T-cell receptor α chain plays a critical role in antigen-specific suppressor cell function

(suppressor factor/T-cell receptor mutants/immunoregulation/T-cell hybridomas)

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ABSTRACT Antigen-specific suppressor T-cell hybridomas release soluble suppressor factors (TsF) in the supernatant that modulate both in vivo delayed-type hypersensitivity and in vitro plaque-forming cell responses in an antigen-specific manner. To study the relationship between the T-cell receptor (TcR) and TsF, we developed a series of TcR α⁺ or TcR β⁻ expression variants from suppressor T-cell hybridomas that expressed the CD3–TcR α/β complex. We demonstrate that loss of TcR α but not TcR β mRNA was accompanied by the concomitant loss of suppressor bioactivity. Homologous transfection of TcR α cDNA into a TcR α⁺ β⁻ clone reconstituted both CD3–TcR expression and suppressor function. Furthermore, suppressor activity from TcR β⁻ variants was specifically absorbed by antigen and anti-TcR α antibodies, but not by anti-CD3 or anti-TcR β affinity columns. These data directly establish a role for the TcR α chain in suppressor T-cell function and suggest that the TcR α chain is part of the antigen-specific TsF molecule.

The T-cell antigen receptors (TcR) on helper and cytotoxic T cells are CD3-associated α/β heterodimers. Initially, donor-derived TcR β-chain rearrangements were not detected in antigen-specific suppressor T-cell (Ts) hybridomas (1, 2). Subsequent analyses of Ts hybridomas indicated that Ts do express TcR α-chain mRNA derived from the Ts donor (3, 4) and that some express the CD3–TcR α/β complex on their surface (5–11). Selection of Ts hybridomas for expression of surface CD3 resulted in an increase in antigen binding (11) and suppressive bioactivity (11, 12). Molecular analysis of three 4-hydroxy-3-nitrophenyl acetate (NP)-specific suppressor effectors (Ts5) hybridomas indicated that each expressed a donor-derived TcR α chain and a TcR β chain derived from the BW5147 fusion partner (4). The absence of a full-length β-chain transcript and the presence of TcR α-chain determinants on the suppressor factor (TsF) from one of these lines suggested that the TcR β chain was not necessary for Ts function and that the TcR α chain might form part of the TsF (4). Data from several independent antigen-specific suppressor systems support this conclusion (9, 10, 13, 14). Here we examine the effects of loss of TcR α- and β-chain expression on TsF bioactivity and use cDNA transfection to demonstrate that TcR α chain is required for Ts function.

MATERIALS AND METHODS

Animals. Mice were purchased from The Jackson Laboratory or were bred at Harvard Medical School.

Antigen. NP-O-succinimide (NP-O-Su) was purchased from Lynx Technologies (San Rafael, CA), p-azobenzene-

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arsionate (ABA), trimethylammonia (TMA), and hapten conjugates were provided by S. Jayaraman.

Antibodies. Hamster anti-CD3 antibody (145-2C11) and anti-Ly6C (144-4B11) were obtained from J. Bluestone (University of Chicago). The anti-TcR α (H28.710) and anti-TcR β (H57.597) hybridomas were a gift from R. Kubo (National Jewish Center for Immunology, Denver). Hybridoma cell lines secreting rat anti-TcR-β6 antibody (44-22-1) and Vpβ (KJ-16) (V, variable region) were obtained from H. Hengartner (Zurich) and P. Marrack (National Jewish Center for Immunology), respectively. A cell line secreting antibody specific for anti-Thy 1.2 (HO-13-4) was obtained from American Type Culture Collection. Fluorescein isothiocyanate-labeled rabbit anti-hamster, goat anti-rat, and goat anti-mouse antibodies were obtained from Organon Teknika-Cappel.

Ts Hybridomas and Expression Variants. Inducer Ts hybridomas (CKB-TS1–38 and CKB-TS1–81) were obtained by fusion of TcR α⁻ β⁻ BW1100 with tolerized spleen cells from CKB (H2B) mice (10). We generated expression variants from these hybridomas following the method of antigen- or antibody-mediated growth inhibition (10). The Ts hybridomas were incubated at 37°C with antigen (NP–KLH, 100 μg/ml) or anti-CD3 antibody (undiluted culture supernatant) plus H-2 compatible spleen cells (SCs) as a source of antigen presenting cells (APCs). The few surviving cells that grew out after 5–10 days were cloned by limiting dilution. Clones were tested for expression of CD3 and TcR α/β by flow cytometry. Clones that lacked expression of the CD3–TcR complex were further analyzed for expression of TcR α and TcR β mRNA by Northern analysis.

In Vitro Growth Inhibition Assays. For antigen-mediated growth inhibition, 1–2 × 10⁴ hybridoma cells were incubated with 5 × 10⁵ irradiated SCs (as a source of APCs) with various hapten protein conjugates (100 μg/ml) as a source of antigen (10). After 18–20 hr at 37°C, 1 μCi of [³H]thymidine (1 Ci = 37 GBq) was added and cultures were harvested 6 hr later and assayed by standard scintillation techniques. The data are presented as mean cpm incorporated into insoluble DNA of triplicate wells.

Antibody-mediated growth inhibition was performed by binding the antibodies to a 96-well tissue culture plate. Microtiter plates were coated with 0.1 ml of protein A (100 μg/ml) (Sigma) or goat anti-rat antibody (100 μg/ml) (Organon Teknika–Cappel) overnight at 4°C. The plates were washed twice and 100 μl of antibody-containing supernatant was added to each well for 4 hr. After three washings, 1–2 × 10⁵
10^4 hybridoma cells were added in a total vol of 200 μl and incubated at 37°C as described above.

**Flow Cytometry.** Cells were stained with hybridoma culture supernatants with followed by appropriate fluorescein isothiocyanate-labeled secondary antibody as described (11). Samples were analyzed on an Epics 752 cell sorter (Coulter).

**Northern Analysis.** RNA was isolated, electrophoresed into formaldehyde gels, transferred to nitrocellulose, and hybridized at 42°C to 32P-labeled probes (15).

**PCR Analysis.** Double-stranded cDNA was prepared as described (16) and amplified for 25 cycles (1 min at 94°C, 2 min at 51°C, 4 min at 72°C) with Taq polymerase (17) using either a 5′ Vb3 oligonucleotide (5′-GGTTCACGCCAC-TCTCCATAAG-3′) and a 3′ Cb oligonucleotide (C, constant region) (5′-CTCACTGACACACGCTCATG-3′) or a 5′ Vb2 oligonucleotide (5′-ACTATGAACAAGTGGGT1T-TGCGCTGAT-TGATGCTCAACAA-3′). After electrophoresis, the gel was blotted and hybridized simultaneously with 32P-labeled oligonucleotides for the amplified TcR α and β fragments. For each cell line, reactions were carried out using only TcR α or β oligonucleotides on 250 pg of cDNA; dilutions of cDNA contained both pairs of oligonucleotides.

**Transfections.** One TcR α′ expression variant (Δ38.3.G7) was transfected by electroporation (CellZap-II; Andersen Electronics, Brookline, MA; 1250 μF, 200 V) with full-length CKB-Ts1-38 (J.W. and M.C.; sequence available on request) or BW5147 TcR α cDNA in the HindIII site of vector CDMRREN. TcR α-chain cDNA clones were isolated and the DNA sequence was determined as described (4). CDMRREN is a modification of CDM8 (18), made by removing the Xho I fragment (nucleotides 2221–2601) and replacing it with a fragment containing the 5′ untranslated region from the EMC virus (nucleotides 260–834 of the viral genome) linked to neomycin phosphotransferase cDNA (neo). cDNA is expressed in the vector as the 5′ part of a bicistronic mRNA containing neo (19). Stable transfecants were selected in G418 (1.5 mg/ml) (GIBCO). Five clones were obtained after transfection with CKB-Ts1-38 TcR α cDNA, three of which showed suppressor activity in bioassays. Clones 38α1 and 38α5 were selected for further functional analysis. After transfection with BW5147 TcR α cDNA, eight clones grew out. Clone B′Wα5 was used as a control since it expressed the highest percentage (2%) of CD3–TcR-bearing cells.

**Affinity Absorption of TsF.** TsF-containing supernatants were adsorbed by batch methods and eluted with 2 M KCl as described (4, 20).

**Suppression of Delayed-Type Hypersensitivity (DTH) Responses.** Mice were immunized with 200 μg of NP-O-Su or 200 μl of 10 mM ABA (diazonium salt) as described (10). To assay for suppressive bioactivity, mice were injected i.v. with 0.5 ml of conditioned medium on days 0 and 1 after antigen priming. On day 6, mice were challenged in the left footpad with the appropriate hapten and footpad swelling responses were measured by caliper 24 hr later.

**Suppression of Plaque-Forming Cell (PFC) Responses.** TsF1 activity was assayed as described (21). Briefly, naive C3HBL/6 or B10.BR responder spleen cells were immunized in vitro with NP-coupled horse or uncoupled sheep erythrocytes. Conditioned media from Ts were added on day 0 at final dilutions of 1:20 or 1:200. Direct anti-NP or anti-sheep erythrocyte PFCs were detected on day 5 by using a modified Jerne plaque assay (21). All DTH and PFC assays were conducted independently at separate institutions.

**RESULTS**

**Generation of Expression Variants and Transfectants.** We recently derived a series of NP-specific Ts1 hybridomas that share phenotypic properties (Thy1+, CD3+, CD4+, CD8+, Ig−, Mac1+) with the Ts1 cell populations that mediate inducer-phase suppression in cell transfer experiments (10, 22). Among this group of 14 NP-specific, CD4+ hybridomas with donor-derived TcR α/β chains, 3 constitutively released TsF and specifically responded to antigen in the presence of H-2 (IE)-compatible APCs (10). These Ts hybridomas may be related to the IE- or DQ-restricted Ts clones reported in other murine and human systems (6, 23). Two of the Ts1 hybridomas, CKB-Ts1-38 and CKB-Ts1-81, were used to develop TcR expression loss variants. Parental CKB-Ts1-38 cells express TcR-Vβ8, JβL2 and Vβ6, Dβ2, and Jγ2.6 and the parental CKB-Ts1-81 line expresses TcR-Vγ4, JγTA19, and Vγ8.3, Dγ2, and Jγ2.4 (M.J.W. and M.C., unpublished data). Three TcR α−β− (Δ38.3.G5, Δ38.3.G7, and Δ38.3.H4) and two TcR α′β′ (Δ38.4.C2 and Δ38.4.D2) variants were selected by growth inhibition.

Northern analysis (Fig. 1a) shows that the RNA from CKB-Ts1-38 hybrids with Vγ8 and Vβ6 probes, while RNA from the fusion partner BW1100 does not. Lines Δ38.3.G7 and Δ38.3.H4 (plus Δ38.3.G5; data not shown) lack detectable Vγ8 mRNA, whereas Δ38.4.C2 and Δ38.4.D2 show a loss of Vβ6 mRNA. PCR amplifications were used to confirm the loss of expression of TcR α- or β-chain mRNA in the expression variants (Fig. 1b). PCR amplifications were carried out on a series of diluted cDNA samples from CKB-Ts1-38, Δ38.3.G7, or Δ38.4.D2. TcR α chain was amplified by using a 5′ Vb8 oligonucleotide and a 3′ Cb oligonucleotide. Similarly the TcR β chain was amplified by using a 5′ Vb6 oligonucleotide and a 3′ Cb oligonucleotide. Titration of the cDNA used to carry out the PCRs indicated that TcR α or β mRNA expression was reduced by a factor of at least 250 in the TcR α′ expression variant Δ38.3.G7 and the TcR β′ expression variant Δ38.3.D2, respectively, when compared with donor-derived lines (lane a), whereas parental Δ38.3.G7 and Δ38.3.H4 lines (lanes c and h) expressed both TcR α and β. The TcR α′ expression variant Δ38.3.G7 and the TcR β′ expression variant Δ38.3.D2 were expressed at lower levels as indicated by the relative intensity of bands as compared with donor-derived lines.

**FIG. 1.** Expression of TcR α and β mRNA. (a) Northern analysis. Total RNA (20 μg) from either BW1100 (lane A); parental Ts1 hybridoma CKB-Ts1-38 (lane B); expression variants Δ38.4.C2, Δ38.4.D2, Δ38.3.G7, or Δ38.3.H4 (lanes C–F); or Δ38.3.G7 TcR α transfecant 38α5 (lane H) were analyzed by Northern blot with a 32P-labeled Vβ6 probe (HindIII/EcoRI; 200-base fragment), Vγ8 (EcoRI/Pst I; 206-base fragment) probe or a full-length CD3ε probe provided by C. Terhorst (Dana–Farber Cancer Institute, Boston). (b) PCR analysis. Quantitation of TcR α and β mRNA in CKB-Ts1-38 and TcR expression variants. PCR using cDNA from parent CKB-Ts1-38, TcR α′ subclone Δ38.3.G7 or TcR β′ subclone Δ38.4.D2 is shown. PCR mixtures contained 100 ng of oligonucleotides specific for either the Vγ8 (α) or Vβ6 (β) rearrangement or both pairs of oligonucleotides (α + β); 0–250 pg cDNA was used per reaction.
to the parental line (Fig. 1b). Expression variants of CKB-TsF-81 were isolated similarly, and one TcR $\alpha^{-}\beta^{-}$ (Δ38.3.E11) clone and one TcR $\alpha^{-}\beta^{-}$ (Δ38.4.F8) clone were identified by Northern analysis (data not shown).

One of the TcR $\alpha^{-}$ expression variants (Δ38.3.G7) was transfected with a full-length CKB-TsF-38 TcR $\alpha$ cDNA. Two independent transfectants, 38a1 and 38a5, that expressed the CKB-TsF-38 TcR $\alpha$ were selected for detailed analysis. A full-length TcR $\alpha$-chain cDNA from BWS147 (V1,1.1 and J1,1.T11) was transfected into Δ38.3.G7 and one transfectant from this series (BWa5) was included as a control. Δ38.3.G7 transfected with homologous CKB-TsF-38 TcR $\alpha$ cDNA hybridizes with both V$\alpha$8 and V$\gamma$6 probes in transfected 38a5 (Fig. 1a) and transfectant 38a1 (data not shown). The V$\gamma$8 mRNA in these transfectants is expressed as a bicistronic 4.4-kb message and this transcript also hybridizes with a neo probe. A smaller mRNA that contains V$\alpha$8 is also transcribed from this vector and possibly results from differential polyadenylation. The latter sequence contains the complete TcR $\alpha$ coding sequence and can be distinguished from endogenous TcR $\alpha$ mRNA by the presence of 5' vector sequences using RNase protection (data not shown). No endogenous V$\gamma$5 mRNA is detected in the transfectant by RNase protection (data not shown).

**Functional Expression of Antigen Receptors.** Flow cytometry and growth inhibition assays were used to determine whether functional antigen receptors were expressed on the variants and transfected cell lines. The CKB-TsF-38 parental line stained with anti-CD3, anti-TcR-C$\beta$, and anti-V$\gamma$6 (Fig. 2). In contrast, the TcR $\alpha^{-}$ (Δ38.3.G7) and TcR $\beta^{-}$ (Δ38.4.D2) expression variants failed to stain with these reagents. Transfection of Δ38.3.G7 with the CKB-TsF-38-derived TcR $\alpha$ cDNA reconstituted surface expression of the CD3–TcR complex in line 38a5 (Fig. 2). Transfectant 38a1 and BWa5, however, showed little or no detectable CD3–TcR complex on their surface by flow cytometry, although each contained transcripts of the transfected cDNA (data not shown).

To evaluate whether the TcRs transduced antigen-specific signals, we compared growth inhibition of the parental, expression variant, and transfected cell lines with antigen (NP–KLH) or anti-TcR antibodies. The parental and transfected (38a5) cell lines were growth inhibited upon stimulation with either specific antigen (NP–KLH), anti-CD3, anti-TcR-C$\beta$, or anti-V$\gamma$6 antibodies (Table 1). In contrast, neither antigen (NP–KLH) nor anti-CD3–TcR antibodies affected the growth of TcR $\alpha^{-}$ or TcR $\beta^{-}$ expression variants (Table 1). These data indicate that a functional CD3–TcR complex was reconstituted in the transfected cell line (38a5) and that NP recognition is associated with the TcR $\alpha/\beta$ complex.

**TsF Activity from Expression Variants and Transfectants.** Conditioned media from parental CKB-TsF-38, TcR $\alpha^{-}$, or $\beta^{-}$ expression variants and transfectants were tested for suppressive bioactivity. Supernatant containing TsF from the parental CKB-TsF-38 hybridoma suppresses NP responses in both DTH and PFC assays (Table 2). The suppressor activity was antigen specific since DTH responses to the control hapten ABA and PFC responses to control antigen (sheep erythrocyte) were not affected. Three different TcR $\alpha^{-}$ expression variants derived from line CKB-TsF-38 were tested and all lost suppressive activity (Table 2), whereas both TcR $\beta^{-}$ expression variants that retain expression of TcR $\alpha$ mRNA (Δ38.4.C2 and -D2) produced antigen-specific TsF activity. In addition, another TcR $\alpha^{-}\beta^{-}$ expression variant (Δ81.4.F8) from a second NP-specific Ts hybridoma (CKB-TsF-81) also produced antigen-specific TsF activity. Expression variant Δ81.3.E11 lost expression of both TcR chains (data not shown) and also lost the ability to produce TsF. Thus, the loss of TcR $\alpha$ expression correlated with lack of suppressive bioactivity (Table 2).

To prove that the TcR $\alpha$ chain plays a role in antigen-specific suppressor cell function, the ability of TcR $\alpha$ transfectants to produce antigen-specific suppressor activity was examined. Both homologous TcR $\alpha$ transfectants (38a1 and 38a5) specifically suppressed DTH and PFC responses to NP (Table 2). Conditioned medium from control transfrectants, carrying either an empty vector or the BWS147 derived TcR $\alpha$ cDNA (BWa5) did not suppress DTH responses (Table 2), demonstrating the specificity of NP-specific TcR $\alpha$ cDNA in mediating NP-specific suppression.

**Absorption of Suppressor Activity from Expression Variants.** Previous studies have shown that TsF activity obtained in supernatants of Ts cell lines can be absorbed by antigen or anti-TcR $\alpha$ affinity columns (4, 8, 9, 13). However, absorption by anti-TcR $\beta$ columns was inconsistent, thus questioning the role of TcR $\beta$ chain in NP-specific suppressor activity (4, 9, 10, 13). We evaluated the serological determinants expressed on the suppressor molecules produced by the three TcR $\alpha^{-}\beta^{-}$ expression variants. Culture supernatants from the TcR $\beta^{-}$ variants were passed over antigen, anti-TcR-C$\beta$, or anti-TcR-C$\alpha$ affinity columns. Suppressor activity was specifically absorbed by the antigen and anti-TcR-C$\alpha$ column and was recovered after salt elution. The TMA–BSA control column and anti-TcR-C$\beta$ failed to absorb TsF activity (Table 3). Thus, TsF molecules

### Table 1. TcR $\alpha$ transfectant expresses functional CD3–TcR complex

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<tr>
<th>Treatment</th>
<th>CKB-TsF-38</th>
<th>Δ38.3.G7</th>
<th>Δ38.4.D2</th>
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<td><strong>α</strong></td>
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**Experiment 1**

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**Experiment 2**

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<td>SC + TMA–KLH</td>
<td>52</td>
<td>81</td>
<td>134</td>
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**Hybridoma cells (1 × 10^6)** were incubated with antigen or antibody at 37°C for 18 hr and then 1 μCi of [3H]thymidine was added. The cultures were harvested 6–8 hr later. Data are presented as mean cpm × 10^3 in triplicate wells. Standard deviation was <10%.

$*p<0.05$ growth inhibition compared to controls with medium only.
share serological determinants with TcR α chain and bind antigen in the absence of TcR β-chain expression.

**DISCUSSION**

We have examined the role of TcR α and β chains in antigen recognition and antigen-specific suppressor cell function. Utilizing the strategies of TcR α or β expression loss variants followed by reconstitution with homologous TcR α cDNA, a critical role for TcR α chain in antigen-specific Ts function was directly demonstrated.

Table 3. Anti-TcR α and antigen specifically absorb TsF from TcR β" Ts variants

<table>
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<tr>
<th>Reagent on coated beads</th>
<th>Column fraction</th>
<th>TcR mRNA</th>
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<th>NP</th>
<th>ABA</th>
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C57BL/6j mice were immunized with NP-O-Su. Mice were injected with 0.5 ml of conditioned medium from TcR β" expression variants (Δ38.4.C2, Δ38.4.D2, Δ81.4.F8) or the indicated column fractions on days 0 and 1 and challenged on day 6 to elicit DTH responses. Data were from 8-10 mice per group. NT, not tested. Positive control responses were 55.9 ± 3.8 × 10⁻³ cm.

*Significant suppression (P < 0.05).

Early reports (1, 2) using molecular approaches determined that antigen-specific Ts hybridomas frequently deleted donor-derived TcR α/β chains, suggesting that components of the conventional TcR α/β receptor may not be responsible for antigen specificity or function. When we reinspected our original series of Ts1 hybridomas (made by fusion with the TcR α/β+ BW5147 thymoma) for TcR expression, we showed that CD3 or antigen-selected Ts hybridomas expressed TcR α/β on their surface (11). This observation has been confirmed in other suppressor T-cell systems (12, 24, 25). Recently, a series of Ts hybridomas was prepared by fusion with the TcR α/β+ BW1100 thymoma; all Ts1 hybridomas from this fusion express Ts donor-derived TcR α/β complexes (10). Thus, data from our and other laboratories using different antigen systems (5, 6, 8, 9, 25) now firmly support that Ts utilize TcR α/β for antigen recognition.

To study the role of each TcR chain in Ts function, we made a series of TcR α or β loss variants from two Ts1 hybridomas that express defined TcR α/β genes. All three TcR α/β expression variants obtained from these Ts1 hybridomas continued to produce antigen-specific suppressor activity (Table 2) but lost surface expression of the CD3–TcR complex and the ability to recognize antigen (Table 1 and Fig. 2). We have made an analogous observation with a subclone derived from a NP-specific Ts3 hybridoma, which lost the expression of TcR β chain but continued to produce antigen-specific suppressor activity (4). These data indicate that TcR β chain, although essential for TcR surface expression, is not necessary for Ts function and that TsF does not consist of solubilized TcR α/β complexes. The data also help reconcile the observation that Ts frequently delete donor-derived TcR β chains while retaining the ability to produce antigen-specific TcF activity. Furthermore, these data clearly demonstrate that in the NP system, TcR β chain is not a necessary component of antigen-specific TsF. This is
in contrast to the findings of Fairchild et al. (13, 24), who reported that a TsF released by a 2,4-dinitrophenyl-specific hybridoma shares serological determinants with both TcR α and β chains. The factors studied by Fairchild et al. (13, 24) have other distinguishing features; i.e., they are produced by CD8-derived cells and display class I restriction, which is presumably determined by TcR β chain (24). In contrast, the NP-specific factors described here are derived from CD4+ cells and lack H-2 restriction.

The current study demonstrates that antigen-specific TsF activity from all three TcR α β mutants can be absorbed by an anti-TcR α (but not anti-TcR β) chain-specific antibody (Table 3), supporting the concept that TcR α chain forms part of the antigen-specific TsF molecule. This is consistent with the observations made in other suppressor systems in which it has been shown that antisense oligonucleotides to TcR α (but not TcR β chain) block production of a component of suppressor factor (14) and that suppressor activity can be absorbed by anti-TcR-Cα antibody (4, 9, 10, 13, 24, 25). The shared antigen specificity of the cell surface TcR α/β and TsF implies that the TcR α chain alone is sufficient for antigen binding by TsF. These assumptions are supported by data from other hapten systems in which TcR α chains are involved in antigen recognition (24, 26).

Previous studies demonstrated that TsF is targeted to APCs, and that TsF-pulsed APCs induce a second population of Ts (Ts2) with antidiotypic specificity (22). It has been difficult to envision the mechanism by which antidiotypic specificity could be achieved if the specificity was directed to a conformational determinant on a heterodimeric TcR α/β, since each protein chain is processed separately. The current studies imply that in the NP system only the TcR α chain is involved in network regulation; thus, APC processing of a single chain is consistent with the idiotypic specificity observed. Such a mechanism may also account for the network regulation noted in other systems (27).

It is not clear at this stage how the TcR α chain is released from Ts. The cDNA expressed in the transfectedants contains a C region that is identical in sequence to that of the published cell-surface TcR α chain (data not shown; see ref. 28 for Cα sequence). Thus, alternative splicing of the TcR α does not appear to be required for TsF production. Furthermore, it is unclear whether the TsF is released from viable cells or the 1-7% dead cells present when the TsF-containing supernatants are harvested. Data obtained from COS cell transfection models suggest that unless the TcR α chain is stabilized by other components of the CD3–TcR complex, the TcR α chain will be rapidly degraded or trapped in the endoplasmic reticulum (29, 30). We postulate that alternative chain(s), other than TcR β, interact with TcR α and help in stabilization and release of this molecule from Ts. The postulated second chain may either modify TcR α and/or complex with it. Evidence from multiple Ts systems suggests that TsF molecule may be a heterodimer (22, 25, 31). If the TcR α chain controls antigen specificity, the postulated second chain could mediate bioactivity. Recent studies with TcR β chain transgenic mice have provided evidence for a chain that complexes with TcR β chain in the absence of CD3 and the TcR α, thus demonstrating that other TcR-associated proteins remain to be characterized (32).

In summary, the critical role of TcR α chain in Ts antigen recognition and TsF function has been directly demonstrated. Loss of TcR α, but not TcR β, RNA results in loss of suppressor bioactivity, which can be specifically restored by transfection with homologous TcR α cDNA. Further analysis of the TcR α chain, its mechanism of release, and its potential association with alternative chains may help us understand the mechanism by which this molecule mediates suppressor function.

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