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Immunology

Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the γ or ζ subunits of the immunoglobulin and T-cell receptors

(single-chain Fv domain/chimeric receptors/immunotargeting/T cell)

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ABSTRACT **The generation of tumor-specific lymphocytes and their use in adoptive immunotherapy is limited to a few malignancies because most spontaneous tumors are very weak or not at all immunogenic. On the other hand, many anti-tumor antibodies have been described which bind tumor-associated antigens shared among tumors of the same histology. Combining the variable regions (Fv) of an antibody with the constant regions of the T-cell receptor (TCR) chains results in chimeric genes endowing T lymphocytes with antibody-type specificity, potentially allowing cellular adoptive immunotherapy against types of tumors not previously possible. To generalize and extend this approach to additional lymphocyte-activating molecules, we designed and constructed chimeric genes composed of a single-chain Fv domain (scFv) of an antibody linked with γ or ζ chains, the common signal-transducing subunits of the immunoglobulin receptor and the TCR. Such chimeric genes containing the Fv region of an anti-trinitophenyl antibody could be expressed as functional surface receptors in a cytolytic T-cell hybridoma. They triggered interleukin 2 secretion upon encountering antigen and mediated non-major-histocompatibility-complex-restricted hapten-specific target cell lysis. Such chimeric receptors can be exploited to provide T cells and other effector lymphocytes, such as natural killer cells, with antibody-type recognition directly coupled to cellular activation.**

Many clinical attempts to recruit the humoral or cellular arms of the immune system for passive anti-tumor immunotherapy have not fulfilled expectations. While it has been possible to obtain anti-tumor antibodies, their therapeutic potential has been limited so far to blood-borne tumors (1, 2), primarily because solid tumors are inaccessible to sufficient amounts of antibodies (3). On the other hand, the use of effector lymphocytes in adoptive immunotherapy, although effective in selected solid tumors, suffers from a lack of specificity [such as in the case of lymphokine-activated killer cells (LAK cells) (4)] or from the difficulty in recruiting tumor-infiltrating lymphocytes (TILs) and expanding such specific T cells for most malignancies (5). Yet, the observations that TILs can be obtained in melanoma and renal cell carcinoma tumors, that they can be effective in selected patients, and that foreign genes can function in these cells (6) demonstrate the therapeutic potential embodied in these cells.

A strategy which we and others have recently developed (7–11) allows us to combine the advantage of the antibody's specificity with the homing, tissue penetration, and target-cell destruction of T lymphocytes and to extend, by *ex-vivo* genetic manipulations, the spectrum of anti-tumor specificity of T cells. In this approach a chimeric T-cell receptor (cTCR) gene composed of the variable region domain (Fv) of an antibody molecule and the constant region domain of the TCR were expressed in T cells. Upon encountering antigen, such cTCR could transmit signals for T-cell activation, secretion of lymphokines, and specific target cell lysis in a major histocompatibility complex (MHC) nonrestricted manner. Moreover, the cTCR-bearing cells undergo stimulation by immobilized antigen, proving that receptor-mediated T-cell activation is not only nonrestricted but also independent of MHC expression on target cells.

Broad application of the cTCR approach is dependent on efficient expression of the cTCR genes in primary T cells. Although retroviral vectors have been demonstrated to be effective for transgene expression in human T cells due to the fact that two genes have to be introduced to express functional cTCR and the very low efficiency of transduction of a single cell with two separate retroviral vectors, new vectors have to be tried which will allow the transduction of two genes in tandem.

To overcome these problems and to extend the applicability of the "T-body" approach to other cells and receptor molecules, we developed a single-chain approach to the cTCR. It is based on the demonstrated ability to express in bacteria an antibody single-chain Fv (scFv) (15, 16). Such scFv domains, which join the antibody's heavy and light variable (V_H and V_L) gene segments with a flexible linker, have proven to exhibit the same specificity and affinity as the natural Fab' fragment. In the present study we constructed chimeric molecules composed of the scFv linked to receptor subunits that might serve to transduce the signal from the scFv and confer antibody specificity to T cells as well as other lymphocytes. Among the polypeptides of the TCR/CD3 (the principal triggering receptor complex of T cells), especially promising are the ζ and its η isoform, which appear as either homo- or hetero-S—S-linked dimers and are responsible for mediating at least a fraction of the cellular activation programs triggered by the TCR recognition of ligand (17, 18). These polypeptides have very short extracellular domains, which can serve for the attachment of the scFv. A most attractive candidate in the group of natural killer (NK)-stimulatory receptors is the low-affinity receptor for IgG, FcγRIII (CD16). Occupancy or cross-linking of FcγRIII activates NK cells for cytokine production, expression of surface molecules, and cytolytic activity (19, 20). In

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Abbreviations: TCR, T-cell receptor; cTCR, chimeric TCR; Fv, variable region domain of antibody; scFv, single-chain Fv; NK, natural killer; FcγR and FcεR, immunoglobulin γ- and ε-chain constant region receptors; scFvR, chimeric molecule between a scFv and a receptor chain; V, variable; V_H and V_L, heavy and light variable; C, constant; TNP, 2,4,6-trinitrophenyl; IL-2, interleukin 2; MHC, major histocompatibility complex; CTL, cytolytic T lymphocytes; mAb, monoclonal antibody; FγG, fowl gamma globulin.

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NK cells, macrophages, and B and T cells, the FcγRIIIA appears as a heterooligomeric complex consisting of a ligand-binding α chain associated with a disulfide-linked γ or ζ chain. The FcγRIIIA signaling γ chain (21) serves also as part of the FcεRI complex, where it appears as a homodimer, is very similar to the CD3 ζ chain, and in fact can form heterodimers with it in some cytolytic T lymphocytes (CTL) and NK cells (22, 24). Remarkably, most recently prepared chimeras between these polypeptides and the extracellular domains of CD4 (25), CD8 (26), the interleukin 2 (IL-2) receptor α chain (27), or CD16 (28) proved to be active in signaling T-cell stimulation even in the absence of other TCR/CD3 components.

To endow T cells and NK cells with antibody-type recognition, we constructed chimeric genes composed of a scFv of an anti-2,4,6-trinitrophenyl (TNP) antibody and either the FcR γ chain or the CD3 complex ζ chain. Cytotoxic lymphocyte hybridomas expressing such chimeric scFvRγ or scFvRζ genes interacted specifically with TNP-modified target cells, underwent activation as monitored by IL-2 secretion, and subsequently killed specifically TNP-modified target cells. These data suggest a functional expression in a continuous polypeptide of antigen-binding and signal-transducing properties which can be utilized as a targeting receptor on lymphocytes.

MATERIALS AND METHODS

Cell Lines and Antibodies. MD.45 is a CTL hybridoma of BALB/c mice allospecific to H-2^b (29). MD.27J is a TCR α^- mutant of MD.45. A.20 is a B lymphoma of BALB/c origin (ATCC T1B 208). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum. Sp6, an anti-TNP monoclonal antibody (mAb), and 20.5, an anti-Sp6 idiotype mAb, were provided by G. Köhler (Max-Planck-Institut für Immunobiologie, Freiburg, Germany) (30). Polyclonal antibodies to human FcεRIγ chain were provided by J.-P. Kinet (National Institutes of Health), and mAbs to the same protein (4D8) (31) were provided by J. Kochan (Hoffmann–La Roche). Rabbit antibodies to murine ζ chain were from M. Baniyash (Hebrew University Medical School, Jerusalem).

Construction of Chimeric Genes. The specific genes encoding the V_H and V_L domains of the Sp6 anti-TNP antibody were derived from the genomic constructs described previously for the preparation of the cTCR (32, 33) by PCR amplifications using oligodeoxynucleotide primers designed according to the 5′ and 3′ consensus amino acid sequences of immunoglobulin V regions (34) introducing the *Xba* I and *Bst*EII restriction sites at the ends of the scFv. In constructing the scFv we used the V_L-linker-V_H design containing a linker sequence similar to linker 212 described by Colcher *et al.* (12). Accordingly, the V_L-3′ and the V_H-5′ primers include sequences comprising the 5′ and 3′ parts of the linker, introducing *Sal* I sites in their 3′ and 5′ ends, respectively. Following digestion of the purified PCR products with *Xba* I and *Sal* I (V_L) and *Sal* I and *Bst*EII (V_H), the fragments were ligated into the *Xba* I and *Bst*EII sites of a pRSV2neo-based expression vector containing the leader of SC15 κ light chain (kindly provided by S. Levy, Stanford University Medical School) and TCR constant region β chain (Cβ), prepared for the expression of anti-38C.13 cDNA cTCR genes (unpublished work and ref. 33). The Cβ of this plasmid was then replaced with either the γ chain amplified from a human cDNA clone (13) or the ζ chain amplified from Jurkat cDNA by using primers introducing *Bst*EII and *Xho* I at the 5′ and 3′ ends. A diagram of the final scFvRγ expression vector is shown in Fig. 1. The sequences of the oligodeoxynucleotide primers used for the construction of the chimeric scFvRγ and scFvRζ are delineated in the legend to Fig. 1.

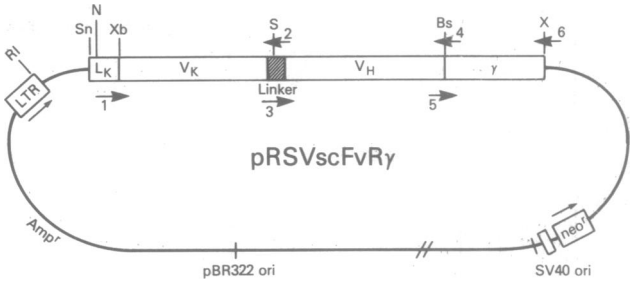

FIG. 1. Scheme of the chimeric scFvR expression vector. For other vectors, R can represent any receptor chain, such as the ζ subunit of CD3 described in this study. The boxes from left to right represent DNA segments corresponding to the Rous sarcoma virus long terminal repeat promoter (LTR), κ light chain leader (L_κ) and variable region (V_κ), the linker (hatched box), heavy chain variable region (V_H), the human γ chain, the G418-resistance gene (neo^r), and the simian virus 40 origin of replication. Restriction sites indicated are *Eco*RI (RI), *Sna*BI (Sn), *Nco* I (N), *Xba* I (Xb), *Sal* I (S), *Bst*EII (Bs), and *Xho* I (X). The arrowheads represent the flanking regions amplified by using the following oligonucleotide primers: 1, V_κ-5′-CCCG**TCTAGA**GGAGAYATYGTWATGACCCAGTCTCCA; 2, V_κ-3′-CCC**GTCGAC**CCTTTWATTTCCAGCTTWGTSCC; 3, V_H-5′-CGG**GTCGAC**TTCCGGTAGCGGCAAATCCTCTGAAGGCAAAGGTSAGGTSCAGCTGSAGSAGTCTGG; 4, V_H-3′-TGMRGAGACGGTGACCGTRGTYCCTTGGCCCCAG; 5, γ-5′-CC**GGTCAC**CGTCTCTTCAGCGGATCCTCAGCTCTGCTATATCCTGGATG; 6, γ-3′-GGCAGCTG**CTCGAG**TCTAAAGCTACTGTGGTGG; 7, ζ-5′-GCT**GGATCC**CAAACTCTGCTACC; and 8, ζ-3′-CGC**CTCGAG**CTGTTAGCGAGGGGGC. These primers were designed to match the consensus sequences of V_H and V_κ. The relevant restriction sites are in bold letters.

Expression of the Chimeric scFvRγ/ζ Genes. Transfection of 20 μg of pRSVscFvRγ/ζ DNA into 20 × 10^6 MD.45 or MD.27J hybridoma cells was performed by electroporation using an ISCO power supply at 1.9 kV (32). Transfectants were selected in G418 at 2 mg/ml. Expression of scFvRγ/ζ on the surface of transfected cells was evaluated by immunofluorescence staining using the 20.5 anti-Sp6 idiotype and fluorescein isothiocyanate (FITC)-labeled anti-mouse Fab′ antibody. Functional assays included an IL-2 production assay and a cytotoxicity assay in which the ability of transfectants to respond specifically to TNP-modified A.20 target cells was evaluated as detailed in ref. 9. The amount of IL-2 was determined by using an IL-2-dependent CTL line and methyl tetrazolium acid staining (14). Cytotoxicity was assayed by ^{51}Cr release (29). All determinations were performed in triplicate.

Immunoprecipitation and Immunoblotting. Washed pellets containing 10^8 cells were lysed in 1 ml of 1% digitonin as described (32). Aliquots of nuclei-free supernatants were incubated with antibodies and then precipitated with second antibodies and protein G-Sepharose (Pharmacia). Alternatively, cell lysates were mixed with sample buffer to a final concentration of 1% $NaDodSO_4$ and either 10 mM iodoacetamide (for nonreducing gels) or 15 mM dithiothreitol (for reducing gels). The washed immunoprecipitates were dissociated in sample buffer under the same conditions. To avoid destruction of the Sp6 idiotope, the samples were incubated at 20°C for 30 min before $NaDodSO_4$/PAGE through a 5–20% gel gradient. Separated proteins were blotted onto nitrocellulose paper and allowed to react with anti-Sp6, anti-γ, or anti-ζ antibodies followed by peroxidase-labeled anti-immunoglobulin antibodies. Washed blots were developed by using a chemiluminescence kit (ECL, Amsterdam).

RESULTS

Construction and Expression of the Chimeric scFvRγ/ζ Chain Genes. To produce a chimeric receptor with an antigen

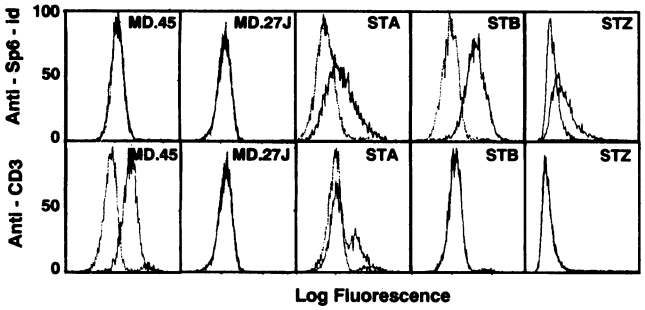

FIG. 2. Fluorescence-activated cell sorter (FACS) analysis of immunofluorescence staining of MD.45 hybridoma and its TCR α^- MD.27J mutant, their corresponding scFvRγ-transfected STA and STB clones, or STZ cells, which result from transfection of the scFvRζ chimeric gene into MD.27J. Solid line, staining with anti-Sp6 idiotypic antibody 20.5 or anti-CD3 mAb 145.2C11. Broken line, control irrelevant antibody.

binding site of a given antibody and the signaling γ or ζ chains, we have adopted the scFv design (15, 16), which allows combining both entities into one continuous molecule. In engineering the pRSVscFvRγ/ζ expression vector (Fig. 1), harboring the V_L and V_H of the Sp6 anti-TNP mAb (23), we introduced elements that enable its usage as a modular expression cassette to accommodate scFvs from different antibodies in combination with γ, ζ, or other chains. This was achieved by using oligonucleotide primers composed of sequences common to the majority of the 5′ and 3′ sequences of either V_L or V_H regions, flanked by relatively unique restriction sites, which allow both in-frame ligation of the different units and removal to other vectors. We have chosen to use the 5′-V_L-linker-V_H-3′ design, which was found suitable for the expression of a variety of single-chain antibodies and their fragments in bacteria (16), although we believe that the converse, 5′-V_H-linker-V_L-3′, alignment (15) can be used as well.

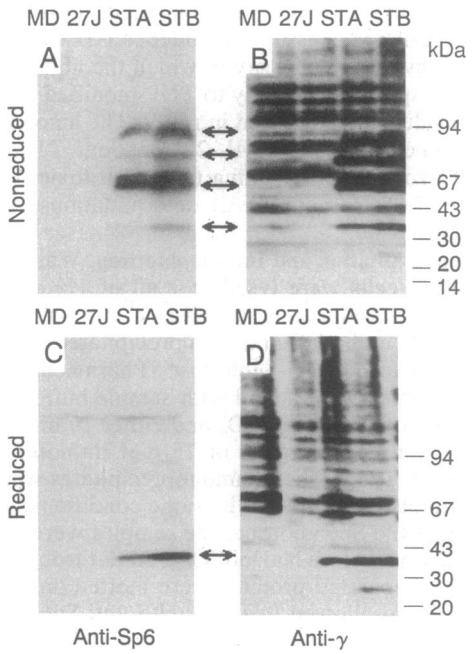

FIG. 3. Immunoblotting analysis of lysates prepared from scFvRγ transfectants and parental hybridomas developed by anti-Sp6 idiotypic mAb 20.5 (*A* and *C*) and rabbit anti-human γ chain (*B* and *D*). Electrophoresis was on four separate gels. The molecular mass scales are related to *B* and *D*; the arrows point to the same bands in *A* and *B* or *C* and *D*.

Introduction of the chimeric scFvRγ gene into the MD.45 murine CTL hybridoma (STA series of transfectants) or its MD.27J TCR α^- mutant, which does not express surface TCR/CD3 complex (STB series), resulted in the expression of the chimeric molecule on the cell surface of selected clones as revealed by staining with the anti-Sp6 idiotypic antibody (Fig. 2). Similar staining was observed for STZ, which was derived by transfecting MD.27J with the scFvRζ chimeric gene. The surface expression of the scFvRγ or scFvRζ molecule was independent of the TCR/CD3 complex: it did not restore surface expression of the CD3 in MD.27J transfected STB or STZ cells, and some subclones of the STA that initially expressed both scFvRγ and TCR/CD3 on their surface lost, upon a prolonged culture period, the TCR/CD3 expression without any apparent effect on the scFvRγ expression and function (not shown).

Immunoblotting analysis of cell lysates prepared from representative STA and STB transfectants using either anti-idiotypic mAb 20.5 or polyclonal anti-human γ antibodies revealed four distinct bands of apparent molecular mass of 36, 54–62, 74–80, and 85–90 kDa, which did not appear in the parental cells (Fig. 3 *A* and *B*). Under reducing conditions (Fig. 3 *C* and *D*) one species, which corresponds to the predicted 36-kDa monomeric form of the scFvRγ, was apparent, indicating the multimeric nature of the molecule. The band with apparent 75-kDa molecular mass corresponds to the homodimeric molecule, and the nature of the 90-kDa species is unknown. It might be a novel γ-associated polypeptide, analogous to the one recently reported (31). This species can be detected only in immunoblots of cell lysates and is not apparent after surface iodination and immunoprecipitation (Fig. 4*B*), suggesting the intracellular origin of the molecule. The appearance of bands in the range of 54–62 kDa was more pronounced in the STB transfectant. It might

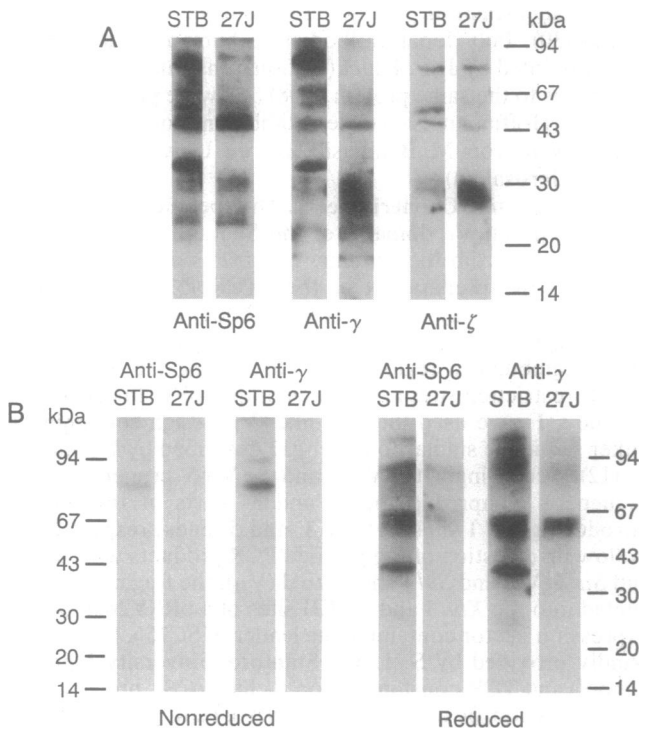

FIG. 4. Composition of the scFvRγ dimers. (*A*) Immunoblot analysis of anti-Sp6 precipitates prepared from STB (scFvRγ transfectant cells) and their parent (MD.27J hybridoma cells). After electrophoresis under nonreducing conditions and blotting, the blot was allowed to react with anti-Sp6, anti-human γ, or anti-mouse ζ antibodies. (*B*) Immunoprecipitation of lysates made of surface-iodinated STB cells (scFvRγ transfectant cells) and their parent (MD.27J hybridoma cells).

represent heterodimers between the chimeric scFvRγ chain and endogenous ζ and probably η chains of the CD3 complex. We therefore electrophoresed anti-Sp6 immunoprecipitates made from STB lysates, blotted the gels, and developed the blots with anti-Sp6, anti-γ, or anti-mouse ζ/η antibodies (Fig. 4*A*). Both the anti-idiotypic and the anti-γ antibodies revealed the four bands from the transfected cells; however, the anti-ζ (which cross-reacts with the mouse η chain) differentially developed only the 60-kDa species. Immunoprecipitation of surface-iodinated proteins with either anti-Sp6 or anti-γ antibodies (Fig. 4*B*) demonstrated a main species of 75 kDa under nonreducing conditions. This is the homodimer of the chimeric chain.

Expression of scFvRγ/ζ as Functional Receptors. To test whether the chimeric scFvRγ or scFvRζ can function as an active receptor molecule, we studied the ability of the transfected hybridomas to undergo antigen-specific stimulation. The MD.45 T cell hybridoma can be triggered through its TCR to produce IL-2, IL-3, or granulocyte/macrophage colony-stimulating factor (GM-CSF). It specifically recognizes and responds to H-2^b target cells (29), while its mutant, MD.27J, cannot be stimulated through its TCR due to the absence of an α chain. Upon introduction of the chimeric Sp6-scFvRγ, both of these cells could be specifically triggered to produce IL-2 after incubation with TNP-modified stimulator cells (Fig. 5*A*) or plastic-immobilized TNP-fowl γ globulin (TNP-FγG)(Fig. 5*B*). Nonmodified A.20 cells or FγG did not activate the transfectants, demonstrating the specificity of the response toward TNP. Stimulation of the various transfectants with immobilized antigen resulted in different degrees of reactivity. While STA responded to plastic-bound TNP-FγG in consistent manner, STB and STZ (transfected with scFvRγ and scFvRζ respectively) lost their ability to undergo stimulation with immobilized antigen but not with hapten-modified cells. Such behavior suggests the necessity of additional synergistic signal for these cells. Indeed, costimulation with TNP-FγG plus either phorbol 12-myristate 13-acetate (PMA) or Ca^{2+} ionophore resulted in enhancement of IL-2 production (data not shown). Incubation with soluble TNP-proteins even at high hapten-to-protein ratios did not result in activation but rather specifically inhibited triggering by immobilized antigen (Fig. 5*B*) or cell-bound hapten. The activation of GTAe.20, a transfectant expressing a two-chain cTCR (9), was also inhibited by soluble TNP-FγG. Identical concentrations of antigen were needed to cause 50% inhibition (IC_{50}) of STA and GTAe.20 (Fig. 5*B*), indicating that the single-chain and the double-chain Fv display the same relative affinity to TNP.

Finally, we tested the ability of the chimeric receptors to mediate specific target cell lysis by incubating them with ^{51}Cr-labeled cells. As shown in Fig. 6, only the cells transfected with the Sp6-scFvRγ or scFvRζ could lyse TNP-modified target cells in a dose-related fashion. This cytolytic activity was specific to TNP, as soluble TNP-FγG blocked it (not shown) and unmodified A.20 cells were not affected by the transfectants.

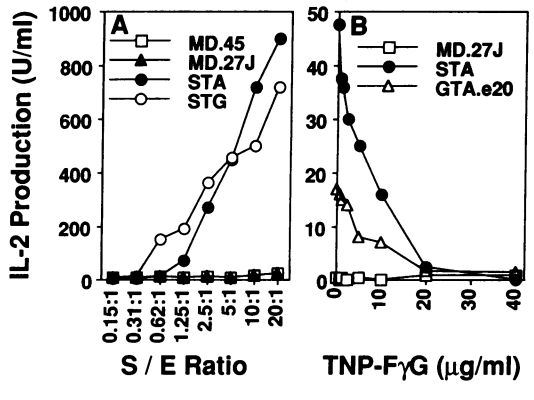

FIG. 5. Transfectants expressing scFvR are stimulated to produce IL-2 after stimulation with TNP-A.20 (*A*) or plastic-immobilized TNP-FγG, without or with different concentrations of soluble TNP-FγG (*B*). GTAe.20 is an Sp6 double-chain cTCR transfectant from our previous study (9). The scFvRζ-expressing STZ produced about 200 units (U) of IL-2 per ml after coculture with TNP-A.20 at 8:1 stimulator-to-effector (S/E) cell ratio. Not shown are the responses of the transfectants to nonmodified A.20 or FγG controls, which were completely negative, exactly like the background responses of the MD.45 and MD.27J cells to TNP-antigen.

DISCUSSION

In this study we demonstrate that a single-chain Fv of an antibody molecule fused to the γ chain of the immunoglobulin Fc receptor or to the ζ chain of the CD3 complex can be expressed in T cells as an antigen-specific receptor. The chimeric scFvRγ/ζ endowed T cells with antibody-type specificity, transmitted a signal for IL-2 production, and mediated target cell lysis. The demonstration that the scFvRγ/ζ fusion protein could mediate antigen-specific stimulation of T cells not expressing the TCR/CD3 receptor complex [as shown for the STB and STZ transfectants derived from the TCR-negative MD.27J mutant (Figs. 5 and 6)], strongly suggests that the γ and ζ chains are capable of autonomous activation of T cells. Yet, because of the low level of heterodimers between the scFvRγ and the endogenous ζ and η chains (Figs. 3 and 4), we cannot exclude some contribution by the residual ζ (or η) chain in the signaling process. Nonetheless, our results clearly indicate that the TCR chains do not take part in this process, thus confirming and complementing recent observations in which antibody crosslinking through the extracellular domains of CD4, CD8, IL-2 receptor, or CD16 joined to the cytoplasmic tail of either one of the γ/ζ family members resulted in T-cell activation (25–28). Like scFvRγ/ζ, chimeric CD4 or CD16-γ/ζ molecules expressed in cytotoxic lymphocytes could direct specific cytolysis against appropriate target cells (25, 28). Interestingly, analysis of mutations within the intracellular 18-residue motif, which has been recently assigned to account for the activity of the γ/ζ chain, revealed that the ability to mediate calcium responsiveness can be separated from the ability to support cytolysis (28). This opens new possibilities in which the chimeric chain, composed of scFv and genetically modified ζ or γ chains, can be used not only to direct the

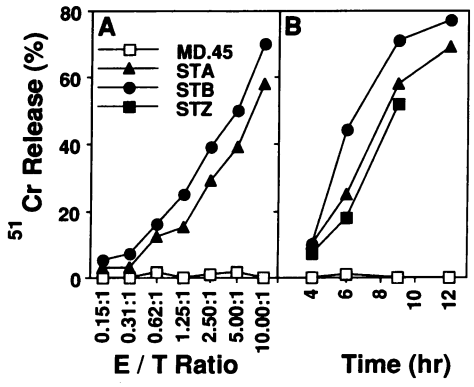

FIG. 6. Specific ^{51}Cr release of TNP-A.20 cells after incubation with transfectants expressing scFvR. Effector cells were incubated with plastic-immobilized TNP-FγG for 8 hr before the killing assay. Kinetic assay was done at an effector-to-target (E/T) cell ratio of 10:1; dose–response was determined in a 9-hr assay. Control nonmodified A.20 target cells incubated with the same effector cells in identical conditions did not release more ^{51}Cr than the spontaneous release (not shown).

specificity but also to dictate the selected reactivity of lymphocytes.

The finding that immobilization of antigen is needed for efficient stimulation through scFvRγ/ζ and that soluble multimeric ligand (such as TNP-protein) did not trigger but rather inhibited receptor-mediated activation through cell- or plastic-bound TNP (Fig. *5B*) indicates that mere engagement or even crosslinking of adjacent γ or ζ chains does not result in T-cell activation (as manifested by IL-2 release). The dependence on ligand immobilization for efficient T-cell triggering has been reported also for cTCR-mediated signaling (8, 9), and the mechanisms underlying this are as yet unclear.

Additional potential applications offered by the antibody scFvR design involve retargeting lymphocytes *in vivo*. As manifested by the TNP-specific cytolysis of target cells mediated by transfectants expressing the scFvRγ/ζ (Fig. 6), the chimeric protein endowed the CTL hybridomas with MHC-nonrestricted specificity. As we previously suggested for the cTCR, this can be exploited to retarget T cells *in vivo* to tumor cells or to any other target of choice, toward which antibodies can be raised. In this regard, the scFvR design is advantageous over the cTCR one. It requires the expression of only one gene instead of the gene pair required for the cTCR, thereby providing simpler construction and transfection. Second, the scFvR design can be employed to confer antibody specificity on a larger spectrum of signaling molecules composed of only one chain. Third, the scFv maintains both V_H and V_L together in one chain; thus, even upon mixed pairing of chimeric with endogenous chains, the antigen-binding properties of the molecule are conserved. Finally, the fact that γ and ζ constitute the signaling chains of the TCR/CD3, FcγRIII, and FcεRI expands the feasibility of exploiting the chimeric receptor for redirecting other effector cells, such as NK cells, basophils, or mast cells in addition to T cells.

We believe that the chimeric scFvRγ/ζ described here (or any of the simple modifications of it as suggested below) that combines the specificity of an antibody as a continuous single chain and the effector function of cytotoxic T cells and NK cells or regulatory function of helper T cells could offer important consequential development for targeted immunotherapy. This approach exploits the scFv as the antigen-recognition unit and the potent cytotoxic responses of NK cells and T cells and/or the ability of T cells to secrete lymphokines and cytokines upon activation at the target site, thus recruiting, regulating, and amplifying other arms of the immune system. The chimeric scFv receptors can confer on the lymphocytes the following functions: antibody-type specificity toward any predefined antigen; specific "homing" to their targets; specific recognition, activation, and execution of effector function as a result of encountering the target; and specific and controlled proliferation at the target site. Potential receptor or signaling molecules that can be used in the single-chain design are γ, ζ, and η subunits of the TCR/FcR complexes; any of the α, β, γ, or δ of the TCR chains; CD16 α chain of the FcγRIIIα; CD2 and CD28 surface molecules; and α, β, and γ chains of the IL-2 receptor as well as any of the chains of the cytokine receptors which take part in stimulation of lymphocyte activation and/or proliferation. Candidate lymphocytes to be endowed with antibody specificity by using this approach are NK cells, lymphokine-activated killer cells (LAK), cytotoxic T cells, helper T cells, and the various subtypes of the above. These cells can execute their authentic natural function and can serve, in addition, as carriers of foreign genes designated for gene therapy, and the chimeric receptor shall serve in this case to direct the cells to their target. Finally, this approach can be applied to anti-idiotypic vaccination by using helper T cells expressing chimeric receptors made of Fv of anti-idiotypic antibodies. Such "designer lymphocytes" will interact and stimulate idiotype-bearing B cells to produce antigen-specific antibodies, thus bypassing the need for active immunization with toxic antigens.

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