Selective Inhibition of Tumor Cell Growth by a Recombinant Single-Chain Antibody-Toxin Specific for the erbB-2 Receptor

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ABSTRACT

A high percentage of human breast and ovarian tumors display amplified c-erbB-2 gene copies, leading to overexpression of the growth factor receptor. Its membrane location and elevated expression make the erbB-2 protein an appropriate target for a directed tumor therapy. We have used recombinant DNA technology to produce a single-chain antibody-exotoxin A (scFv-ETA) fusion protein which specifically binds the human erbB-2 receptor. The scFv portion is composed of the heavy- and light-chain variable domains of a monoclonal antibody which recognizes the extracellular domain of the human erbB-2 receptor. The bacterially produced scFv-ETA protein was shown to bind specifically to cells expressing the human erbB-2 protein. The scFv-ETA inhibits protein synthesis in erbB-2-expressing tumor cells at doses ranging from 2 to 200 ng/ml and is cytotoxic for these cells at equivalent doses. In athymic nude mice, administration of the scFv-ETA inhibited the growth of erbB-2-overexpressing human ovarian carcinoma cells.

INTRODUCTION

Multiple genetic alterations are involved in the development of human breast and ovarian cancer (1, 2); c-erbB-2 gene amplification and elevated levels of the erbB-2 protein have been found in the tumor cells of up to 30% of both cancers (3–8). The erbB-2 protein is a member of the receptor tyrosine kinase family (9, 10). The extracellular accessibility of this growth factor receptor and the fact that high levels of the protein appear to correlate with a bad prognosis (11–15) make the erbB-2 receptor an appropriate target for directing growth-inhibiting or toxic agents to tumor cells.

MAbs2 directed toward tumor cell surface antigens have been shown to inhibit tumor cell growth (16). They can also be coupled to cytotoxic agents, which has been shown to enhance their cytotoxic potential (17, 18). We have recently described several MAbs which bind to the extracellular domain of the human erbB-2 protein (19). Two of these antibodies inhibit the growth in athymic nude mice of erbB-2-transformed cells.3 The therapeutic potential of intact MAbs is limited by, e.g., their immunogenicity, large size, or subunit structure. The antigen-binding domain of the antibody, the Fv, may suffice for many applications. Recently it has been shown that single-chain antigen-binding proteins (scFv), constructed by joining the variable domains of the heavy chain (VH) and light chain (VL) via a short peptide linker, can be produced in bacteria and that they appear to correlate with a bad prognosis (11–15) make the erbB-2 receptor an appropriate target for directing growth-inhibiting or toxic agents to tumor cells.

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2 The abbreviations used are: MAb, monoclonal antibody; ETA, exotoxin A; PBS, phosphate-buffered saline; HMGN buffer, 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.6), 100 mM KCl, 12.5 mM MgCl2, 10% glycerol.

3 I-M. Harwerth, unpublished observations.

2.1. Construction, Expression, and Purification of scFv(FRP5)-ETA.

The scFv(FRP5) gene fragment was isolated as a 739-base pair HindIII/Xbal fragment from pWW615-5, a pUC19 derivative containing a scFv(FRP5)-PhoA fusion gene (23), and inserted 5' of the truncated ETA gene into HindIII/Xbal-digested plasmid pWW20. The resulting plasmid, pWW25, has an open reading frame for the scFv(FRP5)-ETA gene.

The plasmid pFLAG-1 (IBI Biochemicals) (26) was used for the expression of scFv(FRP5)-ETA in Escherichia coli. The expression plasmid was prepared as follows: pFLAG-1 was digested with HindIII and treated with the Klenow enzyme to create blunt ends, and the linearized fragment was digested with EcoRI. The scFv(FRP5)-ETA encoding sequences, contained within a 1916-base pair fragment, were
isolated from plasmid pWW20-5 by HindIII cleavage, Klenow fill-in, and subsequent EcoRI digestion. This blunt ended EcoRI fragment was inserted into the blunt-ended EcoRI pFLAG-1 vector. The resulting plasmid, pWW215-5, was transformed into E. coli strain CC118 (27). A single colony was grown overnight at 37°C in LB medium containing 0.6% glucose and 100 μg/ml ampicillin. The culture was diluted 30-fold in the same medium, grown at 37°C to an A550 of 0.5, and induced with 1 mM IPTG for 1 h at 28°C. Cells were harvested by centrifugation at 4000 × g for 15 min at 4°C and were lysed by freeze/thaw and sonication in 10 ml of PBS containing 10% glycerol, 1 mM CaCl2, 0.3 mM phenylmethylsulfonyl fluoride, and 1 μg/ml DNase I. The lysate was clarified by ultracentrifugation at 45,000 × g for 45 min at 2°C. The supernatant was diluted 10-fold in 1X HMGN buffer and dialysed four times against 1X HMGN buffer to remove the guanidine-HCl. NaCl, CaCl2, and Na2HPO4 (pH 7.5) were added to final concentrations of, respectively, 150, 1, and 10 mM. The protein solution was clarified by centrifugation at 48,000 × g for 15 min at 2°C, and the supernatant was filtered through a 0.45-μm filter and applied to a FLAG affinity column (IBI Biochemicals), following the manufacturer’s recommendations. After binding of the scFv(FRPS)-ETA protein the column was washed three times with 10 bed volumes of PBS containing 1 mM CaCl2. Bound protein was eluted in 1-ml fractions of PBS containing 10 mM EDTA. The fractions containing scFv(FRPS)-ETA were pooled and concentrated by ultrafiltration through a YM10 membrane (Amicon).

The proteins in the pellet were solubilized by suspension in 0.5 ml/g of pellet of 2X HMGN buffer containing 8 M guanidine-HCl. The suspension was centrifuged at 48,000 × g for 45 min at 2°C. The supernatant was diluted 10-fold in 1X HMGN buffer and dialysed four times against 1X HMGN buffer to remove the guanidine-HCl. NaCl, CaCl2, and Na2HPO4 (pH 7.5) were added to final concentrations of, respectively, 150, 1, and 10 mM. The protein solution was clarified by centrifugation at 48,000 × g for 15 min at 2°C, and the supernatant was filtered through a 0.45-μm filter and applied to a FLAG affinity column as described above.

The characteristics of the scFv(FRPS)-ETA protein purified from the soluble or insoluble fraction of the cell were the same.

Immunoprecipitation and Immunoblotting. Cell lysates were prepared, and the proteins were separated by SDS-PAGE and electroblotted onto PVDF membranes as described (28). The erbB-2 protein was detected using the 21N antiserum (29) and 125I-protein A.

The human erbB-2 protein was specifically immunoprecipitated from [35S]methionine-labeled R111 and R2#11 cells using MAb FRPS (19). The immunoprecipitated proteins were analyzed by SDS-PAGE, and the gel was fluorographed after salicylic acid treatment to detect the radioactive proteins.

scFv(FRPS)-ETA Binding Assay. The binding of scFv(FRPS)-ETA to erbB-2 on SKBR3 human breast tumor cells was measured by ELISA. SKBR3 cells were grown on 96-well microtiter plates, fixed with 2% formaldehyde in PBS, and blocked with 3% bovine serum albumin in PBS. All additions to the cells were carried out in 3% bovine serum albumin in PBS. One hundred μl of scFv(FRPS)-ETA at concentrations from 0.2 to 150 nm were added to the wells, and the plates were incubated for 1 h at 37°C. Unbound scFv(FRPS)-ETA was removed, the cells were washed three times with PBS, and the cells were incubated with 100 μl of rabbit anti-erbB-2 antibody, followed by 100 μl of goat anti-rabbit IgG coupled to alkaline phosphatase (Sigma). The specifically bound scFv(FRPS)-ETA was detected by incubation with a solution of 1 μl Tris-HCl (pH 8.0), 0.4 mg/ml p-nitrophenylphosphate disodium (Sigma) for 30 min at 37°C, and then the absorbance at 405 nm was measured.

scFv(FRPS)-ETA Activity Assays. The ADP-ribosylation activity of scFv(FRPS)-ETA was measured using EF-2 isolated from wheat germ as described (30). Duplicate samples containing 500 ng of affinity-purified scFv(FRPS)-ETA or scFv(FRPS)-PhoA (23) as a control were incubated for 30 min at 30°C with 10 μCl [3H]adenosine (NAD (0.01 mCi/ml) (NEN) and approximately 50 units of EF-2. Total proteins were precipitated with TCA, and the transfer of [3H]adenosine from NAD to EF-2 was determined in a liquid scintillation counter.

The effect of scFv(FRPS)-ETA on protein synthesis was measured by seeding cells in 48-well tissue culture plates at a density of 1 × 103 cells/well. After 4 h the medium was replaced by normal growth medium containing various concentrations of scFv(FRPS)-ETA. After incubation at 37°C for 16 h the medium was removed, and the cells were washed and incubated for 4 h in normal growth medium containing 4 μCi [3H]leucine/ml. The cells were washed twice, and the protein was recovered on Whatman GFC filters following TCA precipitation. The rate of protein synthesis in immunotoxin-treated cells was determined and compared to that in untreated control cells. All points were done in triplicate.

The cell killing activity of scFv(FRPS)-ETA was measured with the Cell Titer 96 kit (Promega, Madison, WI) as recommended by the manufacturer. The cells were seeded in 96-well plates at a density of 1 × 104 cells/well in normal growth medium. Various concentrations of scFv(FRPS)-ETA were added to triplicate samples, and the cells were incubated for 40 h. Fifteen μl of dye solution containing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide were added to each well, and plates were incubated for 4 h. Cells were lysed for 16 h by the addition of 100 μl SDS-dimethyl formamide solution/well. All steps were carried out at 37°C. The relative number of viable cells was determined by measuring the absorbance at 570 nm as described (31, 32).

Serum Half-Life of scFv(FRPS)-ETA. scFv(FRPS)-ETA (4.25 μg) in PBS was injected i.v. into Balb/c mice, and blood samples were taken 1, 10, 30, and 45 min after injection. After clotting on ice the serum concentration of scFv(FRPS)-ETA was determined by ELISA as described above.

In Vivo Antitumor Activity of scFv(FRPS)-ETA. In vivo antitumor activity of the scFv(FRPS)-ETA was tested using HTB77 ovariian carcinoma xenografts in athymic nude mice. Approximately 25 mg of tumor tissue were implanted s.c. in each mouse (5 mice/group). Ten days later an Alzet minipump (model 2001), containing different concentrations of scFv(FRPS)-ETA, was implanted s.c. in each animal. The control group received a minipump containing PBS. The pumps contain 200 μl of solution, which was continuously delivered for approximately 7 days. Tumor growth was followed by measuring two perpendicular tumor diameters, the tumor volumes were calculated, and the data were statistically analyzed as described (33). There were no signs of toxic side effects such as weight loss or acute toxic symptoms observed in any of the animals treated with the recombinant scFv(FRPS)-ETA, even when 10 μg/day were injected (data not shown). The intact exotoxin A from P. aeruginosa has a median lethal dose in mice of 0.2 μg (34).

RESULTS

Construction and Expression of a Gene Encoding the Chimeric scFv(FRPS)-ETA Protein. A gene encoding a chimeric protein consisting of the scFv of the erbB-2-specific MAb FRPS (19) fused to domains II and III of the ETA of P. aeruginosa was constructed in the pFLAG-1 expression vector. Domain I of ETA is responsible for cell recognition but is not necessary for its enzymatic activity, which is the ADP-ribosylation of EF-2 (35). The pFLAG-1 vector has an IPTG-inducible tac promoter followed by sequences encoding the ompA signal peptide and the 8-amino acid FLAG epitope which allows the isolation of recombinant protein via antibody affinity chromatography (26). Fig. 4A shows the gene fragments which have been linked to construct the scFv(FRPS)-ETA expression plasmid pWW215-5. The sequence of the toxin gene has been previously published (36). The construction and the sequence of the scFv(FRPS) have been described (23). Fig. 18 shows the sequence of the linker region which spans the junction of the scFv(FRPS) and ETA coding sequences.

The scFv(FRPS)-ETA fusion protein under the control of the IPTG-inducible tac promoter was expressed in E. coli strain CC118. The ompA signal peptide present on the amino terminus of the fusion protein promotes its secretion into the periplasmic space. Lysates were made from induced cultures, and
the soluble scFv(FRP5)-ETA was purified by affinity chromatography using the M1 MAb which recognizes the FLAG epitope. Fig. 2 shows a SDS-PAGE analysis of the soluble proteins from a lysate of cells expressing pWW215-5 (Fig. 2, Lane 1) and the scFv(FRP5)-ETA protein isolated from pWW215-5 lysates following affinity chromatography (Fig. 2, Lane 2). The protein has the expected size of M, 66,000. The scFv(FRP5)-ETA protein is greater than 90% pure after one passage of the lysates over the M1 MAb affinity column. The same protocol was used to purify scFv(FRP5)-ETA from the insoluble fraction of the bacterial lysate. The protein was solubilized and renatured before M1 affinity chromatography (data not shown).

The enzymatic activity of the purified, recombinant scFv(FRP5)-ETA was analyzed by examining its ability to add an ADP-ribosyl group to EF-2. Five hundred ng of scFv(FRP5)-ETA were incubated with wheat germ EF-2 in the presence of [14C]adenosineNAD. The transfer of [14C]adenosine from NAD to EF-2 was measured by incorporation into TCA-insoluble material. In a control experiment, we compared the ADP-ribosylation activity of scFv(FRP5)-ETA to the activity of a single-chain antibody-alkaline phosphatase fusion protein, scFv(FRP5)-PhoA (23). Fig. 3 shows that the activity of scFv(FRP5)-ETA is 40-fold higher than the activity of the control protein.

Binding Properties of the scFv(FRP5)-ETA Protein. The affinity of the recombinant scFv(FRP5)-ETA for the erbB-2 protein was determined by measuring its binding by ELISA. The assay was carried out on SKBR3 breast tumor cells which express approximately 1 x 10^6 molecules of erbB-2 receptor/cell (29). The cells were grown in 96-well dishes, scFv(FRP5)-ETA protein at concentrations of 0.2 to 150 nM was added to the wells, and the plates were incubated for 1 h at 37°C. The specifically bound scFv(FRP5)-ETA was detected by incubation with rabbit anti-ETA serum followed by goat anti-rabbit IgG coupled to alkaline phosphatase. The phosphatase reaction product was measured as absorbance at 405 nm, and the results are shown in Fig. 4. The binding affinity of scFv(FRP5)-ETA to the erbB-2 receptor, measured as the half-maximal saturation value, is 6.5 nM. When the same assay was used to determine the affinity of MAb FRP5 to the erbB-2 receptor, a value of 0.82 nM was observed (23). Thus the affinity of scFv(FRP5)-ETA for erbB-2 is approximately 8-fold lower than that of the MAb FRP5. A similar reduction in the apparent affinities was found for the scFv(FRP5) molecule and a scFv(FRP5)-alkaline phosphatase fusion protein (23), indicating that not the exotoxin A fusion partner but the scFv(FRP5) domain itself is responsible for this effect.

scFv(FRP5)-ETA Displays Selective Toxicity on erbB-2-expressing Cells in Culture. We have shown in an in vitro assay that the recombinant scFv(FRP5)-ETA retains the ability to ADP-ribosylate EF-2. The cytotoxic activity of the protein was examined by measuring its effect upon cellular protein synthesis. The specificity of the scFv(FRP5)-ETA was analyzed. The binding domain of the natural ETA has been replaced by a scFv which recognizes the human erbB-2 receptor. The specificity of
scFv(FRP5)-ETA activity was tested on HC11 mouse mammary epithelial cells transfected with human erbB-2 expression vectors (24). R1#11 and R2#11 are clones of HC11 cells which express, respectively, the normal and the activated allele of the human erbB-2 receptor. The activated allele has a point mutation at position 659, which causes a valine—glutamic acid substitution (37). The HC11 cells which were used as a control express the mouse homologue of the erbB-2 receptor (Fig. 1B; Ref. 28). The HC11 cells and the two clones were incubated with scFv(FRP5)-ETA at concentrations ranging from 0.1 to 1000 ng/ml. Cells were treated for 16 h at 37°C with scFv(FRP5)-ETA, and protein synthesis was measured by pulsing the cells with [3H]leucine. Untreated cultures served as controls. The results are shown in Fig. 5A. The rate of protein synthesis in HC11 cells was not affected by up to 1000 ng/ml of scFv(FRP5)-ETA. In contrast, in the HC11 transfectant cell lines R1#11 and R2#11 expressing the human erbB-2 receptor, protein synthesis was inhibited, with an ID50 of, respectively, 5.3 and 1.6 ng/ml scFv(FRP5)-ETA (Table 1). These results show that scFv(FRP5)-ETA specifically binds to and selectively inhibits protein synthesis in cells which express the human erbB-2 protein.

The effect of the parental MAb FRP5 on the cytotoxic activity of scFv(FRP5)-ETA on R1#11 cells was determined in a competition experiment. We used an assay based upon the cellular conversion of a tetrazolium salt into a blue reaction product (31, 32). The cells were incubated for 16 h at 37°C with [35S]-methionine. The human erbB-2 protein was immunoprecipitated with MAb FRP5 and analyzed by 7.5% SDS-PAGE. The position of the M, 185,000 erbB-2 protein is indicated.

protein is not visible on the exposure shown in Fig. 1C but is present at low levels (29). The cells were incubated with increasing amounts of scFv(FRP5)-ETA, and the rate of protein synthesis was measured (Fig. 1A). Protein synthesis was inhibited in MDA-MB231, SKBR3, and HTB77 cells at an ID50 of, respectively, 5.5, 29, and 195 ng/ml scFv(FRP5)-ETA (Fig. 1A; Table 1). Despite the fact that MDA-MB231 cells have low levels of the erbB-2 receptor, they were not affected by treatment even with high doses of scFv(FRP5)-ETA.

The effect of scFv(FRP5)-ETA on protein synthesis and cellular toxicity was measured on human tumor cells expressing various levels of the erbB-2 receptor. SKBR3, MDA-MB453, and MDA-MB231 are human breast tumor cell lines which express, respectively, high, moderate, and low levels of the erbB-2 protein. HTB77 are ovarian tumor cells with high levels of the erbB-2 receptor (Fig. 7C). The MDA-MB231 erbB-2

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<th>Table 1 In vitro toxicity of scFv(FRP5)-ETA</th>
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*50% inhibitory concentrations were determined in a protein synthesis inhibition assay and a cell viability assay. The data are from Figs. 5 and 6.

ND, not done.
products are consistently altered in breast and ovarian tumors (1, 2). Based on the information about the physical and biochemical properties of these molecules, it now becomes possible to design specific agents which might be combined with standard chemo- and hormonal adjuvant therapies and increase the chances of successful treatment of cancer patients. The c-erbB-2 gene is amplified, and the receptor is overexpressed in up to 30% of breast and ovarian tumors (2–8). Patients whose tumors display elevated erbB-2 levels appear to have a worse prognosis (11–15). The high level of receptor expression, its extracellular accessibility, and its implication in the malignancy of the cancer make it an excellent target for specific cytotoxic reagents. The experiments in this paper describe the results obtained with a recombinant single-chain antibody-toxin protein targeted to the erbB-2 receptor. The scFv(FRP5)-ETA consists of the antibody-binding domain of an erbB-2-specific MAb linked to a modified P. aeruginosa ETA. A similar approach has recently been described (39). We have shown that the scFv(FRP5)-ETA protein specifically binds to cells expressing the human erbB-2 receptor and, at low doses, is able to inhibit the in vivo growth of tumor cells which have high levels of the erbB-2 receptor.

**DISCUSSION**

Approximately one-third of newly diagnosed cancers in women are breast and ovarian (8). A recent metaanalysis of the value of adjuvant chemo- or hormonal therapy in breast cancer treatment has shown that these treatments are beneficial for patients (38). Studies on genetic alterations found in cancer cells have shown that certain protooncogenes and their protein
The recombinant scFv(FRP5)-ETA, produced in bacteria, was found to have an 8-fold reduced affinity for the erbB-2 receptor when it was compared with the original MAb. In other experiments we have examined the characteristics of the scFv(FRP5) and scFv(FRP5)-PhoA recombinant proteins. These proteins also display an approximately 8-fold reduced affinity for the erbB-2 receptor (23). A reduced affinity for the antigen has been described for other scFv proteins (20, 22, 40-42). It is possible that the reduced affinity is due to the peptide linker connecting the two variable domains or to the order of these domains in the protein. The affinity of the recombinant scFv(FRP5)-ETA is still very high and allows specific binding to erbB-2-expressing cells.

The effects of scFv(FRP5)-ETA on cellular protein synthesis and viability were examined in a number of cell lines. The ID50 for protein synthesis inhibition ranged from 1.6 ng/ml for the HC11 R2#11 cells to 195 ng/ml for the HTB77 cells. The differences in susceptibility are not strictly proportional to the amount of erbB-2 protein present in the cells. The HTB77 and SKBR3 cells have the highest level of erbB-2 protein but were the least sensitive to the scFv(FRP5)-ETA. Despite the fact that the cells have approximately the same amount of erbB-2, there was a 6.7-fold difference in the sensitivity of the HTB77 and SKBR3 cells toward the scFv(FRP5)-ETA. There are a few possible explanations for these results. Since the toxin must be internalized to inhibit protein synthesis, the turnover of the receptor may play an important role in the process. We have measured the half-life of erbB-2 receptor in HC11 cells expressing the normal and the activated human alleles and have found that the activated erbB-2 receptor turns over twice as fast as its normal counterpart. This has also been observed in fibroblasts expressing both types of receptor and most likely reflects the high activity of the kinase in the activated receptor (43). This may explain why the R2#11 cells containing the activated erbB-2 allele are slightly more sensitive to the scFv(FRP5)-ETA than R1#11 cells, despite the fact that they contain approximately 8-fold less erbB-2 receptor.

The HTB77, SKBR3, and MDA-MB453 cells are differentially sensitive to the scFv(FRP5)-ETA despite the fact that the half-life of the erbB-2 receptor in these cell lines is approximately the same. It has been reported that tumor cells with very high levels of the erbB-2 receptor shed the extracellular domain of the protein into the medium (44, 45). Therefore, in these cells the receptor turnover as well as the presence of competing free extracellular domain might influence the sensitivity of the cells to the scFv(FRP5)-ETA. It is interesting to note that MDA-MB231 cells were not inhibited by the scFv(FRP5)-ETA. It has been reported that conditioned medium from MDA-MB231 cells contains an erbB-2 ligand (46, 47); therefore, it is possible that the erbB-2 receptor is occupied by a ligand in these cells. Alternatively, the low level of receptor present on the MDA-MB231 cells may not allow sufficient amounts of the scFv(FRP5)-ETA to be internalized into the cells.

scFv(FRP5)-ETA was tested for its antitumor activity in nude mice transplanted with HTB77 tumor cells. Administration of 6 µg/mouse/day was sufficient to inhibit tumor growth completely in 3 of 5 animals. A 6-fold lower dose inhibited tumor growth for 15 days, but eventually tumors grew in all of the animals. We have determined that administration of the scFv(FRP5)-ETA by continuous infusion is the optimal method of delivery. We found that a dose of 5 µg injected once a day had no effect upon tumor cell growth. This suggests that constant serum levels of the recombinant toxin are more efficient than short-term high doses.

Recombinant scFv-ETA proteins have been used successfully to kill cells with specific cell surface antigens. An anti-TacFv-ETA protein, directed to the T-cell interleukin 2 receptor, was shown to kill peripheral blood cells from patients with adult T-cell leukemia (48). A recombinant B3Fv-ETA protein which is directed against a carbohydrate antigen found on the surface of many carcinomas has been shown to kill human tumor xenografts in nude mice (49).

Recombinant proteins such as the scFv(FRP5)-ETA which we have described in this paper may be useful in the adjuvant treatment of breast or ovarian cancer, but possible obstacles must be considered. One factor which may hinder treatment with scFv-toxin proteins is the development of antibodies against the scFv-toxin. The scFv will most likely be less immunogenic than an intact antibody (see Ref. 22 for a discussion).

Fig. 8. Serum half-life of scFv(FRP5)-ETA. Approximately 4.25 µg of scFv(FRP5)-ETA were injected i.v. into Balb/c mice, and blood samples were taken at the indicated time points. After clotting on ice the serum concentrations of scFv(FRP5)-ETA were determined by ELISA.

Fig. 9. Effect of scFv(FRP5)-ETA on the in vivo growth of HTB77 human ovarian tumor xenografts in athymic mice. Approximately 25 mg of HTB77 tumor tissue were implanted s.c. in each mouse (5 mice/group). Ten days later the mice were treated by continous infusion of 1 µg (O) or 6 µg (C) of scFv(FRP5)-ETA per day for 7 days. The control group received PBS (Φ). The tumor size was measured at the indicated times, tumor volumes were calculated, and the data were statistically analyzed.

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To circumvent the antigenicity of the scFv domain, it is possible to transfer the antigen-binding sites in the variable regions from a mouse to a human backbone (50). This process should lower the immunogenicity of a scFv protein but may not completely inhibit it (see, e.g., Ref. 51). In addition, the toxin portion of the recombinant protein will provoke an immune response. Thus, adjutant treatment with a protein such as scFv(FRP5)-ETA will be most effective if high doses can be given for short periods of time.

Another potential problem is the fact that the erbB-2 protein is not a tumor-specific antigen, since other normal human cells also display this receptor. The results from immunohistochemical studies indicate that embryonic tissues contain the highest levels of erbB-2. In the adult, expression is limited to epithelial cells in the digestive tract, reproductive tract, breast, kidney, and skin (52–54). Since the level of erbB-2 protein in these normal tissues is considerably lower than the level observed in some tumors, there is hope that a therapy based upon differential expression of the erbB-2 protein may be successful.

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erbB-2-SPECIFIC IMMUNOTOXIN


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