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)

VIJAY K. KUCHROO*, MICHAEL C. BYRNE†, YUSAKU ATSUMI*, EDWARD GREENFIELD*, JANE B. CONNOLLY†, MATTHEW J. WHITTERS†, RICHARD M. O'HARA, JR.†, MARY COLLINS†, AND MARTIN E. DORF*

*Department of Pathology, Harvard Medical School, Boston MA 02115; and †Genetics Institute, Cambridge, MA 02140

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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 $\alpha^- \beta^+$ 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 acetyl (NP)-specific suppressor effector (Ts₃) 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-

arsonate (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-V β 6 antibody (44-22-1) and V β 8 (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 Ts₁ hybridomas (CKB-Ts₁-38 and CKB-Ts₁-81) were obtained by fusion of TcR $\alpha^- \beta^-$ BW1100 with tolerized spleen cells from CKB (H2^k) 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 μ g/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, $1-2 \times 10^4$ hybridoma cells were incubated with 5×10^5 irradiated SCs (as a source of APCs) with various hapten protein conjugates (100 μ g/ml) as a source of antigen (10). After 18–20 hr at 37°C, 1 μ Ci 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 μ g/ml) (Sigma) or goat anti-rat antibody (100 μ g/ml) (Organon Teknika-Cappel) overnight at 4°C. The plates were washed twice and 100 μ l of antibody-containing supernatant was added to each well for 4 hr. After three washings, $1-2 \times$

Abbreviations: APC, antigen presenting cell; ABA, *p*-azobenzene-arsonate; BSA, bovine serum albumin; DTH, delayed-type hypersensitivity; KLH, keyhole limpet hemocyanin; NP, 4-hydroxy-3-nitrophenyl acetyl; NP-O-Su, NP-O-succinimide; PFC, plaque-forming cell; SC, spleen cell; TcR, T-cell receptor; TMA, trimethylammonia; Ts, suppressor T cell(s); TsF, T-cell suppressor factor.

Suppression of Plaque-Forming Cell (PFC) Responses. TsF₁ activity was assayed as described (21). Briefly, naive C57BL/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.

Generation of Expression Variants and Transfectants. We recently derived a series of NP-specific Ts₁ hybridomas that share phenotypic properties (Thy1⁺, CD3⁺, CD4⁺, CD8⁻,

Northern analysis (Fig. 1a) shows that the RNA from CKB-Ts1-38 hybridizes with V α 8 and V β 6 probes, while RNA from the fusion partner BW1100 does not. Lines Δ 38.3.G7 and Δ 38.3.H4 (plus Δ 38.3.G5; data not shown) lack detectable V α 8 mRNA, whereas Δ 38.4.C2 and Δ 38.4.D2 show a loss of V β 6 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, Δ 38.3.G7, or Δ 38.4.D2. TcR α chain was amplified by using a 5' V α 8 oligonucleotide and a 3' C α oligonucleotide. Similarly the TcR β chain was amplified by using a 5' V β 6 oligonucleotide and a 3' C β oligonucleotide. Titrations 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 Δ 38.3.G7 and the TcR β^- expression variant Δ 38.4.D2, respectively, when compared

FIG. 1. Expression of TcR α and β RNA. (a) Northern analysis. Total RNA (20 μ g) from either BW1100 (lane A); parental Ts1 hybridoma CKB-Ts1-38 (lane B); expression variants Δ 38.4.C2, Δ 38.4.D2, Δ 38.3.G7, or Δ 38.3.H4 (lanes C-F); or Δ 38.3.G7 TcR α transfectant 38 α 5 (lane H) were analyzed by Northern blot with a 32 P-labeled V_{β} 6 probe (*Hind*III/*Eco*RI; 200-base fragment), V_{α} 8 (*Eco*RI/*Pst*I; 206-base fragment) probe or a full-length CD3 ϵ gene 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 Δ 38.3.G7 or TcR β subclone Δ 38.4.D2 is shown. PCR mixtures contained pairs of oligonucleotides specific for either the V_{α} 8 (α) or V_{β} 6 (β) rearrangement or both pairs of oligonucleotides ($\alpha + \beta$); 0–250 pg cDNA was used per reaction.

to the parental line (Fig. 1b). Expression variants of CKB-Ts₁-81 were isolated similarly, and one TcR $\alpha^- \beta^-$ ($\Delta 81.3.E11$) clone and one TcR $\alpha^+ \beta^-$ ($\Delta 81.4.F8$) clone were identified by Northern analysis (data not shown).

One of the TcR α^- expression variants ($\Delta 38.3.G7$) was transfected with a full-length CKB-Ts₁-38 TcR α cDNA. Two independent transfectants, 38 $\alpha 1$ and 38 $\alpha 5$, that expressed the CKB-Ts₁-38 TcR α were selected for detailed analysis. A full-length TcR α -chain cDNA from BW5147 ($V_{\alpha}1.1$ and $J_{\alpha}TT11$) was transfected into $\Delta 38.3.G7$ and one transfectant from this series (BW $\alpha 5$) was included as a control. $\Delta 38.3.G7$ transfected with homologous CKB-Ts₁-38 TcR α cDNA hybridizes with both $V_{\alpha}8$ and $V_{\beta}6$ probes in transfectant 38 $\alpha 5$ (Fig. 1a) and transfectant 38 $\alpha 1$ (data not shown). The $V_{\alpha}8$ 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 α coding sequence and can be distinguished from endogenous TcR α mRNA by the presence of 5' vector sequences using RNase protection (data not shown). No endogenous $V_{\alpha}8$ 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-Ts₁-38 parental line stained with anti-CD3, anti-TcR- C_{β} , and anti- $V_{\beta}6$ (Fig. 2). In contrast, the TcR α^- ($\Delta 38.3.G7$) and TcR β^- ($\Delta 38.4.D2$) expression variants failed to stain with these reagents. Transfection of $\Delta 38.3.G7$ with the CKB-Ts₁-38-derived TcR α cDNA reconstituted surface expression of the CD3-TcR complex in line 38 $\alpha 5$ (Fig. 2). Transfectant 38 $\alpha 1$ and BW $\alpha 5$, 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 (38 $\alpha 5$) cell lines were growth inhibited upon stimulation with either specific antigen (NP-KLH), anti-CD3, anti-TcR- C_{β} , or anti- $V_{\beta}6$ antibodies (Table 1). In contrast, neither antigen (NP-KLH) nor anti-CD3-TcR antibodies affected the growth of TcR α^- or TcR β^- expression variants (Table 1). These data indicate that a functional CD3-TcR complex was reconstituted in the transfected cell line (38 $\alpha 5$) and that NP recognition is associated with the TcR α/β complex.

TsF Activity from Expression Variants and Transfectants. Conditioned media from parental CKB-Ts₁-38, TcR α^- , or β^-

expression variants and transfectants were tested for suppressive bioactivity. Supernatant containing TsF from the parental CKB-Ts₁-38 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 α^- expression variants derived from line CKB-Ts₁-38 were tested and all lost suppressive activity (Table 2), whereas both TcR β^- expression variants that retain expression of TcR α mRNA ($\Delta 38.4.C2$ and $\Delta 38.4.D2$) produced antigen-specific TsF activity. In addition, another TcR $\alpha^+ \beta^-$ expression variant ($\Delta 81.4.F8$) from a second NP-specific Ts hybridoma (CKB-Ts₁-81) also produced antigen-specific TsF activity. Expression variant $\Delta 81.3.E11$ lost expression of both TcR chains (data not shown) and also lost the ability to produce TsF. Thus, the loss of TcR α expression correlated with lack of suppressive bioactivity (Table 2).

To prove that the TcR α chain plays a role in antigen-specific suppressor cell function, the ability of TcR α transfectants to produce antigen-specific suppressor activity was examined. Both homologous TcR α transfectants (38 $\alpha 1$ and 38 $\alpha 5$) specifically suppressed DTH and PFC responses to NP (Table 2). Conditioned medium from control transfectants, carrying either an empty vector or the BW5147 derived TcR α cDNA (BW $\alpha 5$) did not suppress DTH responses (Table 2), demonstrating the specificity of NP-specific TcR α 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 α affinity columns (4, 8, 9, 13). However, absorption by anti-TcR β columns was inconsistent, thus questioning the role of TcR β 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 β^- variants were passed over antigen, anti-TcR- C_{α} , or anti-TcR- C_{β} affinity columns. Suppressor activity was specifically absorbed by the antigen and anti-TcR- C_{α} column and was recovered after salt elution. The TMA-BSA control column and anti-TcR- C_{β} failed to absorb TsF activity (Table 3). Thus, TsF molecules

Table 1. TcR α transfectant expresses functional CD3-TcR complex

Treatment	Growth of Ts hybridomas: [³ H]Thymidine uptake, cpm $\times 10^{-3}$			
	CKB-Ts ₁ -38 ($\alpha^+ \beta^+$)	$\Delta 38.3.G7$ ($\alpha^- \beta^+$)	$\Delta 38.4.D2$ ($\alpha^+ \beta^-$)	38 $\alpha 5$ ($\alpha^+ \beta^+$)
Experiment 1				
Protein A	322	332	208	241
Anti-CD3 ϵ	50*	290	251	48*
Anti-TcR- C_{β}	120*	318	176	44*
Anti-Ly6C	307	265	242	233
Anti-rat Ig	294	380	187	231
Anti- $V_{\beta}6$	89*	360	199	67*
Anti- $V_{\beta}8$	347	307	231	243
Experiment 2				
SC	51	92	108	374
SC + NP-KLH	14*	82	122	55*
SC + TMA-KLH	52	81	134	328

Hybridoma cells (1×10^4) were incubated with antigen or antibody at 37°C for 18 hr and then 1 μ Ci of [³H]thymidine was added. The cultures were harvested 6–8 hr later. Data are presented as mean cpm $\times 10^{-3}$ in triplicate wells. Standard deviation was <10%.

*>50% growth inhibition compared to controls with medium only.

FIG. 2. Surface TcR analysis of parental CKB-Ts₁-38, TcR expression variants ($\Delta 38.3.G7$ and $\Delta 38.4.D2$), and TcR α transfectant (38 $\alpha 5$) by using monoclonal anti-CD3, anti- C_{β} , anti- $V_{\beta}6$, and anti-Thy 1.2 antibodies. Individual profiles of log fluorescence are shown.

Table 2. Suppressive activity of TcR expression variants and transfected cells

TsF source	TcR mRNA		% suppression of DTH		% suppression of PFC	
	α	β	NP	ABA	NP	SRBC
Expression variants						
CKB-Ts ₁ -38	+	+	56 ± 6*	-6 ± 9	60 ± 8*	-2 ± 12
Δ 38.3.G5	-	+	7 ± 11	NT	-60 ± 38	NT
Δ 38.3.G7	-	+	-10 ± 7	10 ± 9	-69 ± 31	NT
Δ 38.3.H4	-	+	-4 ± 13	-7 ± 7	-39 ± 15	NT
Δ 38.4.C2	+	-	61 ± 3*	0 ± 6	48 ± 15*	-37 ± 26
Δ 38.4.D2	+	-	58 ± 6*	-6 ± 12	25 ± 9*	-6 ± 16
CKB-Ts ₁ -81	+	+	62 ± 5*	-12 ± 9	43 ± 16*	-3 ± 20
Δ 81.3.E11	-	-	-8 ± 21	1 ± 15	NT	NT
Δ 81.4.F8	+	-	67 ± 12*	-8 ± 12	NT	NT
Transfection of Δ 38.3.G7 cells						
38 α 1	+	+	64 ± 6*	-11 ± 20	46 ± 7*	7 ± 29
38 α 5	+	+	54 ± 6*	-14 ± 12	44 ± 12*	-27 ± 15
BW α 5	BW	+	-3 ± 18	-8 ± 20	NT	NT

Suppressive activity of CKB-Ts₁-38, TcR expression variants, and TcR α transfectants. Groups of 10–15 B10.BR or C57BL/6 mice were injected i.v. with 0.5 ml of conditioned medium on days 0 and 1. Control mice were primed but did not receive conditioned medium. Data are presented as mean percentage suppression. NT, not tested. Positive control responses were $70 \pm 3.6 \times 10^{-3}$ cm for NP-O-Su and $31 \pm 2.3 \times 10^{-3}$ cm for ABA. For suppression of PFC responses, SCs were harvested from naive C57BL/6 mice and challenged *in vitro* with NP-coupled horse or sheep erythrocytes (SRBC). On days 0 and 1, 40 μ l of Ts hybridoma conditioned medium was added. Conditioned medium from BW1100-derived hybridomas often enhanced these *in vitro* responses as noted by a negative percentage suppression. Percentage suppression of expression variants was calculated by comparing PFC responses of cultures to which TsF was added against the positive control responses (medium only added). For the TcR α transfectants the positive control was conditioned medium from Δ 38.3.G7 transfected with empty vector (CDM8REN). The transfected cell lines were washed and grown in the absence of G418 before collection of TsF-containing conditioned medium. PFC responses of cultures supplemented with culture medium (14,547 PFC per culture) or conditioned medium from an empty vector (CDM8REN) transfectant (14,992 PFC per culture) were not statistically different.

*Significant suppression ($P < 0.01$).

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

Reagent on coated beads	Column fraction	% suppression		
		Δ 38.4D2	Δ 38.4C2	Δ 81.4F8
None	None	83 ± 8*	50 ± 10*	34 ± 8*
Anti-CD3	Effluent	NT	45 ± 8*	NT
Anti-CD3	Eluate	NT	16 ± 14	NT
Anti-TcR-C α	Effluent	-9 ± 23	-4 ± 10	4 ± 7
Anti-TcR-C α	Eluate	74 ± 7*	59 ± 7*	33 ± 12*
Anti-TcR-C α	Effluent	74 ± 7*	59 ± 6*	53 ± 9*
Anti-TcR-C β	Eluate	-11 ± 17	2 ± 16	4 ± 9
NP-BSA	Effluent	-5 ± 13	NT	-9 ± 8
NP-BSA	Eluate	65 ± 18*	NT	40 ± 4*
TMA-BSA	Effluent	66 ± 15*	NT	41 ± 7*
TMA-BSA	Eluate	-1 ± 13	NT	-3 ± 6

C57BL/6J mice were immunized with NP-O-Su. Mice were injected with 0.5 ml of conditioned medium from TcR β -expression variants (Δ 38.4.C2, Δ 38.4.D2, Δ 81.4.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 $55.9 \pm 3.8 \times 10^{-3}$ cm.

*Significant suppression ($P < 0.05$).

Early reports (1, 2) using molecular approaches determined that antigen-specific Ts hybridomas frequently deleted donor-derived TcR 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 Ts₃ hybridomas (made by fusion with the TcR $\alpha^+\beta^+$ 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 $\alpha^-\beta^-$ 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 $\alpha^+\beta^-$ 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 Ts donor-derived TcR β chains while retaining the ability to produce antigen-specific TsF 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 data demonstrate that antigen-specific TsF activity from all three TcR $\alpha^+ \beta^-$ 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-C α 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 antiidiotypic specificity (22). It has been difficult to envision the mechanism by which antiidiotypic 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 transfectants 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 C α 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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