Effects of Monoclonal Antibodies That Block Transferrin Receptor Function on the in Vivo Growth of a Syngeneic Murine Leukemia

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ABSTRACT

The ability of monoclonal antibodies (MAbs) against the murine transferrin receptor to inhibit the growth of transplanted syngeneic AKR/J SL-2 leukemic cells has been investigated. Two rat IgM antibodies, RI7 208 and REM 17.2, which both block transferrin receptor function, inhibited the growth of SL-2 leukemic cells in vitro at concentrations of 5-10 μg per ml. However, RI7 208 was more effective than REM 17.2 in prolonging survival of tumor-bearing mice. The antitumor effects of RI7 208 MAb were dependent on both the antibody dose and number of leukemic cells inoculated. The serum clearance of L-[35S]methionine-labeled RI7 208 and REM 17.2 antibodies was similar and consisted of an initial rapid phase followed by a slower phase. A single dose of 2 mg of antibody maintained a serum MAb concentration (>10 μg/ml) sufficient to inhibit SL-2 leukemic cell growth in vitro for 2-3 days. The liver, kidney, and spleen were the major sites at which each of the antibodies accumulated regardless of whether trace or saturating amounts of antibody were administered. The specific activity of antibody found in s.c. SL-2 tumors was about 2-fold less than that of liver. It was shown that multiple doses of RI7 208 MAb administered on a schedule aimed at maintaining a therapeutic serum level of MAb for 1-3 weeks were more effective than a single dose. Further, administration of RI7 208 MAb, in combination with the anti-Thy-1.1 MAb 19E12, was more effective than either antibody alone. SL-2 mutant cells were selected that were resistant to growth inhibitory effects of RI7 208 in vitro. The effects of RI7 208 MAb on the growth of these mutant cells in vivo suggests the major mechanism by which the MAb inhibits SL-2 tumor growth is by directly blocking receptor function. Acute toxicity associated with administration of the MAb was minimal. However, assays of myeloid and erythroid colony-forming units in bone marrow and spleen of mice given multiple doses of RI7 208 showed a depression of stem cell activity in bone marrow and elevated numbers of erythroid and cellular colony-forming units in the spleen.

INTRODUCTION

Two major approaches utilizing MAbs in the treatment of malignant disease have been investigated (1-19). One is serotherapy (1-14), the other, targeting by antibodies of covalently-bound toxins, drugs, or radionuclides to malignant tissues (16-19). On occasion, striking therapeutic effects have been obtained using MAbs against tumor-associated differentiation antigens in clinical trials (9, 14). In general, however, clinical responses to serotherapy have been variable and animal model studies indicate current limitations of this approach (1-8, 10-13). Although antibody-toxin conjugates have been shown to have potent and specific antitumor effects in vitro, with the exception of one animal study (17), their efficacy in vivo has been less marked (16, 20). Little is known about the potential toxicity of such antibody conjugates in a clinical setting (21).

A third complementary approach to exploit MAbs as antitumor agents is based on the ability of MAbs directed against functional cell surface receptors to mediate direct biological effects. This concept arose from studies of a murine MAb, 42/6, which blocked human transferrin receptor function and inhibited cell growth in vitro (22). MAbs against a variety of other growth-related receptors including the IL-2 receptor (23), the epidermal growth factor receptor (24), and the cell surface molecule encoded by the Neu oncogene (25) are also known to modulate cell growth. Two rat MAbs, RI7 208 and REM 17.2, that block the function of the murine transferrin receptor and inhibit cell growth have been obtained (26-28). Both are IgMs and on the basis of cross-blocking experiments react with the same epitope on the receptor (28). Neither antibody blocks transferrin binding to the receptor and both appear to inhibit transferrin-mediated iron uptake by extensively cross-linking receptors and interfering with their internalization (28). The major mechanism by which 42/6 MAb blocks receptor function may be similar (29). The MAbs initially arrest cell growth reversibly but continued exposure leads to cell death within 1-3 days (26-29).

In this report, the effectiveness of antitumor transferrin receptor MAbs as antitumor agents and their toxicity have been investigated. RI7 208 MAb has a significant inhibitory effect on the growth of the syngeneic SL-2 leukemia in AKR/J mice. Single versus multiple dose therapy and combination therapy with RI7 208 MAb and anti-Thy-1 antibody have been compared. The antitransferrin receptor MAb is not severely toxic when administered in milligram quantities over several weeks but does induce changes in the erythroid and myeloid stem cell populations in the bone marrow and spleens of treated mice.

MATERIALS AND METHODS

Monoclonal Antibodies. Antitransferrin receptor antibodies RI7 208 and REM 17.2, both IgMs, and RI7 217, an IgG2a, have been described previously (26-28). 19E12, a murine anti-Thy-1.1 MAb (1, 2), was kindly provided by Dr. Irwin Bernstein (Fred Hutchinson Cancer Center, Seattle, WA). For the immunotherapy experiments, the rat MAbs REM 17.2 and RI7 208 were obtained from ascitic fluid of BALB/c mice. The mice were primed with 0.4 ml pristane i.p., then 1-2 weeks later irradiated with 300 rads and injected with 1-2 × 108 hybridoma cells. Ascitic fluid was harvested 1-6 weeks later. The MAbs were purified from the ascitic fluid by euglobulin precipitation. Ascitic fluid was dialyzed exhaustively against 5 mM sodium phosphate buffer pH 6.5 and the precipitate collected by centrifugation at 20,000 × g for 30 min. The precipitate was washed once with 5 mM phosphate buffer and then redissolved at approximately 20 mg/ml in 0.15 M NaCl/0.01 M sodium phosphate buffer, pH 7.2 PBS. 19E12 MAb was purified from BALB/c mouse ascitic fluid by precipitation with 50% (w/v) ammonium sulfate (pH 7.0).

Mice. AKR/J mice 6-8 weeks old were obtained from The Jackson Laboratory, Bar Harbor, ME.

Cells and Tissue Culture. The transplantable SL-2 leukemia was derived from a spontaneous leukemia in an AKR/J mouse (1, 2) and was obtained from Dr. Irwin Bernstein. The SL-2 cells were maintained in vitro in Dulbecco’s modified Eagle’s medium supplemented with 10% horse serum. Cells were retrieved from liquid nitrogen every 2-3 months to initiate fresh cultures. For growth assays, SL-2 cells were plated out at an initial cell density of 2-5 × 104 cells/ml and cell counts determined in duplicate cultures using a Coulter counter.

Immunotherapy Experiments. Cells were inoculated in a volume of
approximately 0.2 ml at a s.c. site on the back. Purified antibodies were injected i.p. in sterile PBS. Groups of 4–6 mice were used in each experiment.

Selection of SL-2 Mutants. Mutants resistant to the growth inhibitory properties of R17 208 were selected from the wildtype SL-2 leukemic cell line by growing either cells which had been previously mutagenized with ethyl methane sulfonate sufficient to kill about 90% of the cells (0.1–1 mg/ml) or unmutagenized cells in the continuous presence of 20 μg/ml of purified R17 208 monoclonal antibody (27). Clones were obtained from four independent selections by limiting dilution at 0.2 cells/well in microtiter plates. Clones were grown up in the presence of 20 μg/ml of antibody.

Flow Cytometry. Cells (1 x 10^6) were incubated with hybridoma supernatant containing the indicated antibody or medium alone for 60 min at 4°C followed by incubation with saturating amounts of a fluorescein-isothiocyanate-labeled anti-rat immunoglobulin (Cappel Laboratories). Cells were analyzed on The Salk Institute flow cytometer as described by Lesley and Schulte (28). Cells to be analyzed were maintained in log-phase growth to minimize changes in transferrin receptor expression related to differences in cell density.

CFU-e and CFU-c Progenitor Cell Assays. Single-cell suspensions were made from bone marrow and spleen of individual mice and assayed for CFU-e and CFU-c as previously described (30).

Serum Clearance and Biodistribution Studies. MAbs were metabolically labeled with [§Se]methionine (124 mCi/mg, Amersham Co.) by incubating hybridoma cells at 5 x 10^6 cells per ml with 50 μCi/ml [§Se]-methionine in methionine-free Dulbecco’s modified Eagle’s medium supplemented with 2% (v/v) diazyl horse serum for 4 h at 37°C in a CO₂ incubator. The culture supernatants were collected by centrifugation and dialyzed exhaustively against PBS. Groups of four mice were injected with the radiolabeled antibody (4 x 10^3–4 x 10^6 cpm per mouse) either i.p. or i.v. in 0.2 ml sterile PBS. For serum clearance studies, mice were tailbled into weighed tubes and the blood volume determined by difference. For biodistribution studies, mice were bled out under anesthesia 24 h after injection of radiolabeled antibody and then sacrificed. Their organs were removed, weighed, and their radioactivity determined in a Beckman gamma counter.

**RESULTS**

Inhibition of Growth in Vitro of SL-2 Leukemic Cells by Antitransferrin Receptor Monoclonal Antibodies. The AKR transplantable leukemia SL-2 has been extensively used by Bernstein and his colleagues (1, 2, 5, 7) as a model to determine the parameters which influence the effectiveness of serotherapy with MAbs against tumor cells. Because of the data which had been accumulated using anti-Thy-1 antibodies, and because T-cell leukemias in humans often express large numbers of transferrin receptors and might be an appropriate disease in which to undertake clinical trials, we considered the transplantable SL-2 leukemia to be an attractive model in which to test whether antitransferrin antibodies might selectively inhibit tumor cell growth in *vivo*. As the growth of different cells vary in their sensitivity to inhibition by antitransferrin receptor antibodies in *vitro*, we first determined the effects of the two rat anti-mouse transferrin receptor antibodies that block receptor function on the growth of SL-2 leukemic cells in *vitro*. As shown in Fig. 1, both R17 208 and REM 17.2, which had previously been shown by Lesley and Schulte to inhibit the growth of a variety of murine hematopoietic cell lines (26–28) at concentrations greater than 5–10 μg/ml, also completely inhibited the growth of SL-2 cells over a similar concentration range.

Dose-dependent Inhibition of Tumor Cell Growth in *Vitro* by Anti-Transferrin Receptor Antibodies. To determine the effect of R17 208 and REM 17.2 MAbs on the growth of SL-2 leukemia cells in *vitro*, groups of 4–6 mice were inoculated with different numbers of leukemic cells at a s.c. site on the back and then within an hour given a single i.p. injection of MAb. Shown in Fig. 2 are the effects of antibody treatment on the survival of the tumor-bearing mice. It can be seen that administration of R17 208 MAbs prolonged the survival of tumor-bearing mice in a dose-dependent manner. The mean survival time of mice given 10 mg of R17 208 antibody was 28 ± 1.7 days compared with the control group which was 18.2 ± 2.3 days. As previously reported (31, 32), R17 208 MAbs significantly delayed the appearance of palpable primary tumors at the site of inoculation (data not shown). The effects of R17 208 MAbs on the survival of mice challenged with 3 x 10^6 leukemic cells were considerably greater than mice given 3 x 10^6 cells. However, a single 10-mg dose of R17 208 MAb did not lead to long-term survivors even in the group of mice challenged with 3 x 10^6 leukemic cells. A reproducible finding was that small doses of R17 208 antibody (0.3 mg or 1 mg) slightly decreased the survival of tumor-bearing mice. Although REM 17.2 MAb was as effective as R17 208 at inhibiting the growth of SL-2 leukemic cells in *vitro*, its effect on the survival of tumor-bearing mice was much less marked.

**Determination of the Rate of Clearance of MAbs from the Serum and Their Tissue Localization.** Antibodies metabolically labeled with [§Se]methionine were used to determine the rate of clearance of the antitransferrin receptor MAbs from the serum and their biodistribution. As shown in Fig. 3, sodium dodecyl sulfate-polyacrylamide gel analysis indicated the [§Se]-methionine-labeled antibodies were of high purity and the use of metabolically-labeled proteins avoided the problems associated with either denaturation of radioiodinated antibodies during labeling or dehalogenation in *vivo* (33). As shown in Fig. 4, 6 h after i.p. injection of 2 mg of either R17 208 or REM 17.2 MAbs, serum levels were approximately 30 μg/ml. The clearance of the antibodies from the serum was biphasic. The initial rapid phase of clearance presumably represents specific binding of the antibody to normal tissues (34). The second slower clearance rate is interpreted to be primarily due to catabolism of unbound antibody. Based on the *in vitro* studies, a single i.p. injection of 2 mg of R17 208 antibody is sufficient to maintain therapeutic levels of antibody in the serum for about 2–3 days. The clearance of REM 17.2 MAb from the serum was similar to that of R17 208. Similar results were also obtained when the antibodies were injected i.v. (data not shown).

The distribution of [§Se]methionine-labeled antitransferrin
Therapy with Antitransferrin Receptor Antibodies

Fig. 2. Determination of the antitumor effects of single doses of antitransferrin receptor monoclonal antibodies. Groups of 5-6 AKR/J mice were challenged s.c. with either $3 \times 10^5$ (top), $1 \times 10^6$ (middle), or $3 \times 10^6$ (bottom), SL-2 leukemic cells. Within 1-2 h, groups of mice were treated with a single i.p. dose of either RI7 208 or REM 17.2 monoclonal antibodies in the milligram amounts indicated. Shown are the survival times of individual mice.

Receptor antibodies in normal tissues was also determined when either trace amounts (0.1-1 μg) or saturating (2 mg) doses of radiolabeled antibody were given. The latter dose was selected so that the localization of the MAbs could be assessed under conditions similar to those used in the actual immunotherapy trials. As shown in Fig. 5 (top), 24 h after administration of trace amounts of antitransferrin receptor antibodies, the tissues in which most radioactivity accumulated on a per milligram tissue basis were liver, kidney, and spleen. There was no significant difference in the biodistribution of the two IgM antibodies, RI7 208 and REM 17.2. The antitransferrin receptor antibody of the IgG2a class, RI7 217, also showed a similar distribution except that relatively more of the antibody localized in the spleen. If the results are expressed on a per organ basis, the liver is clearly the major site of accumulation of antitransferrin receptor antibodies. Whether this is entirely due to specific binding to transferrin receptors in the liver or also represents uptake by the reticuloendothelial system is not clear. The distribution of antitransferrin receptor antibodies in normal tissues when a 2-mg dose of the IgM antibodies was given was essentially identical to that observed after the administration of trace amounts of antibody. The only difference noted was that, as expected, after 24 h the specific activity of the blood was approximately 2-fold higher than when trace amounts of antibody were given (Fig. 5, middle). Thus, under conditions where therapeutic amounts of antibody are administered, the liver is also the major site of accumulation of antitransferrin receptor antibodies.

In order to determine the extent to which the antitransferrin receptor antibodies accumulated in tumor tissue, mice bearing s.c. tumors approximately 0.5 cm in diameter were given 2 mg of [35S]methionine-labeled antitransferrin receptor antibodies and after 24 h the biodistribution of the antibodies was determined. As shown in Fig. 5 (bottom), although both IgM antibodies were sequestered from the circulation and accumulated in the tumors, the specific activity of the tumor tissue was not as high as that for the liver. The limiting factor in the localization of the IgM antibodies in the tumor does not appear to be their extra-vascularization as the IgG antibody RI7 217 did not localize more efficiently.

Effects of Multiple Doses of Antitransferrin Receptor Antibody on SL-2 Leukemia Growth. Based upon the rate of clearance of RI7 208 MAb from the serum, two different schedules of administration were used aimed at maintaining antibody serum levels that would be sufficient to inhibit SL-2 leukemia cell growth. Tumor-bearing mice were either given 3 mg of antibody at 3-4 day intervals or 1 mg of antibody daily. As shown in Fig. 6, multiple doses of antitransferrin receptor antibody were found to be much more effective than the single dose used in the experiment shown in Fig. 1. Administration of antibody over several weeks using either schedule resulted in the long-term survival of a majority of tumor-bearing mice.

Effects of the Combination of Anti-Thy-1 Antibody 19E12 and Antitransferrin Receptor Antibody RI7 208 on SL-2 Leukemia Growth. One reason for selecting the SL-2 leukemia model to
Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of [\(^{75}\text{Se}\)]-methionine-labeled monoclonal antibodies. Samples of the monoclonal antibodies were analyzed on a 7.5% acrylamide gel under reducing conditions. Autoradiography with an enhancing screen was for 14 h at \(-70^\circ\text{C}\).

Fig. 4. Serum clearance of rat antimouse transferrin receptor antibodies. Groups of four AKR/J mice were injected i.p. with 2 mg of either RI7 208 or REM 17.2 monoclonal antibodies metabolically labeled with [\(^{75}\text{Se}\)]methionine (2.5 \(\times\) 10^8 cpm/mg). Mice were tailbled at the time indicated and the specific activity of the blood determined. From this data the concentration of monoclonal antibody in the blood was calculated.

Fig. 5. Biodistribution of [\(^{75}\text{Se}\)]methionine-labeled antitransferrin receptor antibodies in normal and tumor-bearing mice. The figure shows three experiments. At top, data are from an experiment in which groups of four normal AKR/J mice were injected i.p. with trace amounts (\(\sim0.1-1\ \mu\text{g}\) of either 4 \(\times\) 10^8–4 \(\times\) 10^9 cpm of one of the [\(^{75}\text{Se}\)]methionine-labeled antitransferrin receptor MAbs as indicated and then sacrificed 24 h later. The specific activity of radioactivity in various organs was determined as described in "Materials and Methods." At middle, data are from a similar experiment except that an antibody dose of 2 mg (specific activity 2 \(\times\) 10^8 cpm/mg) was given. Finally, at the bottom, data are from an experiment in which mice were injected with 3 \(\times\) 10^6 SL-2 leukemic cells 14 days prior to the experiment and bore tumors of approximately 0.5 cm in diameter. The tumor-bearing mice were then injected with trace amounts of radiolabeled antibodies exactly as described for the experiment shown in the upper panels.

test the therapeutic effects of antitransferrin receptor antibodies was the well-established antitumor effects of anti-Thy-1 antibodies in this system (1, 2). These previous studies had shown that the antitumor activity of Thy-1 antibodies involved host immunological effector mechanisms and that the IgG2a isotype was the most effective (7). It was further shown that the major effect of the Thy-1 antibodies was upon the development of metastases and that the effectiveness of the serotherapy was limited by the metastatic growth of Thy-1.1-negative variants and the failure of the anti-Thy-1 antibodies to eliminate the primary tumor at the s.c. site of inoculation when larger doses of tumor cells were given (2, 5). Because the antitransferrin receptor antibody inhibits the growth of SL-2 cells in vivo (see below) by directly blocking receptor function, we wished to determine whether the combination of anti-Thy-1 antibody and antitransferrin receptor antibody was more effective than either antibody alone. The results of two experiments to investigate this question are shown in Fig. 7. As previously reported (31, 32), the anti-Thy-1 and antitransferrin receptor antibodies
THERAPY WITH ANTITRANSFERRIN RECEPTOR ANTIBODIES

Fig. 6. Effect of multiple doses of RI7 208 monoclonal antibody on survival of AKR/J mice challenged with SL-2 leukemic cells. Groups of six mice were challenged with 1 x 10^6 SL-2 leukemic cells and then multiple doses of RI7 208 monoclonal antibody i.p. on the schedules indicated.

Fig. 7. Combination of therapy with anti-Thy-1 and antitransferrin receptor monoclonal antibodies. Groups of 5-6 mice were challenged with 1 x 10^6 SL-2 leukemic cells and then given 3 mg of RI7 208 or 19E12 monoclonal antibodies on days 0, 4, and 7 in the combinations indicated. Survival of the mice was followed for 90 days.

alone had similar effects on prolonging survival of mice challenged with 1 x 10^6 SL-2 cells. The antitransferrin receptor antibody, however, inhibited tumor cell growth at the primary site of inoculation, whereas the anti-Thy-1 antibody did not. As shown in Fig. 7, combination therapy with both antibodies resulted in significantly greater antitumor effects than either antibody alone leading to long-term survival of 3 of 6 and 5 of 6 of the treated mice in Experiments 1 and 2, respectively.

Mechanism of the Antitumor Effects of Antitransferrin Receptor Antibodies in Vivo. We wished to determine whether the antitumor effects of RI7 208 monoclonal antibody in vivo are mediated by the same mechanism which operates in vitro. It has previously been shown by Lesley and Schulte (27, 28) that stable mutants resistant to the inhibitory effects of MAbs can be derived from mouse lymphoma cell lines by continuous exposure to antibodies that block receptor function. Several classes of these mutants have been extensively characterized, and it appears that the mutations are in one of the two structural genes for the transferrin receptor and lead to the synthesis of a modified receptor which has lost the antigenic site for the MAb used in the selection. When analyzed by quantitative iodine-125-labeled antibody binding assays or by fluorescence-activated cell analysis, such mutants, which express both wildtype and mutant receptors, have approximately half as many antigenic sites recognized by the MAb used for selection as parental cells. It has been shown that internalization of transferrin receptors of the mutant cells is no longer inhibited by the MAb to which they have acquired resistance and it is believed that this is a consequence of less extensive cross-linking of the receptors of the mutant cells by the MAb. We decided to investigate the mechanism of in vivo killing of SL-2 cells by deriving similar SL-2 mutants. As shown in Fig. 8, after selection in 20 µg/ml of RI7 208 monoclonal antibody for about 4 weeks, mutants were obtained which after cloning were stably resistant to the growth inhibitory effects of the MAb. When analyzed on the fluorescence-activated cell sorter, it was found that the mutants were similar to those previously described by Lesley and Schulte (27). Although resistant to the effects of RI7 208 MAb, there was at most a 2-fold difference in the amount of RI7 208 MAb bound to the cells (Fig. 9). We reasoned, therefore, that if the mechanism by which RI7 208 inhibits the growth of wildtype SL-2 cells in vivo is by blocking receptor function, the growth of the mutant cells in vivo should not be affected. In contrast, if antibody-mediated immunological host mechanisms play a significant role in the inhibition of tumor growth, as the mutant cells still bind antibody, their in vivo growth should be sensitive to the MAb.

The results of two
Effects of Antitransferrin Receptor Monoclonal Antibody on Normal Tissues. Although the anti-transferrin receptor monoclonal antibodies bind to some normal tissues, administration of 3 mg of RI7 208 antibody twice weekly for up to 4 weeks produced no evidence of gross toxicity. Histological examination of sections of the small intestine, liver, and spleen failed to reveal signs of cellular damage. No decrease in erythrocyte or white blood cell counts were noted in antibody-treated mice. In order to further investigate the effects of the antibodies on normal tissues, we assayed the myeloid (CFU-c) and erythroid (CFU-e) progenitor cells in the bone marrow and spleens of mice given 1 mg of RI7 208 monoclonal antibody daily for 7 days. The results of one such representative experiment are shown in Fig. 11. The cellularity of the bone marrow in antibody-treated mice was not significantly different from that of the controls but there was a 2-fold decrease in the number of CFU-e per 10^6 cells. Concomitantly, an almost 3-fold increase in CFU-e per 10^6 spleen cells was also noted. Further, splenomegaly was noted in all the antibody-treated mice and there was an approximately 4-fold increase in cellularity. Similar but less dramatic changes were observed for CFU-c in bone marrow and spleen.

DISCUSSION

The results reported in this paper clearly establish that the antitransferrin receptor monoclonal antibody, RI7 208, shows a dose-dependent antitumor effect in the SL-2 leukemia syngeneic mouse model system. The magnitude of the effect appears comparable to that previously reported for the anti-Thy-1 antibody, 19E12, in the same system (1, 2, 5). Although another antitransferrin receptor IgM monoclonal antibody inhibited the in vitro growth of SL-2 cells equally efficiently as RI7 208, its antitumor effects in vivo were much less dramatic. Presently, it is not clear why this is so as it appears that the tissue localization and serum clearance of both antibodies are similar. Both antibodies react with the same epitope on the receptor and appear to inhibit in vitro growth of a variety of murine lymphoma cell lines to the same extent and by a similar mechanism (28). Multiple injections of RI7 208 MAb designed to maintain serum levels of 10 μg/ml or higher significantly increased the antitumor effect of the antibody and led to the long-term survival of mice challenged with 1 x 10^6 SL-2 cells provided antibody therapy was begun immediately. If therapy was delayed for 7 days, although survival of the tumor-bearing mice was increased, multiple doses of RI7 208 MAb did not completely eliminate the tumor (data not shown). Fluorescence-activated cell sorter analysis shows that leukemic cells from these animals still bind the antibody and their growth is inhibited by the antibody in vitro. Thus, it does not appear that resistant mutants of the type that are generated in vitro by prolonged exposure to the antibody can account for this failure. Rather, it is likely that the effectiveness of MAb treatment alone is limited if the tumor burden is large. In addition to multiple doses of antitransferrin receptor antibody being more effective than single doses, a combination of antitransferrin receptor antibody and anti-Thy-1 antibody combined also gave increased therapeutic effects. It is possible that antitransferrin receptor antibodies may also be useful in combination with other MAbs that eliminate tumor cells by activation of host immunological effector mechanisms. Again, however, although treatment with both antibodies in combination inhibited the growth of established tumors, it could not totally eliminate the leukemic cells. We are presently investigating whether MAb treatment in combination with other conventional drug therapy will be more effective.

Previous studies of other MAbs that mediate their antitumor activity by utilizing host effector mechanisms have shown that IgMs are uniformly inactive (2, 6). The fact that RI7 208 MAb enhances the tumor growth of mutant SL-2 cells resistant to its blocking effect in vitro also argues strongly against the possibility that antibody-dependent host immunological effector mechanisms alone inhibit the growth of the wildtype SL-2 leukemic cells in vivo. It is likely, therefore, that the antibody
is mediating its effects in vivo at least in part by blocking transferrin receptor function. Neither the decreased tumorigenicity of the transferrin receptor mutants nor the enhancement of their growth in vivo by RI7 208 monoclonal antibody is clearly understood. There are precedents for other mutants with altered cell surfaces being less tumorigenic than wildtype cells (35). The expression of an altered transferrin receptor on the surface of these cells may decrease their tumorigenicity by adversely influencing the cells' ability to take up iron in vivo under conditions where there is competition for available iron with the cells of other tissues. Alternatively, the modified receptor may be immunogenic and elicit an immune response in the host. If this were so, this might also explain the enhancing properties of RI7 208 which may function as a classical blocking antibody (36). However, we have not detected antibodies reactive with the mutant SL-2 cells in the sera of tumor-bearing mice.

Finally, although RI7 208 antibody binds to transferrin receptors in normal tissues, there is no evidence of acute toxicity. However, assays of myeloid and erythroid stem cells in the bone marrow showed a depression of erythropoiesis and myelopoiesis in the bone marrow and a concomitant increase in CFU-e and CFU-c in the spleen. This suggests that RI7 208 is inhibiting the growth of these progenitor cells in the bone marrow and, as a consequence, hematopoiesis which normally in adult mice does not occur in the spleen to any great extent is turned on. The relative lack of toxicity can be explained in part by the fact that other factors in addition to antibody binding determine whether cell growth is inhibited. It is known that cell lines derived from different tissues show differential sensitivity in vitro to the growth inhibitory effects of the antibodies (37). It is likely, therefore, that many normal cells including epithelial cells and fibroblasts which may express transferrin receptors as they proliferate are less sensitive to RI7 208 than hematopoietic tumors.

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