Monoclonal Anti-MM46 Antibody: Ricin A Chain Conjugate: In Vitro and in Vivo Antitumor Activity

Masao Seto, Naoki Umemoto, Masahiko Saito, Yasuhiko Masuho, Takeshi Hara, and Toshitada Takahashi

Laboratories of Experimental Pathology and Cell Biology, Aichi Cancer Center Research Institute, Chikusa-ku, Nagoya 464, Japan [M. S., T. T., and Teijin Institute for Biomedical Research, Asahigaoka, Hino, Tokyo 191, Japan [N. U., M. S., Y. M., T. H.]

ABSTRACT

In an approach to antitumor agents with improved tumor specificity, the ricin toxin subunit A chain was covalently coupled with a monoclonal IgG2b antibody directed against MM antigen, a tumor-specific antigen on syngeneic mouse mammary tumor MM46 cells (anti-MM46 IgG), using N-succinimidyl 3-(2-pyridyldithio)propionate as cross-linking agent. The conjugate thus prepared (anti-MM46 conjugate) showed potent dose-dependent cytotoxicity against MM antigen-positive MM46 cells in vitro and inhibited the cell growth at concentrations above 1 μg/ml. The immunological specificity was verified by the observation that anti-MM46 conjugate did not show cytotoxicity against MM antigen-negative MM48 cells. Neither nonimmune conjugate similarly prepared from mouse nonimmune IgG nor unconjugated anti-MM46 IgG alone exhibited cytotoxicity against MM46 cells. Anti-MM46 IgG still retained considerable in vitro complement-dependent cytotoxicity against MM46 cells after conjugation with ricin A chain.

In Winn-type tumor-neutralizing assay in which C3H/He mice were inoculated i.p. or s.c. with MM46 cells preincubated with a test material, anti-MM46 conjugate showed greater activity than did anti-MM46 IgG. A markedly enhanced efficacy of anti-MM46 conjugate was also observed in therapeutic experiments. When a group of five C3H/He mice inoculated i.p. with 5 × 10⁴ MM46 cells were treated with an i.p. injection of 1 μg of anti-MM46 conjugate on Days 1, 3, and 5, all five mice survived tumor-free, although those treated with 1 μg of anti-MM46 IgG alone died before Day 20 with a life span similar to those of mice treated with nonimmune conjugate or phosphate-buffered saline (control). Thus, the in vivo efficacy of anti-MM46 conjugate over anti-MM46 IgG alone was demonstrated by therapeutic experiments as well as by tumor-neutralizing assays. Although anti-MM46 conjugate showed no antitumor effect when injected i.p. to C3H/He mice bearing s.c.-inoculated MM46 tumor (inoculum, 4 × 10⁵) on Days 1, 3, 5, and 7 at a dose of 10 μg, it inhibited tumor growth when injected intraregionally to tumor-bearing mice, suggesting that the conjugate is effective also to solid-type MM46 tumor if a sufficient amount of anti-MM46 conjugate reaches the tumor site.

INTRODUCTION

An approach to kill tumor cells selectively by localizing cytotoxic agents onto the target tumor cells by the use of conjugates of cytotoxic agents with antitumor antibodies has been studied for several years (3). However, its optimal potential remains to be exploited. Considering the extraordinary cytotoxic potency of diphtheria toxin and ricin, a plant toxin, and their structure of 2 subunits, each of which has a different function, we prepared previously hybrids of the toxin A chain, intracellularly active toxin subunit with Fab' of IgG of rabbit antiserum against mouse leukemia L1210 and demonstrated that the conjugation of the A chain with an antibody confers cytotoxicity upon the enzymic A chain free from the cell-binding subunit B chain (10, 11). An important basic requirement for the antibody: cytotoxic agent conjugates to exert selective cytotoxicity against the target cells is the high immunological specificity of the antibody portion of the conjugates. In recent years, the hybridoma technique (8) has contributed a great deal to the preparation of antibodies directed against cell-surface antigens (7). Indeed, some of such hybridoma-prepared monoclonal antibodies against tumor-associated antigens or other cell-surface antigens were used in studies on antibody:toxin conjugates (1, 4, 9, 15, 16, 26). However, most of these studies were concerned only with in vitro activity. Furthermore, although selective killing of the target cells in vitro by the conjugates could be demonstrated, their effect in inhibiting tumor growth in vivo was generally much less remarkable.

We reported (18) the preparation of a panel of hybridomas secreting monoclonal antibodies against syngeneic, ascitic C3H/He mouse mammary tumor MM46 cells (an MM antigen-positive tumor cell line) (6, 13, 25). Our investigation showed that MM antigen detected by a monoclonal 3–3–C antibody was closely associated with or identical to Ly-6.2 alloantigen and proved that this antigen is anomalous expression of Ly-6.2 on tumor cells derived from a Ly-6.1 strain C3H/He (18). Therefore, MM antigen should function as tumor-specific target antigen for antibody:toxin A chain conjugates.

As a continuation of our work on antibody:toxin conjugates, we have now prepared a conjugate of ricin A chain with the monoclonal antibody against MM46 cells and investigated the in vivo as well as in vitro antitumor activities of the conjugate. This system using syngeneic mouse tumor should be of value in evaluating the potential of monoclonal antibody:ricin A chain conjugates which may take advantage of both the high tumor specificity of the hybridoma-prepared antibody and the high cytotoxicity of A chain exhibited after internalization into the cell.

1 The abbreviations used are: MM antigen, mouse mammary tumor-specific antigen; PBS, 10 mM phosphate buffer, pH 7.4; containing 0.14 M NaCl; anti-MM46 IgG, mouse monoclonal anti-MM46 IgG2b antibody; 3–3–C; Buffer 1, 0.1 M phosphate buffer, pH 8.0; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SPDP, N-succinimidyl 3-(2-pyridyldithio)propionate; Buffer 2, 0.1 M phosphate buffer, pH 7.5, containing 0.1 M NaCl; anti-MM46 conjugate, anti-MM46 antibody 3–3–C:ricin A chain conjugate; nonimmune conjugate, mouse nonimmune IgG:ricin A chain conjugate; T:C, percentage of tumor weights of treated mice relative to control.
MATERIALS AND METHODS

Mice. Male C3H/He mice were purchased from Charles River Japan, Atsugi, Japan. They were 8 to 10 weeks old (26 to 30 g) at the beginning of the experiments. The mice were given a pelleted diet (CE-2; Clea Japan, Tokyo, Japan) and drinking water freely.

Tumor Cell Lines. The MM antigen-positive tumor cell line MM46 and the negative tumor cell line MM48 were provided by Dr. T. Tachibana, Tohoku University School of Medicine, Sendai, Japan, and were maintained by in vivo passage in C3H/He mice. Five to 7 days after inoculation, ascitic tumor cells were collected in PBS and used for experiments after being washed 3 times with the culture medium or PBS.

Preparation of Anti-MM46 IgG and of Mouse Nonimmune Immunoglobulin. Athymic BALB/c nu/nu mice, which were kindly provided by Dr. S. Suzuki, Institute of Medical Science, University of Tokyo, Tokyo, Japan, were inoculated i.p. with 2 × 10⁷ hybridoma 3-3-C cells (18). Ascites fluid and blood were collected from tumor-bearing mice 10 days after cell inoculation and centrifuged. Cell-free supernatant was purified using Protein A-Sepharose CL-4B (Pharmacia Fine Chemicals, Uppsala, Sweden) (2) as follows. Four ml of the pooled serum and ascites fluid were diazylated against Buffer 1 and applied to a Protein A-Sepharose CL-4B column (1.5 x 22 cm) equilibrated with Buffer 1. After absorbance at 280 nm decreased enough with elution with Buffer 1, protein was eluted with 0.1 M citrate buffer, pH 4.5, to give a mixture of IgG1 and IgG2a and then with 0.1 M citrate buffer, pH 3.5, to give pure IgG2b (anti-MM46 IgG) (10 mg) as confirmed by SDS-PAGE and the Ouchterlony test with rabbit antiserum against mouse IgG subclasses (Miles Laboratories, Elkhart, Ind.). Complement-dependent cytolytic activity against MM46 cells was observed only with the IgG2b fraction.

Nonimmune mouse IgG was prepared from nonimmunized normal mouse serum also by Protein A-Sepharose CL-4B column chromatography. After elution with Buffer 1, the column was eluted successively with 0.1 M citrate buffers of pH 5.5, 4.5, and 3.5 to give IgG1, IgG2a, and IgG2b in this order. IgG2a and IgG2b thus obtained were pooled and used as nonimmune IgG.

Ricin and Its A Chain. Isolation of ricin from Ricinus communis beans (Kinokuniya Pharmacy, Tokyo, Japan) and preparation of its A chain were performed as described by Olsnes and Pihl (14).

Preparation of IgG:Ricin A Chain Conjugate. One-tenth ml of 1.92 mg/ml ethanolic solution of SPDP (Pharmacia Fine Chemicals) was added dropwise to 4.9 ml of anti-MM46 IgG in Buffer 2 (1.18 mg/ml) and the mixture was incubated at 23° for 30 min with stirring at 10-min intervals. After addition of 50 μl of 0.1 M Tris-HCl buffer, pH 8.2, to quench the reaction, the mixture was dialyzed against Buffer 2. To the resulting solution of IgG containing the active disulfide groups was added 0.4 ml of ricin A chain in 5 mM acetate buffer, pH 5.5, containing 0.14 mM NaCl and 1 mM EDTA (5.82 mg/ml), and the reaction was allowed to proceed at 23° for 16 hr. The conjugate (anti-MM46 conjugate) was purified by gel filtration on Sephadex G-150 superfine (Pharmacia Fine Chemicals) in 0.9% NaCl solution, sterilized, and stored at −80°.

Nonimmune conjugate was prepared using nonimmune mouse IgG by the same procedure as is described above.

SDS-PAGE. SDS-PAGE was carried out by the modified method of Weber and Osborn (23). Electrophoresis was conducted using 5% polyacrylamide gel (0.5 × 6 cm) in 40 mM Tris buffer, pH 7.4, containing 20 mM sodium acetate and 2 mM EDTA.

Ouchterlony Test. The Ouchterlony test was carried out in 1% agar in PBS and 0.02% NaN₃ solution.

Guinea Pig Antiserum against Ricin A Chain. The antiserum was prepared as described previously (12).

In Vitro Cytotoxicity Test. In a 96-well flat-bottomed microtiter plate (Nunc No. 167008, A/C Nunc, Roskilde, Denmark) were placed 20 μl of a conjugate or IgG solution serially diluted with 0.9% NaCl solution and 200 μl of a MM46 or MM48 cell suspension (2.5 × 10⁵ cells/ml) in Roswell Park Memorial Institute Tissue Culture Medium 1640 (Nissui Seiyaku, Tokyo, Japan) supplemented with 10% heat-inactivated fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.), 20 μM 2-mercaptoethanol (Nakarai Chemicals, Kyoto, Japan) and 0.1 mg of kanamycin sulfate per ml (Banyu Pharmaceutical, Tokyo, Japan). After cell culture in a humidified atmosphere of 5% CO₂ in air at 37°, the number of viable cells was enumerated with 20 μl of 3% trypan blue in PBS added to each well.

Serological Tumor-neutralizing Assay. A Winn-type serological tumor-neutralizing assay (24) was performed by incubating MM46 cells with various concentrations of a conjugate or IgG in PBS at 37° for 30 min and injecting the PBS cell suspension i.p. or s.c. to C3H/He mice. The tumor-neutralizing activity was determined by following the survival of the mice or by measuring the tumor weight.

RESULTS

Preparation of Conjugates. Anti-MM46 IgG was treated with a 5-fold molar excess of SPDP to give IgG to which, on average, two and three-tenths 3-(2-pyridyldithio)propionyl groups were introduced as determined by treating an aliquot of the product with 5 mM dithiothreitol followed by measurement of absorbance at 343 nm due to liberated pyridine-2-thione (20). Then, 3-(2-pyridyldithio)propionylated IgG was treated with a 2-fold molar excess of ricin A chain having a free sulfhydryl group.

Chart 1 shows the elution profile of Sephadex G-150 superfine column chromatography of the reaction mixture. Peak I protein (pooled Fractions 32 to 37) gave 4 bands in SDS-PAGE (Fig. 1A, Gel 1). Their respective migration positions together with observation of a precipitin line both with anti-mouse IgG2b antiserum and with anti-ricin A chain antiserum in the Ouchterlony test (Fig. 1B) indicated that Peak I protein consisted of the conjugates resulting from coupling of the IgG and ricin A chain in IgG:A chain molar ratios of 1:1, 1:2, and 1:3 and a minor amount of unconjugated anti-MM46 IgG. Peak I protein gave, on reduction with 25 mM 2-mercaptoethanol, ricin A chain and H and L chains of IgG (Fig. 1A, Gel 4), confirming the conjugate structure of Peak I protein. Among the conjugates, one having an equimolar coupling ratio was the major product (Fig. 1A, Gel 1). On average, 1.3 molecules of A chain were conjugated to one molecule of IgG as determined by scanning the SDS-PAGE gel on a Shimadzu Model CS-900 dual-wavelength thin-layer chromatography scanner. This Peak I protein (4.7 mg protein) was used as the conjugate in biological studies. Peak II protein proved to be unreacted ricin A chain by the Ouchterlony test (Fig. 1B). The third small peak was supposed

![Chart 1. Elution profile on Sephadex G-150 superfine column chromatography (1.5 x 22 cm; each fraction, 2 ml) of a reaction mixture of ricin A chain with 3-(2-pyridyldithio)propionylated anti-MM46 IgG. Fractions 32 to 37 (bar) were pooled and used as anti-MM46 conjugate.](attachment:image_url)
Anti-MM46 Antibody: Ricin A Chain Conjugate

**Fig. 1.** Structure analysis of anti-MM46 conjugate. A. SDS-PAGE analysis. Gel 1, anti-MM46 conjugate (Chart 1. Peak I protein); Gel 2, anti-MM46 IgG; Gel 3, ricin A chain; Gel 4, reduced anti-MM46 conjugate; Gel 5, reduced anti-MM46 IgG; and Gel 6, SDS-PAGE Marker I containing 5 subunits of RNA polymerase B (Mitsubishi Petrochemical, Tokyo). The reduction of the conjugate and the IgG was carried out by incubating them with 25 mM 2-mercaptoethanol in the running buffer for SDS-PAGE containing 2 M urea at 80°C for 10 min. B. Ouchterlony test. Well 1, Peak I protein; Well 2, Peak II protein; Well 3, rabbit anti-mouse IgG2b antiserum; and Well 4, guinea pig anti-ricin A chain antiserum.

to be pyridine-2-thione, since it had λmax at 343 nm. Nonimmune conjugate was prepared similarly using nonimmune IgG obtained from nonimmunized mice.

**In Vitro Complement-dependent Cytolytic Activity of Antibody and Conjugate.** The cytolytic activities in the presence of complement of anti-MM46 IgG and of anti-MM46 conjugate were examined (Chart 2). Anti-MM46 IgG exhibited an activity of lysing 50% of 1.1 × 10⁶ MM46 cells/ml at an IgG concentration of 0.1 μg/ml. Essentially all of MM46 cells were lysed when the anti-MM46 IgG concentration was increased to about 0.3 μg/ml. Anti-MM46 conjugate showed almost the same magnitude of complement-dependent lytic activity as did anti-MM46 IgG.

**In Vitro Cytotoxicity of Conjugate.** Cytotoxicity of anti-MM46 conjugate against cultured MM46 cells was examined in comparison with cytotoxicities of nonimmune conjugate and of anti-MM46 IgG (Chart 3). Anti-MM46 conjugate showed potent dose-dependent cytotoxicity and inhibited the cell growth at concentrations above 1 μg/ml. On the other hand, anti-MM46 IgG hardly affected the cell growth of MM46 cells, and nonimmune conjugate affected the growth only to a small extent at a concentration of 30 μg/ml. These results indicate that the potent cytotoxicity of anti-MM46 conjugate is due to both the protein synthesis-inhibitory action of the ricin A chain portion and the antigen-binding activity of the anti-MM46 IgG portion. The weak cytotoxicity of nonimmune conjugate is probably due to nonspecific endocytotic internalization of A chain.

Next, the number of the viable MM46 cells was determined after incubation for various time periods with anti-MM46 conjugate or nonimmune conjugate (Chart 4). The cell number of the 0.9% NaCl solution group (control) reached its maximum at about 72 hr of culture and decreased with increasing culture period. Nonimmune conjugate gave growth curves similar to that of the control; anti-MM46 conjugate showed growth-inhibitory effect at a concentration of 10 μg/ml, and especially at 30 μg/ml, cytotoxic effect was apparent with the lapse of time.

Finally, the target-cell specificity of anti-MM46 conjugate...
action against MM46 cells was confirmed.

Tumor-neutralizing Activity of Conjugates and Antibody. Tumor-neutralizing activity of anti-MM46 conjugate, nonimmune conjugate, and anti-MM46 IgG was first assessed by following the survival of groups of C3H/He mice after i.p. inoculation of 5 x 10⁴ MM46 cells preincubated with one of the above 3 test materials (Chart 6). When the tumor cells were preincubated with 15 µg of a test protein per ml (Chart 6A), anti-MM46 conjugate showed the largest tumor-neutralizing activity, making all 5 mice survive tumor free. At this concentration, anti-MM46 IgG alone also showed a strong activity, having 4 mice surviving tumor free. On the contrary, nonimmune conjugate did not exhibit any activity even at this high concentration. When the concentration of the test protein in the cell-treating solution was lowered to 1.5 µg/ml (Chart 6B), anti-MM46 IgG failed to show activity. However, anti-MM46 conjugate still retained its activity: 2 of 5 mice survived tumor free; and the percentage of increase in life span of the remaining mice over that of the control was 25%. At a concentration of 0.15 µg/ml, none showed antitumor activity (Chart 6C).

Tumor-neutralizing activity of anti-MM46 conjugate, nonimmune conjugate, or anti-MM46 IgG was next assessed by determining inhibition of the growth of 4 x 10⁴ pretreated MM46 cells injected s.c. into the inguinal region of C3H/He mice (Chart 7). When the preincubation of the cells with the conjugates and the IgG was performed at a test protein concentration of 5 µg/ml, anti-MM46 conjugate showed a strong antitumor activity resulting in the complete inhibition of the tumor growth in all 5 mice. At a concentration of 1.5 µg/ml, all 3 proteins showed a moderate activity.

Therapeutic Effect on Ascitic MM46 Tumor. Antitumor effects on an early stage and an advanced stage of ascitic MM46 tumor were assessed in C3H/He mice inoculated i.p. with 5 x 10⁴ MM46 cells per mouse. To assess the effect on an early stage of the tumor, test materials were injected i.p. to different groups of 5 mice, starting 24 hr after tumor inoculation, on
Chart 6. Tumor-neutralizing activity against ascitic MM46 tumor. MM46 cells (2.5 x 10^5 cells in 1 ml of PBS) were incubated with anti-MM46 conjugate (•), nonimmune conjugate (A), or anti-MM46 IgG (O) (1.5, 5, or 0.15 µg of the conjugate or the IgG per ml) at 37°C for 30 min. The PBS cell suspension (0.2 ml) containing 5 x 10^5 treated cells was injected i.p. into a C3H/He mouse (5 mice per group). Survival was followed for 60 days. Mice inoculated with untreated MM46 cells served as control (x).

Days 1, 3, and 5 (Chart 8). At a dose of 1 µg/mouse at each injection (Chart 8A), the survivals of the anti-MM46 IgG and the nonimmune conjugate groups were not significantly different from that of the control 0.9% NaCl solution group in which all of the mice died between Days 16 and 20, but all 5 mice of the anti-MM46 conjugate group survived tumor free. At a dose of 3 µg (Chart 8B), not only anti-MM46 conjugate but also anti-MM46 IgG made all 5 mice tumor free. On the other hand, when the dose was decreased to 0.3 µg (Chart 8C), none showed antitumor activity.

Next, in order to assess the antitumor effect on the advanced stage of MM46 tumor, test materials were injected i.p. to different groups of tumor-bearing mice on Days 5, 7, and 9 at a dose of 10 µg/mouse at each injection (Chart 9). In the anti-MM46 conjugate group, one of 5 mice survived tumor free, and the percentage of increase in life span of the remaining mice over that of the control was 29%. Injections of anti-MM46 IgG and of nonimmune conjugate had no effect on the survival.

Comparison of the Therapeutic Effect of Conjugate with That of a Mixture of Components. In order to compare the therapeutic effect of anti-MM46 conjugate on ascitic MM46 tumor with that of an equimolar mixture of the 2 components of the conjugate, groups of 5 C3H/He mice inoculated i.p. with 5 x 10^6 MM46 cells were treated with an i.p. injection of test materials on Days 1, 3, and 5 (Chart 10). All 5 mice given injections of 1 µg of the conjugate per mouse at each injection survived tumor free, confirming the result in Chart 8, whereas none of the 2 components and a mixture of the 2 showed a significant life-prolonging effect.

Therapeutic Effect on Solid MM46 Tumor. In order to
M. Seto et al.

Chart 9. Antitumor effect on advanced ascitic MM46 tumor-bearing C3H/He mice. Groups of 5 C3H/He mice were inoculated i.p. with 5 x 10⁶ MM46 cells. Different groups received on Days 5, 7, and 9 an i.p. injection of one of the following: anti-MM46 conjugate (O), immununone conjugate (A); anti-MM46 IgG (C); and PBS (X). The dose per injection of the conjugate or the IgG was 10 µg/mouse. Survival was followed for 60 days.

Chart 10. Comparison of the antitumor effect of anti-MM46 conjugate with that of a mixture of the 2 components of the conjugate. Groups of 5 C3H/He mice were inoculated i.p. with 5 x 10⁶ MM46 cells. Starting 24 hr later, different groups received on Days 1, 3, and 5 an i.p. injection of one of the following: 1 µg of anti-MM46 conjugate (O), 0.83 µg of anti-MM46 IgG (C), 0.17 µg of ricin A chain (A); a mixture of 0.83 µg of anti-MM46 IgG and 0.17 µg of ricin A chain (O); and PBS (X). The above amounts of the test materials are expressed as the amount per mouse per injection.

assess the inhibition of solid tumor growth, C3H/He mice inoculated s.c. with 2 x 10⁶ MM46 cells in the inguinal region were treated with an i.p. injection of 10 µg of anti-MM46 conjugate or anti-MM46 IgG on Days 1, 3, 5, and 7. No inhibition of tumor growth was observed as determined by tumor weight on Day 40 (data not shown). Therefore, the administration route of the test materials was changed from i.p. to intraregional route. As shown in Chart 11, at a dose of 3 µg/injection, inhibition of tumor growth was observed only with anti-MM46 conjugate (T:C, 50%). At 10 µg, antitumor effect was shown by both anti-MM46 conjugate (T:C, 22%) and anti-MM46 IgG (T:C, 30%).

DISCUSSION

Mouse monoclonal IgG2b antibody directed against syngeneic mouse mammary tumor MM46 cells (anti-MM46 IgG) was conjugated with ricin toxic subunit A chain via a disulfide bond using SPDP as cross-linking agent. SPDP was chosen based on our previous observation (12) that the conjugates cross-linked by a disulfide bond susceptible to the cleavage by a mercapto reagent showed superior in vitro cytotoxicity compared with the conjugates otherwise cross-linked. The conjugate thus prepared (anti-MM46 conjugate) showed a potent cytotoxicity against cultured MM46 cells at a concentration of 30 µg/ml, at which unconjugated anti-MM46 IgG did not exhibit any cytotoxicity. The specificity of the cytotoxicity of the conjugate was demonstrated by the facts that anti-MM46 conjugate was unable to affect the growth of MM antigen-negative MM48 cells and that nonimmunne conjugate did not show significant cytotoxicity.

As the first step to investigate the in vivo efficacy of the conjugate, in vivo tumor-neutralizing activity was examined. Next, therapeutic experiments were carried out. Since anti-MM46 IgG used in the present study is directed against MM antigen (18), it may be effective in tumor suppression by itself (17, 19). Indeed, the IgG exhibited not only a strong in vitro cytolytic activity against MM46 cells in the presence of complement but also a considerably strong inhibitory activity in vivo. Anti-MM46 conjugate, however, showed even greater antitumor activity. The superior tumor-neutralizing activity of anti-MM46 conjugate over the activity of anti-MM46 IgG was evident when the cells treated with 1.5 µg of the conjugate per ml were injected i.p. into C3H/He mice or when the cells treated with 5 µg of the conjugate per ml were injected s.c. A markedly superior efficacy of anti-MM46 conjugate was also observed in therapeutic experiments in which mice inoculated i.p. with MM46 cells were treated i.p. with a test material. The importance of the covalent coupling of antigen-MM46 IgG and ricin A chain was confirmed by the observation that a mixture of the 2 components of the conjugate failed to show antitumor effect equivalent to that of the conjugate. Therapeutic experiments with an advanced stage of ascitic MM46 tumor showed a moderate but significant effect of the conjugate. This result suggests that the immunne system of the host is probably not required for the action of the conjugate, since C3H/He mice inoculated with MM46 cells were immunosuppressed on Day 5 as determined by delayed-type hypersensitivity against sheep RBC.4 The failure of i.p.-injected anti-MM46 conjugate (4 x 10 µg) to show antitumor activity to solid MM46 tumor is probably due to the problem of reaching of the conjugate to the tumor site, for intraregionally injected conjugate was effective in inhibiting the tumor growth.

Therapeutic efficacy of antibody:ricin A chain conjugates

Anti-MM46 Antibody: Ricin A Chain Conjugate

has been studied in mouse model systems by using monoclonal anti-Thy1.2 antibody and a Thy1.2-positive mouse tumor (1) or by using monoclonal anti-transferrin receptor antibody and human tumors implanted into nude mice (22). The present study differs from these former studies in that our model system uses antibody directed against MM antigen, tumor-specific antigen, and MM antigen-positive mouse mammary tumor cells implanted into syngeneic mice. The advantages which this system can enjoy include the following. (a) Various classes of monoclonal antibodies against MM antigen including an IgG2a antibody, from which F(ab')2 can be obtained efficiently, have been established (18). (b) A series of MM antigen-positive and -negative transplantable mouse mammary tumor cell lines (6, 21, 25) is available. (c) These tumor cell lines can be grown in syngeneic mice as ascites and/or solid form.

Using this system, we showed the therapeutic effectiveness of an antibody:ricin A chain conjugate on an ascites form of tumor at an extremely small dose. These potent in vivo antitumor effects are probably due to the strong cytotoxic action of the toxic portion (A chain) of the conjugate demonstrated in in vitro cytotoxicity study in the absence of complement. However, the antibody portion of the conjugate may still retain the activity of the Fc portion even after conjugation with ricin A chain, as demonstrated by the in vitro complement-dependent cytotoxicity of the conjugate. In addition, it should be noted that the conjugate preparation contains unconjugated antibody, although it is in a small amount (Fig. 1A). Therefore, the possibility still remains that the observed antitumor effect of the conjugate may in part be played by the Fc moiety of the antibody portion of the conjugate in collaboration with the host cells and by the unconjugated antibody. The use of the F(ab')2 fragment for the antibody portion of the antibody:ricin A chain conjugates should enable the evaluation of the net in vivo antitumor activity of the conjugate, because F(ab')2 conjugates can no longer act in cooperation with host cells, and because by using rabbit IgG, we were able to isolate a F(ab')2 conjugate with ricin A chain free from unconjugated F(ab')2 by Sephadex G-200 superfine chromatography. Furthermore, it might be possible to enhance the antitumor activity of the A chain conjugates, because Houston et al. (5) showed the better localization of F(ab')2 onto the tumor compared with the case of intact IgG. Therefore, preparation of F(ab')2:ricin A chain conjugate using monoclonal IgG2a anti-MM46 antibody 3–2H18 is now in progress in our laboratories. Further studies on the potential of antibody:ricin A chain conjugates and studies on antibody conjugates with cytotoxic agents other than ricin A chain are worthy of being carried out using monoclonal antibodies to MM antigen and syngeneic mouse mammary tumors in C3H/He mice.

ACKNOWLEDGMENTS

The authors are very grateful to Dr. Y. Nishizuka, Aichi Cancer Center Research Institute; Dr. Y. Kato, Teijin Institute for Biomedical Research, for valuable discussions; and also N. Matsui and Y. Matsuda for technical assistance.

REFERENCES

9. Krolick, K. A., Villeneme, C., Isakson, P., Uhr, J. W., and Vitetta, E. S. Selection of antibodies by affinity chromatography by using rabbit IgG, we were able to isolate a F(ab')2 conjugate with ricin A chain free from unconjugated F(ab')2 by Sephadex G-200 superfine chromatography. Furthermore, it might be possible to enhance the antitumor activity of the A chain conjugates, because Houston et al. (5) showed the better localization of F(ab')2 onto the tumor compared with the case of intact IgG. Therefore, preparation of F(ab')2:ricin A chain conjugate using monoclonal IgG2a anti-MM46 antibody 3–2H18 is now in progress in our laboratories. Further studies on the potential of antibody:ricin A chain conjugates and studies on antibody conjugates with cytotoxic agents other than ricin A chain are worthy of being carried out using monoclonal antibodies to MM antigen and syngeneic mouse mammary tumors in C3H/He mice.
Monoclonal Anti-MM46 Antibody:Ricin A Chain Conjugate: \textit{In Vitro} and \textit{in Vivo} Antitumor Activity

Masao Seto, Naoji Umemoto, Masahiko Saito, et al.


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/42/12/5209

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, use this link http://cancerres.aacrjournals.org/content/42/12/5209.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.