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 T-lineage negative B-lymphoid cell 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 unmodified antibody, the antibody has been used in pilot studies with AML patients. Replacing the scFv-exotoxin A immunotoxin against melanoma-associated chondroitin sulfate proteoglycan, which is expressed on the cell surface, maintains the specificity for CD7-positive cells and allows for targeted killing of CD7-positive lymphocytes.

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 immunomun conjugate 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). Antibodies can be targeted to T-cell leukemia, because of its high binding affinity to the T-cell receptor (5). 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 x 10^9 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 at least 10% of CD7-positive cells, 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

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 phosphatidylinositol 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 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 x 10^9 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 at least 10% of CD7-positive cells, 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-
specific antibody, showed clinical efficacy in Phase I trials (30, 31). The use of this toxin, however, was limited by its instability and associated vascular toxicity. Therefore, current efforts are directed at linking the ricin A toxin by recombinant DNA procedures to an scFv fragment of a CD7-specific antibody, but results of this effort are still unknown (27). The toxin saporin was linked via a bifunctional cross-linker and a disulfide bridge (32), and pokeweed antiviral protein was chemically linked to CD7-specific antibodies (28). In previous attempts, our group has linked cis-platinum to the TH-69 antibody, and this combination caused an inhibition of proliferation and a direct cytotoxic effect in the T-cell line CEM (33). Therefore, CD7 meets the requirements for a potentially useful target for immunotoxins. However, until now, most CD7-directed immunotoxins were generated by conventional chemical coupling and suffered from instability and accompanying side effects, such as vascular toxicity.

To overcome some of these shortcomings, two key steps were taken in the present study. One was the generation of a stable peptide bond 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 ETA3, 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 ~1,000-fold lower relative affinity to the EV 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 effective at killing both T- and B-cell cancer cell lines and bone marrow blasts from patients with lymphoma or leukemia (34, 37, 38).

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 Micro-organisms 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-MCSF; 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 μg/ml, respectively. COS monkey cells (DMSZ) were maintained in DMEM-Glutamax-I medium containing 10% FCS, penicillin, and streptomycin. The LCL cell line was established from bone marrow lymphoblasts of a 6-month-old female infant diagnosed with ALL (35). LLM cells (44) were cultured in Iscove’s Modified Dulbecco Medium (Life Technologies, Inc.), supplemented with human transferrin and linoleic acid at 3 μg/ml, BSA at 10 mg/ml, α-thioglycolate at 50 mg/ml, bathocuproin-digulonic acid at 20 μM, insulin at 1 μg/ml, and recombinant human interleukin-7 (Strathmann, Inc., Hamburg, Germany) at 10 ng/ml.

Patient-derived Leukemic Cells. HOI 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 Terminal 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 μg 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 cells and COS cells positive for the MCSP antigen. For this purpose, 5 × 105 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 μl 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 μg 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 × YT medium [16 grams/liter tryptone/peptone (Roth, Karlsruhe, Germany), 10 grams/liter yeast extract (Roth), and 5 grams/liter NaCl containing 1% glucose and chloramphenicol] at 30 μg/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 × YT medium 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 μg/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, Euerbergien, 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.)
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 N\textsubscript{H}I terminus and a SfiI-site, two oligonucleotides, oligo 1: 5′-cc cat cac cat cac cac gcc ggc cag cgc gcc g-3′ and oligo 2: 5′-ga tc ggc ggc cag ccc gct atg atg atg atg cag gg-3′ (MWG-Biotech, Munich, Germany) were hybridized and ligated into pet27b (+) digested with MscI and BamHI. In a second step, a DNA fragment coding for a 20 amino acid linker with a second SfiI site was inserted into HindIII/Ncol-digested modified pet27b (+) using oligonucleotides 3 and 4 (oligo 3: 5′-gcc ggc cgc ggt ggg gcc ggc atg gcc ggc atg gcc atg gcc ggc atg gcc ggc atg gcc ggc atg gcc cgc gcc gcc gcc gcc gcc gcc ggc cgc gcc ggc ca-gc). This procedure resulted in the construct pet27b (+)L3HS9. Sequences coding for CD7- and MCSP-specific scFvs were excised from the pAK400-anti CD7 and pAK400-anti MCSP expression constructs harboring the corresponding scFv fragments and cloned as SfiI-cassettes into pet27b (+)L3HS9. The resulting plasmids were digested with NotI/Cel II, and a DNA fragment encoding the truncated ETA was amplified by PCR from plasmid pSW202 (47) using the primers oligo 5′: 5′-gat gcc ggc cgc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gcc gc...
notoxin, 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 $\sim 100$ 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-leukemic 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 attributable 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-MCSF-ETA scFv immunotoxin of similar 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-MCSF-ETA toxin failed to

![Image](cancerres.aacjournals.org)
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 pre-treatment 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...
cell lines but also primary leukemic cells. For this purpose, a low-passage culture of cells derived from a pediatric patient (HOL) with a T-ALL was used. HOL cells had been kept in culture for 30 generations. Their generation time was 3 days as opposed to 22–24 h for the cell lines CEM and Jurkat. Treatment of CD7-positive HOL cells with the immunotoxin also caused apoptosis, although less efficient than the effect seen after treatment of the stable lines (Fig. 10). Compared with the MCSP-ETA-treated controls, 20% specific death (Annexin V positive; \( P = 0.007 \)) was observed 96 h after administration of an initial dose of 100 ng/ml and a second dose after 48 h, given with a renewal of the culture medium. The effect was specific, because it was prevented by pretreatment with an excess of the parental TH-69 antibody but not by pretreatment with a comparable excess of a control antibody of matching isotype (\( P = 0.03 \)). Pretreatment with the parental antibody did not completely block CD7-ETA-induced killing, but the difference in cell death at time point 96 h between MCSP-ETA and CD7-ETA blocked by TH-69 did not reach statistical significance (\( P = 0.08 \)). The anti-MCSP-ETA scFv immunotoxin had no effect on HOL cells, which are MCSP negative. In conclusion, the CD7-ETA immunotoxin was able to induce cell death in short-term cultures of primary leukemic cells but not to the same extent as in established cell lines, although the toxin was added twice.

Similar results were obtained by analyzing freshly drawn blood cells from an adult patient treated for pre-T-ALL. Immunophenotyping revealed 23% blasts before exposure to the CD7-ETA immunotoxin. The agent was added in a single dose of 100 ng/ml, and cell 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-
CD7-EXOTOXIN A 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, 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 a too large 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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