Growth Suppression of Human Colorectal Carcinoma in Nude Mice by Monoclonal Antibody C27-Abrin A Chain Conjugate

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PURPOSE: The aim of this study was to assess an immunotoxin, monoclonal antibody C27-abrin A chain conjugate (MAAC), that might be effective in the treatment of colorectal carcinoma. METHODS: The immunotoxin was prepared by a specific monoclonal antibody against carcinoembryonic antigen (CEA), monoclonal antibody C27, linked to N-succinimidyl-3-(2-pyridyldithio)propionate and then coupled covalently to the toxic abrin-A chain to synthesize MAAC. The therapeutic role of this immunotoxin in suppressing the in vitro and in vivo growth of CEA-secreting human colorectal cancer cells (LS174T) was assayed by methods of protein biosynthesis inhibition, cell colony proliferation, and treatment of tumor cells before and after inoculation in nude mice. RESULTS: We found that MAAC effectively suppressed the growth of LS174T in culture medium and completely eradicated cells in inoculated nude mice. In contrast, irrelevant immunotoxin antiferritin-abrin A chain conjugate and isotype-matched monoclonal immunoglobin (MOPC21 IgG1)-abrin A chain conjugate did not cause such effects. The in vitro toxicity was highly specific because the conjugate (MAAC) inhibited de novo protein biosynthesis, impeded growth, and caused death of cells possessing surface CEA determinants. The 50 percent inhibition dose values of the conjugate for colonogenic survival and for protein biosynthesis in LS174T cells were 0.09 µg/ml and 0.06 µg/ml, respectively. Colony survival was inhibited 96.3 percent after prolonged MAAC treatment. MAAC showed selective cytoxicity; the inhibitory effect of MAAC to the CEA-secreting LS174T cells over the CEA-nonsecreting human embryonic kidney cells was 16-fold. CONCLUSION: These results indicate that MAAC may be of benefit in therapy during or soon after resection of colorectal carcinoma or in patients who have micrometastasis. [Key words: Colorectal carcinoma; Abrin A chain immunoconjugate; Monoclonal antibody; Carcinoembryonic antigen]


Antibody-mediated delivery of toxic agents to cells has been suggested for experimental and therapeutic applications. 1,2 Immunotoxins, hybrid protein molecules consisting of highly potent plant or bacterial toxins or their active subunits linked to an antibody, are designed to achieve cell-specific killing through intracellular action of the toxin. 3,4 Conjugates of toxins with antibodies recognizing tumor-associated antigens have been shown to exert specific toxic effects in cell culture in animal models and, recently, in clinical trials for cancer patients. 5–30 We have previously shown that a highly specific monoclonal antibody (MABC27) against human carcinoembryonic antigen (CEA) can effectively target human colorectal carcinoma (CRC) cells. 3,4 To increase the therapeutic toxicity of this monoclonal antibody, our present study aimed to synthesize an immunotoxin by coupling it to the toxin abrin, a toxic protein isolated from Abrus precatorius. Abrin consists of two moieties, the toxic A chain (abrin A) and the carrier B chain (abrin B), which are joined by a disulfide bond. The B chains can bind the toxin to cell surface receptors and somehow facilitate penetration of the toxic A chains into cells. The internalized A chain acts enzymatically to inhibit cellular protein synthesis and eventually kills the cells. 14 In the present study, we have synthesized an immunotoxin by covalently linking the monoclonal anti-CEA antibody (anti-CEA MAB) to the abrin A chain with a coupling agent, N-succinimidyl-3-(2-pyridyldithio)propionate. The
therapeutic role of this immunotoxin in suppressing in vivo and in vitro growth of CEA-secreting human CRC cells (LS174T) was also studied.

MATERIALS AND METHODS

Preparation of Monoclonal Antibody (MAb)

Preparation of MAb against CEA was described in a previous report. Briefly, CEA was purified from liver metastasis of CRC patients. MAbC27 was obtained by fusion of P3-NS1/1-Ag4 mouse myeloma cells and splenic cells from 10-week-old female BALB/c mice (Charles River Laboratories, Atsugi, Japan), immunized with purified CEA. MAbC27 is of IgG1 subclass, with a high affinity and specificity to CEA.

Purification of Antibody

The anti-CEA antibody (MAbC27) was produced in large amounts in the peritoneal cavity of mice and was purified from ascitic fluids by ammonium sulfate precipitation (45 percent saturation at 4°C) and protein A-Sepharose 4B (Pharmacia, Sollentuna, Sweden) affinity chromatography, as described previously. The ascites to be fractionated were mixed with phosphate buffer (0.5 M, pH 8.1) and applied to a protein A-Sepharose 4B gel column. After elution, positive fractions were collected, dialyzed, and then freeze-dried. Purity and specificity of the antibody were tested by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and immunoblot.

Cell Lines

CEA-secreting human CRC cells (LS174T) and CEA-nonsecreting human embryonic kidney (HEK) cells were purchased from American Tissue Culture Collection (Rockville, MD) and were maintained as a monolayer culture in Dulbecco’s Modified Eagle’s medium (DMEM; GIBCO, Grand Island, NY). Medium was supplemented with 10 percent fetal calf serum (FCS; GIBCO), penicillin (100 units/ml), streptomycin (100 μg/ml), and gentamycin (20 μg/ml).

Isolation of Abrin A chain

Abrin a was sequentially isolated from the seeds of Abrus precatorius by Sepharose 4B affinity chromatography and Sephadex G-100 gel filtration as described by Lin et al. Abrin A chain was then isolated from abrin a by reduction with 2-mercaptoethanol application to a O-(diethylaminoethyl)cellulose column (2.5 X 100 cm) and elution in a Tris elution buffer (0.01 M, pH 7.0) containing 0.3 N sodium chloride. Abrin A chain was freed from residual traces of abrin a by recycling on an acid-treated Sepharose 4B affinity column. The unbound (first peak) and the bound (second peak) proteins were identified as abrin A and B chains, respectively. Purity and molecular mass of the abrin A chain were analyzed by SDS-PAGE. Molecular mass of the abrin A chain was found to be approximately 27 kDa.

Preparation of 2-Pyridyldisulfide Substituted (PDP)-MAbC27 and MAbC27-Abrin A Conjugate

N-succinimidyl-3-(2-pyridyldithio)propionate (5 μl of 20 mM solution) in absolute ethanol was simultaneously added to MAbC27 (2 ml containing 2 mg), normal mouse IgG1 (MOPC21) (Litton Bionetic, Charleston, SC), and rabbit anti-human ferritin antibody (DAKO Corp. Carpinteria, CA) in coupling phosphate-buffered saline (0.1 M, pH 7.5) to react for 30 min at room temperature. After the product of PDP-antibody had been dialyzed against the coupling buffer, its 2-pyridyldisulfide content was further analyzed as described previously. One milliliter each of PDP-anti-CEA, PDP-antiferritin, or PDP-MOPC21 IgG1 was mixed to react for 24 hours at 4°C, at a molar ratio of 1:3 with the abrin A chain. The crude conjugate was then applied to a Sephacryl S-200 column (1.8 X 35 cm) to remove the excess of unbound protein and chemical reagents and was eluted with sodium phosphate buffer (0.01 M, pH 7.2) to obtain MAbC27-abrin A chain conjugate (MAAC), MOPC21-abrin A chain conjugate (MOAC), or antiferritin-abrin A chain conjugate (AFAC). Molecular mass and components of the conjugate were determined by SDS-PAGE.

Experimental Design

LS174T cells were cultured in vitro and in vivo and submitted to different experiments. Each experiment was further divided into 1) MAAC-treated (the experimental group), 2) abrin A-treated (the positive control), 3) AFAC, MOAC, abrin A chain, or an equal mixture of MAbC27 and abrin A chain-treated (the experimental controls), and 4) MAbC27-treated (the negative control).

Cell Colony Test

LS174T and HEK cells (2 X 10⁴ in 0.5 ml) were cultured in 24-well plates (Costar, Cambridge, MA) at