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Cytotoxic T lymphocytes with a grafted recognition specificity for ERBB2-expressing tumor cells

(single-chain antibody/chimeric T-cell receptor/targeted cytotoxicity/gene therapy)

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ABSTRACT **Experimental approaches which exploit the targeted cytolytic activity of lymphocytes are being developed for cancer therapy. We generated cytotoxic T lymphocytes (CTLs) with specificity for ERBB2 receptor-expressing tumor cells. A binding function was conferred directly on the ζ chain of the T-cell receptor (TCR) complex to circumvent major histocompatibility complex-restricted antigen recognition through the α and β chains of the TCR. A chimeric gene was constructed which encoded a single-chain Fv antibody (scFv, consisting of the joined heavy- and light-chain variable domains of a monoclonal antibody against the extracellular domain of the ERBB2 receptor), a hinge region as a spacer, and the ζ chain of the TCR. This gene was introduced into CTLs by retroviral gene transfer. The signaling potential of the scFv/hinge/ζ receptors was demonstrated by secretion of interferon γ upon coincubation with ERBB2-expressing cells. Target cells expressing the *ERBB2* gene were lysed *in vitro* with high specificity by the scFv/hinge/ζ-expressing T cells. The growth of *ERBB2*-transformed cells in athymic nude mice was retarded by adoptively transferred scFv/hinge/ζ-expressing CTLs. Transduced CTLs labeled with a fluorescent dye were specifically detected in tumor sections. Our results suggest that tumor cell lysis by CTLs grafted *in vitro* with a major histocompatibility complex-independent recognition could become a gene-therapy approach to cancer treatment.**

The transfer of immune cells with antitumor activity into cancer patients is the principle of adoptive immunotherapy (1, 2). Various immune cells have proven valuable for this purpose. Lymphokine-activated killer (LAK) cells derived from natural killer (NK) cells (3), tumor-infiltrating lymphocytes (TILs), and *in vitro* sensitized (IVS) lymphocytes derived from cytotoxic T lymphocytes (CTLs) (4) have been shown to be able to mediate tumor regression. Clinical trials with both LAK and TIL cell populations have shown therapeutic responses (5, 6). However, these responses were observed in only a fraction of the patients treated (7). Current research efforts are aimed at the improvement of the frequency and the duration of these responses (8) and at the elucidation of the mechanism of the antitumor activity.

The immune cells utilized so far have been derived from the patients, cultured, and reintroduced after expansion in tissue culture. LAK cells are cytolytic cells which react with a broad spectrum of target cells. They are not major histocompatibility complex (MHC)-restricted and lyse tumor cells but also lyse normal cells *in vitro* (9). TIL populations, derived from tumor tissue, are more potent and show specificity for their tumors of origin (10). The isolation and the antitumor activity of these cells is dependent on their natural occurrence and their *in vitro* expansion. It is possible that such cells are present in only a fraction of the tumor patients. The efficacy of lymphocyte-mediated tumor therapy can potentially be improved by *in vitro* manipulation of the recognition specificity of CTLs and their provision with a defined tumor cell specificity. We used insights and components from three different areas of research to achieve this goal.

(*i*) Consistent alterations of cell surface antigens have been identified in human cancer cells. Overexpression of the ERBB2 receptor is frequently observed in human breast and ovarian carcinomas and provides a target at the cell surface which strongly distinguishes tumor cells from their normal counterparts (11). (*ii*) Mouse monoclonal antibodies (mAbs) have been derived which specifically recognize the extracellular domain of ERBB2 (12). The mRNA of the hybridoma cells has been used to construct a gene combining the cDNA of the variable regions of the mAb into a single-chain antibody, scFv (13, 14). (*iii*) The CTL cell surface components which are important for target-cell recognition and signal transduction have been studied. The clonotypic α and β polypeptide chains of the T-cell antigen receptor (TCR) mediate specific target-cell recognition. These chains are associated with the nonpolymorphic components of the CD3 complex (γ, δ, and ε chains) and the ζ chain (15, 16). The disulfide-linked ζ homodimer is a transmembrane molecule and its cytoplasmic part plays a central role in TCR-mediated signal transduction and induction of cytolysis (17–19). Fusion of the ζ chain with an extracellular ligand-binding domain results in a molecule which can be activated by interaction with the ligand (17, 20, 21).

We have used the scFv derivative of a mAb directed against human ERBB2 (13, 14), and grafted it onto the ζ chain of the mouse TCR/CD3 complex. The immunoglobulin-like hinge region of the CD8 α molecule (22) was inserted as a short, flexible spacer between the scFv and ζ domains. Retroviral gene transfer (23, 24) was used to transduce the scFv/hinge/ζ fusion gene into CTLs. Here we report on the signaling capacities of the scFv/hinge/ζ protein and the cytolytic properties of the CTLs. Transduced CTLs express the scFv/hinge/ζ protein as a functional surface receptor and specifically lyse mouse fibroblast and epithelial cells transfected with the human ERBB2 receptor *in vitro*. In athymic nude mice, transduced CTLs were found to localize specifically to tumor tissue and retard growth of NIH 3T3 mouse fibroblasts transformed by the activated human *ERBB2* oncogene upon adoptive transfer.

MATERIALS AND METHODS

Cell Lines and Culture Conditions. CI96 is a C57BL/6 mouse (H-2^b)-derived CTL line with H-2K^d-restricted spec-

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Abbreviations: CTL, cytotoxic T lymphocyte; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine; IFN, interferon; LAK, lymphokine-activated killer; LDH, lactate dehydrogenase; mAb, monoclonal antibody; MHC, major histocompatibility complex; scFv, single-chain Fv; TCR, T-cell antigen receptor; TIL, tumor-infiltrating lymphocyte.

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ificity for P815 (H-2^d) mastocytoma cells (25). CI96 cells were maintained in Dulbecco's modified Eagle's medium (DMEM; GIBCO) with 10% fetal bovine serum (Boehringer Mannheim) and recombinant interleukin 2 (IL-2, 100 units/ml) (26). P815 cells, retroviral packaging cell lines ΩE (24) and PA317 (23), and infectants, the fibroblast cell line NIH 3T3, its transfectant NIH 3T3#3.7 expressing the activated human ERBB2 receptor, and the human ERBB2-expressing breast cancer line MDA-MB453, were cultured in DMEM with 10% fetal bovine serum. HC11 is a mouse mammary epithelial cell line; its transfectant HC11R1#11 expresses the human *ERBB2* protooncogene (27).

Construction of the scFv/hinge/ζ Fusion Gene. The scFv(FRP5)/hinge/ζ cDNA was subcloned into the *Eco*RI site of the retroviral vector pLXSN (23), in which cDNA expression is controlled by the 5′ long terminal repeat of Moloney murine leukemia virus and expression of the neomycin-resistance gene is driven by the simian virus 40 promoter. The ζ and CD8 α hinge cDNAs were derived from total RNA of the CTL line CI96 (25) by reverse transcription followed by PCR (D.M. and B.G., unpublished work). The cDNA encoding the ERBB2-specific scFv(FRP5), including an immunoglobulin heavy-chain leader peptide (13), was ligated to the ζ cDNA starting from amino acid 28 (numbering according to ref. 28). The CD8 α hinge cDNA encoding amino acids 105–165 (numbering according to ref. 22) was subsequently inserted.

Retroviral Gene Transfer. The ecotropic packaging cell line ΩE (24) was transfected by calcium phosphate precipitation with pL[scFv(FRP5)/hinge/ζ]SN plasmid DNA. Transfected cells were stably selected in the presence of G418 sulfate (Geneticin; GIBCO) at 1 mg/ml. Viral supernatants from pools of G418-resistant packaging cells were used to infect the amphotropic packaging cell line PA317 (23) in the presence of Polybrene at 8 μg/ml. Clonal, high-titer producer lines were derived by selection in medium with G418 at 1 mg/ml. Supernatants of these producer lines were used to infect CI96 cells. Clones were derived by growth in medium with G418 at 1 mg/ml.

Biochemical Characterization of Cell Surface Proteins. Viable cells (3×10^7) were surface biotinylated with sulfosuccinimidobiotin (Pierce). After the reaction was quenched with L-lysine, cells were lysed in 1% (vol/vol) Nonidet P-40/150 mM NaCl/50 mM Tris·HCl, pH 8.0/5 mM EDTA/1 mM phenylmethanesulfonyl fluoride supplemented with protease inhibitors. Immunoprecipitation was performed by addition of 3 μg of the ζ-specific mAb H146-968 (gift from R. Kubo, Cytel, San Diego), followed by incubation with protein A-Sepharose. For deglycosylation, precipitates were incubated with protein N-glycosidase F (New England Biolabs) for 1 hr at 37°C. Samples were boiled in either nonreducing or reducing Laemmli sample buffer and electrophoresed through SDS/5–20% polyacrylamide gradient gels. The proteins were transferred to a poly(vinylidene difluoride) membrane (Millipore), probed with horseradish peroxidase-streptavidin conjugate (1:5000; Southern Biotechnology Associates), and visualized with the enhanced chemiluminescence (ECL) kit (Amersham).

Induction of Interferon γ (IFN-γ) Secretion. CTLs were cocultured with 10^5 stimulator cells which had been irradiated with 3000 rads (1 rad = 0.01 Gy) for 48 hr at 37°C in 0.2 ml of IL-2-containing culture medium. Supernatants were collected and analyzed by ELISA for mouse IFN-γ (Genzyme). The ELISA used a solid-phase hamster mAb specific for mouse IFN-γ. After addition of either sample or recombinant mouse IFN-γ standard, a goat polyclonal anti-hamster antibody was added, followed by a peroxidase-conjugated donkey polyclonal anti-goat antibody.

***In Vitro* Cytotoxicity Assay.** The colorimetric CytoTox 96 assay (Promega) (29) was used to quantitate the release of a cytosolic enzyme, lactate dehydrogenase (LDH), upon target-cell lysis. A constant number of target cells (7500 per well) was added to serial 2-fold dilutions of effectors for an 8-hr incubation at 37°C and 5% CO_2 (experimental). After centrifugation, 50-μl aliquots of cell-free supernatant were assayed for LDH content. To correct for spontaneous LDH release from effector cells, LDH levels were measured for each individual effector-cell concentration used in the experimental setup (effector spontaneous). Target-cell spontaneous LDH release (target spontaneous) was also measured. Maximum target-cell LDH release (target maximum) was measured after cell lysis with 0.4% Triton X-100 and was considered as 100% LDH release. All measured values were assayed in triplicate and corrected for the culture medium LDH background. The percentage of specific LDH release was determined as % cytotoxicity = [(experimental − effector spontaneous − target spontaneous)/(target maximum − target spontaneous)] × 100.

***In Vivo* Antitumor Activity.** Two experimental schedules were used to assess the antitumor activity of the CTLs *in vivo*. (*i*) NIH 3T3#3.7 tumor cells (5×10^5) were mixed with 5×10^6 CFYZ.1 cells or parental CI96 cells (effector/target ratio of 10:1) in 0.1 ml of culture medium and immediately injected subcutaneously into the right flank of BALB/c *nu/nu* mice (30). The growth of the tumors was followed by caliper measurements. NIH 3T3#3.7 tumor cells alone were injected as a control. Each group consisted of five animals. (*ii*) BALB/c *nu/nu* mice were inoculated subcutaneously in the right flank with 4×10^5 NIH 3T3#3.7 tumor cells. On days 4 and 5, when tumors were palpable, parental CI96 cells and CFYZ.1 cells were injected intravenously into the tail vein (10^7 cells in 0.2 ml of culture medium). Five hundred units of recombinant human IL-2 (Hoffmann–La Roche) in 0.2 ml of phosphate-buffered saline was administered intraperitoneally on days 4, 5, and 6. The growth of the tumors was followed by caliper measurements. NIH 3T3#3.7 cells with or without IL-2 were injected as controls. Each group consisted of five animals.

Fluorescence Labeling and Tumor Localization of CTLs. CI96 and CFYZ.1 cells were labeled with the fluorescent dye DiI (Molecular Probes) as described (31). In brief, 2×10^7 CTLs were washed twice in DMEM and stained in 1 ml of serum-free DMEM containing 50 μg of DiI. After 1 hr at 37°C, cells were washed three times in DMEM with 10% fetal bovine serum. BALB/c *nu/nu* mice (two per group) carrying NIH 3T3#3.7-induced tumors of 130–220 mm^3 were intravenously injected with DiI-labeled CI96 or CFYZ.1 infectants. Twenty-four hours later, the mice were sacrificed, NIH 3T3#3.7 tumors were removed, and 8-μm sections were cut at −20°C. The cryosections were analyzed by fluorescence microscopy using a rhodamine filter combination (Zeiss Axiophot epifluorescence microscope).

RESULTS

Construction of the scFv/hinge/ζ Gene. A fusion gene consisting of a recognition function, a spacer domain, and the ζ chain as a signaling component of the TCR/CD3 receptor complex was constructed (Fig. 1). The recognition function is contributed by the scFv domain derived from the mAb FRP5 (12), specific for the extracellular domain of ERBB2. scFv comprises the variable domains of the heavy and light chains (V_H and V_L) of the mAb joined by a 15-amino acid linker sequence. This scFv is able to recognize the extracellular domain of ERBB2 (13, 14). The scFv(FRP5) cDNA was ligated to a short linker sequence encoding 59 amino acids from the immunoglobulin-like hinge region of the CD8 α chain (22). This hinge region provides flexibility and accessibility to the scFv moiety and is necessary for the binding of the extracellular domain of ERBB2 to the scFv (not shown).

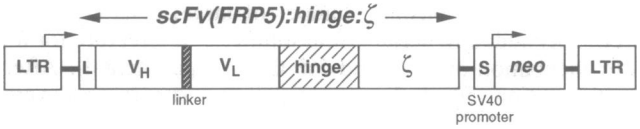

FIG. 1. Schematic representation of scFv(FRP5)/hinge/ζ fusion gene in the retroviral gene transfer vector pL(scFv(FRP5)/hinge/ζ)SN. scFv(FRP5) refers to a fusion of cDNAs encoding the heavy- and light-chain variable domains (V_H and V_L) of mAb FRP5, which is directed against the extracellular domain of the human ERBB2 receptor. V_H and V_L are joined by a short polypeptide linker sequence. L indicates a leader sequence derived from an immunoglobulin heavy-chain leader; hinge refers to a region of the CD8 α gene located in the membrane-proximal region of the extracellular domain which provides accessibility for ligand binding. CD3 ζ is a signaling component of the TCR/CD3 complex consisting of a transmembrane and a cytoplasmic region. The fusion gene is transcriptionally regulated by the Moloney murine leukemia virus 5′ long terminal repeat (LTR), and the vector also encodes a G418-resistance gene (*neo*) regulated by the simian virus 40 (SV40) promoter. Transcriptional start sites are indicated by bent arrows.

The transmembrane and signaling domains of the fusion gene are contributed by the ζ chain of the TCR (19).

Expression of the scFv/hinge/ζ Gene After Retroviral Transduction into T Cells. The pLXSN vector system (23) was chosen for retroviral gene transfer into CTLs (Fig. 1). This vector has been shown to be useful for gene transduction into CTLs (8). It directs the efficient synthesis of the integrated gene product and allows G418 selection of infected cells. An established murine CTL line, CI96 (25), was infected with the recombinant retrovirus. Clones of infected cells selected for high expression of the scFv/hinge/ζ fusion protein were derived and assayed for surface expression of the chimera. Selected clones were surface biotinylated and lysed in 1% Nonidet P-40 for immunoprecipitation with the anti-ζ mAb H146-968. SDS/PAGE analysis of the immunoprecipitates under reducing conditions revealed a series of bands at 48–65 kDa from lysates of infected cells (Fig. 2, lane 2)—but not from lysates of the parental cells (lane 1)—corresponding to the scFv/hinge/ζ protein with a calculated molecular mass of 48.7 kDa. The larger species arose as a consequence of complex glycosylation of the scFv and the hinge region. Deglycosylation with an endoglycosidase, protein N-glycosidase F, resulted in a simplified protein pattern and the reduction of the apparent molecular mass to about 47 kDa (lane 4). The endogenous ζ chain was detected as a 16-kDa band (16.3 kDa predicted) in uninfected and infected cells (lanes 1, 2, and 4). Disulfide-linked scFv/hinge/ζ homodimers of about 96 kDa as well as heterodimers of scFv/hinge/ζ molecules with the endogenous ζ chain with an apparent molecular mass of about 64 kDa were observed under nonreducing conditions (lane 6). The 32-kDa band corresponds to endogenous ζ–ζ homodimers (lanes 5, 6, and 7). Cell surface expression and ERBB2 receptor-binding ability of the scFv/hinge/ζ protein in transduced CI96 CTLs was also confirmed by flow cytometry (data not shown).

Signal Transduction by the scFv/hinge/ζ Fusion Protein. Activation of the TCR by interaction with a specific antigen triggers several signaling pathways and leads, for example, to an increase in intracellular tyrosine phosphorylation, inositol phosphate turnover, intracellular Ca^{2+} release, and production of cytokines such as IL-2, IFN-γ, granulocyte/macrophage-colony-stimulating factor, and IL-3 (15).

Intracellular Ca^{2+} was measured after crosslinking of the recombinant receptor. Crosslinking resulted in a rapid increase of intracellular Ca^{2+} in transduced but not in parental T cells (data not shown). We tested the capability of the scFv/hinge/ζ-expressing CTLs to produce IFN-γ after stimulation with cells expressing human ERBB2 (Fig. 3). Two individual clones expressing the scFv/hinge/ζ chimera,

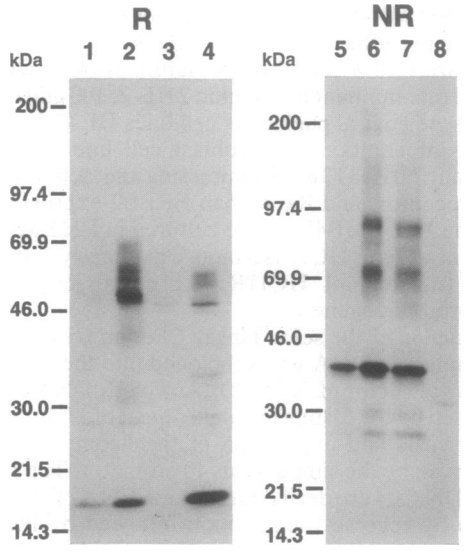

FIG. 2. Expression of scFv/hinge/ζ fusion proteins. Viable CI96 cells (lanes 1 and 5) and CFYZ.1 infectants (lanes 2–4 and 6–8) were surface biotinylated and lysed in 1% Nonidet P-40. Lysates were immunoprecipitated with anti-ζ mAb H146-968 (lanes 1, 2, 4, and 5–7) or a control mAb (lanes 3 and 8). Immunoprecipitates were deglycosylated by protein N-glycosidase F (lanes 4 and 7) and electrophoresed through 5–20% linear gradient gels under reducing (R) or nonreducing (NR) conditions. Positions and molecular masses of standards are indicated.

CFYZ.1 and CFYZ.2, were shown to increase secretion of IFN-γ after coincubation with the *ERBB2*-transfected stimulator cells HC11R1#11, but not with the untransfected parental cells HC11. Nontransduced CI96 cells could not be stimulated to secrete IFN-γ by ERBB2-expressing cells. P815 mastocytoma cells, the cognate target for CI96 CTLs, stimulated CI96 as well as the infectants to produce high levels of IFN-γ. These data indicate that intracellular signaling is triggered upon crosslinking of the scFv/hinge/ζ protein via an extracellular ligand domain and that the scFv/hinge/ζ protein is functionally active.

Cytotoxicity and Antitumor Activity of Transduced T Lymphocytes. The cytolytic activity of infected CI96 (CFYZ.1) cells was determined *in vitro* and *in vivo* (Fig. 4). Oncogenically transformed mouse NIH 3T3 fibroblasts and HC11

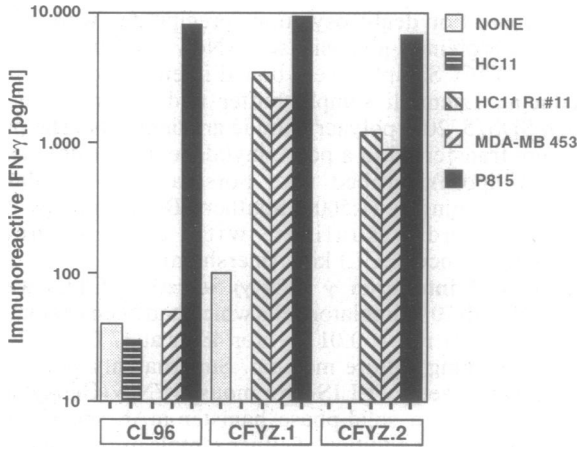

FIG. 3. IFN-γ production by scFv/hinge/ζ-expressing CTLs after stimulation with ERBB2-expressing cells. CTLs (10^5) were cocultured with irradiated stimulator cells (10^5) as indicated in medium with IL-2. After 48 hr at 37°C, supernatants were tested for IFN-γ by ELISA (Genzyme). Mean values obtained from two experiments are shown.

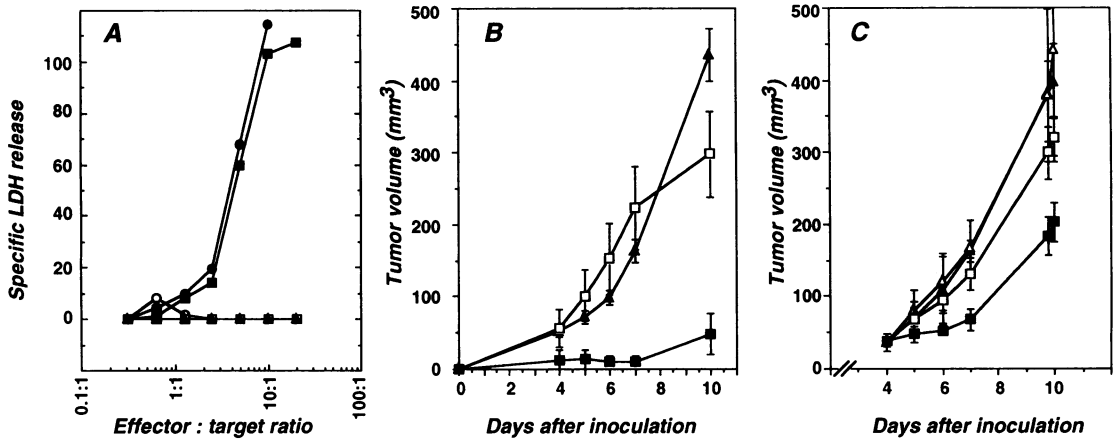

FIG. 4. Cytotoxicity and antitumor effects of scFv/hinge/ζ-expressing CTLs against human ERBB2-expressing target cells *in vitro* and *in vivo*. (*A*) *In vitro* cytotoxicity assay. Infected CFYZ.1 (●, ■, ▲) or parental CI96 (○, □, △) CTLs were incubated with NIH 3T3#3.7 (□, ■) or HC11R.1#11 (○, ●) cells as targets in an 8-hr cytotoxicity assay. NIH 3T3#3.7 cells are fibroblasts transfected with the activated human *ERBB2* oncogene; HC11 R.1#11 cells are breast epithelial cells transfected with the human *ERBB2* protooncogene. The specific release of LDH is plotted versus effector/target ratio. Nontransfected NIH 3T3 (△) and HC11 (▲) cells served as controls and did not release LDH at levels higher than the spontaneous release observed. (*B* and *C*) *In vivo* antitumor activity of CFYZ.1 (*B*) NIH 3T3#3.7 tumor cells were mixed with CFYZ.1 cells (■) or parental CI96 cells (□) at a ratio of 1:10 and immediately injected subcutaneously into the right flank of BALB/c nude mice. Tumor growth was followed by caliper measurements. NIH 3T3#3.7 cells alone (▲) are shown as a control. Values are mean ± SD. (*C*) BALB/c nude mice were inoculated subcutaneously with NIH 3T3#3.7 tumor cells. On days 4 and 5, when tumors were palpable, parental CI96 cells (□) and CFYZ.1 cells (■) were injected intravenously through the tail vein. IL-2 was administered intraperitoneally on days 4, 5, and 6. Tumor growth was followed by caliper measurements. NIH 3T3#3.7 cells without (▲) and with (△) IL-2 are shown as controls. Values are mean ± SD.

epithelial cells expressing human ERBB2 (27) were employed as target cells. The release of LDH from these cells was used as a measure of cell lysis (29). Infected CI96 cells expressing the scFv/hinge/ζ efficiently lysed ERBB2-expressing NIH 3T3 cells or HC11 cells (Fig. 4*A*) at effector/target ratios between 1 and 10. In contrast, no cell lysis was observed when the parental CI96 cells were used as effectors. The mAb FRP5 and the derived scFv domain are specific for human ERBB2 and do not crossreact with the mouse homologue which is expressed at low levels on both cell lines. For this reason, no cell lysis was observed when untransfected NIH 3T3 cells or HC11 cells were incubated with the scFv/hinge/ζ-expressing T cells.

The antitumor activity of infected CI96 cells was determined *in vivo*. NIH 3T3 cells transformed with the human *ERBB2* oncogene led to the rapid formation of tumors after subcutaneous injection into athymic BALB/c nude mice. The simultaneous administration of CFYZ.1 infectants and tumor cells completely suppressed tumor formation for up to 8 days. Administration of the uninfected parental CI96 cells, however, had no effect on tumor cell growth (Fig. 4*B*). A similar result was obtained when nude mice were inoculated first with NIH 3T3 ERBB2-expressing tumor cells and subsequently treated with CFYZ.1 cells in combination with exogenous IL-2. The infectants were injected intravenously into the tail veins on days 4 and 5 after tumor cell inoculation. The administration of the CTLs strongly retarded the growth of the tumor cells, although it did not totally prevent it (Fig. 4*C*). The difference in tumor growth seen on day 10 was statistically significant (Student *t* test, $P < 0.001$).

Transduced CTLs Localize to ERBB2-Expressing Tumor Tissue. The antitumor activity of CFYZ.1 cells implies that these cells can recognize ERBB2-expressing tumor cells *in vivo*. We have directly tested the ability of adoptively transferred CTLs to infiltrate the tumor tissue. Parental and transduced CTLs were labeled with a fluorescent dye (DiI) in cell culture and injected intravenously into mice carrying a transplanted tumor of NIH 3T3#3.7 cells. Tumor sections were prepared and fluorescent cells were visualized. Significant numbers of fluorescent cells were detected in tumors of animals injected with the labeled CFYZ.1 cells, but not in tumors of animals injected with labeled parental CI96 cells. A representative section is shown in Fig. 5. Transduced CTLs have a higher capacity to home to the ERBB2-expressing tumor cells.

DISCUSSION

Tumor formation is based on the mutation of oncogenes and tumor-suppressor genes in somatic cells (32) and on the structural alterations or overexpression of the encoded proteins. Both events might result in alterations in the intracellular processing of these proteins and the presentation of new antigens in association with the major histocompatibility

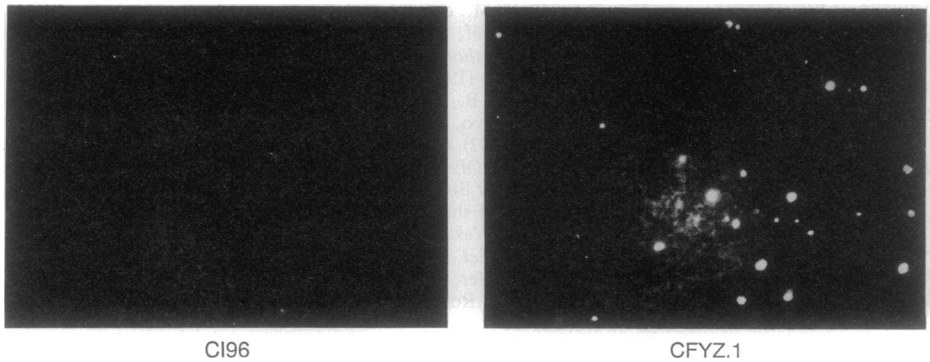

FIG. 5. Fluorescence imaging of tumor sections. Tumors were induced by transplantation of NIH 3T3#3.7 ERBB2-expressing tumor cells in BALB/c nude mice. Cryostat sections (8 μm) of tumor tissue were analyzed. Tumors were removed 1 day after intravenous administration of DiI-labeled CI96 control cells or CFYZ.1 infectants.

antigens on the surface of the cells. The detection of antibodies directed against oncogene products in the serum of tumor patients (33) is an indication that oncogene products can be antigenic. This also seems to apply to the cellular immune response. The occurrence of CTLs which recognize and eliminate tumor cells has been demonstrated in a number of model systems (34, 35). Therapeutic responses in tumor patients treated with *in vitro* expanded TILs have been achieved. These responses are thought to be due to the specific recognition of antigens presented on the surface of the tumor cells. It remains to be clarified which antigens are recognized. The objective response rate of advanced melanoma patients treated with a combination of TILs and IL-2 was 38% (1). Efforts which are aimed at the improvement of these results are underway and involve the genetic manipulation of the TIL cell population. Lymphocytes transduced with the tumor necrosis factor α cDNA might exhibit enhanced antitumor potential (8).

We have chosen genetic manipulation of lymphocytes as an approach to make them more potent antitumor agents. This approach is based on the identification of consistent genetic alterations in tumor cells. ERBB2 is a transmembrane receptor molecule which is overexpressed in a high percentage of human carcinomas (11). Expression of ERBB2 in normal adult tissue is low. This difference in expression identifies ERBB2 as "tumor enhanced" and led us to choose this molecule as a target for directed therapeutic agents (13, 14). We used insights into the molecular structures responsible for TCR signaling and advances in the biotechnology of antibody engineering. The antigen-binding function of a mAb directed against the extracellular domain of the *ERBB2* protooncogene product was fused in a scFv design to the ζ chain of the TCR/CD3 complex. An immunoglobulin hinge-like CD8 α segment served as a spacer region. The recombinant receptors functioned as signal-transducing modules and coupled to the biochemical pathways indicative of TCR activation.

Epithelial and fibroblast target cells transfected with human ERBB2 were effectively lysed in a non-MHC-restricted *in vitro* manner by CTLs expressing the scFv/hinge/ζ chimera. An antitumor effect of CFYZ.1 infectants was observed *in vivo* when these cells were implanted simultaneously with *ERBB2*-transformed fibroblasts into nude mice. Tumor growth was slowed over a period of 10 days. Uninfected parental CTLs did not inhibit tumor growth. The transduced CTLs also had the capability to home to the tumor and displayed cytolytic activity when administered at a different site. The intravenous injection of tumor-specific CTLs in combination with added IL-2 inhibited tumor growth for several days.

This report and recent results from Eshhar's laboratory (36, 37) show that it is possible to design antigen-specific CTLs by grafting the recognition specificity of a mAb onto the signaling components of the TCR/CD3 complex via recombinant DNA and gene transfer techniques. A similar conclusion was reached by Hwu *et al.* (38), who showed that human lymphocytes could be redirected in their recognition specificity by Fc receptor/γ-chain chimeras. Our results show that the specificity and thus the cytolytic effector machinery can be efficiently redirected toward a surface antigen, the ERBB2 receptor, that is important in the etiology of human breast, ovarian, gastric, and colon cancer. It also suggests that the principle of targeted T-cell action might become a useful therapeutic approach and be generally applicable for the elimination of tumor cells which express a surface antigen at higher levels than normal cells. The design potentially permits the generation of CTLs with many desired specificities by exchanging the scFv moiety and replacing it with any existing antigen-recognition function derived from a specific mAb.

The anti-ζ mAb was provided by R. Kubo, the pLXSN plasmid by A. D. Miller, the ΩE cells by H. Land, NIH 3T3#3.7 cells by N. E. Hynes, the CI96 CTL line by K. Eichmann, and the purified ERBB2 extracellular domain by M. Jeschke. We thank F. Gudert and U. Dürmüller for help with the cryosections. We are grateful to K. Karjalainen for valuable discussions and particularly grateful to N. E. Hynes for support of the project. K. Ballmer and W. Filipowicz are acknowledged for critical reading of the manuscript.

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