First publ. in: Proceedings of the National Academy of Sciences of the United States of America : PNAS ; 92 (1995), 7. - pp. 3004-3008

# Immunoregulatory activity of a T-cell receptor α chain demonstrated by *in vitro* transcription and translation

**(immune regulation/T-cell suppressor factor)**

TAKEBUMI ONDA*, THOMAS BRUNNER*, HELEN MESSIER†, REID BISSONNETTE*, FERNANDO ECHEVERRI*, GOTTFRIED BAIER‡, ARUN FOTEDAR†, AND DOUGLAS R. GREEN*§

Divisions of *Cellular Immunology and †Molecular Biology, and ‡Cell Biology, La Jolla Institute for Allergy and Immunology, 11149 North Torrey Pines Road, La Jolla, CA 92037

*Communicated by Kimishige Ishizaka, La Jolla Institute for Allergy and Immunology, La Jolla, CA, November 17, 1994*

**ABSTRACT** **Previous studies from our laboratory and those of others suggested the possibility that the T-cell antigen receptor α (TCRα) chain from some T cells can be released in a soluble form and can have antigen-specific immunoregulatory activity. We have analyzed this phenomenon by *in vitro* transcription and translation (IVTT) of a cDNA encoding a TCRα chain (A1.1 TCRα) suspected of having such activity. We found that TCRα, but not TCRβ, protein produced in this way showed antigen-specific regulatory activity in an *in vitro* immune-response assay. Protein derived from truncated forms of the A1.1 TCRα cDNA had activity providing that, in addition to the variable (V) and joining (J) regions of the α chain (VJα), at least the first 25 amino acids of the α chain of the constant (C) region (Cα) were present. Addition of an irrelevant protein sequence to the VJα failed to impart activity to the molecule, suggesting that the Cα requirement is not simply for stabilization of the resulting protein. These results are discussed in the context of other recent studies on the immunoregulatory activity of soluble TCRα molecules, and the possible physiological relevance of these observations is considered.**

By the late 1960s it was clear to most cellular immunologists that T cells are crucial in the immune response as effector cells in cell-mediated responses and as helper cells in both humoral and cell-mediated responses. It was at about this time that an additional immunologic role for T cells was proposed: the suppression of immune responses. Since the original descriptions by Gershon and Kondo (reviewed in ref. 1), T cells capable of suppressing immune responses have been described in a wide range of systems. One problem that arose in this regard was that the effects were all attributed to "suppressor T cells," implying that there was only one mechanism (and cell type) that could produce such effects. We now know that there are a number of ways in which T cells can inhibit immune responses (e.g., see refs. 1 and 2).

Most T lymphocytes bear T-cell antigen receptors (TCRs) composed of an α and a β chain (TCRα and TCRβ), and it is this receptor that is responsible for recognition of a complex ligand composed of an antigen peptide held in a major histocompatibility molecule on antigen-presenting cells (3). Upon recognition of this specific ligand, $CD4^+$ T cells respond by the production of cytokines that are often capable of inhibiting some types of immune responses (1, 4). This mechanism of antigen-specific T cell-mediated suppression is well established.

A far more controversial mechanism of T cell-mediated immunosuppression involves antigen-specific T cell factors (reviewed in refs. 5–9) that bind antigen and produce an immunoregulatory effect. T-cell hybridomas producing such antigen-specific regulatory molecules were generated and analyzed, and attempts to characterize the antigen-specific factors were made. However, a failure to consistently detect rearrangement of the TCRβ chain genes in such hybridomas (10, 11) contributed to skepticism regarding the existence of such T cells and factors (12). The issue was further confused by the observation that suppressor T-cell hybridomas and transformed T-cell lines rearrange and express TCRα genes (13, 14). Therefore, proposals to the effect that suppressor T cells use a novel T-cell receptor (15) were not substantiated.

Progress has nevertheless been made towards resolving the paradox posed by T cell-derived, antigen-specific immunoregulatory factors. Several laboratories have found that such factors bear determinants recognized by anti-TCRα antibody (16–19), and the molecule resolves as having a molecular mass of 46 kDa, as does TCRα (19). TCRα cDNA from cells capable of producing antigen-specific factors transfers the ability to make such factors when expressed in other T cells (20, 21). Thus, some TCRα molecules appear to have antigen-specific immunoregulatory activity and can apparently exist in a cell-free form, probably in a complex with other molecules. Charged residues within the TCRα transmembrane region permit the molecule to exist in either a membrane form (stabilized by other molecules) or a soluble form (22) (again, probably stabilized by other molecules).

Previously, we reported that a helper T-cell hybridoma, A1.1, specific for a synthetic polypeptide antigen called "poly 18"—poly[Glu-Tyr-Lys-(Glu-Tyr-Ala)$_5$]—plus I-$A^d$ constitutively releases a poly 18-specific immunoregulatory activity detected in an *in vitro* assay (23). An examination of the antigenic fine specificity of this cell-free activity revealed a correspondence to the specificity of the A1.1 TCR (19, 23), and the activity was bound and eluted from an anti-TCRα antibody (19). A relationship with the TCR was further established by the use of antisense oligodeoxynucleotides corresponding to the TCR variable region α chain (Vα) of A1.1 (24). Finally, we found that expression of A1.1 TCRα cDNA in other T-cell hybridomas conferred the ability to produce the soluble, antigen-specific factor (20). These data strongly implicate the A1.1 TCRα molecule as a central component of the soluble antigen-specific immunoregulatory activity from A1.1. Here we examine the immunoregulatory activities of A1.1 TCRα molecules produced by *in vitro* transcription and translation (IVTT).

## MATERIALS AND METHODS

**Peptides and Reagents.** Sheep red blood cells (SRBC) were purchased from Colorado Serum (Denver). Peptides based on

Abbreviations: TCR, T-cell antigen receptor; IVTT, *in vitro* transcription and translation; poly 18, poly[Glu-Tyr-Lys-(Glu-Tyr-Ala)$_5$]; SRBC, sheep red blood cells; cpVIII, phage coat protein VIII; C, constant, V, variable; J, joining.

§To whom reprint requests should be addressed.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

the nonrandom synthetic polypeptide poly 18 were kindly provided by Bhagirath Singh (University of Western Ontario) or by Kirin Pharmaceuticals (Maeabashi, Japan). Peptides were coupled to SRBC by using chromic chloride as described (19, 23).

**TCR Expression.** TCR$\alpha$ and TCR$\beta$ cDNA derived from A1.1 were cloned into Bluescript KS (Strategene). Truncations in TCR$\alpha$ cDNA were generated by digestion with *Xba* I plus *Bst* E II, *Nco* I, or *Pfl*MI; alternatively, truncated forms of TCR$\alpha$ cDNA were generated by polymerase chain reaction with a 5′ primer corresponding to the translation start (in boldface letters): 5′-GAAGAGGGATCC **ATG AAA TCC TTG AGT**-3′. The following 3′ primers were all 5′ to 3′: J (ending at the J/C junction, in boldface letters), **ACG TGC ATT CCA AAC** TAAGAAATTCAACCCA; $C_{10}$ (ending after 10 codons of $C_\alpha$), **GAA CCT GCT GTG TAC** TAAGAATTC-GATCCT; $C_{20}$, **CGG TCT CAG GAC AGC** TAAGAATTC-CTGTTC; $C_{25}$, **ACC CTC TGC CTG TTC** TAAGAATTC-GACTCC; $C_{30}$, **ACC GAC TTT GAC TCC** TAAGAATTCGT-GCCG; and $C_{40}$, **AAA ACC ATG GAA TCT** TAAGAA-TTCATCACT. cDNA (0.05 $\mu$g) was used as template in each PCR amplification on a TwinBlock thermal cycler (Ericomp, San Diego). The mixture consisted of 60 pmol of each primer, dNTPs (200 $\mu$M each), and 100 $\mu$l of *Taq* polymerase buffer (Promega) containing 1.5 mM $MgCl_2$ and 5 units of *Taq* polymerase. After 25 cycles (1 min at 94°C; 1 min at 30°C; and 1 min at 72°C), PCR products were hydrolyzed with *Bam*HI and *Eco*RI and cloned into Bluescript KS. Generation of pComp8-TCR$\alpha$ constructs will be described in detail elsewhere (T.O., D. Laface, G.B., T.B., N. Honma, T. Mikayama, A. Altman, and D.R.G., unpublished data). Briefly, A1.1 $VJ_\alpha$ or $VJC_\alpha$ (where J and C = joining and constant regions) was amplified by PCR and cloned into the pComb8 vector (25) (provided by R. Lerner of Scripps) to produce a fusion protein composed of the TCR$\alpha$ region and coat protein VIII (26). The inserts were cloned into Bluescript SK (Stratagene) for *in vitro* expression. In all cases, RNA was expressed from the phage T7 promoter of Bluescript, and protein was translated using TNT T7-coupled reticulocyte lysate system (Promega). [$^{35}$S]Methionine (DuPont) was added to the reactions, and protein was resolved by SDS/PAGE.

***In Vitro* Assay for Antigen-Specific Immunoregulatory Activity.** "Accessory supernatants" required for A1.1 suppressor activity analysis were prepared as described (19, 23). Briefly, C57BL/6 mice were immunized twice with 0.2 ml of 20% SRBC, and immunoglobulin-free splenocytes ($1 \times 10^7$ cells per ml) were then cultured in RPMI 1640 medium with 10% (vol/vol) FCS. After 48 hr the supernatants were collected, and the material was then extensively absorbed with SRBC at 4°C. To assay for antigen-specific suppressor activity of TCR$\alpha$ or supernatants of A1.1, a simple antigen-specific system was employed as previously described (19, 23). Spleen cells ($1 \times 10^7$) from C57BL/6 mice were placed into 1-ml cultures in RPMI 1640 medium supplemented with 10% FCS and 50 $\mu$M 2-mercaptoethanol. Each culture received 50 $\mu$l of 1% peptide-coupled SRBC. TCR$\alpha$ (diluted 1:1000) with or without accessory supernatant was added to the cultures and incubated at 37°C in humidified 92% air/8% $CO_2$. Anti-SRBC plaque-forming cells (without peptides) were assessed 5 days later. In no case was any suppressive effect observed in the absence of accessory supernatant (not shown). All values (controls and experimental) reported in this paper are from cultures containing accessory supernatant.

**Objectivity of Results.** Assessment of plaque-forming cells is visual and thus necessarily subjective; therefore, the majority of the experiments described in this paper were performed under masked conditions. For antigen-specificity controls, peptides or saline were coded prior to coupling to SRBC. For experiments with truncated or chimeric TCR$\alpha$ molecules, the products of the IVTT (including negative controls) were coded prior to addition to the assay culture.

**Binding of TCR$\alpha$ to Peptide-Coupled SRBC.** Fifty microliters of 10% peptide-coupled SRBC (see above) was mixed with 8 $\mu$l of [$^{35}$S]methionine-labeled, full-length A1.1 TCR$\alpha$ and incubated for 2 hr at room temperature with shaking. Cells were then washed, and cell pellets were directly lysed in reducing SDS sample buffer. Samples were incubated for 5 min at 95°C prior to separation on a SDS/PAGE 10–20% gradient gel (Bio-Rad).

## RESULTS

**IVTT A1.1 TCR$\alpha$ Displays Antigen-Specific Immunoregulatory Activity.** Proteins produced *in vitro* from A1.1 TCR$\alpha$ or TCR$\beta$ cDNA were assessed by SDS/PAGE as shown in Fig. 1 *Upper*. We examined the ability of these proteins to regulate an *in vitro* immune response. A1.1 TCR$\alpha$ displayed the antigen-specific immunoregulatory activity we had previously observed in supernatants of A1.1 cells (19), whereas A1.1 TCR$\beta$ did not (Fig. 1 *Lower*). The antigen-specificity of this material was then tested in a series of experiments with antigenic peptides related to the poly 18 antigen. We observed (Fig. 2 *Upper*) that peptides substituted by alanine at residue 3 or residue 10 failed to act as targets for the TCR$\alpha$ immunoregulatory activity, whereas unsubstituted peptides did. This is the pattern of specificity previously observed for both the A1.1 TCR and for the soluble immunoregulatory activity (19, 20). Radiolabeled A1.1 TCR$\alpha$ was bound by SRBC coupled with the antigenic peptide but not with peptide substituted by alanine at residue 10 (Fig. 2 *Lower*).

This direct binding to antigen was also detected in our bioassay by two methods. First, we found that the antigen-coupled SRBC that were incubated with A1.1 TCR$\alpha$ carried the suppressive activity into the bioassay (i.e., the anti-SRBC response to these was inhibited) (results not shown). Second, we found that free peptide was capable of interfering with the inhibition of the anti-SRBC response in a dose-dependent

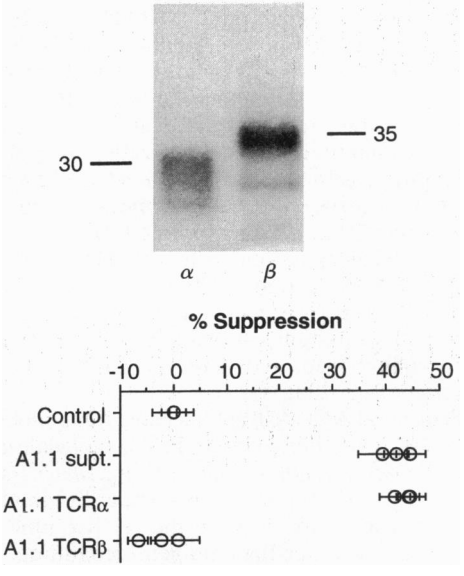


FIG. 1. Activity of A1.1 TCR$\alpha$ and $\beta$ chains. Full-length cDNA encoding the TCR$\alpha$ and TCR$\beta$ chains from the A1.1 T cell hybridoma (20) was transcribed and translated *in vitro* (± [$^{35}$S]methionine), and the activity was examined in an *in vitro* assay of antigen-specific immunoregulation. (*Upper*) SDS/PAGE resolution of [$^{35}$S]methionine-labeled proteins. (*Lower*) Immunoregulatory effect of supernatants from A1.1 versus TCR proteins produced by IVTT. The assay system is described in text. Results are from three experiments (without coding). Size is shown in kDa.

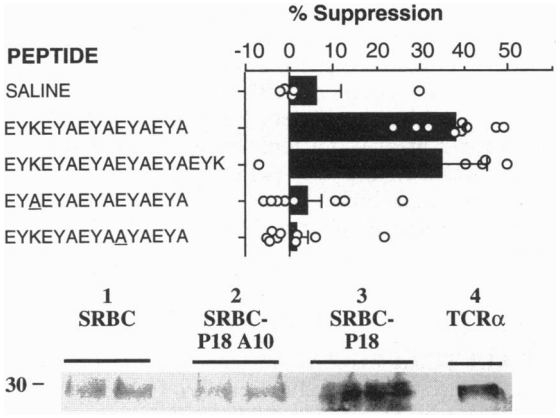


FIG. 2. Antigenic fine-specificity of the immunoregulatory effect and antigen binding of IVTT A1.1 TCR$\alpha$. (*Upper*) The activity of the A1.1 TCR$\alpha$ chain was examined in our *in vitro* assay of antigen-specific immunoregulation. In each experiment, four or more of the indicated peptides (or saline) were coded and coupled to SRBC for the assay culture. All results from nine independent experiments are shown as open circles. Means and SEM from all experiments are shown as bars. (*Lower*) [$^{35}$S]Methionine-labeled TCR$\alpha$ was incubated with peptide-coupled SRBC and washed extensively. The SRBC were then lysed, and the bound protein was resolved by SDS/PAGE. Each condition was performed in duplicate. Lanes 1:, unconjugated SRBC; 2, SRBC coupled with nonantigenic peptide based on poly 18 and substituted with alanine at residue 10 (P18 A10; Glu-Tyr-Lys-(Glu-Tyr-Ala)$_2$-Ala-Tyr-Ala-Glu-Tyr-Ala); 3, SRBC coupled with antigenic peptide based on poly 18 (P18; Glu-Tyr-Lys-(Glu-Tyr-Ala)$_4$-Glu-Tyr-Lys); 4, TCR$\alpha$ directly loaded into gel.

fashion (≈60% inhibition was reduced to 37% by 10 $\mu$g of free peptide per ml and to 2% by 50 $\mu$g/ml) (data not shown).

To determine the amount of A1.1 TCR$\alpha$ that was functional in our system, RNA IVTT was performed in the presence of [$^{35}$S]methionine (0.52 mCi/ml; 1 Ci = 37 GBq) as the only source of methionine; the specific activity on the date used was 646 Ci/mmol. Empirically, we determined that 1 cpm = $0.3 \times 10^{-8}$ mCi. A sample of protein (equivalent to 18.75 $\mu$l of the translated material) was run on SDS/PAGE; the gel was cut, and total radioactivity was determined to be $5.8 \times 10^5$ cpm (essentially all of the cpm was found in a single protein band as expected). Based on this activity of the TCR$\alpha$ protein, the number of methionine residues (nine), and the specific activity of the label, we quantitated the concentration of TCR$\alpha$ protein in the preparation as 16 nM. This was then tested for biological activity in our assay system at known concentrations of TCR$\alpha$. We have assumed that the incorporated radiolabel does not affect bioactivity, an assumption supported by similar titration curves with several different unlabeled TCR$\alpha$ *in vitro* preparations (not shown). Approximately half maximal inhibitory activity in our assay system was observed at 1 pM TCR$\alpha$. In all of the experiments reported here, TCR$\alpha$ was present at a concentration of 10–100 pM.

**Some Truncated A1.1 TCR$\alpha$ Molecules Have Immunoregulatory Activity.** The ability of the TCR$\alpha$ molecule to inhibit immune responses at very low concentrations *in vitro* suggested that this activity is not due to simple competitive inhibition for antigen. The alternative is that the TCR$\alpha$ molecule has biological functions other than antigen recognition, and if so then we would expect such a function to map to discreet regions of the molecule. Presumably, these would lie within the C region or the V framework regions that are conserved among TCR$\alpha$ molecules.

We generated truncated forms of the A1.1 TCR$\alpha$ molecule, as shown schematically in Fig. 3 *Top*. Constructs encoding truncations at *Bst*EII, *Nco* I, and *Pfl*MI were produced, and the resulting *in vitro* translated proteins are shown in Figure 3 *Middle*. When tested for bioactivity, we found that TCR$\alpha$

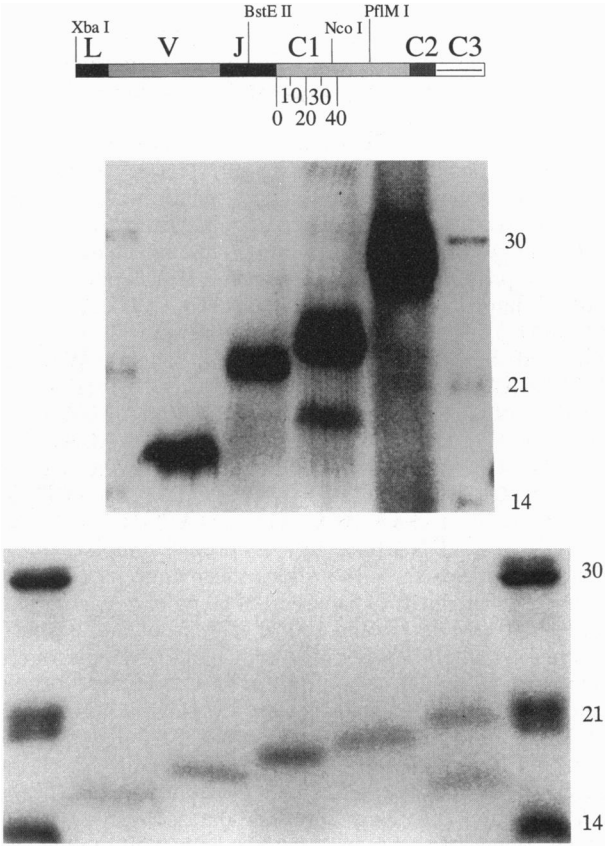


FIG. 3. Truncated A1.1 TCR$\alpha$ polypeptides. (*Top*) A1.1 TCR$\alpha$ cDNA showing relevant regions and domains, restriction sites, and PCR-generated truncations. (*Middle*) The A1.1 TCR$\alpha$ cDNA was truncated at the indicated restriction sites (see *Top*) and cloned into Bluescript for IVTT in the presence of [$^{35}$S]methionine. The resulting protein products were resolved by SDS/PAGE and in each case were of the expected relative molecular mass in kDa. Lanes represent (from left to right) protein produced from the *Xba* I–*Bst*EII fragment, *Xba* I–*Nco* I fragment, and *Xba* I–*Pfl*MI fragment. (*Bottom*) A1.1 TCR$\alpha$ fragments extending from the leader sequence to sites in C$\alpha$ (see *Top*) were generated by PCR and cloned into Bluescript for IVTT in the presence of [$^{35}$S]methionine. The resulting protein products were resolved by SDS/PAGE, and in each case were of the expected relative molecular mass in kDa. Lanes represent (from left to right) size markers, protein produced from VJ, VJC10, VJC20, VJC30, and VJC40, and size markers.

truncated at *Nco* I or *Pfl*MI showed activity, whereas TCR$\alpha$ truncated at *Bst*EII did not (Fig. 4 *Top*). We then constructed additional truncations by PCR, and the proteins resulting from these forms, VJ–VJC40, are shown in Fig. 3 *Bottom*. The bioactivity of these truncated proteins was tested in a series of experiments shown in Fig. 4 *Middle*. We found that in order for the A1.1 TCR$\alpha$ molecule to function in our bioassay, at least 30 amino acids of the C$\alpha$1 domain were required in addition to the VJ regions. TCR$\alpha$ molecules including only the first 20 amino acids of C$\alpha$ consistently failed to function. We then generated a TCR$\alpha$ molecule containing the VJ regions plus the first 25 amino acids of C$\alpha$. This construct yielded a protein of the expected size after IVTT (not shown). As shown in Fig. 4 *Bottom*, it also displayed biological activity in our assay system.

**The Requirement for a Portion of TCR C$\alpha$1 Domain Is Probably Not Due To a Simple Structural Effect.** We engineered chimeric molecules composed of VJ$\alpha$ ± C$\alpha$ in tandem with a phage coat protein, cpVIII (25, 26). The IVTT chimeric proteins were of the predicted sizes, ≈25 kDa for VJ$\alpha$-cpVIII and ≈36 kDa for VJC$\alpha$-cpVIII (Fig. 5 *Upper*). When tested in

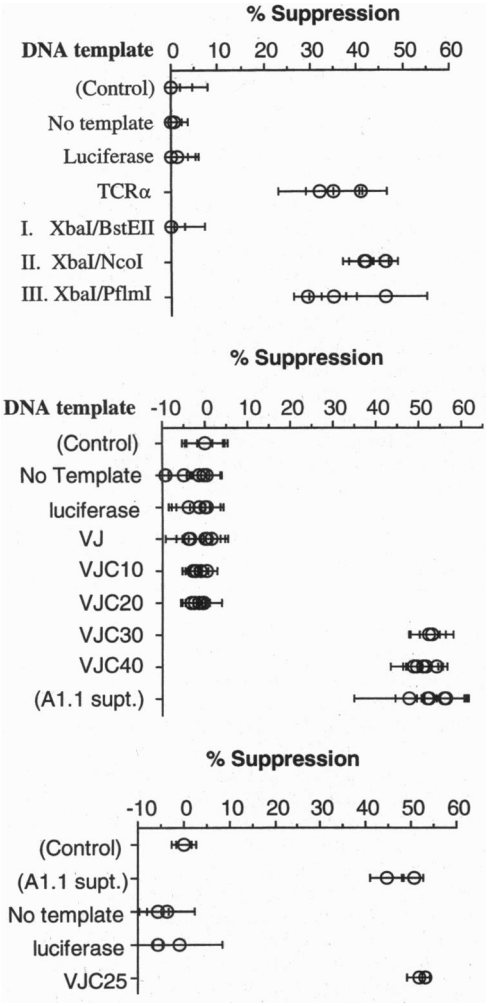


FIG. 4. Immunoregulatory activity of truncated TCRα. (*Top*) Regulatory activity of the truncated TCRα proteins generated as in Fig. 3 *Middle* was tested in our bioassay. All results from three experiments are shown. In each experiment, all products of the IVTT reactions (including no template, luciferase cDNA template, full-length or truncated TCRα) were masked prior to use in the experiment. (*Middle*) Regulatory activity of the truncated TCRα proteins generated as in Fig. 3 *Bottom* was examined. All results from five experiments are shown. In each experiment, all products of the IVTT reactions (including no template, luciferase cDNA template, full-length or truncated TCRα) were masked prior to use in the experiment. (*Bottom*) Activity of TCRα truncated at C25. A TCRα protein extending from the leader to C25 was generated by PCR and IVTT, and the resulting protein resolved at the expected relative molecular mass (not shown). Immunoregulatory activity was assessed as in *Top* and *Middle*, with the indicated translation products masked prior to addition to the culture.

the *in vitro* assay system, the chimeric protein composed of VJCα plus cpVIII displayed function, while that composed of only VJα plus cpVIII did not (Fig. 5 *Lower*). Thus, the addition of an irrelevant protein sequence to VJα failed to impart biological activity.

## DISCUSSION

The ability of IVTT TCRα to regulate immune responses is consistent with older studies on antigen-specific T-cell factors. Several different groups reported that *in vitro* translation of mRNA from T-cell hybridomas producing such factors yielded biologically active, antigen-specific molecules (refs. 27–30; also H. Zheng and D.R.G., unpublished observations). Here we have shown that the A1.1 TCRα protein displays antigen-

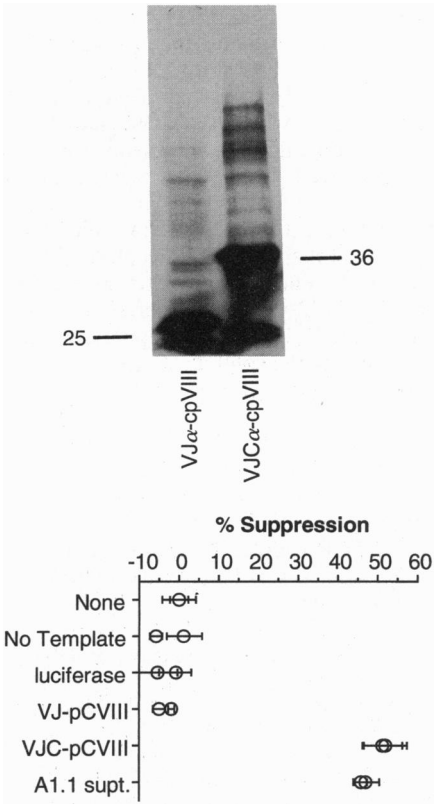


FIG. 5. Characterization and function of TCRα-pCVIII chimeric proteins. Chimeric proteins of A1.1 TCR VJα or VJCα (extending to the start of the transmembrane region) and phage coat protein VIII (26) were generated as outlined in text. (*Upper*) Resolution of [$^{35}$S]methionine-labeled chimeric proteins by SDS/PAGE. Proteins resolved at the expected relative molecule mass in kDa. (*Lower*) Function of chimeric proteins assessed in our *in vitro* assay system. In each experiment, all translation products (including negative and positive controls) were coded.

specific binding and immunoregulatory activity. This direct binding appears to be a requirement for the inhibitory effect, since peptides that are not bound by the TCRα (Fig. 2 *Lower*) do not function as targets in the bioassay (Fig. 2 *Upper*), and free antigenic peptide can compete with the SRBC-bound peptides and thus block the inhibition of the anti-SRBC response in this system (results not shown but described above).

We found that in addition to VJα, the first 25 amino acids of the C region were necessary for the expressed protein to have function (Fig. 4). This requirement for a portion of Cα probably does not represent a simple need for protein stability. Chimeric proteins generated by fusion of TCRα with the cpVIII protein of filamentous phage were functional in our assay system only when the Cα region was present (Fig. 5 *Lower*). However, we have found that phage displaying chimeric proteins composed of either A1.1 TCR VJα or VJCα plus CPVIII (as in Fig. 4) yield phage that bind equivalently to the specific peptides (T.O., D. Laface, G.B., T.B., N. Honma, T. Mikayama, A. Altman, and D.R.G., unpublished data). Since VJα-cpVIII binds peptide but does not function in our assay system and VJCα-cpVIII does both, it is unlikely that the role of the Cα region in the biological activity we observe is simply to stabilize VJα.

An interesting possibility is that the Cα is required for interaction with other proteins to mediate antigen-specific effects. Research from several laboratories suggests that such interactions with molecules likely to be TCRα are a common feature of antigen-specific immunoregulation (16–21, 31). For example, Iwata *et al.* (16) described an antigen-specific immu-

noregulatory factor composed of a molecule bearing a TCRα determinant complexed with an antigen-nonspecific cytokine-like activity, glycosylation inhibiting factor (GIF). Similarly, Bristow and Flood (31) described a molecule bearing a TCRα determinant complexed with an elastase activity.

Such an interaction might be important for release of the TCRα molecule as well. The TCRα transmembrane region, when substituted for the transmembrane region of CD4, results in secretion of a soluble CD4 molecule from cells (21). In the absence of stabilizing CD3 molecules, TCRα is released into the lumen of the endoplasmic reticulum but is degraded there or in the Golgi (22, 32). Nevertheless, TCRα is produced in excess in T cells (32), and it is thus possible that additional molecules can stabilize the soluble TCRα molecules and allow their secretion from the cell.

It remains unclear whether immune regulation by TCRα represents an important physiological mechanism. Although our work relies entirely upon *in vitro* assays, other laboratories have provided support for the idea that TCRα molecules can participate in antigen-specific regulation *in vivo* (17, 21, 33). Whether soluble TCRα molecules can be identified in serum (e.g., see ref. 34) and whether they have important biological functions should be determined.


1. Bloom, B. R., Modlin, R. L. & Salgame, P. (1992) *Annu. Rev. Immunol.* **10,** 453–488.
2. Golub, E. S. & Green, D. R. (1991) *Immunology: A Synthesis* (Sinauer, Sunderland, MA), pp. 494–514.
3. Jorgensen, J. L., Reay, P. A., Ehrich, E. W. & Davis, M. M. (1992) *Annu. Rev. Immunol.* **10,** 835–873.
4. Mosmann, T. R. & Coffman, R. L. (1989) *Annu. Rev. Immunol.* **7,** 145–173.
5. Monroe, J. G., Lowy, A., Granstein, R. D. & Greene, M. I. (1984) *Immunol. Rev.* **80,** 103–131.
6. Murphy, D. B. (1993) *Curr. Opin. Immunol.* **5,** 411–417.
7. Green, D. R. & Zheng, H. (1989) *Ann. Inst. Pasteur* **140,** 294–298.
8. Asherson, G. L., Colizzi, V. & Zembala, M. (1986) *Annu. Rev. Immunol.* **4,** 37–68.
9. Dorf, M. E., Kuchroo, V. K. & Collins, M. (1992) *Immunol. Today* **13,** 241–243.
10. Kronenberg, M., Goverman, J., Haars, R., Malissen, M., Kraig, E., Phillips, L., Delovitch, T., Suciu-Foca, N. & Hood, L. (1985) *Nature (London)* **313,** 647–653.
11. Hedrick, S. M., Germain, R. N., Bevan, M. J., Dorf, M., Engel, I., Fink, P., Gascoinge, N., Heber-Katz, E., Kapp, J., Kaufman, Y., Kaye, J., Melchers, F., Pierce, C., Schwartz, R. H., Sorensen, C., Taniguchi, M. & Davis, M. M. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 531–538.
12. Moller, G. (1988) *Scand. J. Immunol.* **27,** 247–250.
13. DeSantis, R., Givol, D., Hsu, P., Adorini, L., Doria, G. & Appella, E. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 8638–8642.
14. Imai, K., Kanno, M., Kimoto, H., Shigemoto, K., Yamamoto, S. & Taniguchi, M. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 8708–8712.
15. Janeway, C. A. (1988) *Scand. J. Immunol.* **27,** 621–628.
16. Iwata, M., Katamura, K., Kubo, R. T., Grey, H. & Ishizaka, K. (1989) *J. Immunol.* **143,** 3917–3924.
17. Fairchild, R. L., Kubo, R. T. & Moorhead, J. W. (1990) *J. Immunol.* **145,** 2001–2009.
18. Kuchroo, V. K., Steele, J. K., O'Hara, R. M., Jayaraman, S., Selvaraj, P., Greenfield, E., Kubo, R. T. & Dorf, M. E. (1990) *J. Immunol.* **145,** 438–448.
19. Bissonnette, R. P., Zheng, H., Kubo, R. T., Singh, B. & Green, D. R. (1991) *J. Immunol.* **146,** 2898–2907.
20. Green, D. R., Bissonnette, R., Zheng, H., Onda, T., Echeverri, F., Mogil, R. J., Steele, J. K., Voralia, M. & Fotedar, A. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 8475–8479.
21. Kuchroo, V. K., Byrne, M. C., Atsumi, Y., Greenfield, E., Connolly, J. B., Whitters, M. J., O'Hara, R. M., Collins, M. & Dorf, M. E. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 8700–8704.
22. Shin, J., Lee, S. & Strominger, J. L. (1993) *Science* **259,** 1901–1904.
23. Zheng, H., Boyer, M., Fotedar, A., Singh, B. & Green, D. R. (1988) *J. Immunol.* **140,** 1351–1358.
24. Zheng, H., Sahai, B. M., Kilgannon, P., Fotedar, A. & Green, D. R. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 3758–3762.
25. Greenwood, J., Willis, A. E. & Perham, R. N. (1991) *J. Mol. Biol.* **220,** 821–827.
26. Barbas, C. F., Kang, A. S., Lerner, R. A. & Benkovic, S. J. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 4363–4366.
27. Taniguchi, M., Tokuhisa, T., Tanno, M., Yaoita, Y., Shimizu, A. & Honjo, T. (1982) *Nature (London)* **298,** 172–174.
28. Wieder, K. J., Araneo, B. A., Kapp, J. A. & Webb, D. R. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 3599–3603.
29. Beaman, K. D., Ruddle, N. H., Bothwell, A. L. M. & Cone, R. E. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1524–1528.
30. Ricciardi-Castagnoli, P., Robbiati, F., Barbanti, E., Colizzi, V., Pini, C., DeSantis, R., Doria, G. & Adorini, L. (1985) *Eur. J. Immunol.* **15,** 351–355.
31. Bristow, C. L. & Flood, P. M. (1993) *Int. Immunol.* **5,** 79–88.
32. Bonifacino, J. S., Cosson, P. & Klausner, R. D. (1990) *Cell* **63,** 503–513.
33. Yamaguchi, K., Mori, A., Ohno, H., Tagaya, Y. & Ishizaka, K. (1992) *Int. Immunol.* **4,** 337–346.
34. Urbanski, M. & Cone, R. E. (1992) *J. Immunol.* **148,** 2840–2844.