in all control mice (Table 1). When mice were given injections of 3C18 FLC mixed with A9D41 MAb, tumor growth was observed in only 3 of 6 mice (the tumor also grew more slowly in these mice) and there was an increased survival time (Table 1). A control rat MAb (R-FLC) directed against a FLC M, 60,000 antigen did not inhibit tumor growth and did not increase survival time (Table
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Fig. 3. Western blot analysis of sera from DBA/2 mice given injections of in vitro-passaged 3C18 FLC. Sera were harvested from FLC-immunized mice on day 21. Pools of sera were analyzed by Western blot analysis by using plasma membrane fractions from different cell types, as described in “Materials and Methods.” Molecular weight standards were as follows: lysozyme (M, 14,400); soybean trypsin inhibitor (M, 21,500); carbonic anhydrase (M, 31,000); ovalbumin (M, 45,000); bovine serum albumin (M, 66,200); phosphorylase B (M, 92,500).

Control Mice

FLC-immunized Mice

Membranes from:

FLC

RBL-5

K-562

Spleen Cells (DBA/2)

Fig. 4. Fluorescence-activated cell sorter analysis of different FLC lines by indirect immunofluorescence staining with A9D41 MAb. Fluorescence-activated cell sorter analysis was performed as described in “Materials and Methods.”

IgG2a MAb (MOPC12) was ineffective. A9D41 was even effective when administered 2 days after FLC inoculation (Table 2, Experiment 2). In Experiment 1 (Table 2), other mice treated with MAbs or left untreated were sacrificed 6 and 10 days after FLC inoculation. There were far fewer FLC in the liver and spleen of mice treated with A9D41 than in these organs in the 2 groups of control mice (Fig. 8). At 10 days, for example, there was more than a 100,000-fold difference between the number of FLC in the livers of control and MAb-treated mice (Fig. 8). Thus, the increase in the survival time of mice given injections of 3C18 FLC and treated with A9D41 correlated with the inhibition of FLC multiplication in the liver and spleen.

When mice were given injections i.v. of a greater number of FLC (i.e., 2 x 10^6 FLC) and treatment with A9D41 MAb was initiated 4 days later, no increase in survival time was noted (Table 2, Experiment 3) and there was no inhibitory effect on the multiplication of FLC in the liver (data not shown).

We determined the tumor target cell specificity of A9D41 MAb for 3C18 FLC injected i.v. A9D41 MAb did not increase the survival time of DBA/2 mice given injections i.v. of either 1). A9D41 MAb did not inhibit the development of gp70-negative 745 FLC tumors and did not increase mouse survival time (Table 1).

Injection of A9D41 0.5 h after injection of 3C18 FLC also resulted in a marked inhibition of the multiplication of tumor cells in the peritoneum (Fig. 7). In this experiment, the mean survival time of another group of control MOPC12-treated mice was 20.8 ± 1.1 days and 32.5 ± 4.6 days for A9D41 MAb-treated mice. In a second experiment, an inhibitory effect on the multiplication of FLC was observed even 2 days after inoculation of FLC (Fig. 7).

Inhibition of Visceral Metastases after i.v. Injection of 3C18 FLC. In vivo-passaged FLC are highly metastatic to the liver and spleen when injected i.v. and approximately 4 FLC constitute a 50% lethal dose (10). As it has been suggested that passive transfer of antibody to tumor antigens was more effective against systemic disease than against local tumor growth (18, 19), we determined the antitumor effect of A9D41 MAb after i.v. inoculation of FLC. As shown in Table 2, i.v. injection of highly purified A9D41 MAb increased the survival time of DBA/2 mice given injections i.v. of 3C18 FLC. An irrelevant 66 kD-

gp 70+ FLC

gp 70- FLC

Fig. 5. Reactivity of the polyclonal antibody to gp70, the sera from FLC-immunized mice, and the A9D41 MAb to cell membrane fractions from gp70-positive and gp70-negative FLC (Western blot analysis). Western blot analysis was performed by using cell membrane fractions from gp70-positive FLC (in vitro-passaged 3C18 FLC) and from gp70-negative in vivo-passaged 745 cells (6). Western blot assay for the FLC gp70 was performed as previously described (6). Goat anti-gp70 (Lanes 1 and 4); sera from mice immunized with in vitro-passaged 3C18 FLC (Lanes 2 and 5); mouse A9D41 MAb (Lanes 3 and 6). Molecular weight standards were as follows: lysozyme (M, 14,400); soybean trypsin inhibitor (M, 21,500); carbonic anhydrase (M, 31,000); ovalbumin (M, 45,000); bovine serum albumin (M, 66,200); phosphorylase B (M, 92,500).
gp70-negative 745 FLC or syngeneic ESb tumor cells which also metastasize to the liver (Table 3).

**Dose Response of A9D41 MAb in Inhibition of FLC Visceral Metastases.** It was of interest to determine the relationship between the number of FLC injected i.v., the amount of A9D41 injected, and the resulting increase in survival time. DBA/2 mice were given injections of $2 \times 10^3$, $2 \times 10^4$, and $2 \times 10^5$ 3C18 FLC and 24 h later were given injections i.v. of 145, 36.2, 9.1, or 2.2 µg of highly purified A9D41 MAb. The antitumor efficacy of MAb depended on the number of FLC and the amount of MAb injected (Fig. 9). Thus, after injection of $2 \times 10^3$ FLC, only a slight increase in survival time was noted in mice given injections of 145 µg of MAb, whereas a clear-cut effect was noted at 36.2 µg in mice given injections of $2 \times 10^4$ FLC. In mice given injections i.v. of $2 \times 10^6$ FLC, even 2.2 µg proved effective, and all the mice given injections of 145 µg survived. In each group there was a clear-cut dose response.

**Table 1 Effects of A9D41 MAb on development of s.c. tumors in mice given injections of 3C18 or 745 FLC**

<table>
<thead>
<tr>
<th>FLC line</th>
<th>Presence of gp70 surface antigen</th>
<th>MAb</th>
<th>No. of mice with tumor/total no. of mice</th>
<th>Day of death (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3C18</td>
<td>+</td>
<td>A9D41</td>
<td>6/6</td>
<td>27.0 ± 2.5</td>
</tr>
<tr>
<td>3C18</td>
<td>+</td>
<td>A9D41</td>
<td>3/6</td>
<td>48.4 ± 5.6</td>
</tr>
<tr>
<td>3C18</td>
<td>+</td>
<td>R-FLC</td>
<td>6/6</td>
<td>28.2 ± 2.2</td>
</tr>
<tr>
<td>745</td>
<td>-</td>
<td>A9D41</td>
<td>6/6</td>
<td>26.5 ± 1.8</td>
</tr>
<tr>
<td>745</td>
<td>-</td>
<td>A9D41</td>
<td>6/6</td>
<td>25.8 ± 1.2</td>
</tr>
</tbody>
</table>

* P < 0.001.
*NS, not significant.

**Table 2 Antitumor activity of A9D41 MAb in mice given injections i.v. of 3C18 FLC**

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>MAb</th>
<th>Time of MAb injection</th>
<th>No. of mice with tumor/total no. of mice</th>
<th>Mean day of death ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>30 min before FLC</td>
<td>4/4</td>
<td>12.5 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>MOPC</td>
<td>30 min before FLC</td>
<td>4/4</td>
<td>11.5 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>A9D41</td>
<td>30 min before FLC</td>
<td>4/4</td>
<td>24.0 ± 1.6</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>48 and 120 h after FLC</td>
<td>8/8</td>
<td>11.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>A9D41</td>
<td>48 and 120 h after FLC</td>
<td>8/8</td>
<td>14.5 ± 0.5</td>
</tr>
<tr>
<td>3</td>
<td>BSA</td>
<td>4 days after FLC</td>
<td>4/4</td>
<td>7.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>A9D41</td>
<td>4 days after FLC</td>
<td>4/4</td>
<td>8.0 ± 0.0</td>
</tr>
</tbody>
</table>

*Seven-week-old female DBA/2 mice were given injections i.v. of 174 µg of highly purified A9D41 MAb or 166 µg of a control IgG2a MAb (MOPC), or left untreated. Thirty min after MAb injection, mice were inoculated i.v. with $2 \times 10^3$ 3C18 FLC.
*NS, not significant.
* P < 0.001.

**Fig. 7. Inhibition of the multiplication of FLC in the peritoneum of mice treated with A9D41 MAb.** Experiment 1, 7-week-old male DBA/2 mice were given injections i.p. of $2 \times 10^6$ 3C18 FLC, and then given injections i.p. 0.5 h and 24 h later of 448 µg of partially purified A9D41 MAb (•) or the same amount of an unrelated IgG2a (O). At 7 days the peritoneal cavity was washed and the total number of FLC was determined by colony formation in agarose (see "Materials and Methods"). There were 5 mice/group. Experiment 2, 7-week-old female DBA/2 mice were given injections of $5 \times 10^4$ 3C18 FLC, and 0.5 h later were given injections of 448 µg of partially purified A9D41 MAb (•) or BSA (O). There were 4 mice/group, and the total number of FLC was determined 2 and 7 days after tumor inoculation. Each symbol corresponds to an individual mouse.
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A9D41 MAb was also effective in inhibiting the development of FLC metastases, we determined whether MAb was of benefit as adjuvant therapy with or without IFN α/β.

DBA/2 mice were given injections s.c. of 3C18 FLC. When tumor nodules were palpable (about 1 cm in diameter) at 8 or 9 days, the primary s.c. tumor was excised and the mice were distributed into 4 groups: no further treatment; A9D41 MAb; IFN α/β; or A9D41 MAb and IFN α/β. As can be seen from Fig. 10, MAb and IFN α/β were each effective alone as adjuvant therapy in increasing the survival time of FLC-injected mice, but the combination was the most effective therapy.

Studies Designed to Explore Mechanisms of Action of A9D41 MAb in Inhibiting Visceral Metastases

A9D41 MAb Shows Complement-dependent Cytotoxicity for FLC. As shown in Fig. 11 A, highly purified A9D41 MAb was cytotoxic for 3C18 FLC in the presence of rabbit complement.

regardless of the number of FLC injected. (The regression line for each group was significant, P < 0.05.)

Use of A9D41 MAb as Adjuvant Therapy with or without Mouse IFN α/β after Excision of Primary s.c. Tumor. We have previously shown that mouse IFN α/β was very effective as adjuvant therapy in inhibiting the development of FLC visceral metastases after excision of the primary s.c. tumor (20).

Fig. 8 Estimation of the number of FLC in the liver and spleen of DBA/2 mice treated with A9D41 MAb. DBA/2 mice were treated with A9D41 MAb (•, ○), MOPC (□, □), or left untreated (□, □) and given injections i.v. of 3C18 FLC. The experimental conditions are described in the legend to Table 2, Experiment 1. Six and 10 days after inoculation of FLC, 3 mice in each group were sacrificed and the liver (•, ○) and spleen (□, □) were treated with collagenase and the cell suspension was plated in agarose to determine the number of FLC colonies (see "Materials and Methods")

Table 3 Target cell specificity of A9D41 MAb for 3C18 FLC

Experiment 1. 8-week-old female DBA/2 mice were given injections i.v. of 763 μg of partially purified A9D41 or left untreated, and 0.5 h later were given injections of 7.2 × 10⁴ 3C18 FLC or 7 × 10⁴ ESB cells. Experiment 2. 7-week-old female DBA/2 mice were given injections i.v. of 174 μg of highly purified A9D41 or left untreated, and 0.5 h later were given injections i.v. of 2.5 × 10⁵ 745 FLC.

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Tumor cell injected</th>
<th>Treatment</th>
<th>No. of mice with tumor/total no. of mice</th>
<th>Mean day of death ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3C18 FLC (gp70 positive)</td>
<td>None</td>
<td>3/3</td>
<td>13.0 ± 0</td>
</tr>
<tr>
<td>2</td>
<td>ESB</td>
<td>None</td>
<td>3/3</td>
<td>9.0 ± 0</td>
</tr>
<tr>
<td>2</td>
<td>3C18 FLC (gp70 negative)</td>
<td>A9D41</td>
<td>6/6</td>
<td>23.4 ± 1.8</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>A9D41</td>
<td>6/6</td>
<td>8.2 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>A9D41</td>
<td>5/5</td>
<td>13.4 ± 0.2</td>
</tr>
</tbody>
</table>

Fig. 9 Efficacy of A9D41 MAb in inhibiting 3C18 FLC metastases as related to the number of FLC and the amount of MAb injected. Seven-week-old male DBA/2 mice were given injections i.v. of 2.5 × 10⁵ 3C18 FLC (○), 2 × 10⁵ 3C18 FLC (□), or 2 × 10⁴ 3C18 FLC (▲); 24 h later, different amounts of highly purified MAb were injected i.v. There were 4 mice/group. The symbols indicate the mean day of death ± SE. When the SE is not shown, it lies within the symbol. All dead mice had FLC tumors in the liver and spleen. The experiment was terminated 75 days after tumor inoculation. At this time the only surviving mice were in the group receiving 2 × 10⁵ FLC (▲). There were 4 of those surviving mice receiving 145 μg of MAb; 2 of those mice receiving 36.2 μg; 1 of those mice receiving 9.1 μg; and 0 of those mice receiving 2.2 μg. None of these surviving mice had tumors at 75 days.

Fig. 10. Use of A9D41 MAb with or without IFN α/β as adjuvant therapy after excision of the primary s.c. FLC tumor. Seven-week-old male DBA/2 mice were given injections s.c. of 2 × 10⁴ 3C18 FLC. At 8 and 9 days the primary s.c. tumor was excised as previously described (20) and mice were distributed into the 4 groups as indicated. There were 10 mice/group. Treatment was as follows: untreated (x); A9D41 MAb, 766 μg of partially purified immunoglobulin injected i.v. every 7 days for 4 weeks (○); mouse IFN α/β, 2.4 × 10⁴ units injected i.p. 3 times/week for 4 weeks (□); combined MAb and mouse IFN α/β, same schedule as above (▲). All dead mice were autopsied and all had tumor metastases in the liver and spleen.

Fig. 11 A Efficacy of A9D41 MAb in inhibiting 3C18 FLC metastases as related to the number of FLC and the amount of MAb injected. Seven-week-old male DBA/2 mice were given injections i.v. of 2.5 × 10⁵ 3C18 FLC (○), 2 × 10⁵ 3C18 FLC (□), or 2 × 10⁴ 3C18 FLC (▲); 24 h later, different amounts of highly purified MAb were injected i.v. There were 4 mice/group. The symbols indicate the mean day of death ± SE. When the SE is not shown, it lies within the symbol. All dead mice had FLC tumors in the liver and spleen. The experiment was terminated 75 days after tumor inoculation. At this time the only surviving mice were in the group receiving 2 × 10⁵ FLC (▲). There were 4 of those surviving mice receiving 145 μg of MAb; 2 of those mice receiving 36.2 μg; 1 of those mice receiving 9.1 μg; and 0 of those mice receiving 2.2 μg. None of these surviving mice had tumors at 75 days.
was terminated at 60 days. 2 of 5 MAb-treated mice were alive without tumor. Then given injections i.v. of 560 fig of partially purified A9D41 MAb or left untreated 0.5 h prior to i.v. injection of 8 x 10^3 C18 FLC. When the experiment was terminated at 60 days, one MAb-treated mouse was still alive without tumor. DBA/2 mice were given injections i.v. of anti-CD, (1 mg/mouse) 3 days prior to injection of FLC. Mice were 3, 8-week-old female DBA/2 mice were given injections i.v. of anti-CD4 (500 »ig/mouse) 30 min before or 2.5 h after i.v. injection of 7 x 10^3 C18 FLC. Experiment 2, 8-week-old male or female DBA/2 mice were given injections i.v. of 10^3 C18 FLC and 0.5 h later were given injections i.v. of highly purified A9D41 MAb or left untreated. By radioimmunoassay, preincubation of 3C18 FLC with the F(ab')2 blocked the binding of intact A9D41 MAb. A9D41 MAb was not cytotoxic for FLC in the absence of rabbit complement. We prepared by pepsin digestion a F(ab')2 fragment from the A9D41 MAb was not cytotoxic for 3C18 FLC in vitro in the presence of rabbit complement (data not shown). As shown in Fig. 11B, partially purified or highly purified A9D41 MAb (data not shown) did not inhibit the multiplication of 3C18 FLC in vitro in the presence of complement. Purified F(ab')2 of A9D41 Is Inactive in Vivo. As can be seen from Table 4, the F(ab')2 of A9D41 MAb did not exert any anti-FLC activity in mice, whereas the intact MAb was highly effective.

Decreased Efficacy of A9D41 MAb in Immunodeficient Mice. The finding that the F(ab')2 was ineffective in mice suggested the possibility that the immune system of the host participated in the inhibitory effect of A9D41 MAb on the development of FLC visceral metastases. Accordingly, we tested the efficacy of MAb in immunodeficient mice given injections i.v. of 3C18 FLC. As can be seen in Table 5, the MAb was effective in bg/bg and nu/nu mice but less so than in heterozygote bg/+ and nu/+ mice (Table 5, Experiments 1 and 2). Treatment of mice with anti-CD4 antibody markedly reduced the efficacy of MAb in one experiment (Table 5, Experiment 3), and completely abolished its effect in a second experiment (data not shown). By radioimmunoassay it could be shown that antibody to CD4 did not compete with the binding of A9D41 MAb to 3C18 FLC (data not shown). Treatment with anti-CD8 antibody inhibited somewhat the action of MAb in this experiment, but to a much lesser extent than did the antibody to CD4.

A9D41 MAb-treated, 3C18 FLC-injected mice Develop Immune Response to 3C18 FLC. As shown in Fig. 11A, partially purified or highly purified A9D41 MAb (data not shown) did not inhibit the multiplication of 3C18 FLC in vitro in the presence of complement. As can be seen from Table 4, the F(ab')2 of A9D41 MAb did not exert any anti-FLC activity in mice, whereas the intact MAb was highly effective.

Experiment 1, 4-month-old female bg/bg or bg/dba/2 mice were given injections i.v. of 180 μg of highly purified A9D41 MAb 30 min before or 2.5 h after i.v. injection of 7 x 10^3 C18 FLC. Experiment 2, 8-week-old male or female DBA/2 mice were given injections i.v. of 10^3 C18 FLC and 0.5 h later were given injections i.v. of 460 μg of partially purified A9D41 MAb or MOPC, Experiment 3, 8-week-old female DBA/2 mice were given injections i.v. of anti-CD4 (500 μg/mouse) or anti-CD8 (1 mg/mouse) 3 days prior to injection of FLC. Mice were then given injections i.v. of 560 μg of partially purified A9D41 MAb or left untreated 0.5 h prior to i.v. injection of 8 x 10^3 C18 FLC. When the experiment was terminated at 60 days, 2 of 5 MAb-treated mice were alive without tumor. Days

Table 4 F(ab')2 of A9D41 MAb does not protect DBA/2 mice given injections i.v. of 3C18 FLC

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice with tumor/total no. of mice</th>
<th>Mean day of death ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>5/5</td>
<td>11.8 ± 0.2</td>
</tr>
<tr>
<td>F(ab')2</td>
<td>5/5</td>
<td>12.4 ± 0.4</td>
</tr>
<tr>
<td>A9D41 MAb</td>
<td>9/10</td>
<td>24.9 ± 3.1</td>
</tr>
</tbody>
</table>

* NS, not significant.  
* P < 0.001.

Table 5 Efficacy of A9D41 MAb in immunodeficient mice

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Group</th>
<th>Treatment</th>
<th>No. of mice with tumor/total no. of mice</th>
<th>Mean day of death ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>bg/+</td>
<td>None</td>
<td>3/3</td>
<td>12.0 ± 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A9D41</td>
<td>6/6</td>
<td>23.2 ± 2.7</td>
</tr>
<tr>
<td>2</td>
<td>bg/bg</td>
<td>None</td>
<td>3/3</td>
<td>10.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A9D41</td>
<td>6/6</td>
<td>17.7 ± 0.9</td>
</tr>
<tr>
<td>2</td>
<td>nu/+</td>
<td>MOPC,</td>
<td>4/4</td>
<td>11.8 ± 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A9D41</td>
<td>4/4</td>
<td>23.0 ± 0.7</td>
</tr>
<tr>
<td>3</td>
<td>nu/nu</td>
<td>MOPC,</td>
<td>3/3</td>
<td>10.6 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A9D41</td>
<td>3/3</td>
<td>17.7 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>None</td>
<td>6/6</td>
<td>12.0 ± 0</td>
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<td></td>
<td></td>
<td>A9D41</td>
<td>6/6</td>
<td>30.9 ± 6.2</td>
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<tr>
<td></td>
<td>anti-CD4</td>
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<td>6/6</td>
<td>11.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A9D41</td>
<td>6/6</td>
<td>13.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>anti-CD8</td>
<td>None</td>
<td>5/5</td>
<td>11.8 ± 0.2</td>
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<tr>
<td></td>
<td></td>
<td>A9D41</td>
<td>6/6</td>
<td>21.6 ± 1.7</td>
</tr>
</tbody>
</table>

* P < 0.001.  
* P < 0.01.  
* P < 0.05.

A9D41 MAb was not cytotoxic for FLC in the absence of rabbit complement.

We prepared by pepsin digestion a F(ab')2 fragment from the highly purified A9D41. By radioimmunoassay, preincubation of 3C18 FLC with the F(ab')2 blocked the binding of intact A9D41 MAb (data not shown). The F(ab')2 was, however, not cytotoxic for 3C18 FLC in vitro in the presence of rabbit complement (data not shown).
Table 6  A9D41 MAb-treated FLC-injected mice develop an immune response to FLC as demonstrated by presence of antibody to FLC in their sera and capacity of these sera to transfer anti-FLC activity

<table>
<thead>
<tr>
<th>Groups (total no. of mice)</th>
<th>Mice given injections of FLC</th>
<th>Treated with A9D41 MAb</th>
<th>Serum pool letter</th>
<th>Antibody titer*</th>
<th>Mean day of death ± SE of FLC-injected mice receiving sera#</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (12)</td>
<td>+</td>
<td>-</td>
<td>A₁</td>
<td>1:12</td>
<td>13.5 ± 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A₂</td>
<td>&lt;1:6</td>
<td>13.0 ± 0.7</td>
</tr>
<tr>
<td>B (12)</td>
<td>-</td>
<td>-</td>
<td>B₁</td>
<td>&lt;1:6</td>
<td>13.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B₂</td>
<td>&lt;1:6</td>
<td>13.0 ± 0.4</td>
</tr>
<tr>
<td>C (12)</td>
<td>-</td>
<td>+</td>
<td>C₁</td>
<td>1:96</td>
<td>17.2 ± 0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C₂</td>
<td>1:96</td>
<td>16.2 ± 0.8</td>
</tr>
<tr>
<td>D (22)</td>
<td>+</td>
<td>+</td>
<td>D₁</td>
<td>1:96</td>
<td>15.5 ± 1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>D₂</td>
<td>1:96</td>
<td>15.5 ± 0.6</td>
</tr>
</tbody>
</table>

7 days

<table>
<thead>
<tr>
<th>Groups (total no. of mice)</th>
<th>Mice given injections of FLC</th>
<th>Treated with A9D41 MAb</th>
<th>Serum pool letter</th>
<th>Antibody titer*</th>
<th>Mean day of death ± SE of FLC-injected mice receiving sera#</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A₁</td>
<td>1:12</td>
<td>13.5 ± 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A₂</td>
<td>&lt;1:6</td>
<td>13.0 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B₁</td>
<td>&lt;1:6</td>
<td>13.5 ± 0.3</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>B₂</td>
<td>&lt;1:6</td>
<td>13.0 ± 0.4</td>
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<tr>
<td></td>
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<td>C₁</td>
<td>1:96</td>
<td>17.2 ± 0.9</td>
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<td></td>
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<td>C₂</td>
<td>1:96</td>
<td>16.2 ± 0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>D₁</td>
<td>1:96</td>
<td>15.5 ± 1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>D₂</td>
<td>1:96</td>
<td>15.5 ± 0.6</td>
</tr>
</tbody>
</table>

28 days

Contrast, the sera of mice given injections of A9D41 MAb alone (Group C) or of MAb and 3C18 FLC (Group D) had high levels of complement-mediated cytotoxicity (titers of 1:96), reflecting the presence of residual A9D41 MAb in the circulation. At 28 days, there was no longer any residual MAb activity in 2 pools of sera from 6 mice in Group C as shown by the absence of complement-mediated cytotoxicity and the incapacity of these sera to transfer protection. In contrast, 4 of 4 pools of sera from 12 mice given injections of 3C18 FLC and treated with MAb (D₁, D₄, D₅, and D₆) had cytotoxic antibodies and transferred protection against 3C18 FLC (Table 6).

These results were confirmed in a second experiment in which 5 pools of sera from 13 MAb-protected mice were tested 28 days after i.v. injection of 3C18 FLC. All 5 pools of sera showed complement-mediated cytotoxicity to 3C18 FLC ranging in titer from 1:96 to >1:192, and these sera transferred protection against 3C18 FLC (data not shown).

Discussion

Previous experiments on the importance of antibodies to FLC in mediating part of the IFN α/β-induced suppression of FLC visceral metastases (4), and the experiments reported herein (Figs. 1 and 2) on the role of antibody in protecting FLC-immunized mice against challenge with highly tumorigenic FLC, stimulated us to isolate a MAb against FLC antigens. The availability of a syngeneic mouse IgG2a MAb to FLC, stimulated us to isolate a MAb against FLC antigen (27). Our A9D41 MAb was particularly effective in inhibiting the growth of gp70-positive 3C18 FLC tumors transplanted by different routes into syngeneic mice, but it was ineffective in mice given injections of gp70-negative 745 FLC or an unrelated tumor cell (Tables 1 and 3). Several investigators have already emphasized the particular in vivo efficacy of MAbs of the IgG2a isotype compared with other IgG isotypes (21–26). The A9D41 was target cell specific and irrelevant IgG2a MAbs did not inhibit 3C18 FLC growth in DBA/2 mice.

The FVL gp70 antigen has been shown to be immunogenic and immunized mice were protected against FVL challenge (27, 28). Sera from mice with dormant FVL infections contained cytolytic antibodies directed against virion gp70 antigen (29). Britt and Chesebro (30) showed that two IgG2a MAb directed against gp70 reduced the proliferation of FLC in lethally irradiated mice. We have shown that 3C18 FLC-injected mice treated effectively with IFN α/β developed cytotoxic IgG antibodies which recognized the gp70 antigen (4), and that in adoptive transfer experiments sera from these surviving mice protected other mice against challenge with 3C18 FLC (4).
when administered hours to days after i.v. inoculation of FLC (Table 2; Fig. 8), i.e., at a time when these tumor cells were already present in these organs (10). The efficacy of MAb in inhibiting FLC visceral metastases was directly related to the number of FLC and the dose of MAb injected. Thus, even small amounts (≤2.2 μg) of MAb were effective in inhibiting visceral metastases when only a few FLC were injected i.v. (Fig. 9). On the other hand, large amounts of MAb (5 mg) were ineffective in increasing survival time when injected into mice with extensive liver and spleen metastases (Table 2, Experiment 3) or into established solid s.c. FLC tumors (data not shown).

Experiments designed to explore the mechanisms of action of A9D41 MAb on the development of visceral metastases suggested that, although MAb was cytotoxic for FLC in vitro in the presence of rabbit complement (Fig. 11A) (but not in the presence of mouse serum alone without rabbit complement), it probably acted in vivo together with host mechanisms. Vollmers et al. (38, 41) reported that a MAb which inhibited mouse B16 melanoma pulmonary metastases acted directly on the tumor cells, inhibiting their multiplication in soft agar, inducing "a more normal behavior in vitro," and blocking the adhesion of melanoma cells to tissue culture dishes. Trauth et al. (36) also described a MAb directed against a cell surface antigen which acted directly on human tumor cells in vitro or on xenografts in nu/nu mice. However, in our experiments, A9D41 MAb did not inhibit the multiplication of 3C18 FLC in vitro (Fig. 11B).

Furthermore, the F(ab')2 fragment of A9D41, which bound to FLC, was not cytotoxic for FLC in the presence of complement and was ineffective in inhibiting the development of FLC visceral metastases (Table 4), in accord with the results of others who found the F(ab')2 fragment ineffective in their systems (23, 25, 26).

The finding that A9D41 MAb was less effective in bg/bg and athymic nu/nu mice and virtually ineffective in mice treated with antibody to CD4, indicated that host lymphoid cells participated in some manner in the antitumor action of A9D41 MAb (Table 5), and suggested that the immune system played an essential role in the inhibitory effect of MAb on the development of visceral metastases.

(a) MAb may have acted in an antibody-dependent cell-mediated cytotoxicity manner on FLC together with macrophages (22-24) or lymphoid cells (35, 40, 42). We have, however, not been able to demonstrate any spleen cell cytotoxicity for FLC (effectortarget cell ratios 100:1) in 6- or 18-h radioactive chromium release assays, using either normal DBA/2 spleen cells or spleen cells from FLC-injected MAb-protected mice with or without MAb in the assay.

(b) The results presented in Table 6 clearly showed that MAb-protected, FLC-injected mice developed an immune response to 3C18 FLC, as determined by the serum antibody level and the capacity of these sera to transfer anti-3C18 FLC activity. This immune response probably contributed to the inhibition of FLC metastases. The experiments showing that antibody to CD4 markedly reduced the efficacy of MAb (Table 5) are in accord with these results, as CD4 cells may constitute an essential cell population for an anti-FLC immune response.

The possibility that injection of an antitumor cell antibody exerts some of its effects by modifying the immune response has been previously suggested, without, however, any experimental evidence (19, 43). Kirch and Hammerling (37) and Herlyn and Koprowski (23) found no evidence that an immune response contributed to the antitumor effects of MAb in their experimental systems. To the best of our knowledge, our results represent the first indication that the immune response of the host to the tumor may contribute to the antitumor activity of a MAb directed against a tumor antigen. These findings may be relevant to the use of MAb in the treatment of patients with cancer. Thus, an immune response developing in the course of MAb treatment may explain why tumor regression was observed in patients with B-cell lymphomas treated with antidiotyope antibodies even after discontinuation of therapy (44).

A9D41 MAb was also effective as adjuvant therapy in inhibiting the development of FLC liver and spleen metastases after excision of the primary established s.c. tumor (Fig. 10). Bernstein et al. (19) had previously reported the efficacy of MAb as adjuvant therapy after surgery in inhibiting tumor metastases. In our previous experiments, IFN α/β was also effective as adjuvant therapy after excision of the s.c. FLC tumor (20). The results presented herein showed that combination MAb-IFN α/β was more effective than either therapy alone (Fig. 10). Basham et al. (26, 31, 32) showed a synergistic antitumor effect of IFNs α, β, and γ and MAb on the i.p. growth of a murine B-cell lymphoma. It would seem worthwhile utilizing combination therapy of MABs with IFNs or other cytokines such as interleukin 2 (25, 40) or chemotherpay (43) in the treatment of metastatic disease in patients when MABs, directed against specific tumor antigens become available (44).

ACKNOWLEDGMENTS

We are grateful to Dr. C. Carnaud (Hôpital Necker, Paris, France) for helpful discussion, and to Dr. E. De Maeyer (Institut Curie, Orsay, France) for the gift of nu/+ and nu/nu DBA/2 mice.

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Inhibition of Friend Leukemia Cell Visceral Metastases by a New Monoclonal Antibody and Role of the Immune System of the Host in Its Action

Arturo Sala, Ion Gresser, Daniele Chassoux, et al.