A Recombinant CD7-specific Single-Chain Immunotoxin Is a Potent Inducer of Apoptosis in Acute Leukemic T Cells

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ABSTRACT

A recombinant immunotoxin was constructed from the hybridoma antibody TH-69 directed against human CD7, a surface antigen of leukemic T cells. The antibody was subcloned as a single chain Fv (scFv) fragment and genetically linked to a truncated Pseudomonas exotoxin A fragment containing the catalytic domains II and III but lacking the receptor binding domain I. Domain I was replaced by the scFv, thus conferring restricted specificity for CD7-positive cells. The bacterially expressed and purified toxin retained binding specificity for CD7-positive cells. It promoted apoptosis in two CD7-positive cell lines derived from T-lineage acute lymphoblastic leukemias, CEM and Jurkat, but not in the CD7-negative B-lymphoid lines REH, Nalm-6, and SEM. Maximum killing in excess of 95% was reached after 96 h in CEM and Jurkat cells with a single dose of 100 ng/ml. Cells treated with a similarly constructed scFv-exotoxin A immunotoxin against melanoma-associated chondroitin sulfate proteoglycan, an antigen absent from leukemic T cells, remained unaffected. Lysis of target cells occurred via apoptosis as evidenced by staining with Annexin V and specific cleavage of poly(ADP-ribose) polymerase; PI, propidium iodide; scFv, single-chain fragment from the variable region.

INTRODUCTION

Antibodies have attracted renewed strong interest as therapeutics in clinical oncology. The CD20 antibody rituximab (1), the anti-Her2/neu breast cancer drug Herceptin (2), and the CD33 immunocoagglutinate gemtuzumab ozogamicin (3) all have impressive activity in tumor patients. However, patients suffering from T-cell leukemias and lymphomas still have very limited treatment options. The 5-year survival for most of these patients is <30%, and this category ranks as the worst in lymphoma prognosis (4). The CD52 antibody CAMPATH-1H (Alemtuzumab), recently approved by the Food and Drug Administration for the treatment of B-cell chronic lymphocytic leukemia, is also effective against certain T-cell neoplasias (5). However, this antibody leads to a prolonged and almost complete depletion of T and B lymphocytes, with a considerable frequency of opportunistic infections. Prognosis of childhood T-ALL has improved with modern chemotherapy, but T-ALL patients with remission induction failure after induction chemotherapy or with relapse of T-ALL still have a very poor prognosis (6).

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The abbreviations used are: T-ALL, acute T-cell leukemia; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ETA, exotoxin A; FACS, fluorescence-activated cell sorter; MCSP, melanoma-associated chondroitin sulfate proteoglycan; PARP, poly(ADP-ribose) polymerase; PI, propidium iodide; scFv, single-chain fragment from the variable region.

One of the prerequisites for a successful immunotherapy of T-cell neoplasias is the selection of an appropriate target antigen, which ideally should be T cell specific and expressed on most T-cell lymphomas and leukemias but absent on at least a portion of normal T lymphocytes. The CD7 antigen meets these requirements. In addition, a substantial portion of AML of M1/2 type is also CD7 positive (7–10). CD7 is a cell surface glycoprotein of ~40 kDa and a member of the immunoglobulin superfamily (11). Galectin I is a ligand for the extracellular domain of CD7, and interaction of T-lymphoid cells with this ligand promotes apoptosis (12). Therefore, CD7 may be viewed as a novel type of “death receptor,” and other specific functions of CD7 are still unknown. The protein participates in signaling processes to the inside of cells, demonstrated by its association with phosphoinositide 3-kinase after binding of CD7-specific antibodies (13). On activation of T cells, the surface density of CD7 is increased, and CD7 has been proposed to participate in the activation and surface adhesion of mature T cells and natural killer cells (14, 15). CD7 is a marker for very early stages of T-cell maturation and is already present on lineage-committed hematopoietic progenitors in the fetal liver and on pluripotent progenitors of T cells in the thymus, bone marrow, and cord blood (16, 17). CD7 is further expressed on a majority of human thymocytes and a large subset (~85%) of peripheral blood T cells and natural killer cells (11, 18–23). The remaining subset of CD7-negative peripheral T cells maintains immune functions needed for the prevention of opportunistic infections and the engraftment of hematopoietic stem cells. Therefore, this subset may become relevant for therapeutic purposes, because it may serve to repopulate the T-cell compartment at least in part after a CD7-directed therapy.

A key property of CD7 for therapeutic applications is its rapid internalization after binding by an antibody, even after binding by monovalent antibody fragments (24). This property makes the antigen well suited for targeting by immunotoxins, which are internalized together with the antigen and subsequently poison the cell from inside. Here, the CD7 hybridoma antibody TH-69 (25), generated by one of the authors (M. G.; Ref. 26), was used for the construction of a CD7-specific immunotoxin, because of its high binding affinity (7.6 × 109 Mol−1). Binding of this antibody to the human T-cell lines CEM, Jurkat, and MOLT-16, derived from ALL, did not affect the proliferation of these cells in culture and did not induce apoptosis to a measurable extent. However, in athymic (“nude,” nu/nu−/−) and SCID mice xenotransplanted with CEM or MOLT-16 cells, the same antibody produced significant antitumor effects (26). Binding of the antibody caused a rapid down-regulation (“modulation”) of the antigen, and the Fc portion of the antibody was essential for the antitumor effect in vivo. Because of these promising results obtained with the unmodified antibody, the antibody has been used in pilot studies with patients with advanced T-cell tumors.4

Several investigators have targeted CD7, because the rapid internalization of the antigen after antibody binding makes this molecule attractive for the design of immunotoxins (27–30). The DA7 immunotoxin, consisting of the ricin A toxin chemically linked to a CD7-
out to be the case. In this report, we describe the construction of the active against CD7-positive cells derived from T-ALLs, and this turned CD25-specific scFvs linked to truncated ETA were remarkably effi-

truncated ETA becomes a potent immunotoxin, antibody directed against an antigen capable of internalizing, the MATERIALS AND METHODS domain I is not toxic as long as it remains in the extracellular space. Consequently, the truncated version of ETA lacking inhibiting protein synthesis via a block of the translation elongation active center of the toxin, respectively, which causes apoptosis by between the scFv antibody fragment and the toxin by recombinant genetics. The other was the use of a truncated toxin, which was unable to enter human cells on its own, in case it should be cleaved from the antibody moiety. This truncated toxin was devoid of its own binding domain for receptors on mammalian cells and, therefore, was expected to generate significantly fewer side effects. The recombinant toxin created here can only be absorbed by CD7-positive cells by virtue of its scFv portion and the CD7 internalization mechanisms of these cells.

The toxin used in our approach was Pseudomonas ETA\(^3\), specifically the truncated version lacking domain I and containing only domains II and III (34). Domain I is the binding domain for a cell surface receptor present on most mammalian cells. The intact toxin enters mammalian cells via binding of domain I to this receptor. Domains II and III are required for intracellular transport and carry the active center of the toxin, respectively, which causes apoptosis by inhibiting protein synthesis via a block of the translation elongation factor-2 (35). Consequently, the truncated version of ETA lacking domain I is not toxic as long as it remains in the extracellular space. In addition, the truncated form of Pseudomonas ETA is expected to have fewer side effects on vascular endothelial cells, because it has an \(\sim\)1,000-fold lower relative affinity to the LDV receptor on human vascular endothelial cells than, e.g., ricin A (36). Once coupled to an antibody directed against an antigen capable of internalizing, the truncated ETA becomes a potent immunotoxin, e.g., CD22- and CD25-specific scFvs linked to truncated ETA were remarkably efficient immunotoxins for the treatment of hairy cell leukemias and CD25-positive hematological malignancies, respectively (37, 38). Here, we asked whether scFv-ETA immunotoxins may also be effective against CD7-positive cells derived from T-ALLs, and this turned out to be the case. In this report, we describe the construction of the immunotoxin and its initial functional characterization in vitro with leukemic cell lines and long-term cultures of primary leukemic cells.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. Escherichia coli XL1-Blue (Stratagene, Amsterdam, the Netherlands) was used for the amplification of plasmids and cloning and E. coli TG1 (from Dr. G. Winter, MRC, Cambridge, United Kingdom) for the screening of antibody libraries. Libraries were generated in the phagemid vector pAK100, and pAK400 was used for the expression of soluble scFvs (39). E. coli BL21 (DE3; Novagen, Inc., Madison, WI) served for the expression of scFv-ETA fusion proteins.

Culture of Eukaryotic Cells. Leukemia-derived SEM cells (40), CEM cells (41), Jurkat cells (42), REH cells (DSMZ, German Collection of Microorganisms and Cell Lines, Braunschweig, Germany), Nalm-6 cells (DSMZ), MV4;11 cells (American Type Culture Collection, Rockville, MD), and the two hybridomas TH-69 (26) and 9.2.27 (anti-MCSP; Ref. 43) were cultured in RPMI 1640-Glutamax-I (Invitrogen, Karlsruhe, Germany) containing 10% FCS and penicillin and streptomycin at 100 units/ml and 100 \(\mu\)g/ml, respectively. COS monkey cells (DSMZ) were maintained in DMEM-Glutamax-I medium containing 10% FCS, penicillin, and streptomycin. The LLC cell line was established from bone marrow lymphoblasts of a 6-month-old female infant diagnosed with ALL\(^5\). LLC cells (44) were cultured in Iscove’s Modified Dulbecco Medium (Life Technologies, Inc.), supplemented with human transferrin and linoleic acid at 3 \(\mu\)g/ml, BSA at 10 mg/ml, and thiogalactoside at 20 nm, insulin at 1 \(\mu\)g/ml, and recombinant human interleukin-7 (Strathmann, Inc., Hamburg, Germany) at 10 ng/ml.

Patient-derived Leukemic Cells. HIL cells were derived from a 10-year-old patient, diagnosed with precursor T-ALL (CD1a\(^+\), CD2\(^−\), CD4\(^−\), CD7\(^+\), CD8\(^−\), and CD3\(^+\)) at the Children’s Hospital of the University of Erlangen. He was treated according to ALL-BFM-86 protocol and reached remission at day 33 of protocol I. The culture was established from peripheral blood, and the cells proliferated with a doubling time of 3 days. They were cultured in the same media as described for the cultivation of LLM cells. After 60 generations, the cells entered a proliferative crisis, and the majority of the cells died. Sufficient aliquots were frozen from early passages (generation 30) to allow reproducible work with cells of a defined number of generations in culture. The cultured cells continued to express high levels of CD7. In addition, fresh cells from a 27-year-old male patient with pre-T-ALL were tested. The immunophenotype of bone marrow blasts (80% marrow infiltration) initially was CD7\(^−\)/CD5\(^+\)/cytoplasmic CD3\(^+\)/CD34\(^−\)/CD1\(^−\)/CD3\(^+\). Cells showed Termi- nal deoxynucleotidyl transferase positivity. Tests were performed after the patient was referred to our university hospital after initially being treated elsewhere with one course of cyclophosphamide-Adriamycin-vincristine-prednisone chemotherapy for aggressive lymphoma.

Preparation of scFv Phage Display Libraries. Total RNA was prepared with Trizol (Invitrogen) from the CD7 antibody producing hybridoma, clone TH-69, and the hybridoma 9.2.27, producing a control antibody against MCSP. First-strand cDNA was prepared from 10–15 \(\mu\)g of total RNA (39). PCR amplification of immunoglobulin variable region cDNAs and cloning into the phagemid vector pAK100 was performed as described (39, 45). Propagation of combinatorial scFv libraries and filamentous phages was performed after published procedures (45).

Panning of Phage Display Libraries with Intact Cells. Panning of phage display libraries with intact cells was carried out using CD7-positive CEM cells and COS cells positive for the MCSP antigen. For this purpose, 5 \(\times\)10\(^5\) cells were incubated in PBS (Invitrogen, Groningen, the Netherlands) containing 2% nonfat dry milk (NM-PBS) to block nonspecific binding sites and then incubated with 500 \(\mu\)l of the scFv phage library for 1.5 h at 25°C under slow agitation. Cells were washed 10 times with 2% NM-PBS and twice with PBS. Bound phages were eluted by adding 50 \(\mu\)g of the parental antibody as competitor. After incubation for 10 min at 25°C, cells were sedimented by centrifugation, and the supernatant was used to infect 10 ml of exponentially growing E. coli TG1 cells. A total of 20 ml of 2 \(\times\) 10\(^6\) cell/ml (16 g/liter tryptone/peptone (Roth, Karlsruhe, Germany), 10 g/liter yeast extract (Roth), and 5 g/liter NaCl containing 1% glucose and chloramphenicol at 30 \(\mu\)g/ml was added, and the cells were incubated for 2 h at 37°C under vigorous shaking (250 rpm). The cells were then superinfected with helper phage. A total of 70 ml of 2 \(\times\) 10\(^6\) cell/ml was then added, and isopropyl-
thiogalactoside was added at a final concentration of 0.5 mm. Two h after infection, kanamycin was added to a final concentration of 25 \(\mu\)g/ml, and the culture was grown overnight at 30°C. On the next day, phages were prepared as described (45). After two rounds of panning, individual phages were purified, and the inserts were sequenced (46) using an Applied Biosystems automated DNA sequencer (ABI Prism 310 Genetic Analyzer; Perkin-Elmer, Eberlingen, Germany).

Bacterial Expression and Purification of Soluble scFv Fragments. For the soluble expression of antibody fragments, cDNAs coding for CD7- and MCSP-specific scFvs were subcloned into the expression vector pAK400, and the plasmids were propagated in E. coli HB2151 (from Dr. G. Winter; MRC,

\(^5\) J. Greil, unpublished data.

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Construction and Expression of scFv-ETA Fusion Proteins. For periplasmic expression of scFv fragments fused to truncated ETA under the control of the inducible T7 promoter, the plasmid pet27b(+) (Novagen, Inc.) was modified as follows. To introduce a 6 × His-tag at the NH2 terminus and a Stbl-site, two oligonucleotides, oligo 1: 5′-cc cat cac cat cac cat cac ggc gcc cag gcc ggc g-3′ and oligo 2: 5′-ggc agc gcc cgg ctt gcc ggc ggc gat gtt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc gat ggt gcc ggc 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CD7-EXOTOXIN A scFv IMMUNOTOXIN

Fig. 2. Construction scheme for the recombinant immunotoxin. 6xHis: NH2-terminal hexa-histidine tag; VL and VH, variable regions of the L- and H-chains of the TH-69 monoclonal antibody. Linkers 1 and 2: flexible linkers consisting of glycine and serine residues (Materials and Methods); ETA1, truncated ETA fragment, consisting of domains II and III (amino acids 253–613) but lacking the receptor-binding domain I of the intact Pseudomonas toxin. Molecular masses of the various fragments in Da were calculated from their amino acid sequence.

Fig. 3. ScFv fusion proteins CD7-ETA and MCSP-ETA are capable of binding to antigen-positive cells. Cells were stained with purified scFv-ETA fusion proteins (black) or a nonrelated scFv-ETA fusion protein (white) at the same concentration and analyzed by FACS. A, CD7-positive CEM cells stained with CD7-ETA. B, CD7-negative SEM cells stained with CD7-ETA. C, MCSP-positive LLM cells stained with MCSP-ETA. D, MCSP-negative MV4-11 cells stained with MCSP-ETA. The data presented here are representative of three separate experiments.

gate the cytotoxic properties of the recombinant CD7-specific immuno-
toxin, the agent was added to CEM and SEM cells, and viable cells were counted by using trypan blue exclusion. Cell death was antigen specific, because it was blocked by pretreatment with excess concentrations of the parental monoclonal CD7 antibody, TH-69, but not with similar concentrations of an isotype control antibody (Fig. 4). Next, the extent of cell death was measured as a function of time and the dose of the agent by staining of nuclei with PI and FACS. The fraction of cells with subdiploid DNA content was equated with the fraction of dead cells. The immunotoxin was added to the cultures in a single dose, and culture aliquots were analyzed for cell death at different times after addition. The toxin promoted death of CEM cells in a time- and dose-dependent manner (Fig. 5A) but failed to affect SEM cells at the same concentrations (Fig. 5B). Significant cell death was already obtained at concentrations of 1 ng/ml, compared with untreated cells (P = 0.002), and maximum death was reached at ~100 ng/ml, corresponding to a concentration of 1.3 nM. Thus, the agent was capable of killing CD7 antigen-positive cells with high efficiency within 96 h after the addition of a single dose in the nanomolar concentration range. The immunotoxin also killed cells from a second leukemia-derived T-ALL line, Jurkat, at similar concentrations. At concentrations causing maximum death of Jurkat cells, the B-leuke-
imic cell lines REH, Nalm-6 (Fig. 6), and SEM remained unaffected (Fig. 5B). Thus, it can be excluded that the toxin killed nonspecifically, and the data support the conclusion that the cytotoxic effect was antigen-specific. The fraction of dead cells measured by this procedure did not reach 100%, although microscopic analysis suggested near complete lysis. This apparent discrepancy was resolved and found to be the result of this particular method of quantitating cell death. When a different method was used, the fraction of dead cells exceeded 95% under identical conditions (see below).

Cell Death Induced by the CD7-ETA Immunotoxin Occurs via Apoptosis. To investigate whether the observed cell death was attrib-
utable to apoptosis, CEM and Jurkat cells were treated with CD7-ETA or with the control toxin and then stained with Annexin V and PI. After the exposure (48 h), 30% of CEM cells and 24% of Jurkat cells stained positive for Annexin V and negative for PI, a characteristic sign of death by apoptosis (Fig. 7A, bottom right quadrant). As a control, the recombinant anti-MCSPT-ETA scFv immunotoxin of simi-
lar design failed to induce Annexin V staining and cell death. As an additional sign of death by apoptosis, the CD7-ETA toxin induced cleavage of PARP from its intact form (116 kDa) to its characteristic 85 kDa proteolytic fragment in CEM cells. Treatment of these cells with equivalent concentrations of the anti-MCSP-ETA toxin failed to

Fig. 4. Killing by CD7-ETA is blocked specifically by the parental TH-69 antibody. CEM cells were left untreated (○) or were treated with 100 ng/ml CD7-ETA (△), 100 ng/ml CD7-ETA + TH-69 (△), 100 ng/ml CD7-ETA + isotype control antibody (●), TH-69 (■), and isotype antibody (●). At given time points, viable cells were counted by trypan blue staining. Each time point was measured in triplicate, and SDs are indicated by error bars. The data are representative of four separate experiments.
induce PARP cleavage (Fig. 7B). Thus, by two independent criteria, the CD7-specific immunotoxin was shown to induce cell death via apoptosis for both the CEM and Jurkat lines (Fig. 7, A and B).

A direct comparison of the two methods used to quantitate cell death was performed. After (96 h) the addition of the toxin at 100 ng/ml, cell death was measured either by staining with PI in hypotonic solution (method 1; Fig. 8A) or by staining with PI (PI uptake) and Annexin V (method 2; Fig. 8B). Method 1 showed ~80% of dead cells (subdiploid DNA content), whereas measurement by method 2 showed a significantly higher number of dead cells (P = 0.04). Thus, measurement by method 1 underestimated the number of dead cells relative to determination by microscopic counting and method 2 (Fig. 8, A and B). Apoptotic death induced by the toxin and evidenced by method 2 was inhibited specifically by pretreatment with an excess of the parental monoclonal TH-69 antibody (Fig. 9, middle two panels). Treatment with a comparable excess of an isotype control antibody (Fig. 9, bottom two panels) showed cell death to the same extent as cells treated with the CD7-ETA toxin alone (Fig. 9, top two panels). These results confirm the conclusion that killing was antigen specific and that induction of apoptosis was attributable to receptor-mediated uptake of the immunotoxin.

The Recombinant CD7-ETA Immunotoxin Promotes Specific Lysis of Short-term, Cultured Leukemic Cells. To be of therapeutic interest, the toxin needs to lyse not only stable leukemia-derived...
death was measured by FACS analysis using Annexin V staining and PI uptake. After 24 h, 12% specific cell death was observed and 20% after 48 h. Fresh peripheral blood mononuclear cells from a healthy donor showed 18% specific lysis. These data provide additional evidence that this agent is capable of eliminating at least a subset of fresh CD7-positive peripheral blood cells. Additional studies will have to be performed to provide definitive evidence for the killing of primary leukemic blasts.

**DISCUSSION**

The central finding of this report was that the recombinant CD7-ETA scFv immunotoxin effectively killed cultured leukemic cells. Viewed from today’s state of knowledge, this may not appear unex-

**Fig. 8.** Comparison of two different methods for the measurement of cell death. A, flow cytometric analysis of DNA content by PI staining of nuclei. Using this method, 81% of cell death was obtained for the same sample that gave rise to 97.8% with the second method (B). B, measurement by Annexin V and PI staining and FACS analysis. Aliquots from the same batch of treated cells were analyzed with both methods. Using method 2 (Annexin and PI staining), the combined number of events in the bottom and top right panels was 97.8%. The data are representative of three separate experiments.

**Fig. 9.** Killing by the CD7-ETA scFv toxin is antigen-specific as determined by competition with the parental TH-69 hybridoma antibody and appropriate controls. Top two panels, CEM cells treated with the CD7-ETA toxin in a single dose of 100 ng/ml analyzed after 40 and 64 h of exposure to the agent; middle two panels, cells treated with the same amount of the CD7-ETA toxin plus a 100-fold molar excess of the parental TH-69 antibody for 40 and 64 h, respectively. Bottom two panels, cells treated with the same amount of the agent plus a 100-fold molar excess of a nonrelevant isotype-matched control antibody for 40 and 64 h, respectively. The data are representative of three separate experiments.

**Fig. 10.** Long-term primary cultures of leukemia-derived cells can be killed specifically by the CD7-ETA scFv immunotoxin. HOL cells at generation 30 were treated with a first dose (100 ng/ml) of the CD7-ETA immunotoxin or the anti-MCSP-ETA control toxin at time 0 and a second dose at 48 h. The percentage of dead cells was determined by Annexin V and PI staining and FACS analysis. ◆ treated with the CD7-ETA toxin alone; x, treated with the CD7-ETA toxin plus an isotype-matched control antibody; ●, treated with the CD7-ETA toxin plus a 100-fold molar excess of the parental TH-69 antibody; ■, treated with the anti-MCSP-ETA toxin alone. Numerical values plotted are the arithmetic means from two independent experiments. Error bars represent the SDs from the mean as calculated by the Microsoft Excel computer program.
pected. It was known before this study that CD7 internalizes after binding of a specific antibody and that the unmodified parental TH-69 antibody had antitumor efficacy in a xenotransplanted mouse model (26). It was further known that conventionally produced CD7-specific immunotoxins were effective against cultured leukemia cells (27–32). Finally, recombinant scFv immunotoxins (CD22-ETA and CD25-ETA) carrying the same truncated ETA variant that was used here had been reported to be effective in patients with relapsed, chemotherapy-refractory hairy cell leukemia and CD25-positive hematological malignancies (37, 38). These latter reports established that scFv-ETA immunotoxins constructed by a similar principle as the one used here are efficient against certain forms of human leukemias in vivo.

Contrasted with this background, the results of the present study offer several new elements of knowledge: (a) the construction principle of recombinant scFv-ETA molecules used here works not only for the CD22 and CD25 antigens (37, 38) but also for CD7; it may also work for a number of other cell surface antigens expressed in similar concentrations and internalized with similar kinetics after binding of an antibody; (b) several of these scFv-ETA toxins induce efficient lysis in the concentration range of ng/ml (37, 47). This is true both for the CD22-ETA scFv toxin (37) and the CD7-ETA chimeras reported here, as well as other published constructs (27, 47). Although different methods were used to quantitate cell death, and the results are not strictly comparable, it is probably still fair to state that several of these scFv-ETA toxins work with similar potency on human leukemic cells and are effective in the concentration range of <100 ng/ml. A precise comparison of the various agents can only be achieved in a side-by-side evaluation using the same assay for cellular toxicity; (c) the attachment of a truncated ETA domain did not alter the binding specificity of the scFv portion in agreement with the results reported by others (37, 38, 47); in our construct, a different linker was used than in other published constructs, and, thus, a new element of information is that the precise sequence of the linker does not appear to be critical; (d) cell death occurred by apoptosis as opposed to other mechanisms; this was shown in our study by Annexin V staining and PARP cleavage; and (e) our CD7-specific toxin worked not only for the rapidly dividing T-ALL cell lines CEM and Jurkat but also for long-term cultures of leukemia-derived HOL cells, although with lower efficiency. The reasons for the reduced efficiency are unknown, but probably the difference is attributable to the fact that stable lines differ from primary cells by the gain of additional mutations. This often leads to the selection of cells with a shorter generation time that may also be more susceptible to the agent.

The finding that ~20% of specific lysis was obtained in long-term primary cultures and cultures of fresh biopsy material as opposed to >95% in stable lines is not a disappointment. In other studies published to date, the effect of immunotoxins was evaluated primarily by using stable lines and xenograft models, in which human leukemic cells were transplanted into immunocompromised mice and were then treated with the agent (26). Studies describing the efficacy of scFv immunotoxins for primary patient cells (54) are limited. Therefore, other authors may also have experienced difficulties in evaluating such agents in primary cells. In view of these difficulties, it is encouraging that 20% of cellular lysis was reproducibly obtained in the present study in long-term primary cultures. The observation that only 20% of lysis was obtained in a 96-h period does not preclude the potential future usefulness of this agent in vivo, because in vivo it may be administered in several successive doses, and, thus, a higher percentage of killing may be achieved. The usefulness of this agent in vivo may further depend on a number of parameters that have not been evaluated in our studies with cultured cells and that can only be assessed by in vivo experiments.

In summary, in the present study, a CD7-ETA scFv immunotoxin was constructed with a design aiming at circumventing some of the problems encountered with earlier CD7-directed immunotoxins generated by chemical coupling of the toxin to the antibody. The new agent bound to CD7-positive T-lymphoid cells and killed them by the induction of apoptosis. In view of the known fact that CD7 internalizes rapidly after ligand binding and the unpublished observation that clinical application of the native unmodified TH-69 antibody leads to antigen modulation resulting in CD7-negative cells,4 a CD7-specific immunotoxin appears promising against T-cell malignancies. This expectation is further supported by the recent findings that other immunotoxins constructed by fusions to a truncated ETA produced encouraging clinical results. Treating patients with hairy cell leukemia with the immunotoxin BL22 (CD22-ETA), Kreitman et al. did not observe any pulmonary edema, a complication encountered after treatment with corresponding immunotoxins containing a deglycosylated ricin A chain (30, 31). The CD7-ETA scFv immunotoxin reported here may therefore circumvent some of the problems seen with older immunotoxins.

Although a CD7-positive subpopulation of CD34+CD38− cord blood cells with differentiation capabilities beyond the T-cell lineage has been described (16, 17) and the expression of CD7 on immature AML (7–10) could even be interpreted as an indication for progenitors to myeloid and T lineage, no significant CD7 expression is found on most hematopoietic progenitor cells (16). Therefore, an immunotoxin directed against CD7 has a promising range of prospective applications against T-cell neoplasms. Although a greater fraction of T-cell leukemias and some cases of AML express CD7 on their surface than CD3, CD4, CD5, or CD25, the CD7 construct still avoids eliminating too many normal T cells important for the maintenance of relevant immune functions, because it spares the CD7-negative subset of normal T-lymphoid cells (23). Elimination of too large a subset of normal T cells is a problem accompanying the clinical use of the CD52 antibody Campath-1H. Therefore, the CD7 immunotoxin reported here deserves further testing in in vivo models, and such experiments are under way.

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A Recombinant CD7-specific Single-Chain Immunotoxin Is a Potent Inducer of Apoptosis in Acute Leukemic T Cells

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