

Antitumor Effects of B3-PE and B3-LysPE40 in a Nude Mouse Model of Human Breast Cancer and the Evaluation of B3-PE Toxicity in Monkeys

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ABSTRACT

B3 is a tumor-reactive monoclonal antibody (mAb) that binds to a limited number of normal tissues. Immunotoxins made with B3 coupled to either *Pseudomonas* exotoxin (PE) or recombinant forms of PE with a deletion of the cell-binding domain (LysPE40) have been shown to cause complete tumor regression in nude mice bearing a rapidly growing A431 (L. H. Pai *et al.*, Proc. Natl. Acad. Sci. USA, 88: 3358-3362, 1991) human epidermoid carcinoma. In this study we show that an immunotoxin composed of mAb B3 when chemically coupled to LysPE40 (B3-LysPE40) led to complete regression of a slowly growing breast cancer, MCF-7, in nude mice when given i.v. every other day for five doses. mAb B3 coupled to native PE also produced significant regression of the MCF-7 tumor. The reactivity of mAb B3 was evaluated using an immunohistochemical method on the two responsive tumors, MCF-7 and A431, and compared with a typical human colon carcinoma specimen that has B3 antigen on its surface. The results showed that both A431 and MCF-7 xenograft tumors have similar reactivity to B3 when compared with the human colon carcinoma specimen. To evaluate the toxicity of B3-PE in primates, Cynomolgus monkeys received escalating doses of B3-PE i.v. on Days 1, 3, and 5. Based on antibody localization studies using frozen sections of normal human and monkey tissue, gastric, trachea, and bladder mucosal injury could have occurred. However, no clinical signs of injury or histological damage to these organs were seen at the doses administered. Chemical hepatitis due to PE was transient and well tolerated at doses up to 50 μ g/kg for three doses. The lethal dose was about 100 μ g/kg, and the cause of death was liver necrosis, as shown by necropsy. We conclude that mAb B3, when coupled to PE40 or PE, can produce strong antitumor activity *in vivo*. The similar level of reactivity of the B3 antibody in our tumor models with a surgical specimen of a human colon carcinoma and the toxicity study in monkeys indicate that therapeutic doses of B3-PE and B3-LysPE40 can be delivered without causing toxicity to normal organs that express B3 antigen. Although both B3-PE and B3-LysPE40 have antitumor activity in nude mice bearing a human xenograft, B3-LysPE40 is better tolerated and should be further evaluated as a therapeutic agent for cancer patients.

INTRODUCTION

In the past few decades monoclonal antibodies against human cancer-associated antigens have allowed specific tumor targeting to be explored as a new therapeutic modality for cancer. Radioactive nuclides, chemotherapeutic agents, and bacterial or plant toxins coupled to monoclonal antibodies are presently being tested in cancer patients (1-4).

Our laboratory has focused on immunotoxins made with PE.³ It is a M_r 66,000 protein that kills cells by ADP ribosylation and inactivation of elongation factor 2 which causes the

arrest of protein synthesis. PE is composed of three major structural domains each with a different function (5, 6). Domain I is responsible for cell recognition; Domain II, for translocation across the cell membrane; and Domain III, for the ADP-ribosylation activity of the toxin. Recently, the last five amino acids of the carboxyl terminus of Domain III have also been shown to have a role in translocation (7). LysPE40 is a M_r 40,000 recombinant form of PE that lacks the cell-binding domain and has a chemically reactive lysine residue near the amino terminus which facilitates coupling to antibodies.

We have recently described a murine monoclonal antibody, termed B3, that is directed against a carbohydrate antigen of the Le^v family that is present on the surface of many human carcinomas. Immunohistochemical studies of human carcinomas show that 12 of 12 adenocarcinomas of the colon react with mAb B3, and 75% of these react strongly and homogeneously. Similar strong uniform reactivity was found in gastric (3 of 4) and esophageal (7 of 9) carcinomas. B3 also reacts strongly with 65% of the breast carcinomas and 55% of the ovarian carcinomas tested (8). Most recently, we found that 10 of 15 adenocarcinomas of the lung also react strongly and uniformly with mAb B3.⁴ Immunotoxins made with monoclonal antibody B3 and native PE or with LysPE40 have been shown to have cytotoxic activity against human tumor cell lines that express the B3 antigen on their surface. These immunotoxins were also shown to cause complete tumor regression in nude mice bearing a rapidly growing human epidermoid carcinoma, A431 (9). In this study, we initially evaluated the antitumor activity of immunotoxins B3-PE and B3-LysPE40 when administered to nude mice bearing a slowly growing human breast carcinoma, MCF-7. Both the MCF-7 tumor and the A431 tumor are appropriate models of human cancer because the amount of B3 antigen on these tumors, as shown by the immunohistochemical reactivity, is equal to or less than the amount of B3 antigen that is present in many human carcinoma specimens tested.

We also evaluated the toxicity of B3-PE in monkeys. Because the B3 antibody does not react with mouse tissues but does react with the stomach, trachea, and bladder of humans and monkeys with equal intensity (8), we administered B3-PE to monkeys. At a dose of 50 μ g/kg that caused complete regression of A431 tumors in mice (9), no damage to stomach, trachea, or bladder was observed. Our data indicate that B3-PE and B3-LysPE40 merit further evaluation as therapeutic agents for cancer.

MATERIALS AND METHODS

Monoclonal Antibody B3. The properties of mAb B3 (IgG1 κ) have been previously described (8). The mAb used for these experiments was purified from serum-free culture medium by ammonium sulfate precipitation and chromatography on Mono Q and TSK-250 gel filtration columns. Its purity was established by sodium dodecyl sulfate/polyacrylamide gel electrophoresis. Monoclonal antibody MOPC-21 (Sigma Chemical Co.) was used as control antibody in our experiments.

⁴ Unpublished data.

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³ The abbreviations used are: PE, *Pseudomonas* exotoxin A; LysPE40, recombinant form of PE with a deletion of the cell-binding domain; mAb, monoclonal antibody; IgG1, immunoglobulin G1; PCR, polymerase chain reaction; PBS, phosphate-buffered saline; HSA, human serum albumin; LD₅₀, 50% lethal dose; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; ID₅₀, 50% inhibitory dose.

It is also a IgG1 κ mouse monoclonal antibody with no known reactivity with mouse or human tissues.

Pseudomonas Exotoxin. PE, purified from the culture medium of *Pseudomonas aeruginosa*, was purchased from Swiss Serum (Berne, Switzerland).

Construction of Plasmid-expressing LysPE40. Fig. 1 shows the plasmid pJB8L1 f+t used for the expression of LysPE40 in *Escherichia coli*. pJB8L1 carries a *LysPE40* gene under control of a T7 promoter and also contains a T7 transcriptional terminator and an f1 phage origin. Earlier, for LysPE40 expression we used the plasmid pJY85L in which the *LysPE40* gene is preceded by an OmpA signal sequence. OmpA is cleaved upon secretion of LysPE40, leaving a three-amino acid extension Ala-Asn-Leu at the amino terminus of LysPE40 (10, 11). To construct pJB8L1 f+t, a 180-base pair fragment was amplified by PCR using two oligonucleotides and pJY85L as the template. One oligonucleotide was the same as the 5' end of DNA encoding for LysPE40 and also contained an *Nde*I site at the 5' end of the codon for the first alanine. The other oligonucleotide was complementary to the sequence in Domain II and contained a *Sal*I site. The PCR-amplified fragment was restricted by *Nde*I and *Sal*I and purified by electrophoresis. After purification the PCR fragment was ligated with a 4.8-kilobase pair dephosphorylated *Nde*I-*Sal*I fragment of pVC 8 f+t. pVC 8 f+t is a plasmid containing a gene for PE40 under the control of T7 promoter.

Expression and Purification of LysPE40. BL21 (λDE3) cells were transformed with the plasmid pJB8L1 f+t. The cells were grown in superbroth containing 100 μ g/ml of Ampicillin at 37°C. At an A_{650} of 1.0, cells were induced with 1 mM isopropyl-B-D-thiogalactopyranoside. The cells were harvested 1 h later, and protein was localized as described elsewhere (10). The periplasm was used as the source of LysPE40 because most of the expressed protein was secreted into the periplasm. After filtration through a 0.45- μ m filter, periplasm was applied onto an 8-ml Q-Sepharose column. Proteins were eluted by a linear gradient from 0 to 0.5 M NaCl in 0.02 M Tris-HCl (pH 7.4). The fractions containing LysPE40, as determined by sodium dodecyl sulfate/polyacrylamide gel electrophoresis, were pooled and applied on a Mono Q 10/10 fast protein liquid chromatography (FPLC, Pharmacia) column. The recombinant protein was eluted using the same linear gradient described for the Q-Sepharose column. Further purification was achieved by gel filtration chromatography on a TSK 250 column. The N-terminal sequence of LysPE40 was found to be Ala-Asn-Leu-Ala-Glu-Ala-Phe-Lys-Gly-Gly-Ser-Leu.

Construction of Immunoconjugates. The antibody and toxin were linked by a thioether bond. PE and LysPE40 were incubated with 3-fold molar excess of succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (Pierce Chemical Co., Rockford, IL) at room temperature for 30 min. Monoclonal antibody B3 and MOPC-21 were incubated with 2-iminothiolane hydrochloride (Pierce Chemical Co., Rockford, IL) at 37°C for 1 h. Details of conjugation and purification methods have been described elsewhere (9).

Protein Synthesis Assay. The cytotoxic activity of the immunotoxins was evaluated in cultured tumor cell lines by measuring [3 H]leucine incorporation as previously described (9). The mean values of triplicates were expressed as a percentage of controls that did not receive immunotoxin.

LD₅₀. Regarding toxicity of B3-PE and B3-LysPE40 in mice, groups of female BALB/c mice were given injections i.p. with escalating doses of immunotoxin diluted in 200 μ l of PBS-HSA (0.2%). Animals were observed for 72 h for toxicity and death. The LD₅₀ is the dose of immunotoxin that kills 50% of the animals in 48 h.

Antitumor Activity of B3-PE and B3-LysPE40 in Nude Mice Bearing a Human Breast Carcinoma. Human MCF-7 cells were grown as subcutaneous tumors in female athymic nude mice. On Day 0 of each experiment, subcutaneous tumors were surgically removed from carrier mice and cut in pieces of approximately 1 mm in size. The tumor was implanted in the right flank using a sterile trochar. A 17 β -estradiol pellet (0.72 mg/pellet, 60-day release) from Innovative Research of America, Toledo, OH, was placed in the posterior cervical region to maintain tumor growth. Treatment of mice with B3-PE or B3-LysPE40 was started 13 to 15 days after tumor implantation, when the tumor

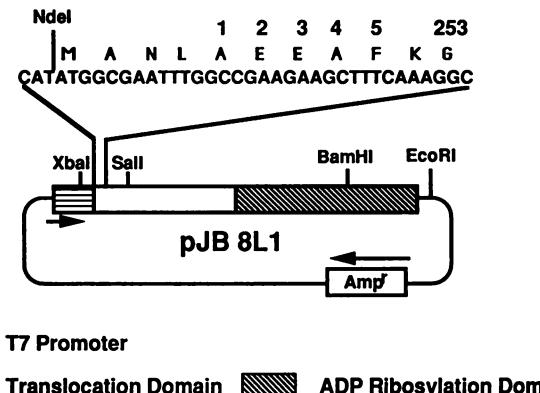


Fig. 1. Expression plasmid for LysPE40. Amino acids are shown using single-letter symbols. The numbers refer to the respective number of amino acid in native PE.

had reached 5 x 5 mm in size (50 mm³). Control groups received MOPC-PE, mAb B3, or PBS containing 0.2% HSA. Each treatment group consisted of six to eight animals. Tumors were measured with a caliper every 4 days, and the volume of the tumor was calculated by using the following formula: tumor volume (mm³) = length \times (width)² \times 0.4.

Immunohistochemistry. A frozen sample from a human colon carcinoma specimen and a MCF-7 subcutaneous tumor resected from nude mice 20 days after tumor implantation were cryostat sectioned and processed for peroxidase immunohistochemistry as previously described (12). To minimize cross-reaction with endogenous mouse IgG in the tumor transplant, the initial immunoreactive step utilized a B3-PE conjugate, followed by rabbit anti-PE, then an anti-rabbit-horse-radish peroxidase conjugate.

Toxicity of B3-PE in Cynomolgus Monkeys. This study was performed at the Hazleton Laboratories (Vienna, VA), Department of Toxicology, by principal scientist Dr. Dan W. Dalgard (HWA Project 421-158) from December 18, 1990, through March 11, 1991, under an approved animal protocol. B3-PE was prepared at the Laboratory of Molecular Biology, National Cancer Institute, NIH. Eight Cynomolgus monkeys, 2 in each group, received 10, 30, 50, and 100 μ g/kg of B3-PE i.v. on Days 1, 3, and 5. All monkeys were screened and were found not to contain antibodies against PE in the serum prior to the study. Animals were observed for morbidity and mortality after dosing for toxic or pharmacological effects, body weight, appearance, neurological changes, appetite, and changes in excreta. Blood samples were collected, and the serum chemistries performed included SGOT, SGPT, alkaline phosphatase, bilirubin, lactate dehydrogenase, total protein, albumin, glucose, blood urea nitrogen, creatinine, electrolytes, and amylase. Hematology included standard indices and platelet estimation. Urinalysis and examination of the stool for the presence of occult blood were performed. Necropsy was performed in the 2 animals in the 100- μ g/kg group.

Assay of Blood Levels of B3-PE in Monkeys. Blood samples were collected 10 min after each dose on Days 1, 3, and 5. The level of the immunotoxin was measured by bioassay in which serum samples were incubated with A431 cells, and the ability of the serum sample to inhibit protein synthesis was measured. A standard curve was made for each immunotoxin.

RESULTS

Immunotoxins made from monoclonal antibody B3 have been shown to be cytotoxic for various human cancer cell lines that express the B3 antigen on their surface (9). B3-PE is a potent immunotoxin active against human epidermoid carcinoma, A431, and breast carcinoma, MCF-7; the ID₅₀ for inhibition of protein synthesis for these cell lines was consistently between 0.1 and 0.5 ng/ml (9). B3-LysPE40 is also active

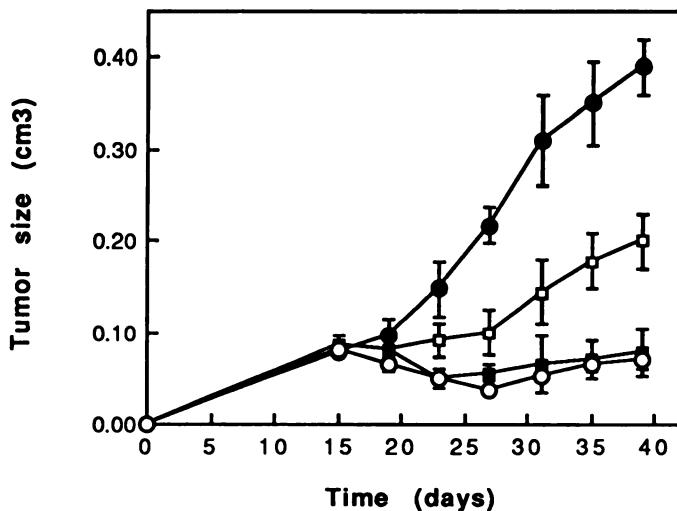


Fig. 2. Effect of B3-PE on the growth of MCF-7 tumors in nude mice. Tumors were implanted on Day 0 and treated with 0.25 (□), 1 (■), or 1.5 µg (○) of B3-PE i.v. on Days 15, 17, 19, and 21. The control group received MOPC-PE (●). Points, mean; bars, SD.

against these cell lines with ID_{50} s between 1 and 4 ng/ml. This 10-fold decrease in toxicity is due to the absence of Domain I which decreases cytotoxic activity but also decreases nonspecific toxicity in animals. The cytotoxic effects of the immunotoxins are specific because they are eliminated when excess antibody is added (50 µg/ml). No competition was seen when an excess of an irrelevant antibody (MOPC-21) was used. Both B3-PE and B3-LysPE40 did not intoxicate cells that do not express the B3 antigen when tested up to 500 ng/ml (9).

To determine the toxicity of B3-PE and B3-LysPE40 in mice, increasing doses of immunotoxins were injected i.p. into BALB/c mice. The LD_{50} of B3-PE was found to be 125 µg/kg, and that of B3-LysPE40, 6250 µg/kg per single dose. No detrimental effects were noted in the control group that received PBS-HSA (0.2%).

The antitumor activity of B3-PE and B3-LysPE40 was tested in an animal model of breast cancer using the transplantable estrogen-dependent MCF-7 tumor. On Day 0 of each experiment, athymic female nude mice were implanted with MCF-7 tumors (≈ 1 mm in size) removed from carrier mice. The tumor was implanted in the right flank. A 17 β -estradiol pellet (0.72 mg/pellet, 60-day release) was also implanted to maintain tumor growth. Treatment of mice with B3-PE or B3-LysPE40 was started 13 to 15 days after tumor implantation, when the tumor reached 5×5 mm in size (50 mm^3). As shown in Fig. 2, in animals that were given four doses of 1.5 µg of B3-PE on Days 15, 17, 19, and 21, a very significant antitumor effect was evident; the tumor stopped growing, regressed substantially in size, but then resumed growth 8 to 10 days later. In animals treated with 0.5 and 1 µg, lesser effects were seen. Control animals treated with MOPC-PE, B3, or PBS-HSA (0.2%) had no antitumor activity and were sacrificed 1 mo later when the tumor size achieved 1.0×1.0 cm in size.

Fig. 3 shows the effect of B3-LysPE40 on the growth of MCF-7 tumors in nude mice at various dose levels. Animals were treated every other day i.v., with the immunotoxin at dose levels of 20, 50, and 75 µg. Treatment was started on Day 13. At a dose level of 75 µg/injection, complete regression of the tumor was observed, which lasted for more than 1 mo. Control animals treated with PBS or mAb B3 alone were sacrificed after 4 wk when tumors had reached 1.0×1.0 cm in size.

To determine if tumors growing in B3-PE-treated mice failed to fully respond because they had become "antigen-negative," tumor was removed from a mouse treated with 1.5 µg of B3-PE. By immunohistochemistry, these tumor cells had a positive pattern of reactivity with B3 that is similar to cells maintained in tissue culture (results not shown). We conclude that the failure of B3-PE to cure the MCF-7 tumor was not due to the emergence of antigen-negative clones, but due to the presence of antigen-positive cells which might have been killed if larger amounts of immunotoxin could have been delivered to the tumor.

To determine if the MCF-7 tumors represent a model that is relevant for the treatment of human cancers, we examined the reactivity of B3 with a MCF-7 tumor removed from a nude mouse 20 days after tumor implantation and an A431 tumor removed 10 days after implantation and compared their reactivity with that of a human colon carcinoma sample. Immunohistochemical studies showed that the colon tumor reacted with a stronger intensity than did the MCF-7 or A431 tumor growing in mice (Fig. 4). Therefore we conclude that the model is relevant for the study of human cancers.

To determine whether the specific (monoclonal antibody B3 related) or the nonspecific (PE related) toxicities would be dose limiting, B3-PE was administered to Cynomolgus monkeys. In addition, we wished to determine the maximum tolerated dose in primates. These issues were especially relevant because monkey tissues react in a similar manner and intensity as do human tissues with the B3 antibody (8). Eight Cynomolgus monkeys, two in each group, received i.v. injections of B3-PE at dose levels of 10, 30, 50, and 100 µg/kg on Days 1, 3, and 5. No major clinical abnormalities were noted in the animals that received 10, 30, and 50 µg/kg except transient loss of appetite and mild weight loss (less than 8% of the initial body weight) during the first week. Elevation of SGOT, SGPT, alkaline phosphatase, and lactate dehydrogenase occurred in all animals. The magnitude of the enzyme elevation ranged from 3- to 15-fold from the pretreatment level. Fig. 5 shows the SGOT, SGPT, and alkaline phosphatase levels of one animal from each group. Elevation occurred by Day 3, generally peaking by Day 5, and then returning to pretreatment values by the end of 2 wk

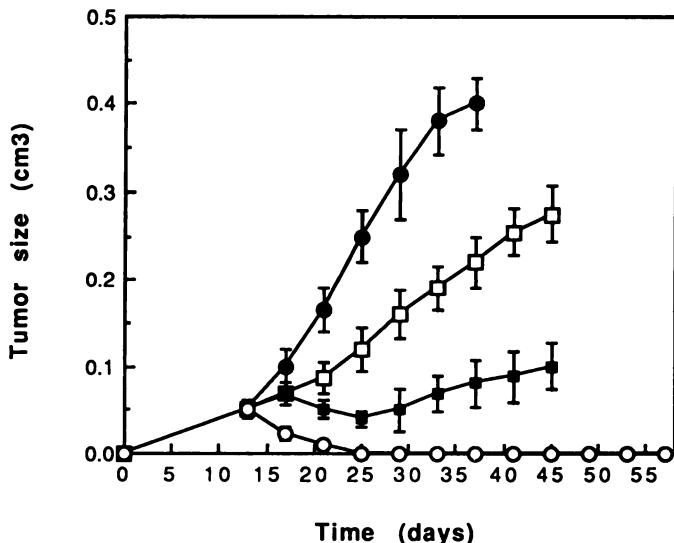


Fig. 3. Effect of B3-LysPE40 on the growth of MCF-7 tumors in mice. Animals were treated on Days 13, 15, 17, 19, and 21 with 20 µg (□), 50 µg (■), or 75 µg (○) of B3-LysPE40 i.v. The control group received mAb B3 alone (●). Points, mean; bars, SD.

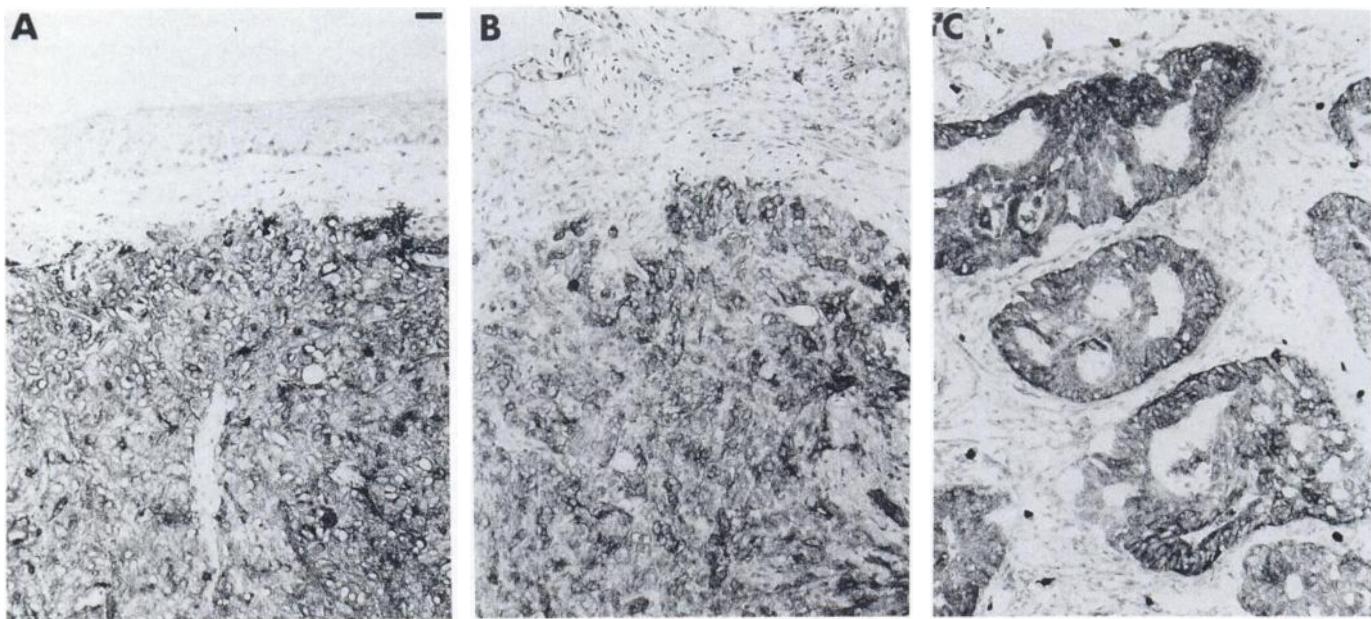


Fig. 4. Immunohistochemical localization of B3 antigen. Frozen sections of A431 tumor (A), MCF-7 tumor (B) resected from nude mice, and a human colon carcinoma (C). Bar, 10 μ m. \times 238.

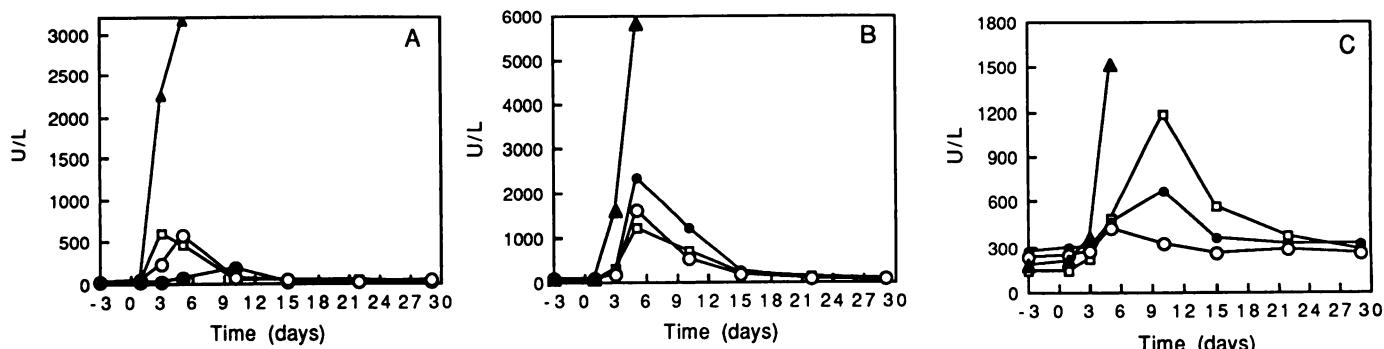


Fig. 5. Serum SGOT (A), SGPT (B), alkaline phosphatase (C) of monkeys after receiving 10 (□), 30 (●), 50 (○), and 100 (▲) μ g/kg of B3-PE. Blood samples were obtained on Days -3, 1, 3, 5, 10, 21, and 29 of the study.

Table 1 Serum level of B3-PE in monkeys

Dose (μ g/kg)	Monkey	Serum level (ng/ml)		
		Day 1	Day 3	Day 5
10	1	12.5	21	27
	2	5	12.5	13
30	1	60	78	130
	2	46	62	100
50	1	172	384	294
	2	166	178	384
100	1	833	909	227
	2	625	806	83

^a Third dose omitted because of toxicity.

in the animals that received 10, 30, and 50 μ g/kg of B3-PE. Bilirubin remained normal in these groups. There were no significant changes in the other blood chemistries (electrolytes, blood urea nitrogen, creatinine) or cell blood count. Serum amylase levels remained normal. Urinalysis showed no evidence of bladder injury during therapy. However, occult blood was noted occasionally in the urine of female monkeys due to menses contamination. There were no occult blood found in the stool.

Monkeys that received 100 μ g/kg developed massive liver

failure. Both animals died on Day 5 of the study. Liver enzymes were elevated more than 100-fold from pretreatment levels, and serum bilirubin ranged from 4 to 5 mg/dl on Day 5. At necropsy, the liver was found to be very pale in both animals. Histological examination of the liver showed diffuse hepatic necrosis characterized by cleared, vacuolated cytoplasm in most hepatocytes with condensed, pyknotic nuclei and perivascular acute inflammatory infiltrates. Histological examination revealed no significant abnormalities in the heart, kidney, trachea, spleen, lymph node, pancreas, stomach, urinary bladder, or lungs.

Blood samples were obtained approximately 10 min after dosing on Days 1, 3, and 5. Serum levels of B3-PE were measured by bioassay, and results from each individual monkey are shown on Table 1. Results are consistent with the amount of immunotoxin administered. The serum level of B3-PE increased after each dose. Monkeys that received 10, 30, and 50 μ g/kg on Days 1, 3, and 5 achieved serum levels as high as 27 ng/ml, 130 ng/ml, and 384 ng/ml by Day 5, respectively. These animals had liver injury that was reversible, as shown by normalization of liver enzymes. The two monkeys that received 100 μ g/kg on Days 1 and 3 achieved blood levels as high as 909 and 806 ng/ml of B3-PE after the second dose. The third

dose was omitted in this group due to toxicity. Plasma levels obtained prior to necropsy were 227 and 83 ng/ml.

DISCUSSION

In the present study, monoclonal antibody B3 was linked to native PE or LysPE40, a recombinant form of PE, and shown to have a significant antitumor effect in nude mice bearing an established human breast carcinoma, MCF-7. B3-PE was able to cause substantial tumor regression and delay of growth at a dose level of 1.5 μ g every other day for 4 days. B3-LysPE40 at a dose level of 75 μ g every other day for 4 days caused complete regression of MCF-7 tumor. We have previously shown that both immunotoxins were capable of causing complete disappearance of A431 tumors in nude mice at a lower dose level. Because both A431 and MCF-7 cells *in vitro* have a similar ID₅₀, 0.1 to 0.3 ng/ml for B3-PE and 1 to 4 ng/ml for B3-LysPE40, this difference in tumor response in mice may be due to the difference in the growth rate of the tumors in mice or the ability of the immunotoxin to penetrate into the tumor (9). A431 tumors grow very rapidly in mice, taking only 7 to 10 days to achieve 0.5 cm³, while it takes approximately 4 wk for MCF-7 tumors to achieve a similar volume. Because PE kills cells by inhibiting protein synthesis, cells that are actively growing may be more sensitive to these immunotoxins.

When compared with the reactivity of a human colon carcinoma specimen removed directly from a patient, the MCF-7 tumor and the A431 tumor grown in mice had a lower reactivity with the B3 antibody as shown by immunohistochemical analysis. Since the intensity of staining of the colon tumor is typical of many human carcinomas studies (8), we believe that similar or greater antitumor effects can be achieved in patients with tumors reactive to B3 antibody.

To determine if B3 linked to PE or PE40 could be developed for use in humans, we next evaluated the toxicity of B3-PE in Cynomolgus monkeys. B3-PE rather than B3-LysPE40 was selected because B3-PE is about 10-fold more potent, and limiting amounts of LysPE40 were available for preparation of immunotoxin. Monkeys were chosen because the B3 antigen is present in similar locations and at similar amounts in human and monkey tissues. These locations are in glands of the stomach and epithelium of the bladder, trachea, and tonsils (8). Based on these findings respiratory, gastrointestinal, or bladder injury was anticipated. However, no specific B3 antigen-targeted toxicity was observed at the doses administered. Except for liver toxicity, related to PE itself, no clinical signs of injury or histological abnormalities in organs that express B3 antigen were seen. Limiting toxicity was liver failure related to PE at the 100- μ g/kg dose level. This confirms our previous study with another PE-containing immunotoxin, anti-Tac-PE, when administered to monkeys in preclinical studies; at doses of 66 μ g/kg every day for 7 days, liver damage was the major toxicity observed.⁵ Because B3-LysPE40 is better tolerated than is B3-

PE, we anticipate that in monkeys and humans that therapeutic levels can be achieved without significant liver toxicity. Previously we described a method of preparing LysPE40 from the bacterial culture medium (11). The toxin contained an OmpA signal sequence to promote secretion. Recently, we have found it is easier to purify LysPE40 from the periplasm where it accumulates at much higher concentrations than in the medium. When the OmpA signal sequence was removed, a large proportion of LysPE40 was found in the periplasm from which it could be readily purified.

We conclude that monoclonal B3 when coupled to PE or PE40 has strong antitumor activity in nude mice bearing xenograft tumors that express B3 antigen. The similar reactivity of mAb B3 with immunotoxin-sensitive MCF-7 and A431 tumors and a human colon carcinoma sample makes us believe that a similar effect can be achieved in patients. Toxicity studies in monkeys demonstrate that therapeutic doses of B3-PE can be delivered without injury to normal organs that express B3 antigen. These data indicate that immunotoxins composed of B3 and PE or LysPE40 warrant further evaluation as antitumor agents in cancer patients.

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REFERENCES

1. Vitetta, E. S., Fulton, R. J., May, R. D., Till, M., and Uhr, J. W. Redesigning nature's poisons to create anti-tumor reagents. *Science* (Washington DC), **238**: 1098-1104, 1987.
2. Pastan, I., Willingham, M. C., and FitzGerald, D. J. Immunotoxins. *Cell*, **47**: 641-648, 1986.
3. Goldenberg, D. M. Future role of radiolabeled monoclonal antibodies in oncological diagnosis and therapy. *Semin. Nucl. Med.*, **19**: 332-339, 1989.
4. Apelgren, L. D., Zimmerman, D. L., Briggs, S. L., and Bumol, T. F. Antitumor activity of the monoclonal antibody-Vinca alkaloid immunoconjugate LY203725 (KS1/4-4-desacetylvinblastine-3-carboxyhydrazide) in a nude mouse model of human ovarian cancer. *Cancer Res.*, **50**: 3540-3544, 1990.
5. Allured, V. S., Collier, R. J., Carrole, S. F., and McKay, D. B. Structure of exotoxin A of *Pseudomonas aeruginosa* at 3.0 Angstrom resolution. *Proc. Natl. Acad. Sci. USA*, **83**: 1320-1324, 1986.
6. Hwang, J., FitzGerald, D. J., Adhya, S., and Pastan, I. Functional domains of *Pseudomonas* exotoxin identified by deletion analysis of the gene expressed in *E. coli*. *Cell*, **48**: 129-136, 1987.
7. Chaudhary, V. K., Jinno, Y., FitzGerald, D., and Pastan, I. *Pseudomonas* exotoxin contains a specific sequence at the carboxyl terminus that is required for cytotoxicity. *Proc. Natl. Acad. Sci. USA*, **87**: 308-312, 1990.
8. Pastan, I., Lovelace, E. T., Gallo, M. G., Rutherford, A. V., Magnani, J. L., and Willingham, M. C. Characterization of monoclonal antibodies B1 and B3 that react with mucinous adenocarcinomas. *Cancer Res.* **51**: 3781-3787, 1991.
9. Pai, L. H., Batra, J. K., FitzGerald, D. J., Willingham, M. C., and Pastan, I. Anti-tumor activities of immunotoxins made of monoclonal antibody B3 and various forms of *Pseudomonas* exotoxin. *Proc. Natl. Acad. Sci. USA*, **88**: 3358-3362, 1991.
10. Kondo, T., FitzGerald, D., Chaudhary, V. K., Adhya, S., and Pastan, I. Activity of immunotoxins constructed with modified *Pseudomonas* exotoxin A lacking the cell recognition domain. *J. Biol. Chem.*, **263**: 9470-9475, 1988.
11. Batra, J. K., Jinno, Y., Chaudhary, V. K., Kondo, T., Willingham, M. C., FitzGerald, D. J., and Pastan, I. Antitumor activity in mice of an immunotoxin made with anti-transferrin receptor and recombinant form of *Pseudomonas* exotoxin. *Proc. Natl. Acad. Sci. USA*, **86**: 8545-8549, 1989.
12. Willingham, M. C. Immunocytochemical methods. *Focus*, **12**: 62-67, 1990.

⁵ R. Kirkman, D. FitzGerald, and I. Pastan, unpublished data.