

Adoptive Transfer of Chimeric Fc_εRI Gene-Modified Human T Cells for Cancer Immunotherapy

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ABSTRACT

Immunotherapeutic approaches involving genetic modification of T cells show promise in generating highly specific tumor-reactive effector cells for cancer treatment. Given the high affinity of Fc_εRI (the subtype I Fc receptor for IgE) for IgE monoclonal antibody (mAb), modification of T cells with chimeric Fc_εRI in combination with tumor-specific IgE mAbs is potentially a powerful and effective strategy to specifically target T cells to tumor cells. In this study, we retrovirally transduce human primary T cells with a cDNA encoding the extracellular domain of Fc_εRI linked to the hinge and transmembrane domains of Fc_γRII and the cytoplasmic domains of CD28 and T cell receptor ζ chain (Fc_εRI-CD28- ζ). We demonstrate that human T cells expressing Fc_εRI-CD28- ζ , in the presence of tumor-specific IgE mAb recognizing mouse CD8 antigen (Ly-2.1⁺), can specifically secrete cytokine, proliferate, and mediate cytotoxic function after antigen ligation. Furthermore, adoptive transfer of Fc_εRI-CD28- ζ cells incubated with anti-Ly-2.1 IgE mAb significantly enhances the survival of irradiated nonobese diabetic-severe combined immunodeficiency mice bearing Ly-2.1⁺ tumor compared with control mice. Thus, this set of experiments demonstrates that Fc_ε gene-engineered human T cells mediate effector function *in vitro* and *in vivo* in an IgE-dependent manner and thus a novel and valid approach for cancer therapy can now be further developed.

OVERVIEW SUMMARY

We have retrovirally transduced human primary T cells with a chimeric receptor comprising the extracellular domain of Fc_εRI (the high-affinity Fc receptor for IgE) linked to Fc_γRII (the low-affinity Fc receptor for IgG) hinge and transmembrane regions and the cytoplasmic domains of CD28 and T cell receptor ζ chain (Fc_εRI-CD28- ζ). Human T cells expressing Fc_εRI-CD28- ζ , in the presence of tumor-specific IgE mAb recognizing mouse CD8 antigen (Ly-2.1⁺), could specifically secrete cytokine, proliferate, and mediate cytotoxic function after antigen ligation. Transfer of Fc_εRI-CD28- ζ T cells incubated with anti-Ly-2.1 IgE mAb significantly enhanced the survival of irradiated nonobese diabetic-severe combined immunodeficiency mice bearing established Ly-2.1⁺ tumors. Thus, the combination of chimeric Fc_ε receptors with tumor-specific IgE is a novel approach for enhancing specific targeting of T cells for cancer therapy.

INTRODUCTION

IMMUNOTHERAPY OF CANCER represents a promising approach for harnessing the natural power and specificity of the immune system. Current immunotherapeutic strategies involving adoptive transfer of tumor-specific T cells have led to some encouraging responses, particularly in melanoma patients (Dudley *et al.*, 2002; Yee *et al.*, 2002), and have exceeded the effectiveness of other approaches involving vaccination using tumor peptide-pulsed dendritic cells or tumor extracts (Rosenberg *et al.*, 2004; Kershaw *et al.*, 2005). Nevertheless, the utility of these types of therapy has not been demonstrated against more refractory cancers including breast, colon, and ovarian carcinoma. T cells reactive against these tumors are difficult to isolate and downregulation or loss of MHC-peptide complexes on many tumors has precluded success of these various treatment regimens (Yee *et al.*, 2002; Bubenik, 2004; Watson *et al.*, 2006).

A strategy to potentially overcome these limitations involves the genetic modification of effector T lymphocytes with chi-

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meric receptors that can specifically recognize tumor-associated antigen (TAA) in a non-MHC dependent manner. A novel chimeric receptor design comprising an extracellular single-chain variable fragment (scFv)-binding domain fused to the CD28 costimulatory and T cell receptor ζ chain (TCR- ζ) signaling chains linked in tandem (scFv-CD28- ζ) has shown promise (Finney *et al.*, 1998; Hombach *et al.*, 2001; Haynes *et al.*, 2002; Teng *et al.*, 2004). Nevertheless, a potential limitation of this approach includes the need to construct a new scFv for every tumor antigen targeted, which may be ineffective given that tumors are often heterogeneous in terms of antigen expression (Correale *et al.*, 2003; Zhang *et al.*, 2003; Luborsky *et al.*, 2005). In addition, the scFv may exhibit lower affinity for antigen than the natural Fab' from which it is derived (Ravetch and Kinet, 1991). Previous studies have also demonstrated that T cells can be specifically directed to target cells after modification with tumor-reactive TCR $\alpha\beta$ genes (Kessels *et al.*, 2001; Zhao, 2005). Nevertheless, several issues concerning application of this approach in the clinic involve mispairing of TCR $\alpha\beta$ in transduced T cells with endogenous TCR in T cells isolated from patients and the need to isolate TCRs that are specific for all MHC haplotypes.

An alternative approach involves genetic modification of effector T cells with Fc receptors capable of binding any antitumor monoclonal antibody (mAb) of the correct isotype. This potentially enables a number of tumor-specific IgEs recognizing multiple target antigens to be employed, which may overcome the problem of tumor cells escaping lysis as a result of tumor antigen heterogeneity, and enables greater flexibility of treatment. The high-affinity Fc receptor for IgE (Fc ϵ RI) is present on basophils, mast cells, and eosinophils (Metzger *et al.*, 1986; Kawakami and Galli, 2002) and appears to be the best candidate for therapeutic application given its high-affinity binding constant for IgE ($2.5 \times 10^{10} M^{-1}$) (Kershaw *et al.*, 1996b). In addition, the use of tumor-specific IgE mAb in this setting may attract various Fc ϵ effector cells to the tumor site, which in combination with Fc ϵ RI-modified T cells may induce a potent antitumor effect. Furthermore, the fact that serum IgE levels are low (unlike for IgG) (Gould *et al.*, 2003) makes this a promising form of immunotherapy and also supports this approach over those based on the use of Fc γ receptors (Kershaw *et al.*, 1996b; Clemenceau *et al.*, 2006).

Fc ϵ RI comprises a ligand-binding α subunit, a β subunit, and a pair of intracellular γ chains (Metzger *et al.*, 1986; Ravetch and Kinet, 1991; Hulett and Hogarth, 1994). IgE binds in the ectodomain of the α chain and a chimeric receptor consisting of only the α subunit and the γ chain can be expressed and functions in the absence of the β chain (Hakimi *et al.*, 1990). To extend this idea, chimeric Fc ϵ RI containing an intracellular TCR- ζ signaling domain has previously been engineered and found to endow a mouse CTL line, CTLL-R8, with the ability to lyse tumor cells in an IgE-dependent manner (Kershaw *et al.*, 1996a,b). Nevertheless, for effective translation into the clinic, this approach using both primary and costimulatory signaling motifs must be tested in primary human T lymphocytes.

In this study, we have generated a novel chimeric Fc ϵ RI containing both CD28 and TCR- ζ signaling domains. Primary human T cells engineered with this chimera were found to effectively lyse tumor cells *in vitro* and significantly suppress tumor growth *in vivo* in an IgE-specific manner. Thus, this series of

experiments has clearly demonstrated that chimeric Fc ϵ receptors and tumor-specific IgE may be effective tools for specific targeting of T cells for cancer therapy.

MATERIALS AND METHODS

Cell culture and antibodies

The mouse thymoma cell line E3, which expresses the Ly-2.1 antigen, was maintained in Dulbecco's modified Eagle's medium (DMEM) with the following additives: 10% (v/v) fetal calf serum (FCS), 2 mM glutamine, penicillin (100 U/ml), and streptomycin (100 μ g/ml) (Invitrogen GIBCO, Grand Island, NY). Lewis lung carcinoma (3LL) cells were grown in RPMI 1640 medium supplemented with 10% (v/v) FCS, 2 mM glutamine, penicillin (100 U/ml), and streptomycin (10 μ g/ml) (Invitrogen GIBCO). The PG13 retroviral producer cell line (American Type Culture Collection [ATCC], Manassas, VA), expressing the gibbon ape leukemia viral envelope, was cultured in complete medium consisting of RPMI 1640, 10% heat-inactivated fetal calf serum, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, penicillin (100 U/ml), streptomycin (100 μ g/ml), $5 \times 10^{-5} M$ 2-mercaptoethanol, and 2 mM glutamine (all from Invitrogen, Carlsbad, CA). The following mAbs were used in this study: 3B4 (mouse anti-human Fc ϵ RI α , IgG1) (kindly provided by P.M. Hogarth, Helen Macpherson Smith Trust Inflammatory Diseases Laboratory, Burnet Institute at Austin, Heidelberg, Australia); 1D10 (mouse anti-human granzyme B, IgG1); YTS169.4 (chimeric human/rat anti-Ly-2, IgE mAb) (Isaacs *et al.*, 1992), and human anti-250-30.6 (colon carcinoma antigen) IgE mAb.

Construction of chimeric Fc ϵ RI receptor

Human Fc ϵ RI α , the hinge and transmembrane regions of the low-affinity Fc receptor for IgG (Fc γ RII α) (derived from the pCMV5 expression vector encoding Fc ϵ RI- ζ) (Kershaw *et al.*, 1996a), and the cytoplasmic domain of CD28 (derived from pRSV encoding the anti-erbB2-CD28 receptor) (Haynes *et al.*, 2002) were amplified by polymerase chain reaction (PCR), using the following primers:

Sense, Fc ϵ RI:	5'-GCTCTAGAGTCCCTCAGAACCTAAGGT-3'
Antisense, Fc γ RII α :	5'-CTTACTCCTGCAGTAGATCAAGGCCACTA-3'
Sense, CD28:	5'-ATCTACTGCAGGAGTAAGAGGAGCAGGC-3'
Antisense, CD28:	5'-GACTCGAGGGAGCGATAGGCTGCGAAGTC-3'

These fragments (5' and 3' sequences containing, respectively, *Xba*I and *Xho*I restriction sites are underlined) were fused by PCR splice overlap extension. The resulting Fc ϵ RI-CD28 cDNA was subcloned into *Xba*I- and *Xho*I-digested pRSV (a kind gift from Z. Eshhar, Weizmann Institute of Science, Rehovot, Israel) containing the L κ leader (i.e., the secretory leader sequence of the immunoglobulin κ chain). The entire fragment was then excised from pRSV and subcloned into the *Sna*BI and *Xho*I sites of the retroviral vector pSamEN (Royal *et al.*, 2002).

Finally, the cytoplasmic human TCR- ζ signaling domain was subcloned into the *Xho*I site to generate the entire Fc ϵ RI-CD28- ζ receptor.

Generation of PG13 retrovirus-producing packaging cells

The generation of PG13 retrovirus-producing cell lines harboring the pSamEN vector encoding the Fc ϵ RI-CD28- ζ receptor or pSamEN alone was performed as previously described (Teng *et al.*, 2004). Briefly, plasmid DNA was transfected into the amphotropic cell line PA317 and the ecotropic cell line GP+E86, using the ping-pong technique as previously described (Hoatlin *et al.*, 1995). PG13 packaging cells were then infected with viral supernatant derived from transfected PA317 packaging cells in the presence of Polybrene (8 μ g/ml; Sigma, St. Louis, MO). This bulk-transduced PG13 line was single-cell cloned to generate a high-expressing PG13 line (designated CL9). Primary human T lymphocytes were then transduced with supernatant derived from control or CL9 PG13 retrovirus producer cells.

Transduction of primary human T lymphocytes

Transduction of human T lymphocytes was performed as described previously (Teng *et al.*, 2004). Briefly, peripheral blood mononuclear cells (PBMCs) from healthy donors (obtained from the Australian Red Cross Blood Service) were isolated by Ficoll-Isopaque density centrifugation (GE Healthcare Bio-Sciences, Uppsala, Sweden). Before transduction, T cells were generated from human PBMCs by activation with anti-CD3 (ORTHOCONE OKT3, 30 ng/ml; Ortho Biotech, Bridgewater, NJ) and recombinant human interleukin-2 (IL-2 [Proleukin], 600 IU/ml; National Cancer Institute, Frederick, MD) for 72 hr. Stimulated T cells were collected, medium was removed, and cells were resuspended at 1×10^6 cells/ml in supernatant from PG13 retrovirus producer cell lines (CL9 or control PG13 line) containing IL-2 (600 IU/ml) and Polybrene (8 μ g/ml; Sigma). T cells were aliquoted into 24-well tissue culture plates (2 ml/well) and centrifuged ($1000 \times g$) at room temperature for 1 hr. After centrifugation, T cells were cultured overnight at 37°C in a 95% air–5% CO₂ atmosphere. The next day, 1.5 ml of medium was removed and the transduction was repeated. After a further 24 hr, cells were passaged 1:2 with fresh medium containing IL-2. Seventy-two hours later, cells were harvested and resuspended at 1×10^6 cells/ml in fresh medium containing IL-2 and G418 (600 μ g/ml). After 5 days of selection in G418, cells were harvested and resuspended in fresh medium containing IL-2, but no G418. IL-2 (600 IU/ml) was added every 2–3 days of subsequent culture.

Flow cytometry

Receptor expression on human T lymphocytes after transduction was detected by staining with 3B4 (mouse anti-human Fc ϵ RI α antibody), or isotype-matched control 1D10 mAb followed by staining with a phycoerythrin (PE)-conjugated anti-mouse immunoglobulin mAb (Chemicon, Melbourne, Australia). Cell surface phenotyping of transduced cells was determined by direct staining with PE-conjugated anti-human CD8, PE-Cy5-conjugated anti-human CD4 (Dako, Glostrup,

Denmark), or fluorescein isothiocyanate (FITC)-conjugated anti-human CD56 (BD Biosciences Pharmingen, San Diego, CA).

Cytotoxicity assay

The cytolytic capacity of transduced T cells was assessed in a standard 4-hr ⁵¹Cr release assay as previously described (Haynes *et al.*, 2002). Briefly, gene-engineered T cells (1×10^7 /ml) were incubated for 60 min at 4°C in a 1:1000 dilution of anti-mouse Ly-2.1 chimeric human/rat IgE or control human anti-250-30.6 IgE mAb, before being used immediately in the assay.

Cytokine production assays

Cytokine secretion by transduced T cells after receptor ligation was detected by enzyme-linked immunosorbent assay (ELISA) (BD Biosciences Pharmingen). Briefly, gene-engineered human T lymphocytes (10^6) (T-CL9 or mock-transduced T cells) were incubated with anti-mouse Ly-2.1 chimeric human/rat IgE antibody or control human anti-30.6 IgE mAb for 1 hr at 4°C and then cocultured with E3 tumor cells (5×10^5) in 12-well plates for 20 hr. Transduced T cells were also stimulated with plate-bound 3B4 (anti-Fc ϵ RI α , 1 μ g/ml) or isotype control 1D10 (anti-granzyme B, 1 μ g/ml) mAb. No exogenous IL-2 was added. Supernatants were harvested and interferon (IFN)- γ and granulocyte-macrophage colony-stimulating factor (GM-CSF) secretion by transduced T cells was determined by ELISA. Cytokine secretion after stimulation of endogenous CD3/CD28 receptors were assessed for each effector T cell population, using plate-bound anti-CD3 (ORTHOCONE OKT3, 1 μ g/ml) and anti-CD28 (1 μ g/ml; BD Biosciences Pharmingen) mAbs.

Proliferation assay

To assess the proliferative capacity of gene-engineered T cells, an [³H]thymidine incorporation assay was employed. Transduced human T lymphocytes (10^5 cells per well) were cultured in triplicate in 96-well flat-bottom plates with medium alone or with plate-bound anti-Fc ϵ RI mAb (3B4, 1 μ g/ml) or isotype control 1D10 mAb (anti-granzyme B, 1 μ g/ml) for 3 days. In addition, T cells were stimulated with plate-bound anti-CD3 and anti-CD28 mAbs (1 μ g/ml). No exogenous IL-2 was added. The cultures were pulsed with [³H]thymidine (0.5 μ Ci/well [0.0185 MBq]; GE Healthcare UK, Little Chalfont, UK) for the final 16 hr of the culture period and the incorporation of radioactivity was measured in a TRI-CARB 2100TR liquid scintillation counter (PerkinElmer Life and Analytical Sciences, Boston, MA).

Adoptive transfer model

Inbred nonobese diabetic-severe combined immunodeficiency (NOD-SCID) mice were purchased from the Animal Resource Centre (Perth, Australia). The antitumor efficacy of Fc ϵ RI-modified human T cells was assessed against the Ly-2.1⁺ E3 thymoma cell line. Tumor cells (1×10^4) were injected intraperitoneally into groups of irradiated (2.5 Gy) NOD-SCID mice ($n = 5$ –10). Primary human T cells (1×10^7) (T-CL9 or mock-transduced T cells) were incubated with anti-Ly-2.1⁺ IgE

mAb (1:1000 dilution) for 1 hr at 4°C before intraperitoneal injection into mice at the following times after tumor inoculation: 6 hr (day 0), 24 hr (day 1), 48 hr (day 2), and 96 hr (day 4). In some experiments T-CL9 cells incubated with control human anti-250-30.6 IgE mAb were injected or mice were injected with a 1:1000 dilution of anti-Ly-2.1 IgE mAb alone on days 0, 1, 2, and 4. In the delayed adoptive T cell transfer experiment, groups of irradiated NOD-SCID mice ($n = 5-8$) were injected intraperitoneally with E3 thymoma tumor cells (1×10^4). Primary human T cells (T-CL9, 1×10^7) were incubated with either anti-Ly-2.1⁺ IgE mAb or control human anti-250-30.6 IgE mAb for 1 hr before intraperitoneal injection into mice at the following times after tumor inoculation: 3 days after tumor inoculation (day 3), day 4, day 5, and day 7. After adoptive transfer of T cells, mice were monitored daily for tumor growth. Mice that were morbid were killed and the day of death was recorded. Data were recorded as percentage survival after tumor inoculation.

RESULTS

High-level expression of chimeric Fc ϵ RI receptor in primary human T lymphocytes

Chimeric Fc ϵ RI was composed of the Fc ϵ RI ligand-binding subunit linked via the Fc γ RII hinge and transmembrane region

to the cytoplasmic domains of CD28 and TCR- ζ (Fig. 1A). The generation of a PG13 retrovirus-producing packaging cell line harboring the chimeric receptor was performed in a two-step process as described in Materials and Methods. Briefly, high-level expression of chimeric Fc ϵ RI was detected in transfected PA317 retrovirus-packaging cells after specific staining with anti-Fc ϵ RI (3B4) mAb, which recognizes an epitope located in domain I of Fc ϵ RI α . Supernatant produced from transfected PA317 cells was subsequently used for transduction of PG13 retrovirus-producing packaging cells. Bulk-transduced PG13 cells were single-cell cloned to generate a high-expressing clone (CL9), with expression of the chimera detected after staining with anti-Fc ϵ RI (3B4) mAb (data not shown). Primary human T lymphocytes preactivated with OKT3 and IL-2 were transduced with retroviral supernatant generated from stably transfected PG13 packaging cells (CL9) or from control PG13 packaging cells. Expression of chimeric Fc ϵ RI on transduced human T lymphocytes was detected by staining with 3B4 mAb (Fig. 1B) (30–40%, $n = 4$). No expression of chimeric Fc ϵ RI was detected on T cells transduced with packaging cells containing empty pSamEN retroviral vector (Fig. 1C). Consistent with previous results (Teng *et al.*, 2004), expression of chimeric Fc ϵ RI was detected in CD8⁺ T cells and CD4⁺ T cells (data not shown). After transduction/selection in G418, the culture consisted predominantly of T cells (CD3⁺CD8⁺, $56 \pm 15\%$, $n = 3$; CD3⁺CD4⁺, $37 \pm 12\%$, $n = 3$), with only a small proportion of CD56⁺CD3[−] cells ($3 \pm 1\%$, $n = 3$).

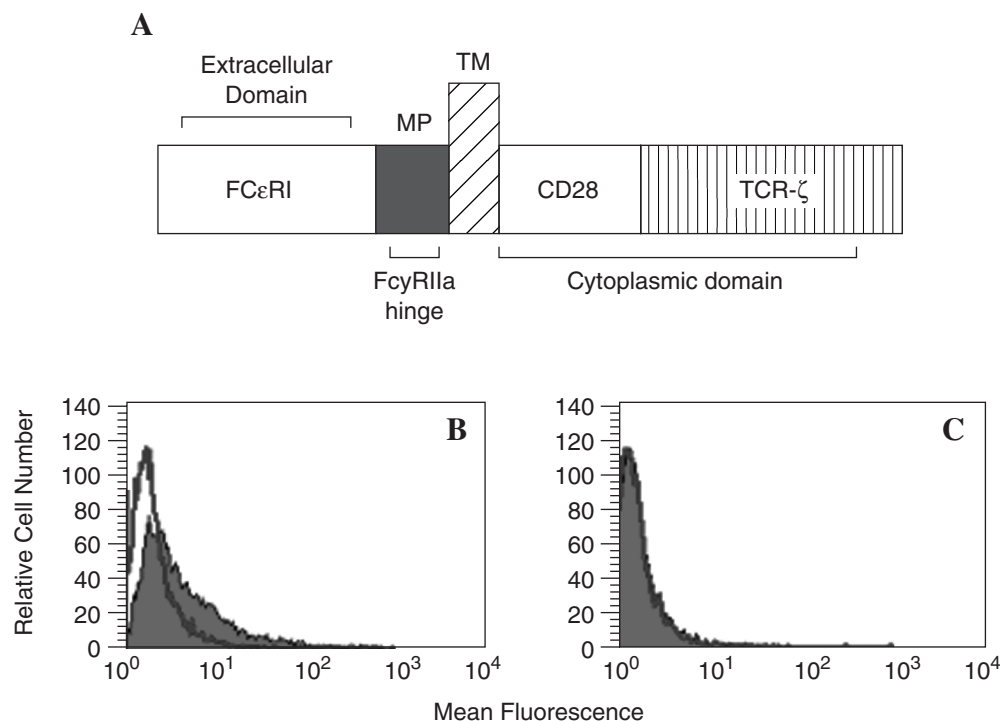


FIG. 1. Expression of chimeric Fc ϵ RI in primary human T lymphocytes. (A) Schematic representation of the Fc ϵ RI-CD28- ζ receptor. The construct is composed of Fc ϵ RI linked via a membrane-proximal (MP) hinge region of Fc γ RII α to the transmembrane (TM) and cytoplasmic regions of human CD28 and TCR- ζ as described in Materials and Methods. Expression of the pSamEN-Fc ϵ RI-CD28- ζ chimeric receptor (B) or pSamEN retroviral vector alone (C) was analyzed by flow cytometry. Cells were stained with anti-Fc ϵ RI mAb (3B4) (solid histogram) or an anti-IgG1 isotype control mAb followed by PE-labeled sheep anti-mouse mAb.

Gene-modified T cells: IgE-dependent lysis, cytokine production, and proliferation

The ability of gene-engineered human T cells expressing the Fc ϵ RI-CD28- ζ chimeric receptor (T-CL9 cells) to mediate IgE-specific effector function was assessed in a number of *in vitro* assays. A standard ^{51}Cr release assay was first used to test the capacity of Fc ϵ RI-modified T cells to mediate specific tumor lysis. T-CL9 cells incubated in the presence of anti-Ly-2.1 IgE mAb specifically lysed Ly-2.1 $^{+}$ E3 thymoma cells (Fig. 2A) but not Ly-2.1 $^{-}$ Lewis Lung carcinoma 3LL tumor cells (Fig. 2B). Lysis of E3 tumor cells was dependent on both expression of chimeric Fc ϵ RI and the presence of anti-Ly-2.1 IgE mAb because T-CL9

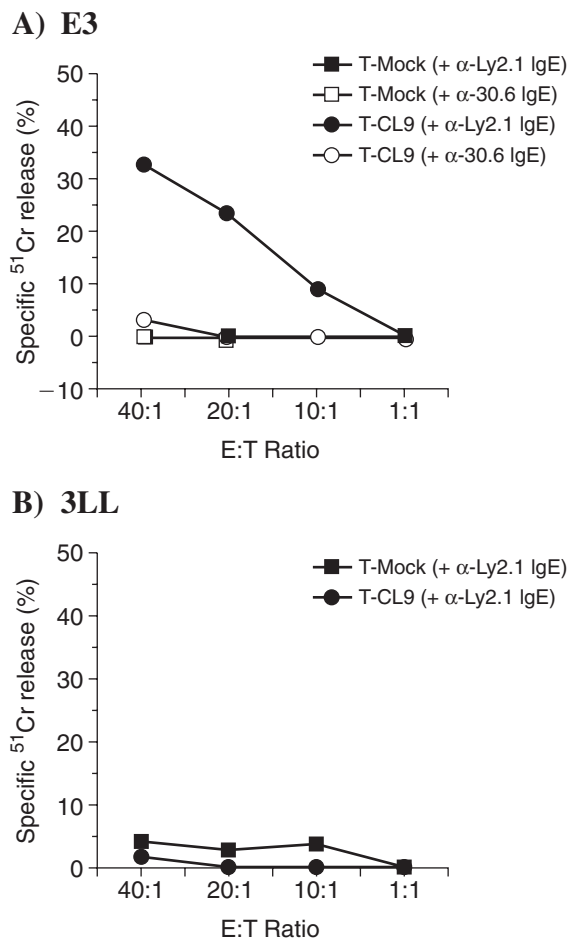


FIG. 2. IgE-specific lysis of E3 tumor cells by gene-engineered human T cells. The cytolytic function of transduced T lymphocytes was evaluated in a 4-hr ^{51}Cr release assay. T cells transduced with the Fc ϵ RI-CD28- ζ receptor (T-CL9 cells) and incubated with anti-Ly-2.1 IgE mAb (solid circles) but not with anti-30.6 control IgE mAb (open circles) specifically lysed Ly-2.1 $^{+}$ tumor E3 (A). Lysis was Ly-2.1 specific because T-CL9 cells incubated with anti-Ly2.1 IgE mAb could not lyse the Ly2.1 $^{-}$ tumor line 3LL (B). T cells transduced with pSamEN vector alone (T-Mock) and incubated with anti-Ly2.1 IgE mAb (solid squares) and/or anti-30.6 control IgE mAb (open squares) were unable to lyse either Ly-2.1 $^{+}$ E3 tumor cells or Ly-2.1 $^{-}$ 3LL cells (A and B). Results are expressed as means $\pm$ SE of triplicate samples and are representative of five experiments.

cells incubated with control IgE mAb recognizing colon carcinoma antigen 30.6 resulted in only background lysis of E3 tumor cells (Fig. 2A). In addition, mock-transduced T cells incubated with anti-Ly-2.1 IgE mAb did not lyse either Ly-2.1 $^{+}$ or Ly-2.1 $^{-}$ tumor cells (Fig. 2A and B). To determine the duration that IgE mAb remains bound to chimeric receptor-expressing T cells, we examined the lytic capacity of chimeric Fc ϵ RI-transduced human T cells at various time points *in vitro* after incubation with anti-Ly-2.1 IgE mAb. Interestingly, our results demonstrated that transduced T cells could still mediate killing of E3 target cells after 72 hr of incubation, indicating that the anti-Ly-2.1 IgE mAb remained bound to the chimeric receptor (data not shown). Thus, the kinetics of IgE antibody bound to Fc ϵ RI-transduced T cells would appear not to be a significantly limiting factor for this approach.

An important function of T cells is their ability to secrete immune-stimulatory cytokines. Hence, we tested whether T-CL9 cells could specifically secrete the cytokines IFN- γ and GM-CSF after Ly-2.1 antigen ligation or receptor cross-linking with plate-bound anti-Fc ϵ RI (3B4) mAb. T-CL9 cells cocultured with E3 tumor cells in the presence of anti-Ly-2.1 IgE mAb or stimulated with immobilized anti-Fc ϵ RI mAb specifically produced IFN- γ and GM-CSF (Fig. 3A and B). In contrast, T-CL9 cells cocultured with E3 target cells in the presence of control anti-30.6 IgE mAb or stimulated with immobilized control anti-granzyme B 1D10 mAb secreted negligible levels of these cytokines (Fig. 3A and B). Furthermore, only background levels of cytokine were produced by mock-transduced T cells cocultured with E3 tumor cells in the presence of either anti-Ly-2.1 or anti-30.6 IgE mAbs or after stimulation with plate-bound 3B4 or control mAb 1D10. The lower activity of mock-transduced T cells was not due to an inherent lack of activity because these cells responded to a similar extent as T-CL9 cells after incubation on immobilized anti-CD3 and anti-CD28 mAbs. The increased level of cytokine secreted by T-CL9 cells after stimulation with plate-bound anti-Fc ϵ RI (3B4) mAb or control anti-CD3/CD28 mAbs was most probably due to a stronger stimulus being provided to transduced T cells compared with antigen-positive tumor targets, as we and others have reported previously (Hombach *et al.*, 2001; Moeller *et al.*, 2005).

Last, we tested the ability of T-CL9 cells to specifically proliferate after receptor ligation, using an [^3H]thymidine incorporation assay. T-CL9 cells but not mock-transduced T cells proliferated after receptor cross-linking by plate-bound 3B4 mAb and was comparable to stimulation through endogenous CD3/CD28 receptors (Fig. 4). Proliferation of T-CL9 cells was dependent on expression and triggering of the chimeric Fc ϵ RI because no proliferation of mock-transduced T cells was observed on incubation with plate-bound anti-Fc ϵ RI (3B4) mAb. In addition, no proliferation of T-CL9 or mock-transduced T cells was observed on incubation with control 1D10 mAb. Overall, these experiments have demonstrated the capacity of primary human T cells expressing the Fc ϵ RI-CD28- ζ chimeric receptor to mediate cytotoxicity, cytokine release, and proliferation in a tumor-specific IgE-dependent manner.

Human T cells expressing chimeric Fc ϵ RI mediate IgE-dependent suppression of tumor growth

The ability of chimeric Fc ϵ RI gene-modified human T cells to specifically reject tumor was examined with Ly-2.1 $^{+}$ E3

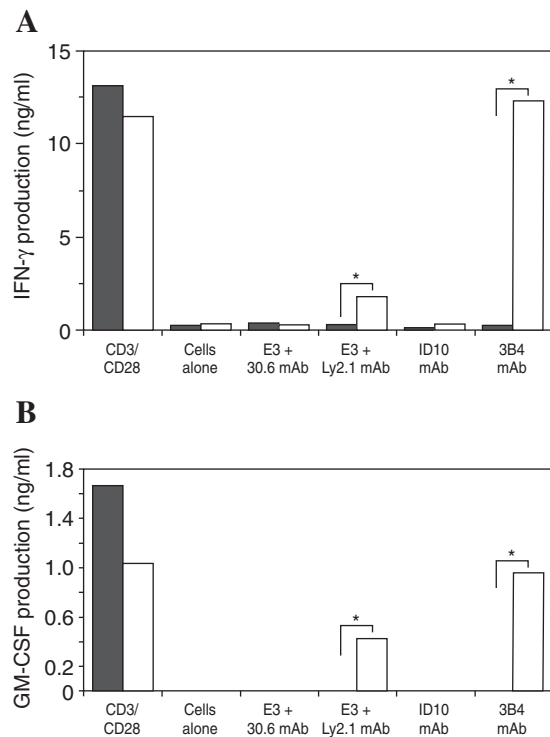


FIG. 3. Antigen-specific cytokine production by gene-engineered T cells expressing the Fc ϵ RI-CD28- ζ chimeric receptor. Human T cells transduced with pSamEN vector alone (solid columns) or pSamEN-Fc ϵ RI-CD28- ζ (T-CL9 cells; open columns) were incubated with either anti-Ly-2.1 IgE mAb or anti-30.6 control IgE mAb and cocultured with E3 tumor cells or stimulated with plate-bound anti-CD3 and anti-CD28 mAb or anti-Fc ϵ RI mAb 3B4 or isotype control IB10 (1 μ g/ml). Harvested supernatants were analyzed for IFN- γ (A) and GM-CSF (B) production by ELISA. The level of cytokine produced between Fc ϵ RI-modified T cells and mock-transduced T cells was significant (* p < 0.03, as statistically assessed by the Mann-Whitney test). Results are expressed as nanograms of cytokine secreted per milliliter $\pm$ SEM of duplicate samples and are representative of two experiments.

thymoma target cells in irradiated NOD-SCID mice. For this experiment, 1×10^4 E3 tumor cells were injected intraperitoneally into groups of 5 to 10 mice. This was followed by four intraperitoneal injections of T-CL9 or mock-transduced T cells (1×10^7 cells per injection) previously incubated with anti-Ly-2.1 IgE mAb on days 0, 1, 2, and 4. Tumor-bearing mice that received T-CL9 cells incubated with anti-Ly-2.1 IgE mAb demonstrated enhanced survival compared with mice that received no T cell treatment (Fig. 5A). The antitumor effect was dependent on expression of chimeric Fc ϵ RI in T cells because mock-transduced T cells incubated with anti-Ly-2.1 IgE mAb died similarly to untreated control mice (Fig. 5A). As a test of antibody specificity, we performed another experiment comparing the antitumor effect of transduced T-CL9 cells incubated with anti-Ly-2.1 IgE or control human anti-30.6 IgE mAb. The enhanced antitumor response by chimeric Fc ϵ RI-transduced T cells was specifically dependent on the presence of anti-Ly-2.1 IgE, as mice receiving transfer of T-CL9 cells incubated with

control anti-30.6 IgE mAb died similarly to untreated mice (Fig. 5B). In addition, treatment of mice with anti-Ly-2.1 IgE mAb alone on days 0, 1, 2, and 4 had no effect on tumor growth, indicating that the transfer of Fc ϵ RI gene-modified T cells in the presence of tumor-specific IgE mAbs was needed to mediate the observed antitumor effect (Fig. 5B). Although T-CL9 cells could significantly impact tumor growth, the absence of complete tumor rejection and long-term survival of mice suggested that our T cells were not persisting. In fact, using an anti-human CD45 monoclonal antibody (a pan-human leukocyte marker), we were unable to detect either mock or gene-modified T cells by flow cytometry in either the peripheral blood, spleen, or intraperitoneal cavity of mice beyond 1 week after T cell injection (data not shown). Given the nonpersistence of our adoptively transferred T cells in tumor-bearing mice, it is unlikely that the cause of death in any of our mice were caused by graft-versus-host disease (GvHD). Rather, all mice died from disseminated E3 tumor growth in the intraperitoneal cavity.

To assess the ability of chimeric Fc ϵ RI-transduced T cells to impact on established disease, a similar tumor model was set up whereby transduced T-CL9 cells incubated with anti-Ly-2.1 or control 30.6 IgE mAb were adoptively transferred into E3 tumor-bearing mice on days 3, 4, 5, and 7 after tumor inoculation (Fig. 6). Mice receiving T-CL9 cells incubated with anti-Ly-2.1 IgE mAb again demonstrated enhanced survival compared with similar groups of mice that received T-CL9 cells incubated with control 30.6 IgE mAb (Fig. 6). Thus, these experiments have demonstrated for the first time that chimeric Fc ϵ RI-transduced primary human T cells could suppress established tumor growth *in vivo* in a tumor antigen IgE-specific manner.

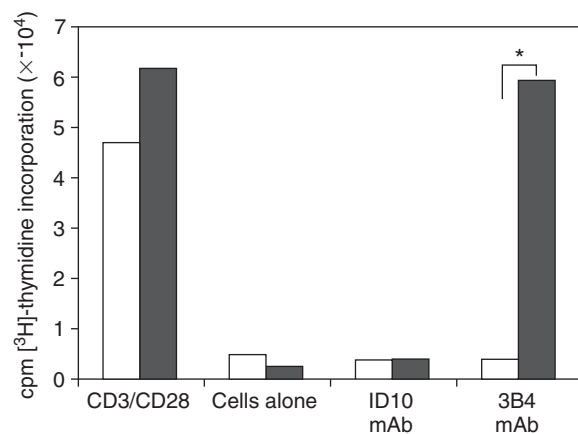


FIG. 4. Specific proliferation of gene-engineered human T cells expressing the Fc ϵ RI-CD28- ζ chimeric receptor. Proliferation of gene-engineered T cells was assessed in a 72-hr [^{3} H]thymidine incorporation assay. Mock-transduced T cells (open columns) and T cells expressing the Fc ϵ RI-CD28- ζ chimeric receptor (T-CL9 cells; solid columns) were incubated in medium alone or with plate-bound 3B4 mAb, isotype control ID10 mAb, or anti-CD3/CD28 mAb for 3 days. Results are expressed as means $\pm$ SEM of triplicate samples and are representative of three experiments (p = 0.002, Mann-Whitney test).

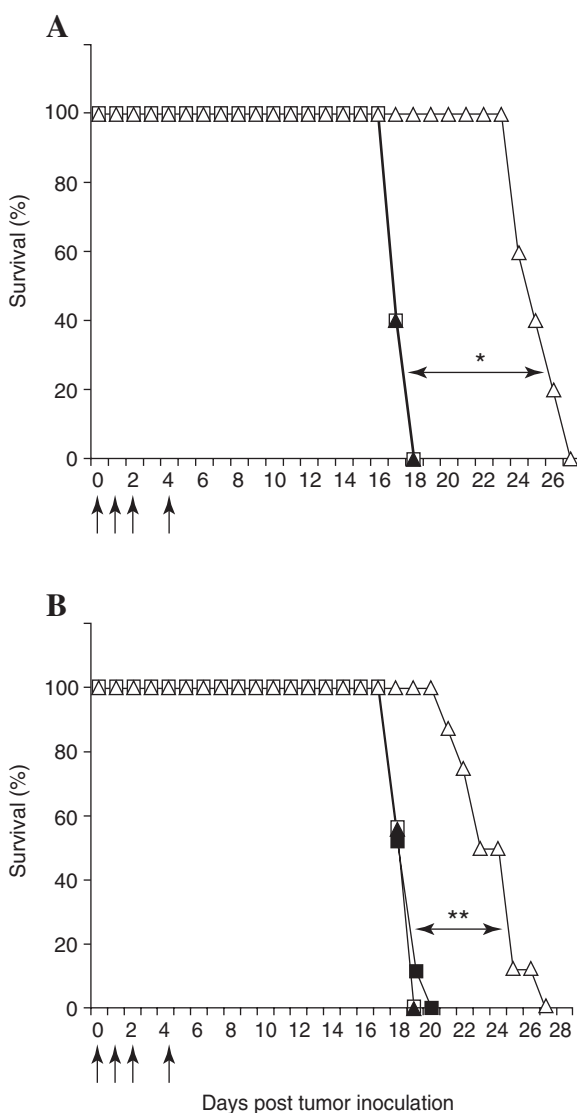


FIG. 5. Increased survival of tumor-bearing NOD-SCID mice after adoptive transfer of T-CL9 cells. Groups of 5–10 mice were injected intraperitoneally with 1×10^4 Ly-2.1⁺ E3 thymoma cells. (A) Tumor-bearing mice were left untreated (solid triangles) or treated intraperitoneally with either mock-transduced T cells (open squares) or T-CL9 cells (open triangles) incubated with anti-Ly-2.1 IgE mAb 6 hr, 1 day, 2 days, and 4 days after tumor inoculation (1×10^7 T cells per injection). Mice treated with T-CL9 cells incubated with anti-Ly-2.1 IgE mAb had significantly enhanced survival compared with mice treated with mock-transduced T cells incubated with anti-Ly-2.1 mAb (* $p = 0.0079$, Mann–Whitney test). (B) As a further test of specificity, mice were treated with T-CL9 cells incubated with anti-Ly-2.1 IgE mAb (open triangles) or anti-30.6 control IgE mAb (open squares). Mice receiving T-CL9 cells incubated with anti-Ly-2.1 IgE mAb survived significantly longer than mice receiving T-CL9 cells incubated with anti-30.6 control IgE mAb (** $p = 0.0001$, Mann–Whitney test). No enhanced survival was observed in untreated mice (solid triangles) or mice treated with anti-Ly-2.1 IgE mAb alone at 6 hr, 1 day, 2 days, and 4 days (solid squares). Arrows indicate number of injections, and results are representative of two experiments performed.

DISCUSSION

Gene modification of T cells to enhance their recognition and activation by tumor-associated antigen (TAA) is a promising approach for adoptive immunotherapy. At present, T cells engineered with scFv chimeras containing the CD28 costimulatory molecule linked in tandem with the TCR- ζ signaling chain have been demonstrated to optimally induce T cell effector function. Despite this, a potential limitation of the scFv approach is that TAA expression on any one tumor cell can be heterogeneous, therefore the use of gene-modified T-scFv cells against a single TAA may result in generation of tumor escape variants. A novel approach to potentially address this issue involves redirecting T cells by genetic modification with Fc ϵ receptors. The advantages of this approach include (1) the high affinity of IgE for the Fc ϵ receptor; (2) the ability to target different TAAs on tumor cells, using a cocktail of tumor-specific IgE mAbs, which may prevent tumor escape through loss and/or downregulation of TAAs; and (3) the recruitment of other Fc ϵ receptor-expressing effector cells (e.g., mast cells, eosinophils, and basophils) to the tumor site, which may contribute to the antitumor response. In this study, we have constructed a chimeric Fc ϵ RI consisting of the Fc ϵ RI extracellular domain linked to the Fc γ RII hinge and the signaling domains of CD28 and TCR- ζ (Fc ϵ RI-CD28- ζ). The design of this construct represents a further optimization of a first-generation Fc ϵ RI chimera containing only the TCR- ζ signaling domain (Kershaw *et al.*, 1996a,b). Our results demonstrate, for the first time, the ability

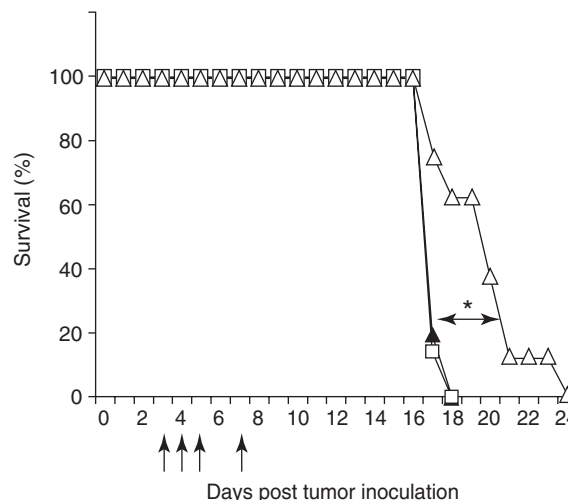


FIG. 6. Enhanced survival of established tumor-bearing NOD-SCID mice after adoptive transfer of T-CL9 cells. Groups of five to eight mice were injected intraperitoneally with 1×10^4 Ly-2.1⁺ E3 thymoma cells. Tumor-bearing mice were left untreated (solid triangles) or treated intraperitoneally with T-CL9 cells incubated with anti-Ly-2.1 (open triangles) or anti-30.6 control IgE mAb (solid squares) 3, 4, 5, and 7 days after tumor inoculation (1×10^7 T cells per injection). Mice treated with T-CL9 cells incubated with anti-Ly-2.1 IgE mAb had significantly enhanced survival compared with T-CL9 cells incubated with control 30.6 IgE mAb (* $p = 0.0205$, Mann–Whitney test). Arrows indicate number of T cell injections.

to effectively express this novel chimeric construct in human primary T cells and the capacity of this chimera to induce specific T cell cytokine production, proliferation, and cytolytic function in an IgE-dependent manner. Furthermore, adoptive transfer of T-Fc_εRI-CD28-ζ cells incubated with an anti-Ly-2.1 IgE mAb significantly enhanced the survival of irradiated NOD-SCID mice bearing established Ly-2.1⁺ tumors. Importantly, we show that the antitumor response observed required the combination of adoptively transferred T-Fc_εRI-CD28-ζ cells incubated with the anti-Ly-2.1 IgE mAb because no such response occurred when similar groups of tumor-bearing mice were treated with T-Fc_εRI-CD28-ζ cells incubated with anti-30.6 control IgE mAb or injected with anti-Ly-2.1 IgE mAb alone.

Previous studies have demonstrated that T cells could be specifically directed to tumor targets by gene modification with chimeric Fc_γR, which binds antibody of the IgG isotype. A chimeric human receptor consisting of the extracellular domain of human Fc_γRI and the transmembrane and intracellular domains of TCR-ζ endowed a cytotoxic T cell line, CTLL-R8, with the ability to lyse tumor targets in an mAb isotype-dependent manner (Smyth *et al.*, 1994). In a more recent study, genetic modification of primary human T cells with a chimeric receptor consisting of Fc_γRIIIα linked to Fc_εRIγ demonstrated specific lysis of target cells coated with an anti-CD20 mAb (Clemenceau *et al.*, 2006). Despite these encouraging results, high serum IgG levels and relatively low affinities of Fc_γR for monomeric IgG complicate the use of Fc_γR for immunotherapy. By contrast, the high-affinity receptor for IgE, Fc_εRI, is a far more attractive candidate given that IgE serum levels are much lower than those of IgG (Gould *et al.*, 2003).

A number of studies have aimed at exploiting the inflammatory response induced by IgE mAbs for tumor immunotherapy. Indeed, the ability of IgE mAb treatment to suppress the growth of a human ovarian tumor cell line in SCID mice has been demonstrated by Gould *et al.* In this study, the authors compared the antitumor efficacy of MOv-18-IgG or MOv-18-IgE mAb targeting the TAA folate-binding protein (FBP), overexpressed in ovarian carcinoma. Tumor-bearing mice injected with peripheral blood mononuclear cells (PBMCs) followed by MOv-18 IgE mAb demonstrated inhibition of tumor growth compared with mice treated with PBMCs and MOv-18 IgG mAb (Gould *et al.*, 1999). The antitumor effect observed required combined treatment with human PBMCs and MOv-18 IgE mAb because no antitumor response was observed after treatment of tumor-bearing mice with PBMCs alone or in combination with a control IgE mAb. In a further study, the authors validated that human monocytes in the PBMC culture were responsible for the observed antitumor response and their effector mechanism was found to involve IgE antibody-dependent cell-mediated cytotoxicity (ADCC) (Karagiannis *et al.*, 2003). In another study, administration of an IgE mAb targeting the 30.6 antigenic determinant expressed on the surface of colorectal adenocarcinoma cells was demonstrated to transiently inhibit the growth of established COLO 205 tumor growing subcutaneously in SCID mice (Kershaw *et al.*, 1998). In contrast to these studies, the antitumor effects in our model were mediated solely through engineered human T cells because our culture did not contain any significant proportion of monocytes. Our approach combined the specificity and affinity of IgE for its receptor and antigen with the potent effector function and

trafficking ability of T cells. An additional advantage of our strategy is the potential effect mediated by other Fc_ε⁺ effector cells, although this was not tested in our model. Future work may include the administration of human leukocytes (basophils, eosinophils, mast cells, macrophages) combined with Fc_εRI-modified T cells into tumor-bearing mice, or the development of a mouse anti-Ly-2.1 IgE mAb that can trigger endogenous Fc_εR-expressing effector cells. Alternatively, engrafting NOD-SCID mice with a human hematopoietic compartment, using human CD34⁺ stem cells, may provide a good model with which to test the contribution of other Fc_ε⁺ effectors. This model may also provide optimal conditions for the function of Fc_εRI-modified human T cells given that in our current model, antitumor effects of cytokines such as IFN-γ produced by gene-modified human T cells may be compromised because of their lack of cross-reactivity with murine cytokine receptors. Nevertheless, the results from our study and others have demonstrated proof of principle that IgE mAb can be used in different ways to mediate antitumor responses through their ability to activate immune cell effector mechanisms.

Despite its promise, a potential problem that might be encountered in using IgE mAb for tumor immunotherapy is the development of systemic anaphylaxis, which may occur if soluble TAAs are shed by tumors into the circulation. In our study, no toxicity was observed in NOD-SCID recipient mice. However, we were unable to properly assess the potential risk of anaphylaxis developing in our tumor model system given that the anti-Ly-2.1 mAb used in our study was a chimeric human/rat anti-IgE mAb, which was unable to bind to murine Fc_εRI receptors on potential reactive cells (Ravetch and Kinet, 1991). The use of a murine tumor-specific IgE mAb targeting a tumor that shed TAA into the circulation would be a more useful model to address this issue. Encouragingly, a previous study involving the use of a murine IgE antibody to target a viral tumor antigen in an *in vivo* mouse model system reported no development of systemic anaphylaxis (Nagy *et al.*, 1991). Nevertheless, the only real way to assess whether the use of chimeric Fc_εRI-modified T cells and tumor-specific IgE mAb is problematic is in a phase I clinical trial.

In summary, we have demonstrated successful gene modification of human primary T with a chimeric Fc_εRI. T-Fc_εRI-CD28-ζ cells were successfully redirected to Ly-2.1⁺ tumors, using an anti-Ly-2.1 IgE mAb, which resulted in the activation of T cell effector function. Furthermore, adoptive transfer of T-Fc_εRI-CD28-ζ cells incubated with anti-Ly-2.1 IgE mAb significantly enhanced the survival of Ly-2.1⁺ tumor-bearing NOD-SCID mice. The availability now of a number of different tumor-specific IgE mAbs targeting multiple TAAs (Kershaw *et al.*, 1998; Gould *et al.*, 1999; Karagiannis *et al.*, 2003) enhances the feasibility of using this approach to address the issue of antigen heterogeneity on tumor cells, which is a current problem for other immunotherapeutic strategies. Thus, redirecting T cells expressing Fc_ε chimeric receptors potentially broadens the utility of gene-modified T cells for cancer therapy.

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REFERENCES

- BUBENIK, J. (2004). MHC class I down-regulation: Tumor escape from immune surveillance? [review]. *Int. J. Oncol.* **25**, 487–491.
- CLEMENCEAU, B., CONGY-JOLIVET, N., GALLOT, G., VIVIEN, R., GASCHET, J., THIBAUT, G., and VIE, H. (2006). Antibody-dependent cellular cytotoxicity (ADCC) is mediated by genetically modified antigen-specific human T lymphocytes. *Blood* **107**, 4669–4677.
- CORREALE, P., AQUINO, A., GIULIANI, A., PELLEGRINI, M., MICHELI, L., CUSI, M.G., NENCINI, C., PETRIOLI, R., PRETE, S.P., DE VECCHIS, L., TURRIZIANI, M., GIORGI, G., BONMASSAR, E., and FRANCINI, G. (2003). Treatment of colon and breast carcinoma cells with 5-fluorouracil enhances expression of carcinoembryonic antigen and susceptibility to HLA-A(*)02.01 restricted, CEA-peptide-specific cytotoxic T cells *in vitro*. *Int. J. Cancer* **104**, 437–445.
- DUDLEY, M.E., WUNDERLICH, J.R., ROBBINS, P.F., YANG, J.C., HWU, P., SCHWARTZENTRUBER, D.J., TOPALIAN, S.L., SHERRY, R., RESTIFO, N.P., HUBICKI, A.M., ROBINSON, M.R., RAFFELD, M., DURAY, P., SEIPP, C.A., ROGERS-FREEZER, L., MORTON, K.E., MAVROUKAKIS, S.A., WHITE, D.E., and ROSENBERG, S.A. (2002). Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. *Science* **298**, 850–854.
- FINNEY, H.M., LAWSON, A.D., BEBBINGTON, C.R., and WEIR, A.N. (1998). Chimeric receptors providing both primary and costimulatory signaling in T cells from a single gene product. *J. Immunol.* **161**, 2791–2797.
- GOULD, H.J., MACKAY, G.A., KARAGIANNIS, S.N., O'TOOLE, C.M., MARSH, P.J., DANIEL, B.E., CONEY, L.R., ZURAWSKI, V.R., JR., JOSEPH, M., CAPRON, M., GILBERT, M., MURPHY, G.F., and KORNGOLD, R. (1999). Comparison of IgE and IgG antibody-dependent cytotoxicity *in vitro* and in a SCID mouse xenograft model of ovarian carcinoma. *Eur. J. Immunol.* **29**, 3527–3537.
- GOULD, H.J., SUTTON, B.J., BEAVIL, A.J., BEAVIL, R.L., McCLOSKEY, N., COKER, H.A., FEAR, D., and SMURTHWAITE, L. (2003). The biology of IGE and the basis of allergic disease. *Annu. Rev. Immunol.* **21**, 579–628.
- HAKIMI, J., SEALS, C., KONDAS, J.A., PETTINE, L., DANHO, W., and KOCHAN, J. (1990). The α subunit of the human IgE receptor (Fc ϵ RI) is sufficient for high affinity IgE binding. *J. Biol. Chem.* **265**, 22079–22081.
- HAYNES, N.M., TRAPANI, J.A., TENG, M.W., JACKSON, J.T., CERRUTI, L., JANE, S.M., KERSHAW, M.H., SMYTH, M.J., and DARCY, P.K. (2002). Single-chain antigen recognition receptors that costimulate potent rejection of established experimental tumors. *Blood* **100**, 3155–3163.
- HOATLIN, M.E., KOZAK, S.L., SPIRO, C., and KABAT, D. (1995). Amplified and tissue-directed expression of retroviral vectors using ping-pong techniques. *J. Mol. Med.* **73**, 113–120.
- HOMBACH, A., WIECZARKOWIECZ, A., MARQUARDT, T., HEUSER, C., USAI, L., POHL, C., SELIGER, B., and ABKEN, H. (2001). Tumor-specific T cell activation by recombinant immunoreceptors: CD3 ζ signaling and CD28 costimulation are simultaneously required for efficient IL-2 secretion and can be integrated into one combined CD28/CD3 ζ signaling receptor molecule. *J. Immunol.* **167**, 6123–6131.
- HULETT, M.D., and HOGARTH, P.M. (1994). Molecular basis of Fc receptor function. *Adv. Immunol.* **57**, 1–127.
- ISAACS, J.D., WATTS, R.A., HAZLEMAN, B.L., HALE, G., KEOGAN, M.T., COBBOLD, S.P., and WALDMANN, H. (1992). Humanised monoclonal antibody therapy for rheumatoid arthritis. *Lancet* **340**, 748–752.
- KARAGIANNIS, S.N., WANG, Q., EAST, N., BURKE, F., RIFFARD, S., BRACHER, M.G., THOMPSON, R.G., DURHAM, S.R., SCHWARTZ, L.B., BALKWILL, F.R., and GOULD, H.J. (2003). Activity of human monocytes in IgE antibody-dependent surveillance and killing of ovarian tumor cells. *Eur. J. Immunol.* **33**, 1030–1040.
- KAWAKAMI, T., and GALLI, S.J. (2002). Regulation of mast-cell and basophil function and survival by IgE. *Nat. Rev. Immunol.* **2**, 773–786.
- KERSHAW, M.H., DARCY, P.K., HULETT, M.D., HOGARTH, P.M., TRAPANI, J.A., and SMYTH, M.J. (1996a). Redirected cytotoxic effector function: Requirements for expression of chimeric single chain high affinity immunoglobulin E receptors. *J. Biol. Chem.* **271**, 21214–21220.
- KERSHAW, M.H., DARCY, P.K., TRAPANI, J.A., and SMYTH, M.J. (1996b). The use of chimeric human Fc ϵ receptor I to redirect cytotoxic T lymphocytes to tumors. *J. Leukoc. Biol.* **60**, 721–728.
- KERSHAW, M.H., DARCY, P.K., TRAPANI, J.A., MACGREGOR, D., and SMYTH, M.J. (1998). Tumor-specific IgE-mediated inhibition of human colorectal carcinoma xenograft growth. *Oncol. Res.* **10**, 133–142.
- KERSHAW, M.H., TENG, M.W., SMYTH, M.J., and DARCY, P.K. (2005). Supernatural T cells: Genetic modification of T cells for cancer therapy. *Nat. Rev. Immunol.* **5**, 928–940.
- KESSELS, H.W., WOLKERS, M.C., VAN DEN BOOM, M.D., VAN DER VALK, M.A., and SCHUMACHER, T.N. (2001). Immunotherapy through TCR gene transfer. *Nat. Immunol.* **2**, 957–961.
- LUBORSKY, J.L., BARUA, A., SHATAVI, S.V., KEBEDE, T., ABRAMOWICZ, J., and ROTMENSCH, J. (2005). Anti-tumor antibodies in ovarian cancer. *Am. J. Reprod. Immunol.* **54**, 55–62.
- METZGER, H., ALCARAZ, G., HOHMAN, R., KINET, J.P., PRIBLUDA, V., and QUARTO, R. (1986). The receptor with high affinity for immunoglobulin E. *Annu. Rev. Immunol.* **4**, 419–470.
- MOELLER, M., HAYNES, N.M., KERSHAW, M.H., JACKSON, J.T., TENG, M.W., STREET, S.E., CERUTTI, L., JANE, S.M., TRAPANI, J.A., SMYTH, M.J., and DARCY, P.K. (2005). Adoptive transfer of gene-engineered CD4⁺ helper T cells induces potent primary and secondary tumor rejection. *Blood* **106**, 2995–3003.
- NAGY, E., BERCZI, I., and SEHON, A.H. (1991). Growth inhibition of murine mammary carcinoma by monoclonal IgE antibodies specific for the mammary tumor virus. *Cancer Immunol. Immunother.* **34**, 63–69.
- RAVETCH, J.V., and KINET, J.P. (1991). Fc receptors. *Annu. Rev. Immunol.* **9**, 457–492.
- ROSENBERG, S.A., YANG, J.C., and RESTIFO, N.P. (2004). Cancer immunotherapy: Moving beyond current vaccines. *Nat. Med.* **10**, 909–915.
- ROYAL, R.E., KERSHAW, M.H., REEVES, M.E., WANG, G., DALY, T., TREISMAN, J., LAM, J., and HWU, P. (2002). Increased functional expression of transgene in primary human lymphocytes using retroviral vectors modified with IRES and splicing motifs. *Gene Ther.* **9**, 1085–1092.
- SMYTH, M.J., KERSHAW, M.H., HULETT, M.D., MCKENZIE, I.F., and TRAPANI, J.A. (1994). Use of the 5'-flanking region of the mouse perforin gene to express human Fc γ receptor I in cytotoxic T lymphocytes. *J. Leukoc. Biol.* **55**, 514–522.

- TENG, M.W., KERSHAW, M.H., MOELLER, M., SMYTH, M.J., and DARCY, P.K. (2004). Immunotherapy of cancer using systemically delivered gene-modified human T lymphocytes. *Hum. Gene Ther.* **15**, 699–708.
- WATSON, N.F., RAMAGE, J.M., MADJD, Z., SPENDLOVE, I., ELLIS, I.O., SCHOLEFIELD, J.H., and DURRANT, L.G. (2006). Immunosurveillance is active in colorectal cancer as downregulation but not complete loss of MHC class I expression correlates with a poor prognosis. *Int. J. Cancer* **118**, 6–10.
- YEE, C., THOMPSON, J.A., BYRD, D., RIDDELL, S.R., ROCHE, P., CELIS, E., and GREENBERG, P.D. (2002). Adoptive T cell therapy using antigen-specific CD8⁺ T cell clones for the treatment of patients with metastatic melanoma: *In vivo* persistence, migration, and antitumor effect of transferred T cells. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 16168–16173.
- ZHANG, J.Y., CASIANO, C.A., PENG, X.X., KOZIOL, J.A., CHAN, E.K., and TAN, E.M. (2003). Enhancement of antibody detection in cancer using panel of recombinant tumor-associated antigens. *Cancer Epidemiol. Biomarkers Prev.* **12**, 136–143.
- ZHAO, Y. (2005). Primary human lymphocytes transduced with NY-ESO-1 antigen-specific TCR genes recognize and kill diverse human tumor cell lines. *J. Immunol.* **174**, 4415–4423.

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