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Human Gene Therapy answers the need for a central forum dealing with all aspects of gene transfer in mammals.

Human Gene Therapy (ISSN: 1043-0342) is published 18 times a year (monthly, except semi-monthly in January, March, May, July, September, and November) by Mary Ann Liebert, Inc., 2 Madison Avenue, Larchmont, NY 10538-1962. Periodicals postage paid at Larchmont, NY, and at additional mailing offices. **Postmaster:** Send address changes to *Human Gene Therapy*, c/o Subscription Department, Mary Ann Liebert, Inc., 2 Madison Avenue, Larchmont, NY 10538-1962. Mailed in Canada under CPC IPM #1269712.

Subscriptions should be addressed to the Publisher and are payable in advance. Rates for subscriptions are \$973 per volume of 18 issues in the United States and Possessions and \$1,078 elsewhere. The student post-doctorate/fellow rate is \$100. Subscriptions begin with the first issue of the current volume. Bulk subscriptions available upon request from the Publisher.

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Reprints, except special orders of 100 or more, are available from the authors.

Information for Manuscript Submission is given elsewhere in the publication.

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Human Gene Therapy is indexed in *Index Medicus*, *MEDLINE*, *Current Contents/Life Science*, *EMBASE/Excerpta Medica*, *Cambridge Scientific Abstracts*, *Reference Update*, and *BIOSIS*.

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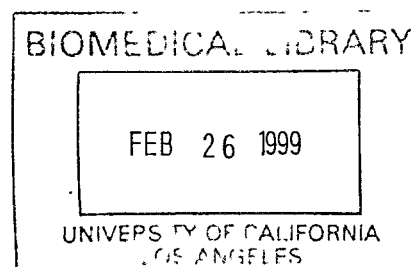
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2nd Annual Meeting

American Society of Gene Therapy

June 9 – 13, 1999
Washington, DC



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Anti-Tumor Activity of Human T Cells Expressing the CC49- ζ Chimeric Immune Receptor

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ABSTRACT

A chimeric immune receptor consisting of an extracellular antigen-binding domain derived from the CC49 humanized single-chain antibody, linked to the CD3 ζ signaling domain of the T cell receptor, was generated (CC49- ζ). This receptor binds to TAG-72, a mucin antigen expressed by most human adenocarcinomas. CC49- ζ was expressed in CD4⁺ and CD8⁺ T cells and induced cytokine production on stimulation. Human T cells expressing CC49- ζ recognized and killed tumor cell lines and primary tumor cells expressing TAG-72. CC49- ζ T cells did not mediate bystander killing of TAG-72-negative cells. In addition, CC49- ζ T cells not only killed FasL-positive tumor cells *in vitro* and *in vivo*, but also survived in their presence, and were immunoprotective in intraperitoneal and subcutaneous murine tumor xenograft models with TAG-72-positive human tumor cells. Finally, receptor-positive T cells were still effective in killing TAG-72-positive targets in the presence of physiological levels of soluble TAG-72, and did not induce killing of TAG-72-negative cells under the same conditions. This approach is being currently being utilized in a phase I clinical trial for the treatment of colon cancer.

OVERVIEW SUMMARY

Tumor-specific T cells are generated in cancer patients, but often fail to eradicate the tumor. *Ex vivo* expansion of these tumor-infiltrating lymphocytes (TILs) followed by reinfusion into the patient has shown some efficacy, but fails to provide significant long-term responses. This may be a result of the low frequency of high-affinity tumor-specific T cells. To circumvent these issues, we have generated T cells bearing a chimeric immune receptor composed of an extracellular, TAG-72 tumor antigen-specific scFv domain and an intracellular CD3 ζ domain (CC49- ζ). Genetically modified human T cells expressing CC49- ζ lyse TAG-72-positive tumor cells and are immunoprotective in xenograft tumor models with TAG-72-positive human tumor cells.

INTRODUCTION

TUMOR-DIRECTED CYTOLYTIC CELLS have been observed in patients that have later succumbed to their disease. To increase the numbers of these nascent cytolytic cells, interleukin

2 (IL-2) was used to drive the *ex vivo* expansion of lymphokine-activated killer (LAK) cells or tumor-infiltrating lymphocytes (TILs). LAK cell- and TIL-based therapies for most tumor types did not demonstrate significant long-term clinical responses, perhaps owing to the low frequency of high-affinity tumor antigen-specific T cells (Herberman *et al.*, 1987; Blasse, 1995; Yannelli *et al.*, 1996). *Ex vivo* antigen-driven expansion of syngeneic T cells can produce large numbers of antigen-specific T cells (Riddell and Greenberg, 1995; Rooney *et al.*, 1995; Riddell *et al.*, 1996). Even though impressive clinical efficacy has been observed in the treatment of cytomegalovirus (CMV) infections and Epstein-Barr virus (EBV) lymphoproliferative disease with this approach, the use of antigen-specific T cells is not without difficulties, including patient-to-patient variability in cytotoxic T lymphocyte (CTL) frequency, the prolonged culture times required to generate therapeutic numbers of CTLs, and the low frequency of antigen-specific T cells for non-virus-associated malignancies.

The genetic modification of human T cells to express high-affinity, tumor antigen-specific chimeric immune receptors (CIRs) offers an alternative method to produce large numbers of tumor-specific T cells. These receptors are created through

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the use of tumor- or virus-specific single-chain antibodies (scFvs) or ligands fused to the intracellular signaling domains of the FcR γ or the T cell receptor (TCR) CD3 ζ chains. T cells expressing a variety of scFv-based receptors have been shown to lyse tumor- or virus-infected targets *in vitro* (Stancovski *et al.*, 1993; Moritz *et al.*, 1994; Roberts *et al.*, 1994; Hwu *et al.*, 1995). In some cases, the chimeric immune receptor-bearing T cells extended the survival of tumor-bearing mice.

T cell immunotherapy of cancer patients will likely entail repeat administrations of the engineered T cells. All scFv-based receptors constructed previously utilized murine antibodies for ligand binding. Multiple administrations of T cells expressing these receptors will likely be prohibited owing to generation of a human anti-mouse antibody (HAMA) response and subsequent elimination of the genetically modified T cells. For clinical development, chimeric immune receptors constructed from human ligands or humanized scFvs will be necessary. We describe here a CIR specific for the panadenocarcinoma surface antigen, TAG-72, constructed from the humanized scFv of the CC49 antibody (Shu *et al.*, 1993; Kashmiri *et al.*, 1995). The murine CC49 antibody (Molinolo *et al.*, 1990) has been used in clinical trials in more than 500 patients as either a radiotherapeutic agent or radiodiagnostic tool for colon, ovarian, prostate, and breast cancers, and has shown no significant secondary organ toxicity (Greiner *et al.*, 1992; Meredith *et al.*, 1994; Murray *et al.*, 1994). Primary human T cells were genetically modified with high efficiency using a retrovirus encoding the CC49- ζ CIR (hereafter referred to as CC49- ζ). The CC49- ζ T cells demonstrated antigen-specific lysis of all TAG-72-positive tumor cell lines and primary cells from patient isolates tested, produced immunostimulatory cytokines on receptor stimulation, and were immunoprotective in murine xenograft tumor models. In addition, CC49- ζ T cell activity was retained in the presence of Fas ligand (FasL)-expressing tumor targets and in the presence of physiologic levels of soluble TAG-72.

MATERIALS AND METHODS

Generation of CC49- ζ receptor retrovirus

The CC49- ζ TAG-72 antigen recognition domain consists of an scFv of the V_H and V_L regions from the humanized CC49 antibody (Shu *et al.*, 1993; Kashmiri *et al.*, 1995) joined through a (Gly₄-Ser)₃ linker. A 788-bp fragment was obtained by digestion with *Nco*I and *Rsr*II from plasmid pTAHU-CC49SCIgDCH1 and ligated to the [γ]₁ hinge and CH3 domain of human IgG₁ (residues 221–230 and 341–444 of immunoglobulin [Ig] γ ₁), using an oligonucleotide linker flanked by *Rsr*II and *Nsp*I sites. The extracellular portion of CC49- ζ is attached to the human CD3 ζ intracellular region (residues 31–142 of CD3) through the human membrane-associated IgG M1 transmembrane-spanning domain and human CD4 transmembrane-spanning region (residues 372–395 of CD4). The CIR backbone, hinge- γ 1(CH3)- γ M1-CD4 TM-CD3 ζ , was generated at Cell Genesys (Foster City, CA). CC49- ζ was cloned into the retroviral rkat43.2 vector backbone, which has been previously described (Finer *et al.*, 1994). The anti-HIV_{env} gp120, 447D- ζ chimeric immune receptor is similar in struc-

ture to CC49- ζ , with the CC49 scFv replaced with the 447D scFv (Patel *et al.*, 1999).

Virus was generated by transient CaPO₄-mediated transfection of 293 cells as described previously (Roberts *et al.*, 1994), with the following modification; three different plasmids, the kat retroviral plasmid encoding CC49- ζ and two plasmids expressing the retroviral Gag-Pol and Env proteins, necessary for packaging functions, were used to decrease the likelihood of generating replication-competent retrovirus through recombination between the CC49- ζ -viral and packaging vectors.

Tumor cells

The LS174T, KLE-B, CCRF-CEM, and MIP-1 cell lines were obtained from J. Schlom (National Cancer Institute, Bethesda, MD). LS180, Snu-1, Jurkat, NCI H716, and NCI H508 cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD). Human colon carcinoma liver metastases were a generous gift of R. Warren (University of California, San Francisco). Tumor metastases were disassociated in Dulbecco's modified Eagle's medium (DMEM) with collagenase (1 mg/ml) and 0.002% DNase in MEM at 37°C with mechanical agitation. The tumor cell suspensions were washed extensively and cultured for 4–18 hr at 37°C in 5% CO₂ in MEM supplemented with 10% fetal calf serum (FCS), 2 mM sodium pyruvate, penicillin-streptomycin, and L-glutamine.

Culture and transduction of human T cells

Ficoll-purified peripheral blood mononuclear cells (PBMCs), depleted of adherent cells, were resuspended at 3×10^6 cells/ml in T cell medium (TCM) (AIM-V [Gibco, NY] and RPMI [1:1] with HEPES, sodium pyruvate, glutamine, penicillin-streptomycin, and 10% human serum) and stimulated with anti-CD3 and anti-CD28 antibody-coated Dynal (Lake Success, NY) beads at a density of three beads per cell in TCM for 48 hr at 37°C in 5% CO₂. On day 2, the beads were removed by magnetic separation. The PBMCs were then resuspended at 1×10^6 cells/ml in TCM with IL-2 (100 IU/ml). One day later, the T cells were resuspended at 10^6 cells/ml in TCM with IL-2 and mixed with an equal volume of supernatant containing the CC49- ζ retrovirus and supplemented with IL-2 (100 IU/ml) and Polybrene (4 μ g/ml). This step was repeated twice: 24 and 48 hr later. On day 6 the cells were removed from the transduction mix and resuspended in TCM with IL-2 at 10^6 cells/ml. The culture was then split every 1–2 days to 2×10^6 cells/ml for an additional 4–10 days.

FACS analysis

CC49- ζ or nontransduced T cells (5×10^5) were incubated with 100 ng of a CC49 anti-idiotypic antibody, AI49-3, in the presence of 3 mg of normal mouse IgG at room temperature for 20 min, and then incubated with 1 mg of goat anti-mouse IgG-PE (phycoerythrin) in the presence of 3 mg of normal goat IgG in 100 ml of phosphate-buffered saline (PBS)–2% FCS at room temperature for 20 min. For dual staining for CD4⁺ and CD8⁺ T cells, the cells were then stained with 1 mg of anti-CD8-FITC (fluorescein isothiocyanate) or anti-CD4-FITC antibodies.

⁵¹Cr release assays

⁵¹Cr-labeled target cells (5000) were mixed with various numbers of T cell effectors in a total volume of 200 ml in a round-bottom 96-well plate. In bystander killing analysis, TAG-72-negative cells were labeled and mixed with nonlabeled TAG-72 cells. After a 4-hr incubation at 37°C and 5% CO₂, the cells were pelleted at 1200 rpm for 4 min and 100 ml of the supernatant removed for counting of the released ⁵¹Cr. The percent specific release is calculated as follows: percent specific release = [(cpm_{released} - cpm_{spontaneous release}) / (cpm_{maximum released} - cpm_{spontaneous release})] × 100.

Soluble TAG-72 inhibition assays

CC49- ζ and nontransduced T cells were incubated with soluble TAG-72 (sTAG-72) from bovine submaxillary mucin (Sigma Chemicals, St. Louis, MO) or with sTAG-72-positive patient serum for 1 hr at 37°C and then mixed with an equal volume of medium containing ⁵¹Cr-labeled target cells and used in a 4-hr ⁵¹Cr release assay.

Animal tumor models

Mice were maintained in the Cell Genesys animal facility. Four- to 6-week-old SCID-NOD mice were injected intraperitoneally with 10⁶ KLE-B cells or subcutaneously with 10⁶ LS174T or KLE-B cells in 200 ml of PBS. Prior to injection, tumor cells were trypsinized and washed three times in PBS. Treated mice received 3 × 10⁶ CC49- ζ -transduced or normal human T cells by the same route. The T cells were mixed with the tumor cells immediately prior to injection into the animals. Mice were monitored daily for the development of subcutaneous tumors in the subcutaneous tumor model or sacrificed at 6 or 12 weeks and examined for the presence of tumors in the intraperitoneal tumor model.

RESULTS*Generation and growth of CC49- ζ T cells*

Human T cells were stimulated to proliferate (see below for conditions) and then transduced with retrovirus encoding CC49- ζ (Fig. 1). After a short expansion period, the T cells were stained with anti-CC49 idiotype and anti-CD8 antibodies and the cells analyzed by fluorescence-activated cell sorting (FACS). The average T cell transduction efficiency was 31%, with the range being 21 to 95% (Fig. 2A), without selection of receptor-positive cells. Both CD4⁺ and CD8⁺ T cells were transduced to express CC49- ζ (54 and 56% CC49- ζ positive, respectively, in this representative example) (Fig. 2B). Receptor expression was stable; in one experiment CC49- ζ -positive T cells were maintained for 35 days of continuous culture without any observable loss of receptor expression (Fig. 2C).

Since the state of activated T cells is known to affect T cell function and survival, we tested the effect of culture conditions on T cell responsiveness and function mediated by CC49- ζ . T cells that were stimulated with an anti-CD3 antibody became unresponsive to IL-2 following CC49- ζ stimulation mediated by interaction with TAG-72-expressing target cells (Fig. 3A).

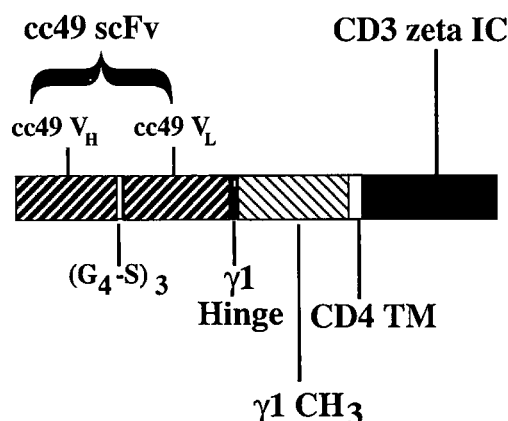


FIG. 1. The CC49- ζ chimeric immune receptor. A CIR consisting of an extracellular antigen-binding domain derived from the CC49 humanized single-chain antibody, linked to the CD3 ζ signaling domain of the T cell receptor, was generated. scFv, Single-chain variable fragment; IC, intracellular domain; V_H and V_L, variable heavy and light chains, respectively; (G₄-S)₃, (Gly₄-Ser)₃ peptide linker; γ 1, immunoglobulin γ 1; TM, transmembrane region.

The responsiveness to IL-2 is measured as proliferation. In contrast, T cells costimulated by anti-CD3 and anti-CD28 treatment were responsive to IL-2-induced proliferation (Fig. 3B). Thus, all further studies were conducted using T cells grown under these conditions.

Antigen-specific lysis of tumor cells by CC49- ζ T cells

The lytic activity and specificity of CC49- ζ -positive T cells were tested against several TAG-72-positive and -negative tumor cell lines. CC49- ζ T cells killed all TAG-72-positive targets tested, including LS174T and KLE-B cells (Fig. 4A and B) and the degree of lysis correlated with the level of TAG-72 expression on the targets (Table 1). CC49- ζ -positive T cells did not kill TAG-72-negative targets, including NCI H716 (Fig. 4C), MIP-1, and Snu-1. Lysis of these TAG-72-negative targets did not increase in the presence of TAG-72-positive tumor cells, indicating that the lytic activity of the CC49- ζ T cells was specific and did not result in any bystander lysis of TAG-72-negative cells (Fig. 4D and E).

CC49- ζ T cells kill patient-derived tumor cells

The sensitivity of tumor-derived cell lines and primary tumor cells to T cell-mediated lysis can be vastly different. However, CC49- ζ -transduced T cells efficiently lysed primary tumor cells from liver metastasis explants from two patients with advanced colon carcinoma. Even though the colon carcinoma cells expressed low, heterogeneous levels of TAG-72 (Fig. 5A), CC49- ζ T cells generated from two independent donors lysed the tumor cells, while nontransduced T cells from the same donors did not kill the colon carcinoma cells (Fig. 5B and C).

CC49- ζ -mediated function is similar to TCR-mediated activity

We analyzed the ability of CC49- ζ to mediate target killing and to generate the production of cytokines following stimula-

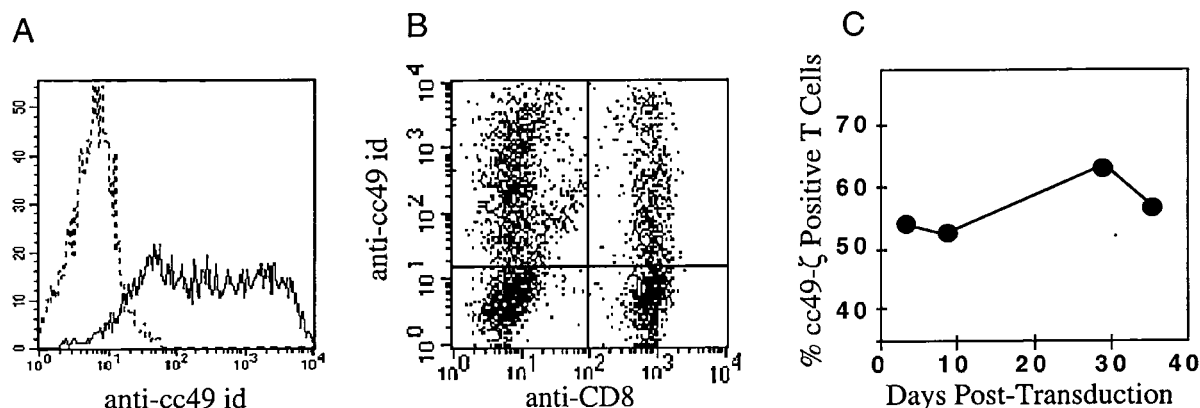


FIG. 2. T cell expression of CC49- ζ . (A) Four days after transduction CD8⁺ T cells were stained for expression of CC49- ζ with an anti-idiotypic antibody (solid line) or an isotype-matched control antibody (dashed line). (B) Ten days after transduction PBMCs were stained for expression of the CC49- ζ chimeric immune receptor and CD8. (C) Over the course of 35 days of continuous culture following transduction, T cells were stained for expression of the CC49- ζ chimeric immune receptor.

tion. T cells that were stimulated through the TCR or the CIR (with anti-CD3 or anti-CC49 idiotypic antibodies) shared a similar increase in activity (Fig. 6).

In addition to killing TAG-72-positive cells, CC49- ζ -positive T cells secreted granulocyte-macrophage colony-stimulating factor (GM-CSF; 150 to 600 pg/ml/10⁶ T cells/24 hr), interferon γ (IFN- γ ; 20 to 8500 pg/ml/10⁶ T cells/24 hr), and tumor necrosis factor α (TNF- α ; 60 to 90 pg/ml/10⁶ T cells/24 hr) following stimulation with tumor targets. IL-2 and IL-4 were not detected. No cytokines were detected in the cultures containing CC49- ζ T cells in medium alone. This pattern of cytokine expression was similar to the cytokine profile of cytolytic T cells stimulated through the T cell receptor (data not shown). Thus, the CC49- ζ -mediated functional effects are similar to those generated on T cell receptor engagement.

Soluble TAG-72 does not affect the function of CC49- ζ T cells

A potential inhibitor of CC49- ζ -positive T cell activity is the presence of soluble TAG-72 (sTAG-72), which can be detected in the serum of patients with advanced disease in concentrations up to 600 U/ml (Molinolo *et al.*, 1990). However, sTAG-72 did not significantly inhibit T cell lysis of TAG-72-positive targets when CC49- ζ T cells were preincubated with sTAG-72 concentrations up to 2000 U/ml (Fig. 7). After longer incubations of 2 days in the presence of sTAG-72 (100 U/ml), 60% of the CC49- ζ T cell lytic activity was retained (data not shown). These results suggest that sTAG-72 does not block sufficient numbers of the T cell CC49- ζ receptors to abrogate CC49- ζ T cell-mediated lysis of tumor targets.

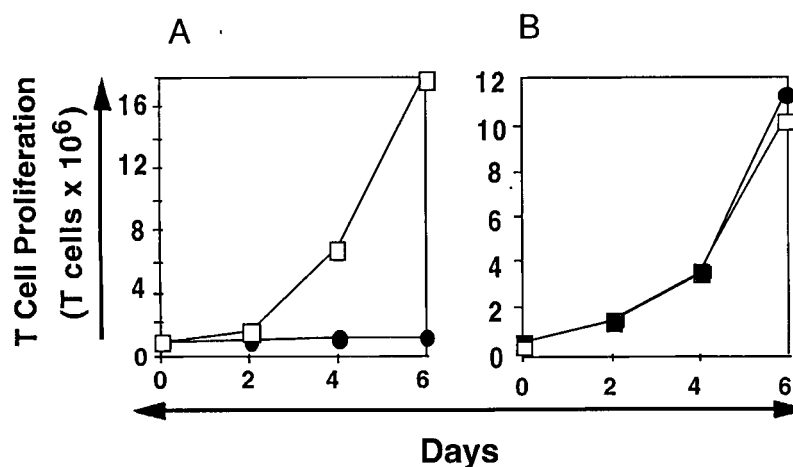


FIG. 3. T cell activation and growth of CC49- ζ T cells (A and B) Culture conditions impact T cell responsiveness. Long-term culture of T cells activated by anti-CD3 treatment induces T cell unresponsiveness to IL-2 on signaling through the CC49- ζ receptor from TAG-72-expressing targets (A, circles). However, short-term culture of T cells activated by anti-CD3 and anti-CD28 costimulation maintains T cell responsiveness to IL-2 following CC49- ζ receptor stimulation from TAG-72-expressing targets (B, circles). Nontransduced T cells proliferate in response to IL-2 irrespective of growth conditions, as they cannot respond to TAG-72-expressing targets (A and B, squares).

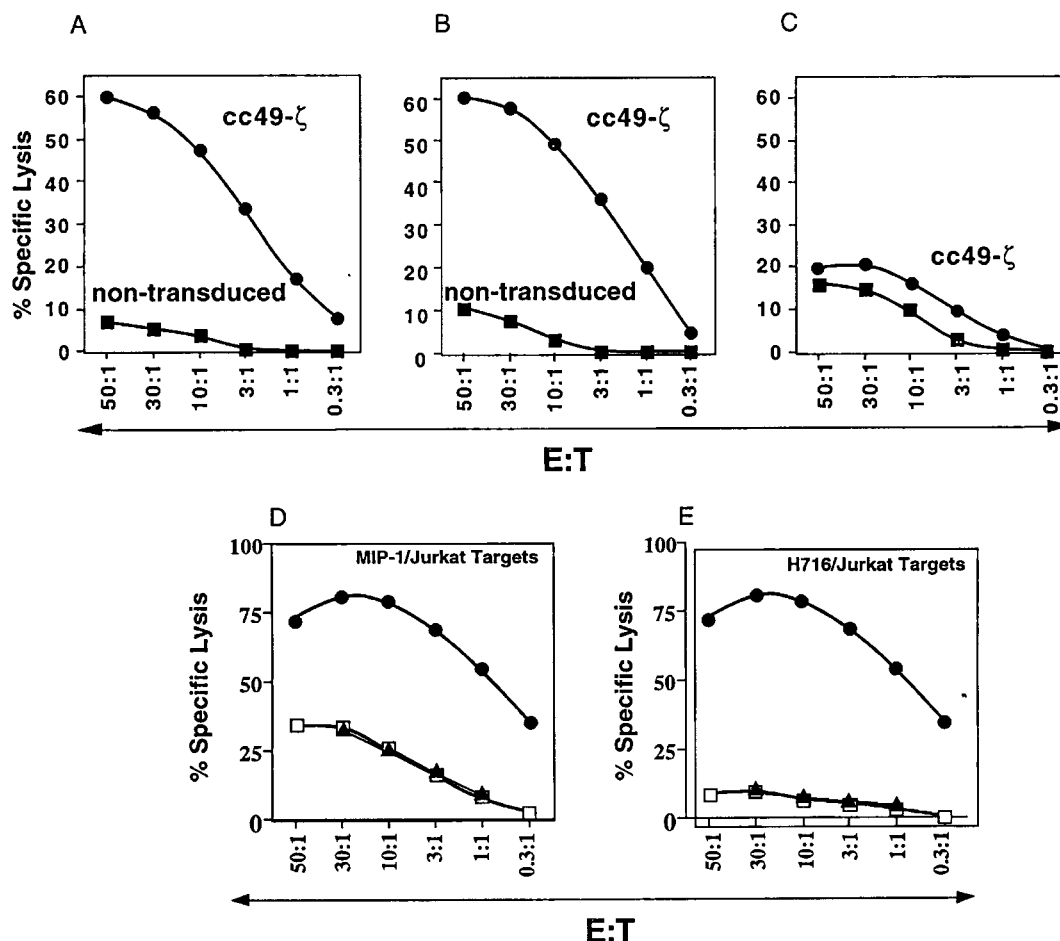


FIG. 4. CC49- ζ T cell antigen-specific lysis of TAG-72-positive and -negative tumor cells. The specificity of CC49- ζ T cells (A–C, circles) was tested with numerous TAG-72-positive targets including the colon carcinoma-derived LS174T cell line (A) and the KLE-B cell line (B). The TAG-72-negative targets included the colon carcinoma-derived NCI H716 (C). Target cell lysis with nontransduced T cells is indicated (A–C, squares). CC49- ζ T cells did not induce bystander killing of TAG-72-negative cells such as MIP-1 and H716. CC49- ζ T cells lysed radiolabeled TAG-72-positive Jurkat cells (D and E, circles) but not radiolabeled TAG-72-negative MIP-1 (D, squares) and H716 (E, squares). Bystander lysis was not observed with radiolabeled TAG-72-negative MIP-1 (D, triangles) and H716 (E, triangles) were mixed with G-72-positive Jurkat cells.

In addition, we investigated the ability of sTAG-72 to induce killing of TAG-72-negative cells, possibly by adhering to those cells. As shown in Fig. 7B, killing of TAG-72-negative cells was negligible in the presence of sTAG-72 (1200 U/ml).

T cell survival in the presence of FasL-expressing tumor targets

The utility of T cell immunotherapy has been questioned with the report that tumor cells can express Fas ligand (FasL) and escape immune surveillance by Fas-positive cytolytic T cells (Shiraki *et al.*, 1997). CC49- ζ cells expressed Fas at high levels, as shown in Fig. 8A, and several of the tumor targets we tested, including KLE-B cells (Fig. 8B) and LS174T cells, were positive for FasL expression as assessed by FACS analysis. However, these targets were efficiently lysed by Fas-positive CC49- ζ T cells (Fig. 4A and B).

In addition to killing FasL-expressing targets, the CC49- ζ T cells survived without IL-2 in the presence of an excess of FasL-

positive tumor cells, in both short-term and 6-day cultures. Moreover, in the 6-day assay, CC49- ζ -positive T cell survival was greater in cultures with tumor cells than without (Fig. 8C). Therefore, tumor cell expression of FasL is not a limitation for the CC49- ζ positive T cells. However, this is in contrast to previous reports that the LS174T cell line mediated lysis of tumor-specific T cell clones (Shiraki *et al.*, 1997).

CC49- ζ T cells prevent the growth of TAG-72 tumors in mice

CC49- ζ T cells were immunoprotective in two different murine tumor models. Subcutaneous injection of 10^6 TAG-72-positive LS174T cells into SCID-NOD mice produced local large tumors in 2 to 4 weeks (Fig. 9A) that were unaffected by treatment with either nontransduced T cells (0 of 4 disease free) (Fig. 9A and B) or T cells expressing an irrelevant chimeric immune receptor, 447D- ζ (0 of 4 disease free) (Fig. 9B). However, coinjection of human CC49- ζ T cells prevented LS174T

TABLE 1. PERCENTAGE OF TARGET CELL LYSIS, AT AN EFFECTOR-TO-TARGET RATIO OF 30 TO 1, OBTAINED FROM A SERIES OF ^{51}Cr RELEASE ASSAYS PERFORMED WITH A VARIETY OF TAG-72-POSITIVE AND -NEGATIVE TUMOR-DERIVED CELL LINES^a

Cell line	Origin	Relative TAG-72 expression	Target cell lysis (%)
Jurkat	T lymphocyte	++++	52
KLE-B	Endometrial adenocarcinoma	+/++++	42
LS174T	Colon adenocarcinoma	+/++	25
NCI H508	Cecum adenocarcinoma	+/++	22
LS180	Colon adenocarcinoma	+	15
MIP-1	Colon carcinoma	—	4
Snu-1	Gastric adenocarcinoma	—	2
BT20	Breast carcinoma	—	1
A549	Lung carcinoma	—	0
NCI H716	Cecum adenocarcinoma	—	0

^aThe relative level of TAG-72 surface expression is also indicated.

tumor establishment in 75% (3 of 4 disease free) ($r > 0.04$) to 100% (4 of 4 disease free) of the mice ($r > 0.01$) in two separate experiments.

In a second murine xenograft tumor model, SCID-NOD mice injected intraperitoneally with 10^6 TAG-72- and FasL-positive KLE-B cells developed tumors disseminated throughout the peritoneal cavity and associated with the site of injection, the intestines, spleen, kidney, and liver. Coinjection of human CC49- ζ cells into the peritoneal cavity protected 100% (4 of 4) of the treated mice while all of the mice receiving either no treatment or nontransduced T cells developed tumors (0 of 4 disease free). These experiments demonstrate that CC49- ζ T cells are immunoprotective in mice that develop either localized or disseminated tumors derived from two distinct human tumor types.

DISCUSSION

Mounting a curative anti-tumor immune response in colon cancer patients will likely require multiple doses of high-affinity tumor antigen-specific T cells. This can be readily achieved through the *ex vivo* genetic modification of a patient's T cells to express the humanized CC49- ζ . The humanized receptor reduces the likelihood of a HAMA response and foregoing the use of a selectable marker in this strategy not only avoids the negative impact of prolonged T cell culture on the biology of T cells observed in previous clinical trials (Yannelli *et al.*, 1996), but also precludes the potential patient immune response against the foreign selectable marker protein (Riddell *et al.*, 1996).

We demonstrate here that CC49- ζ T cells killed TAG-72 antigen-positive target cells derived from colon, gastric, and endometrial carcinomas, as well as primary colon carcinoma patient explants. In response to TAG-72-positive targets, CC49- ζ T cells secreted GM-CSF, TNF- α , and IFN- γ . The lytic activity of CC49- ζ T cells was antigen specific and bystander

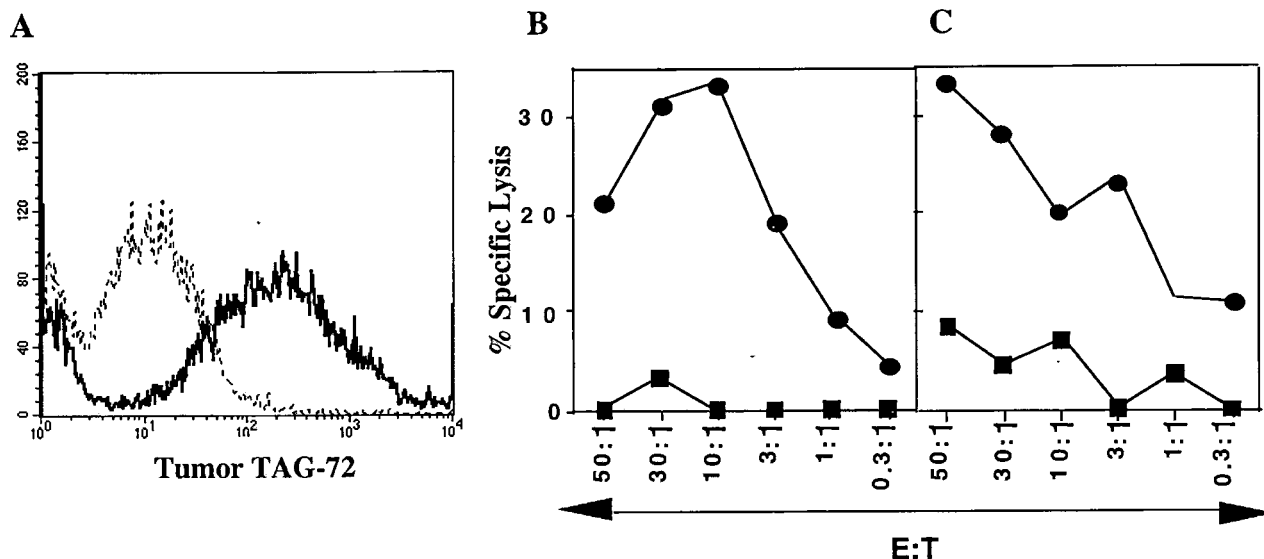


FIG. 5. CC49- ζ T cells kill primary human colon carcinoma cells. (A) Primary human hepatometastatic colon carcinoma cells were stained for expression of TAG-72 with the CC49 MAb (solid line) or an isotype-matched control antibody (dashed line). (B and C) Lysis of primary human hepatometastatic colon carcinoma cells (from Fig. 4A) by CC49- ζ T cells (circles) or non-transduced T cells (squares) from two donors (B and C).

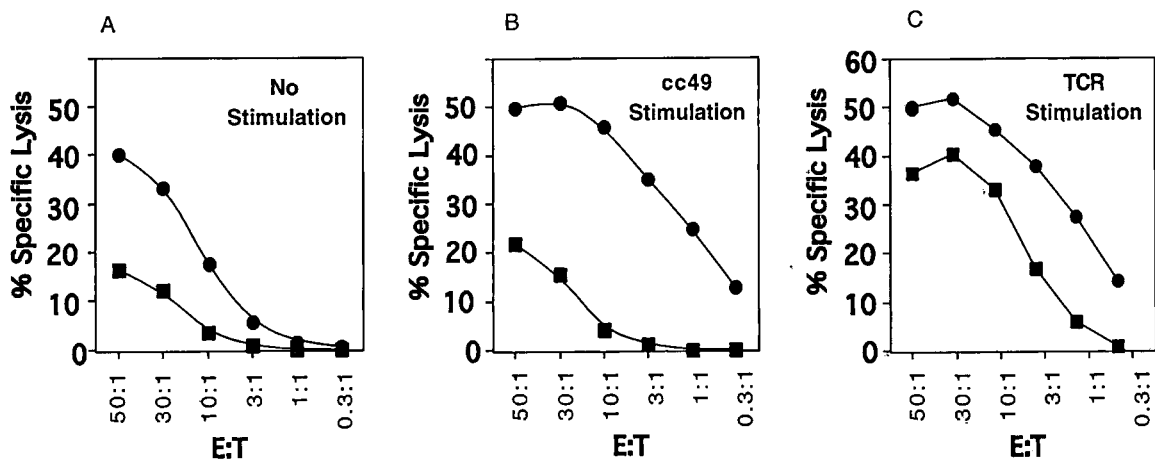


FIG. 6. CC49- ζ receptor stimulation. CC49- ζ T cells (circles) and nontransduced T cells (squares) were cultured in medium without receptor stimuli (A) or alternatively received either CC49- ζ receptor (B) or T cell receptor stimuli (C) for 2 days. The T cells were then reisolated and the lytic activity quantified in a 4-hr ^{51}Cr release assay with TAG-72-positive targets.

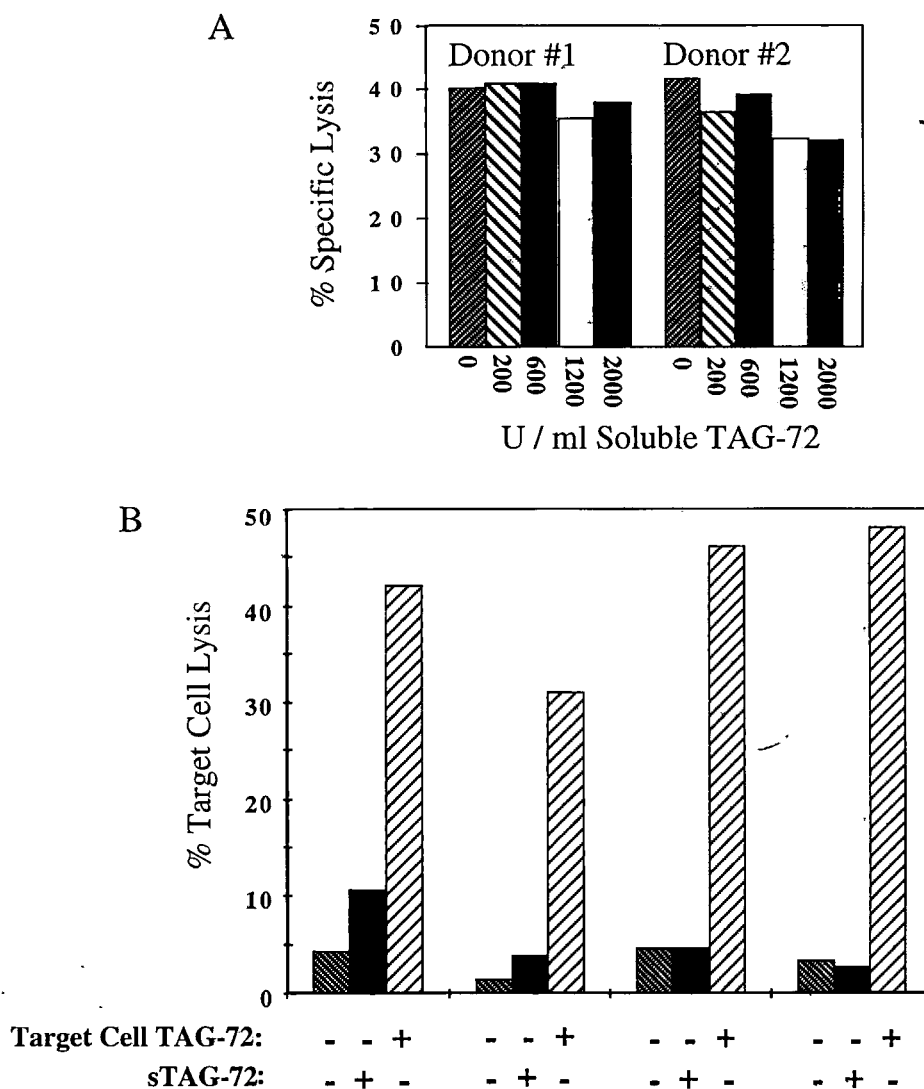


FIG. 7. CC49- ζ T cell lytic activity in the presence of soluble TAG-72. High concentrations of soluble sTAG-72 (sTAG-72) interfered minimally with lysis of TAG-72-positive Jurkat cells by CC49- ζ T cells from two donors (A). The presence of sTAG-72 (1200 U/ml) in the medium did not induce CC49- ζ T cell-mediated lysis of TAG-72-negative cells (B). "Target cell TAG-72" indicates the expression or absence of TAG-72 on the labeled target cells.

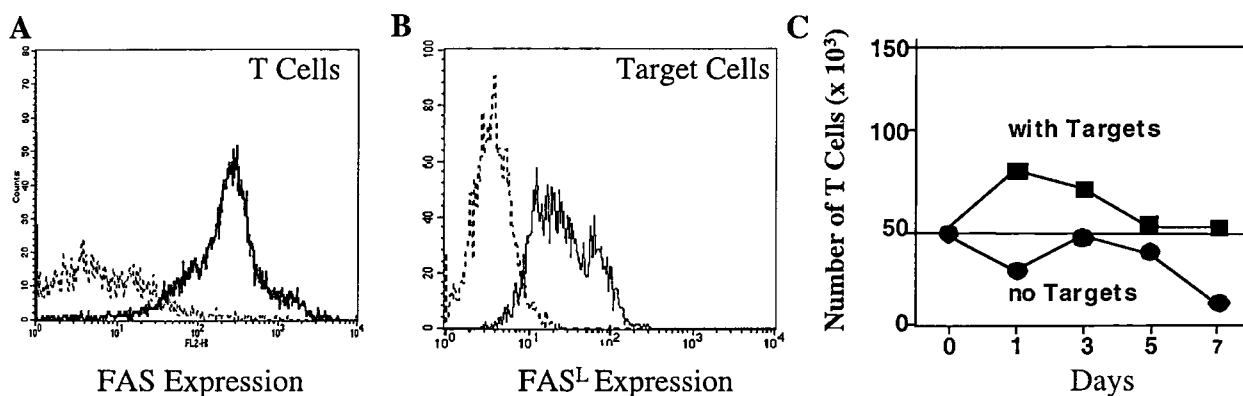


FIG. 8. CC49- ζ T cells are not killed by FasL-positive LS174T tumor cells. (A) CC49- ζ T cells express high levels of Fas. (B) LS174T colon carcinoma cell expression of FasL. (C) CC49- ζ T cells were cultured without IL-2 in the presence (squares) or absence (circles) of LS174T cells and the T cells counted every 2 days.

lysis of TAG-72-negative targets was not observed. Importantly, the CC49- ζ T cells continued to kill TAG-72-expressing tumor cells in the presence of sTAG-72. In addition, the sTAG-72 did not induce CC49- ζ T cell-mediated killing of TAG-72-negative cells, thus ruling out a mechanism whereby sTAG-72-coated cells would form novel targets for CC49- ζ T cells. This potent lytic activity was observed in two different *in vivo* murine tumor models involving either localized subcutaneous tumors arising from human colon carcinoma cells or disseminated intraperitoneal tumors arising from endometrial carcinoma-derived cells. Both tumor models led to extremely aggressive tumors that reached a large size within 1–2 weeks, thus also precluding schemes to prevent established tumors.

The Fas-FasL pathway is a mechanism of normal immune regulation employed both at the level of T cell selection and immune response homeostasis. However, tumor expression of FasL, in particular expression of FasL by colon hepatometastatic adenocarcinomas such as LS174, has been shown to mediate the lysis of T cells (Shiraki *et al.*, 1997). This

mechanism of evading the immune response presents a significant challenge to T cell-adoptive immunotherapy. This is not an obstacle for CC49- ζ T cells as tumor cell expression of FasL did not diminish CC49- ζ T cell survival or activity. T cells from disparate sources have different sensitivities to Fas-mediated lysis (Shiraki *et al.*, 1997; Walker *et al.*, 1997). This phenomenon can be traced in many cases to the level of Bcl-x expression in the cell. The insensitivity of CC49- ζ T cells to Fas-mediated lysis may reflect elevated cell Bcl-x expression.

Annually in the United States gastrointestinal-derived cancers are diagnosed in more than 160,000 people and are responsible for more than 55,000 deaths (NCI, 1997). In light of the anti-tumor activity and specificity of the CC49- ζ T cells, as well as the absence of efficacious conventional therapies for colon cancer, these preclinical studies have led us to initiate a phase I clinical trial for patients with advanced colon cancer. The clinical experience with the murine CC49 antibody in radiotherapy and radioimaging clinical trials in patients with colon, gastric, breast, and prostate cancer has demonstrated an-

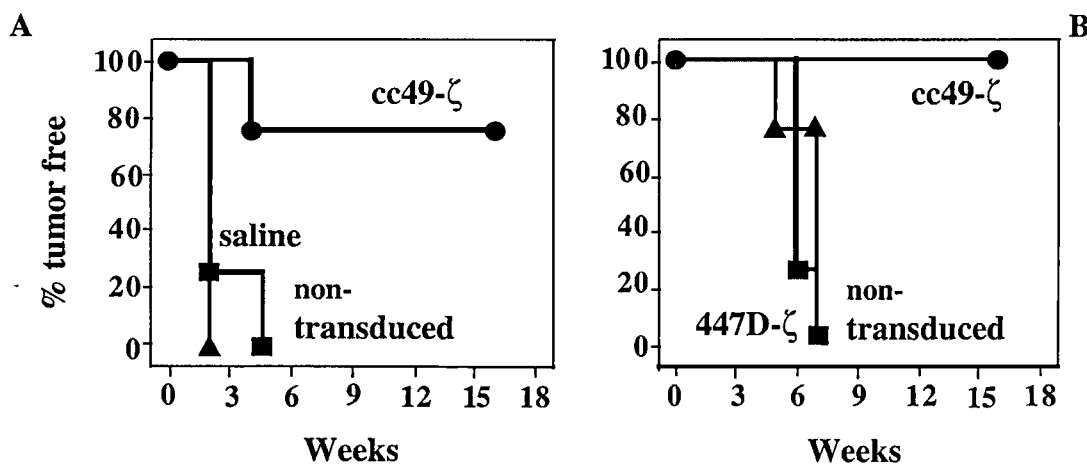


FIG. 9. CC49- ζ T cells kill tumor cells *in vivo*. (A) SCID-NOD mice were injected subcutaneously with TAG-72-positive LS174T tumor cells and coinjected with either CC49- ζ T cells (circles), nontransduced T cells (squares), or saline (triangle) and the development of tumors followed over time. (B) SCID-NOD mice were injected subcutaneously with TAG-72-positive LS174T tumor cells and coinjected with either CC49- ζ T cells (circles), nontransduced T cells (squares), or T cells expressing an irrelevant CIR (anti-HIV_{env} gp120, 447D- ζ) (triangles) and the development of tumors followed over time.

tibody localization to primary and secondary sites of disease, with little nonspecific secondary organ toxicity (Greiner *et al.*, 1992; Meredith *et al.*, 1994; Murray *et al.*, 1994). Few clinical objective responses were observed, largely owing to the induction of a human anti-mouse antibody (HAMA) response resulting in a rapid clearance of the murine CC49 antibody on subsequent administrations. CC49- ζ is derived from a humanized CC49 antibody, and therefore is unlikely to induce an HAMA response even with repeat administrations, with no loss of target specificity of the original antibody. The combination of the lytic and cytokine activities of T cells and the specificity conferred by the humanized CC49 antibody creates an immunotherapy that addresses many of the deficiencies reported previously for T cell immunotherapy of cancer.

ACKNOWLEDGMENTS

We thank Dr. Robert Warren (University of California, San Francisco) for the human colon carcinoma patient samples, Diane J. Poole and Melinda Van Roey for technical assistance, and Drs. Gary Greenburg and Margo Roberts (Cell Genesys) for thoughtful comments.

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Received for publication June 11, 1998; accepted after revision November 2, 1998.