Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 12654–12658, December 1994
Immunology

Functional three-domain single-chain T-cell receptors

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Contributed by Jack L. Strominger, July 19, 1994

ABSTRACT **T-cell receptors (TCRs) are membrane anchored heterodimers structurally related to antibody molecules. Single-chain antibodies can be engineered by linking the two variable domains, which fold properly by themselves. However, proper assembly of the variable domains of a human TCR (V_α and V_β) that recognize the HLA-DR2b/myelin basic protein-(85–99) peptide complex was critically dependent on the addition of a third domain, the constant region of the TCR β chain (C_β), to the single-chain construct. Single-chain molecules with the three-domain design, but not those with the two-domain design, expressed in a eukaryotic cell as chimeric molecules linked either to glycosyl phosphatidylinositol or to the transmembrane/cytoplasmic domains of the CD3 ζ chain were recognized by a conformation-sensitive monoclonal antibody. The chimeric three-domain single-chain TCR linked to CD3 ζ chain signaled in response to both the specific HLA-DR/peptide and the HLA-DR/superantigen staphylococcal enterotoxin B complexes. Thus, by using this three-domain design, functional single-chain TCR molecules were expressed with high efficiency. The lipid-linked single-chain TCR was solubilized by enzymatic cleavage and purified by affinity chromatography. The apparent requirement of the constant domain for cooperative folding of the two TCR variable domains may reflect significant structural differences between TCR and antibody molecules.**

T-cell receptor (TCR) recognition of antigen fragments presented by major histocompatibility complex (MHC) molecules is a critical step in the initiation of a specific immune response (1, 2). The TCR α and β chains are each composed of two immunoglobulin-like domains; most of the amino acid residues that are found to be highly conserved in the variable (V) region of immunoglobulins are also found in TCR V regions, suggesting that the tertiary structure of the TCR may resemble that of immunoglobulins (3, 4). However, TCR V regions have significantly more primary sequence variability, an increased apparent rate of divergence in phylogeny, and peaks of variability in addition to those noted in immunoglobulins (5, 6). In order to understand and control the molecular interactions underlying T-cell recognition of MHC/peptide complexes, complete structural knowledge of the TCR is required.

Several approaches have been employed to produce soluble, recombinant TCRs. In these recombinant TCR molecules, the transmembrane/cytoplasmic regions of α and β chains were replaced with sequences from lipid-linked proteins (7), the CD3 ζ chain (8), or immunoglobulins (9, 10). Soluble TCRs were either recovered as secreted proteins or obtained by enzymatic cleavage of the surface-expressed recombinant proteins. All of these approaches rely, however, on the assembly of the heterodimer, which is inefficient (11). In addition, high-level expression of the human TCR α chain in transfected eukaryotic cells is not stable. These problems can be avoided by the design of a single-chain (sc) recombinant protein in which the V regions of the heterodimer are joined by a short peptide linker. Such a design has been successfully applied to antibody molecules (12). Such recombinant molecules, scFv, have a specificity and affinity similar to that of native antibodies (12). Several reports have described the production of scTCRs in bacterial expression systems using the sc antibody (Fv) design (13–16), but none have presented functional data indicating that these scTCRs could recognize their MHC/peptide complexes or superantigens. Recently, however, the production in bacteria of a scTCR with the two-domain Fv design that could recognize its natural ligands has been reported, although the fraction correctly refolded was extremely low (17).

In the present report, different scTCR designs were evaluated in transfected eukaryotic cells with respect to surface expression of TCR molecules, proper folding, and recognition of the appropriate MHC/peptide ligand. A three-domain sc construct [α-chain V (V_α)–linker–β-chain V (V_β)–β-chain constant (C_β)] was stably expressed on the cell surface when linked to a glycosyl phosphatidylinositol (GPI) anchor and recognized by a conformation-dependent monoclonal antibody (mAb) specific for the $V_\beta 17$ segment. The soluble form of this recombinant protein could be readily obtained by enzymatic cleavage with phosphatidylinositol-specific phospholipase C (PI-PLC). Replacement of the GPI domain with the cytoplasmic portion of the ζ chain resulted in a functional TCR molecule that transduced an intracellular signal following recognition of either the proper MHC/peptide or the MHC/staphylococcal enterotoxin B (SEB) superantigen complex. The production of a functional scTCR directly demonstrates the feasibility of employing sc design to produce soluble TCRs.

MATERIALS AND METHODS

Construction of Recombinant TCR Molecules. cDNAs of TCR α and β chains were prepared from mRNA of Hy.2H9 cells (18) with Superscript reverse transcriptase (BRL) and an oligo(dT) primer (Sigma) and were amplified by PCR using Vent DNA polymerase (New England Biolabs) and primers 5′-GCTCGAGGCGGCGATGGAAACTCTCCTGGGAGT-3′ (A5) and 5′-GGAATTCAGCTGGACCACAGCCGC-3′ for α-chain and 5′-GCTCGAGCTCTGCCATGGACTCCTG-GA-3′ and 5′-GGAATTCAGAAATCCTTTCTCTTGAC-3′ for β-chain. The cDNAs were cloned into the mammalian expression vector pBJ-neo (8). GPI-anchored TCR molecules (α-PI and β-PI) were constructed as follows. A *Ban* I site was

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Abbreviations: TCR, T-cell receptor; MHC, major histocompatibility complex; sc, single chain; GPI, glycosyl phosphatidylinositol; PI-PLC, phosphatidylinositol-specific phospholipase C; SEB, staphylococcal enterotoxin B; MBP, myelin basic protein; V, variable; C, constant; IL-2, interleukin 2; mAb, monoclonal antibody.

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engineered after the fifth amino acid residue beyond the last cysteine by oligonucleotide-directed mutagenesis. The region 3′ of the *Ban* I site was then replaced with a *Ban* I–*Not* I fragment encoding the GPI signal domain from the human placental alkaline phosphatase. For the construction of various scTCRs, variable domains of the 2H9 TCR α and β chains were prepared by PCR using primers A5 and 5′-CAGAGCTCACGGATGAACAATAAGGCTGGT-3′ for the V_α domain in all the scTCR constructs, 5′-TCGGATATCGATGGTGGAATCACTCAGTCC-3′ (B5) and 5′-CAGAGATCAGCACGGTGAGCCGGTTCCC-3′ for the V_β domain in AB-PI-1, 5′-GTGGGAGATCTCTGCTTCTGATGGCTCAAAC and B5 for the V_β domain in AB-PI-2, 5′-CACGGATCCCCGTCTGCTCTACCCCAGGC and B5 for the V_β and C_β domains in ABC-PI, and 5′-CACGGATCCCCGTCTGCTCTACCCCAGGC-3′ and B5 for the V_β and C_β domains in ABC-ζ. The cDNA encoding the transmembrane and cytoplasmic domains of murine CD3 ζ chain (8) was a gift of R. D. Klausner (National Institutes of Health). Convenient restriction sites were engineered at the end of each fragment to aid in the assembly of the construct. The linker was a 15-amino acid motif of GGGGS repeated three times (12) with *Sac* I at the 5′ end and *Eco*RV at the 3′ end. Except for α-PI, all the constructs were cloned into pBJ-neo, which carries the G418-resistance gene. α-PI was cloned into pCEP-4 (Invitrogen), which bears the hygromycin-resistance gene. All constructs were verified by multiple restriction digests and by sequencing with the Sequenase kit (United States Biochemical).

Affinity Purification and Characterization of a Soluble Three-Domain scTCR. After transfection and G418 selection (8), cells expressing a high level of GPI-linked three-domain scTCR (ABC-PI) were isolated by three rounds of sorting. The resulting cells were grown in spinner culture to a density of 10^6 per ml and harvested by centrifugation. The pellet was washed twice with phosphate-buffered saline (PBS) and resuspended in PBS containing 2 mM Pefabloc (Centerchem, Stamford, CT) to a density of 5×10^7 per ml with PI-PLC (Sigma) added at 1 unit/ml. Cells were incubated at 37°C for 1 hr with constant rocking. The supernatant was collected by centrifugation and by passage through a 0.45-μm filter and applied to a column of Acti-gel (Sterogen, Arcadia, CA) with immobilized βF1. The column was washed with 10 volumes of PBS and the soluble TCR was eluted with 0.15 M glycine (pH 2.8). Fractions were immediately neutralized with 0.1 volume of saturated Tris. The soluble TCR was then dialyzed against >100 volumes of PBS at 4°C with at least four changes and concentrated to 0.5 mg/ml by vacuum dialysis against PBS. Five micrograms of purified soluble three-domain scTCR was analyzed by SDS/PAGE under reducing conditions.

Stimulation of Transfectants with Antibodies, SEB, and Peptide/MHC Ligands. ABC-ζ-transfected BW5147β $\alpha^-\beta^-$ (19) cells (5×10^4 per well) were cultured in a 96-well round-bottom plate to which various antibodies had been immobilized (1 μg per well). The supernatants were collected after 24 hr and interleukin 2 (IL-2) production was assessed in a bioassay using an IL-2-dependent cell line (CTLL) and the CellTiter-96 nonradioactive proliferation assay (Promega). In the case of ABC-ζ-transfected RBL-2H3 (8) cells, the cells were incubated with [^{3}H]serotonin (NEN) at 0.5 μCi (18.5 kBq) for 24 hr before they were added to the antibody plate. After incubation at 37°C for 2 hr, radioactivity released into the supernatant was measured in a liquid scintillation counter. The specific serotonin release was calculated as described (8). For SEB stimulation, 5×10^4 transfected cells per well were cultured with various concentrations of SEB (Toxin Technology, Sarasota, FL) in the presence or absence of 2×10^5 B cells. For antigen presentation, 5×10^4 transfected cells per well were cocultured with 2×10^5 B cells which were incubated with or without the myelin basic protein (MBP)-(85–99) peptide for 3 hr before the experiment. The assays were conducted as described above.

RESULTS

mAb C1 Recognizes a Conformational Epitope of TCR. Recombinant TCR molecules were generated by using the TCR α- and β-chain sequences of the human MBP-specific T-cell clone Hy.2H9 (18). This clone TCR is composed of the V_α3.1 and V_β17.1 segments and is specific for the immunodominant MBP peptide MBP-(85–99) in the context of HLA-DR2 (DRA, DRB1*1602) (18). Usage of the V_β17.1 segment allowed the proper folding of recombinant TCRs to be probed with the superantigen SEB (20) and the mAb C1 (21). To confirm the V_β17 specificity of C1, the extracellular domains of TCR α and β chains of Hy.2H9 cells were fused to the C-terminal sequence from human placental alkaline phosphatase for GPI anchorage (Fig. 1) and the DNAs encoding the GPI-anchored β and α chains (β-PI and α-PI) were sequentially transfected by electroporation (8) into a TCR α- and β-chain-deficient murine lymphoma cell line, BW5147 $\alpha^-\beta^-$ (BW$^-$) (19). The surface expression of the GPI-anchored TCR chains was monitored by staining with mAbs αF1 (22), βF1 (23), and C1. αF1 and βF1 recognize nonconformational epitopes located in the C region of the TCR α and β chains, respectively. Surface expression of the GPI-anchored TCR β chain is independent of heterodimer formation and assembly of the CD3 complex (7). In the β-PI-transfected cells (Fig. 2, open curves), high-level expression of β-PI was confirmed by staining with βF1. Interestingly, there was little C1 staining of these transfectants. However, when a GPI-anchored 2H9 α chain was supertransfected into the β-PI transfectant (Fig. 2, shaded curves), C1 reactivity was greatly increased while the level of βF1 staining remained constant. Thus, the V_β17-specific C1 epitope is conformational and dependent on the proper pairing of TCR α and β chains and can therefore be used to assess the proper folding of recombinant TCRs bearing a V_β17 sequence.

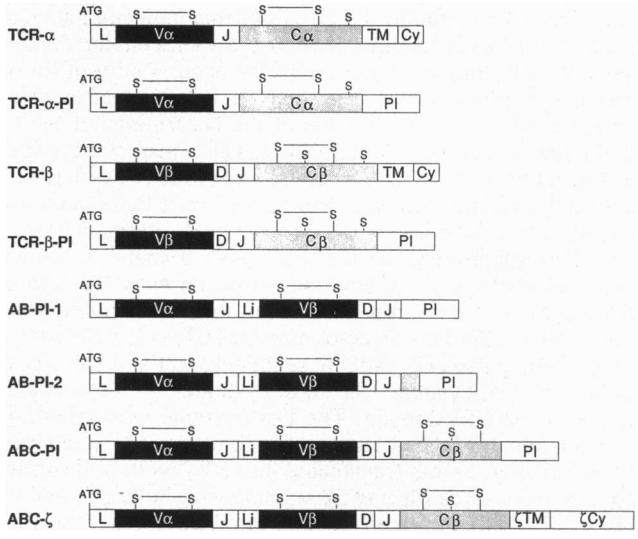

FIG. 1. Schematic representation of TCR α- and β-chain genes and various chimeric constructs. S—S, disulfide bond; L, leader; V, variable segment; J, joining segment; C, constant region; TM, transmembrane region; Cy, cytoplasmic region; ATG, start codon; Li, 15-residue peptide linker containing three repeats of GGGGS; PI, GPI domain of human placental alkaline phosphatase with the sequence LAPPAGTTDAAHPGRSVVPALLPLLAGTLLLL (7). The ζ region contains transmembrane and cytoplasmic domains of the murine CD3 ζ chain starting at position 31 (8).

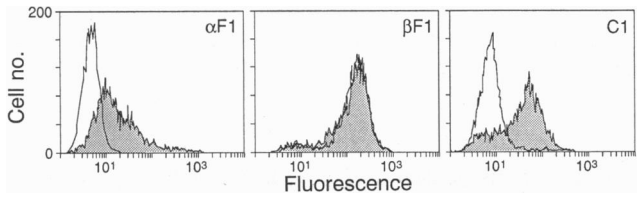

FIG. 2. Coexpression of α-PI and β-PI are required for C1 reactivity. Flow cytomeric analysis of β-PI-transfected BW$^-$ cells stained with mAb αF1, βF1, or C1 before (open curves) and after (shaded curves) the supertransfection of the α-PI construct.

High-level expression of the TCR α chain (α-PI) was, however, not stable either alone or in the presence of β-PI. Attempts were made on several cell lines, including COS-7, CHO-K1, and a TCR-deficient variant of Jurkat cells, JK-β$^-$ (J.RT3-T3.5, American Type Culture Collection). The expression level of α-PI was comparable to that of β-PI after the initial drug selection, but continued culture for less than a month yielded a population of cells with little surface expression of α-PI, whereas β-PI expression was stable (data not shown). The inability to obtain cell lines with stable high-level expression of the PI-anchored human TCR α-chain has been reported by other laboratories as well (24).

Expression and Purification of a Three-Domain scTCR. To overcome the limitations set by the unstable expression of the human TCR α chain, various sc designs were examined. Initially, a design similar to that of sc antibodies (Fv) was chosen (12). A 15-residue flexible linker was used to link the C terminus of the V_α–J_α domain to the N terminus of the β chain. The GPI domain was then ligated to the C terminus of the V_β–J_β domain. The construct (AB-PI-1, Fig. 1) was transfected into several cell lines, including JK-β$^-$, COS-7, CHO-K1, and BW$^-$. Although the expression of the gene was confirmed by the detection of the correct RNA transcripts (Fig. 3*B*), no surface expression was detected, as evidenced by negative C1 antibody staining (Fig. 3*A*). Immunoprecipitation after metabolic labeling failed to recover any C1-reactive sc molecules from these transfectants (data not shown). The inability to identify any C1-reactive protein could have been due to the design of this molecule, such as insufficient linker length between the extracellular domain and the GPI domain. To improve the accessibility of the sc construct, another two-domain scTCR was designed in which an extra 30-amino acid portion of the N terminus of the C_β domain was added as a hinge region. The transfectants of this construct (AB-PI-2, Fig. 1) were still not reactive with the C1 antibody (data not shown). Finally, the entire C_β domain was added to the sc construct. A complete C_β domain should provide enough distance for the V_α–V_β domains to be expressed on the cell surface and, more importantly, should allow surface expression to be monitored with another antibody, βF1 (23). This three-domain scTCR was constructed by extending the TCR β-chain sequences to the residue right before the last cysteine (the sixth cysteine), which was then fused to the GPI domain. The last cysteine was deleted to prevent dimerization between C_β domains. This construct (ABC-PI, Fig. 1) was transfected into BW$^-$ cells and surface expression was confirmed by staining with both βF1 and C1 (Fig. 3*C*, shaded curves). Both antibodies stained the cells with comparable efficiency, suggesting that most of the molecules were expressed in the correct conformation. Moreover, the molecule could be efficiently cleaved from the cell surface with PI-PLC (Fig. 3*C*, open curves).

Soluble three-domain scTCR was purified from transfectants after PI-PLC cleavage followed by affinity chromatography using the βF1 antibody. The purified three-domain scTCR appeared as multiple bands at 50–70 kDa after SDS/PAGE (Fig. 3*D*). The heterogeneity of scTCR is probably the

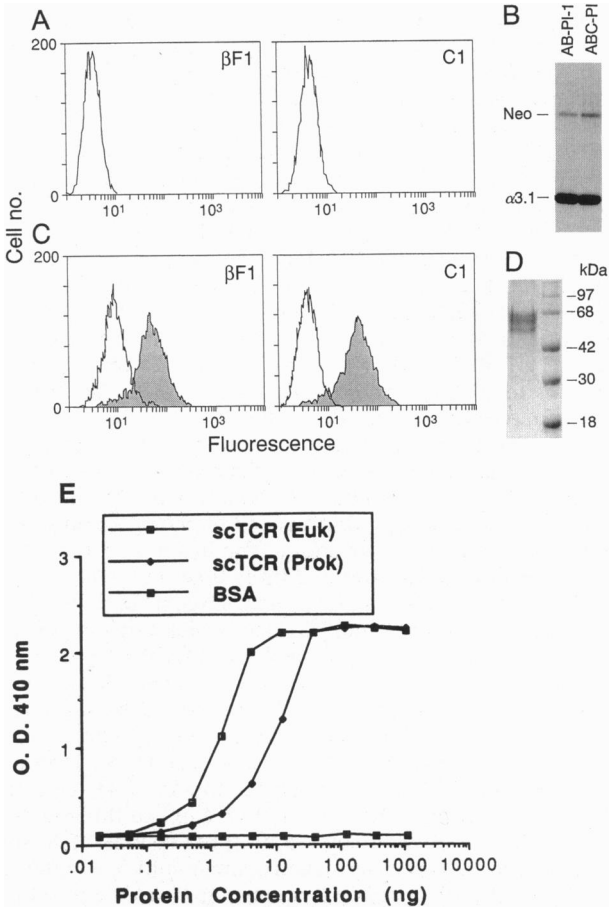

FIG. 3. Flow cytomeric analysis of surface expression of scTCR constructs. (*A*) Lack of C1 reactivity in BW$^-$ cells transfected with two-domain scTCR (AB-PI-1). (*B*) RNA analysis of poly(A)-enriched total cell RNA isolated from BW$^-$ cells transfected with two-domain and three-domain scTCR constructs AB-PI-1 and ABC-PI, respectively. Samples were analyzed in a nuclease S1 protection assay (25) using probes specific for the 5′ end of the transcripts from the TCR constructs. The coexpressed *neo* (G418-resistance gene) transcripts from the vector were analyzed with a probe at the same time as a control. (*C*) Flow cytometric analysis of ABC-PI-transfected BW$^-$ cells with both βF1 and C1 antibodies before (shaded curves) and after (open curves) PI-PLC treatment. (*D*) SDS/PAGE of affinity-purified three-domain scTCR. (*E*) Comparison of C1 reactivity of three-domain scTCRs produced from eukaryotic (Euk) and prokaryotic (Prok) expression systems in a two-antibody ELISA. A plateau is reached because the amount of βF1 attached to the plate became limiting.

result of variable glycosylation; its polypeptide size calculated from amino acid composition is 40 kDa. The structural integrity of the three-domain scTCR was verified by a two-antibody ELISA (Fig. 3*E*). The molecules were first captured by the βF1 antibody immobilized to the plate and then assessed for reactivity with the C1 antibody. When compared with the three-domain scTCR produced in a bacterial expression system (unpublished work), the scTCR from the eukaryotic system gave 10–20 times higher C1 reactivity. The purified three-domain scTCR was stable and could be stored in PBS at 4°C for months without significant loss of C1 reactivity.

Functional Characterization of a Chimeric Three-Domain scTCR. To directly assess the functional integrity of the three-domain scTCR, a self-signaling scTCR was produced by replacing the GPI domain with the transmembrane and cytoplasmic domains of the CD3 ζ chain. These regions have been shown to be sufficient for signal transduction when its

extracellular fusion partner is crosslinked by an antibody or by the proper ligand (8, 26, 27). To enable the recovery of three-domain scTCR as a soluble form, a linker containing a thrombin cleavage site was inserted into the junction of three-domain scTCR and the ζ domain. The construct (ABC-ζ) was transfected into BW^- cells (28) and the rat basophilic leukemia cell line RBL-2H3 (RBL) (8), and the populations displaying high-level expression of three-domain scTCR were isolated by three rounds of cytofluorometric sorting using the antibody βF1. The ABC-ζ-transfected cells were first stimulated with various antibodies to confirm the self-signaling nature of this recombinant molecule. The signal transduced upon the activation of the three-domain scTCR was measured as IL-2 production in BW^- transfectants, whereas serotonin release was measured in RBL transfectants. Both transfectants showed a strong response following βF1 and C1 stimulation but not to purified mouse immunoglobulin or anti-CD8 antibody used as controls (Fig. 4 *A* and *B*). The structural integrity of the scTCR was further examined with the superantigen SEB, which binds to both $V_\beta 17$ and MHC class II molecules, resulting in TCR crosslinking and T-cell activation regardless of the peptide bound to the MHC molecule (20, 29). ABC-ζ transfectants displayed a concentration-dependent response toward SEB (Fig. 4*C*) when the superantigen was presented by transformed B-cell lines with high-level expression of DR1 (DRA, DRB1*0101; cell line LG2) or DR2 (DRA, DRB1*1602; cell line 9016). Thus, the lateral face of the TCR V_β region to which SEB is thought to bind (30) is structurally intact.

To prove that the three-domain scTCR did indeed recognize the MHC/peptide ligand, antigen presentation experiments using the natural ligand for the Hy.2H9 clone, 9016 cells bearing the DRB1*1602 allele of DR2, and MBP-(85–99) peptide were performed. To ensure detection of subtle abnormalities in the structure of the three-domain scTCR, 9009 cells (DRA, DRB1*1601), which also bind MBP-(85–99), were used as a control. DRB1*1601 and DRB1*1602 differ only at position 67 in the $DR_\beta 1$ domain; this TCR contact-residue substitution does, however, abolish recognition of the peptide by the parent T-cell clone (ref. 18; K.W.W.,

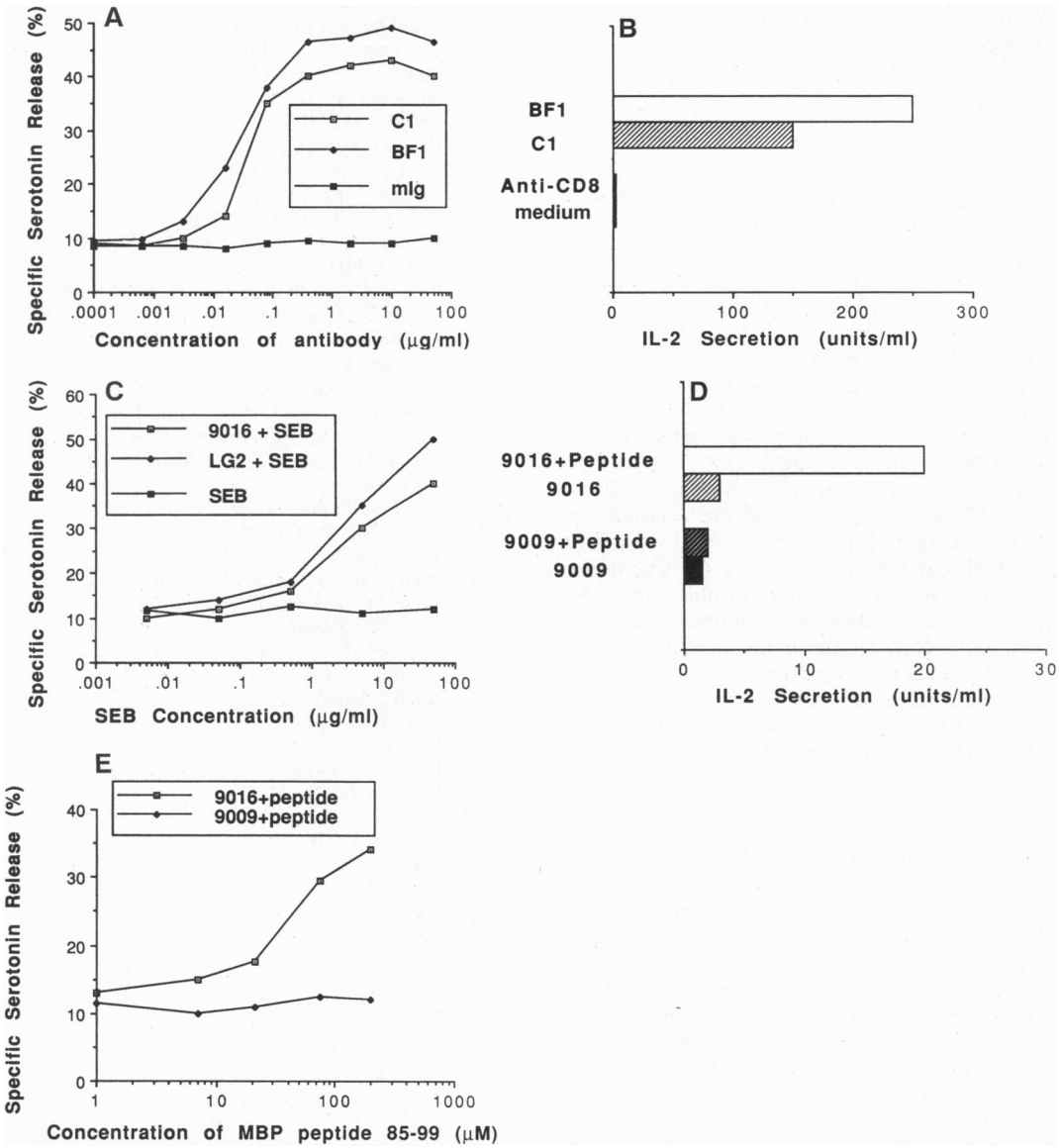

FIG. 4. (*A*) [^{3}H]Serotonin release following TCR crosslinking of three-domain scTCR–ζ chimeric construct (ABC-ζ)-transfected RBL cells. C1 is specific to $V_\beta 17$ and βF1 is specific to the C region of TCR β chain. mIg, mouse immunoglobulin. (*B*) IL-2 release following TCR crosslinking of ABC-ζ-transfected BW^- cells. (*C*) [^{3}H]Serotonin release following SEB stimulation of ABC-ζ-transfected RBL cells. (*D*) MHC-restricted antigen-specific response from the ABC-ζ-transfected BW^- cells. (*E*) Dose–response curve of MHC/peptide recognition of ABC-ζ-transfected RBL cells.

unpublished work). The ABC-ζ-transfected BW$^-$ cells secreted IL-2 in response to peptide-pulsed 9016 cells, but not to peptide-pulsed 9009 cells (Fig. 4*D*). Similar results were obtained with RBL transfectants (Fig. 4*E*), as serotonin release was dependent on the concentration of the MBP peptide used to pulse 9016 cells. The signal appeared to be weak when compared with antibody stimulation. This is not surprising, however, since saturating amounts of antibodies are expected to crosslink the majority of TCR molecules on the target cell, whereas a much smaller fraction of TCR molecules is probably engaged when T cells are cocultured with peptide-pulsed antigen-presenting cells, in which a maximum of 5–15% of the DR molecules bind the peptide. The requirement for a high concentration of peptide or SEB is not due to the sc design, since high concentrations of peptide are also needed to stimulate $\alpha\beta$ heterodimers of the TCR-ζ constructs (8). It is likely that the decrease in sensitivity results from the lack of CD3, CD4, and/or other adhesion/signaling molecules. Nonetheless, these results demonstrate that the three-domain scTCR was correctly folded and functionally competent. In addition, a soluble form of three-domain scTCR could be obtained from the ABC-ζ transfectants by thrombin cleavage and affinity purification (data not shown).

DISCUSSION

A scTCR molecule was designed which contains the V domains of both α and β chains and the C domain of the β chain. This scTCR molecule could be stably expressed at a high level in eukaryotic cells and could be isolated in a soluble form by enzymatic cleavage and affinity chromatography. The V_α and V_β domains appeared to be properly paired, since the scTCR bound to a conformation-dependent mAb, the superantigen SEB, and the proper MHC/peptide ligand. This design of scTCR offers an alternative to the two-chain design of soluble TCRs and has several advantages. (*i*) The sc design avoids the low-efficiency dimerization process which may be the limiting step in the assembly of TCR heterodimers from α and β subunits made in *Escherichia coli*. The sc design therefore allows efficient expression of the recombinant protein in quantities suitable for structural analysis and for some diagnostic or therapeutic applications. (*ii*) The design avoids the problems associated with the unstable expression of the human TCR α chain that have hindered efficient expression of human TCR molecules in eukaryotic cells. (*iii*) The sc design may allow the construction of TCR phage display libraries similar to those made for sc antibodies (28, 31). scTCR phage libraries may be powerful tools for the isolation of TCRs with defined specificities and/or high affinity for selective targeting of malignant and virally infected cells and for analyzing the interactions among TCR, MHC/peptide complexes, and superantigens.

Unlike antibodies, separately expressed V domains of TCR α and β chains have not been reconstituted to form heterodimers (ref. 32; K. L. Hilyard, personal communication). However, despite this success in producing the three-domain scTCR, a two-domain scTCR with detectable C1 reactivity could not be produced either in eukaryotic cells or in bacteria. The presence of the C_β domain (or part of the domain) may be required for the proper folding and/or stabilization of a scTCR molecule. In any event, the three-domain design provides a general means for the efficient production of functional scTCR molecules.

We thank Drs. James Huston and Herman Oppermann for helpful advice and Dr. Basya Rybalov for technical assistance. This work was supported by National Institutes of Health Grant CA47554 (J.L.S.), and National Multiple Sclerosis Society Postdoctoral Fellowship Award FG915-A-1 (S.C.). K.W.W. is a Harry Weaver Neuroscience Scholar of the National Multiple Sclerosis Society.

1. Ashwell, J. D. (1990) *Annu. Rev. Immunol.* **8,** 139–167.
2. Jorgensen, J. L., Reay, P. A., Ehrich, E. W. & Davis, M. M. (1992) *Annu. Rev. Immunol.* **10,** 835–837.
3. Davis, M. M. & Bjorkman, P. J. (1988) *Nature (London)* **334,** 395–402.
4. Chothia, C., Boswell, D. R. & Lesk, A. M. (1988) *EMBO J.* **7,** 3745–3755.
5. Behlke, M. A., Spinella, D. G., Chou, H. S., Sha, W., Hartl, D. L. & Loh, D. Y. (1985) *Science* **229,** 566–570.
6. Becker, D. M., Patten, P., Chien, Y.-H., Yokota, T., Eshaar, Z., Giedlin, M., Gascoigne, N. R. J., Goodnow, C., Wolf, R., Arai, A. & Davis, M. M. (1985) *Nature (London)* **317,** 430–434.
7. Lin, A. Y., Devaux, B., Green, A., Sagerstrom, C., Elliott, J. F. & Davis, M. M. (1990) *Science* **249,** 677–679.
8. Engel, I., Ottenhoff, T. H. M. & Klausner, R. D. (1992) *Science* **256,** 1318–1321.
9. Weber, S., Traunecker, A., Oloveri, F., Gerhard, W. & Karjalainen, K. (1992) *Nature (London)* **356,** 793–796.
10. Gregoire, C., Rebai, N., Schweisguth, F., Necker, A., Mazza, G., Auphan, N., Millward, A., Schmitt-Verhulst, A.-M. & Malissen, B. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 8077–8081.
11. Klausner, R. D., Lippincott-Schwartz, J. & Bonifacino, J. S. (1990) *Annu. Rev. Cell Biol.* **6,** 403–431.
12. Huston, J., McCartney, J., Tai, M.-S., Mottola-Hartshorn, C., Jin, D., Warren, F., Keck, P. & Oppermann, H. (1992) *Int. Rev. Immunol.* **10,** 195–217.
13. Novotny, J., Ganju, R. K., Smiley, S. T., Hussey, R. E., Luther, M. A., Recny, M. A., Siliciano, R. F. & Reinherz, E. L. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 8646–8650.
14. Ward, E. S. (1992) *J. Mol. Biol.* **224,** 885–890.
15. Soo Hoo, W. F., Lacy, M. J., Denzin, L. K., Voss, E. W., Jr., Hardman, K. D. & Kranz, D. M. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 4759–4763.
16. Kurucz, I., Jost, C. R., George, A. J. T., Andrew, S. M. & Segal, D. M. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 3830–3834.
17. Hilyard, K. L., Reyburn, H., Chung, S., Garboczi, D. N., Bell, J. I. & Strominger, J. L. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 9057–9061.
18. Wucherpfennig, K. W., Zhang, J., Witek, C., Matsui, M., Modabber, Y., Ota, K. & Hafler, D. A. (1994) *J. Immunol.* **152,** 5581–5592.
19. White, J., Blackman, M., Bill, J., Kappler, J., Marrack, P., Gold, D. & Born, W. (1989) *J. Immunol.* **143,** 1822–1828.
20. Choi, Y., Kotzin, B., Herron, L., Callahan, J., Marrack, P. & Kappler, J. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8941–8945.
21. Friedman, S. M., Crow, M. K., Tumang, J. R., Tumang, M., Xu, Y., Hodtsev, A. S., Cole, B. C. & Posnett, D. N. (1991) *J. Exp. Med.* **174,** 891–900.
22. Henry, L., Tian, W. T., Rittershaus, C., Ko, J. L., Marsh, H. C., Jr., & Ip, S. H. (1990) *Hybridoma* **8,** 577–588.
23. Brenner, M. B., Mclean, J., Scheft, H., Warnke, R. A., Jones, N. & Strominger, J. L. (1987) *J. Immunol.* **138,** 1502–1509.
24. Devaux, B., Bjorkman, P. J., Stevenson, C., Greil, W., Elliott, J. F., Sagerstrom, C., Clayberger, C., Krensky, A. M. & Davis, M. M. (1991) *Eur. J. Immunol.* **21,** 2111–2119.
25. Chung, S. & Perry, R. P. (1993) *Nucleic Acids Res.* **21,** 3301–3308.
26. Irving, B. A. & Weiss, A. (1991) *Cell* **64,** 891–898.
27. Romeo, C. & Seed, B. (1991) *Cell* **64,** 1037–1045.
28. Clackson, T., Hoogenboom, H. R., Griffiths, A. D. & Winter, G. (1991) *Science* **254,** 624–628.
29. Irwin, M. J., Hudson, K. P., Ames, K. T., Fraser, J. D. & Gascoigne, N. R. (1993) *Immunol. Rev.* **131,** 61–78.
30. Choi, Y., Herman, A., Digiusto, D., Wade, T., Marrack, P. & Kappler, J. (1990) *Nature (London)* **346,** 471–473.
31. McCafferty, J., Griffiths, A. D., Winter, G. & Chiswell, D. J. (1990) *Nature (London)* **348,** 552–554.
32. Ward, E. S. (1991) *Scand. J. Immunol.* **34,** 215–220.