Mod Rheumatol (2002) 12:167–173

ORIGINAL ARTICLE

Kensei Tsuzaka · Natsuko Onoda · Keiko Yoshimoto
Yumiko Setoyama · Katsuya Suzuki · Ming Pang
Tohru Abe · Tsutomu Takeuchi

T-cell receptor ζ mRNA with an alternatively spliced 3′ untranslated region is generated predominantly in the peripheral blood T cells of systemic lupus erythematosus patients

Received: September 17, 2001 / Accepted: December 17, 2001

Abstract To investigate the mechanism of the downregulation of T-cell receptor ζ chain (TCRζ) expression in the peripheral blood T cells (PBTs) of systemic lupus erythematosus (SLE) patients, we analyzed the 3′ untranslated region (3′UTR) of TCRζ mRNA, because the 3′UTR in mRNA is responsible for posttranscriptional regulation. Use of the reverse transcriptase polymerase chain reaction (RT-PCR) to amplify the 917 bp TCRζ 3′UTR cDNA demonstrated that the short variant cDNA (355 bp), expressed as an alternatively spliced 3′UTR with 562-bp deletion, was predominated in the PBTs of 11 of 14 SLE patients, whereas mainly the wild-form cDNA (917 bp) was detected in the PBTs of seven negative controls (two systemic sclerosis patients, five normal controls) and in two T-cell line hybridomas. Semiquantitative PCR also revealed the predominant expression of the TCRζ mRNA with alternatively spliced 3′UTR (TCRζ mRNA/as-3′UTR), and a decreased expression of TCRζ mRNA with the wild form 3′UTR (TCRζ mRNA/w-3′UTR) in SLE T cells. However, there was no difference in the expression of the open reading frame (ORF) TCRζ mRNA between the negative controls and SLE patients. The TCRζ protein expression level according to Western blot analysis correlated well with that of TCRζ mRNA/w-3′UTR ($r = 0.931$) and reversibly with TCRζ mRNA/as-3′UTR ($r = -0.614$), but not with ORF TCRζ mRNA ($r = -0.296$). It can be concluded that the reduced expression of TCRζ mRNA/w-3′UTR and the predominant expression of TCRζ mRNA/as-3′UTR lead to downregulation of the TCRζ protein in SLE T cells.

Key words Alternative splicing · Autoimmune disease · CD3 · Signal transduction · T lymphocytes

K. Tsuzaka (✉) · N. Onoda · K. Yoshimoto · Y. Setoyama · K. Suzuki · M. Pang · T. Abe · T. Takeuchi
Second Department of Internal Medicine, Saitama Medical Center, Saitama Medical School, 1981 Kamoda, Kawagoe 350-8550, Japan
Tel. +81-49-228-3400; Fax +81-49-226-5274
e-mail: kentsu@saitama-med.ac.jp

Introduction

Systemic lupus erythematosus (SLE) is well known as one of the prototype systemic autoimmune diseases.[1] The signal transduction pathway via the T-cell receptor (TCR) has been shown to be altered in MRL *lpr*/*lpr* mice.[2] Although the *lpr* gene is characterized by a retrotranspozone that disrupts *fas* expression and leads to deficient apoptosis and lymphadenopathy, recent studies have demonstrated that background genes in SLE play an important role in developing autoimmune phenomena and autoimmune diseases.[3–8] Defects in signal transduction through the TCR–CD3 complex may cause T-cell dysfunction and autoimmunity in NOD mice as well as in MRL *lpr*/*lpr* mice.[9–11] Several studies, including our own, have demonstrated the functional defect of early signaling molecules on peripheral blood T cells (PBTs) as a cause of T-cell dysfunction in human SLE,[12–14] and it has been suggested that the T-cell receptor ζ chain (TCRζ) is coupled to the signal transduction machinery in T cells, and that decreased or aberrant expression of TCRζ may cause T-cell dysfunction, loss of tolerance, or the development of autoimmunity.[15] We recently reported that tyrosine phosphorylation and expression of TCRζ were significantly decreased or absent in more than half of SLE patients, and that several patients exhibited some alterations in the TCRζ mRNA open reading frame (ORF), such as exon 7 skipping or point mutations.[16,17] However, as the frequency of the alteration in the ORF TCRζ mRNA is low in comparison with the decrease in TCRζ protein expression, we attempted to explore alternative mechanisms for the decreased TCRζ expression in SLE. We particularly focused on the 3′ untranslated region (3′UTR) of TCRζ mRNA, since we had concentrated on the ORF in the TCRζ mRNA in our previous studies.[16,17] Since the 3′UTR of mRNA has been reported to play a pivotal role in posttranscriptional regulation by altering the mRNA stability and affecting the transportation and localization of mRNA, we analyzed the 3′UTR of TCRζ mRNA in SLE patient PBTs in this study. Here we report finding that the TCRζ mRNA with a short spliced variant of the

3′UTR was predominantly generated in SLE, whereas the expression of the TCRζ mRNA with wild-form 3′UTR was reduced. Moreover, TCRζ protein expression was found to be well correlated with the expression of the TCRζ mRNA with wild-form 3′UTR but not with the TCRζ mRNA ORF, suggesting that it might represent a novel mechanism for the decreased expression of TCRζ in SLE.

Methods

Patient selection

Fourteen Japanese systemic lupus erythematosus (SLE) patients who met the revised 1982 American College of Rheumatology (ACR, formerly the American Rheumatism Association) criteria for SLE[18] were selected for analysis of their TCRζ mRNA. Five normal healthy Japanese controls and two Japanese systemic sclerosis (SSc) patients who met the criteria for SSc[19] were included as negative controls.

Isolation of peripheral blood T cells

Peripheral blood (20 ml) was collected in a preheparinized tube, and peripheral blood mononuclear cells were isolated by Ficoll–Hypaque density-gradient centrifugation (Pharmacia Biotechnology, Uppsala, Sweden). The cell suspensions were passed over a nylon-wool column to yield a T-cell-enriched population, and the cell population obtained was more than 92% reactive to anti-CD3 (UCTH1:IgG1).

Western blot

PBTs (1×10^7 cells) were lysed with 1 ml lysis buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% NP-40, 10 mM ethylenediaminetetraacetate (EDTA), 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml aprotinin, and 10 μg/ml leupeptin) at 4°C for 15 min, and disrupted by sonication. After centrifuging at 10000 *g* for 5 min, the supernatant was electrophoresed on a 15% sodium dodecyl sulfate (SDS)-polyacrylamide gel. The proteins were electrophoretically blotted onto PVDF membranes (Millipore, Bedford, MA, USA), and the membranes were soaked at 37°C for 1 h in blocking agents (Blockace; Dainippon Pharmaceuticals, Tokyo, Japan) and incubated with a mouse monoclonal antihuman TCRζ antibody TIA-2 (Coulter Immunology, Hialeah, FL, USA) at 16°C for 1 h. After washing three times, the membranes were incubated with the horseradish peroxidase (HRP)-conjugated antimouse IgG (Amersham, Amersham, UK), and after washing three more times, the signals were detected by chemiluminescence-enhancing reagents (Amersham). Treated membranes were visualized on ECL X-ray film (Amersham). The density of the specific bands was quantified by scanning with a Scan Jet II (Hewlett Packard) and NIH Image Software (version 1.56).

Primer design for PCR

Primers to amplify the TCRζ open reading frame (ORF) cDNA (605 bp of the expected size) were arranged on the upstream and downstream sides of the ORF of TCRζ mRNA according to the nucleotide sequence reported by Weissman et al.[20] [5′-TCAGCCTCTGCCTCCCAGCCTC TTTCT-3′ (+33 to +59) and 5′-ATGCTTCATCCTGTGT CTCATAATCTG-3′ (+638 to +612), respectively]. Primers for the TCRζ 3′UTR cDNA (917 bp of the expected size) were arranged on the 5′ end of exon 6 and the 3′ end of exon 8 [5′-TCTCAGTACAGCCACCAAGGACACC-3′ (+437 to +461) and 5′-CGTGAAGTGAATCAACGG CCTCCTCTT-3′ (+1354 to +1328), respectively] (Fig. 1). As a positive control, glyceraldehyde 3-phosphate dehydrogenase (G3PDH) cDNA (983 bp of the expected size) was amplified by PCR using primers specific for G3PDH (Clontech Laboratories, Palo Alto, CA, USA).

RT-PCR

Messenger RNA was isolated from PBTs with an mRNA isolation kit (Pharmacia Biotechnology) immediately after separating them from peripheral blood. The mRNA was then converted to whole cDNA by reverse transcriptase according to the method described previously.[17] Using 5 μl of the whole cDNA derived from the PBTs as the template, the cDNA was amplified by PCR with specific primers and Taq DNA polymerase (Perkin-Elmer, Foster City, CA, USA). The PCR conditions were denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min for a total of 38 cycles. Five microliters of the PCR product was analyzed by 1.5% agarose gel electrophoresis.

Semiquantitative PCR for TCRζ cDNA

TCRζ ORF cDNA, TCRζ 3′UTR cDNA, and G3PDH cDNA from PBTs were amplified by PCR as described above, and electrophoresed in 1.5% agarose gels under standardized conditions. Aliquots were taken from the reaction mixture every three cycles, starting with cycle 21. After staining with ethidium bromide, the UV-induced fluorescence of specific bands was quantified in relation to the DNA molecular weight marker by scanning with Scan Jet II (Hewlett Packard) and NIH Image Software (version

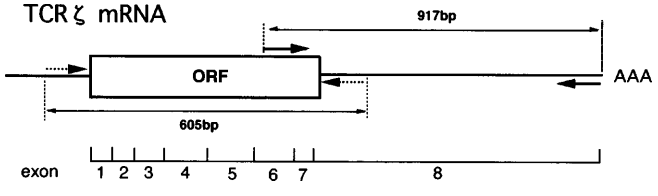

Fig. 1. Primers for amplifying TCR ζ cDNA in reverse transcriptase polymerase chain reaction (RT-PCR). Primers for amplifying TCRζ open reading frame (*ORF*) cDNA were arranged on the upstream and downstream of the ORF of TCRζ mRNA (*dotted arrows*). Primers for TCRζ 3′UTR cDNA were arranged on the 5′ end of exon 6 and the 3′ end of exon 8 (*solid arrows*). The expected sizes of TCRζ ORF cDNA and 3′UTR cDNA were 605 bp and 917 bp, respectively.

1.56), and the relative OD values were graphically evaluated. We calculated the difference in the number of cycles (Δn) needed to reach identical yields of specific PCR products as compared with the internal G3PDH standard according to the method described by Kinoshita et al.[21]

Nucleotide sequencing

PCR products were electrophoresed in 1.5% agarose gels, and the specific DNA bands were eluted from the gels with a DNA elution kit (GENECLEAN II Kit, BIO 101, Vista, CA, USA). The isolated DNA was then ligated into the polylinker regions of pT7Blue T-vector (Novagen, Madison, WI, USA). The nucleotide sequences of the insert DNA were determined using the dideoxychain termination method[22] with T7 DNA polymerase[23] (US Biochemical, Cleveland, OH, USA).

Statistical analysis

Statistical significance was calculated by one-way ANOVA and the Mann–Whitney *U*-test for unpaired data by using Statview software (version 4.5, Abacus, Berkeley, CA, USA). A level of $P < 0.05$ accepted as being statistically significant.

Results

RT-PCR of TCRζ 3′UTR cDNA

Whole mRNA was isolated from PBTs (14 SLE patients, 2 SSc patients, and 5 normal controls) and two human leukemic T cell lines (Jurkat and MOLT4). Whole mRNA was converted to whole cDNA by reverse transcriptase, and the TCRζ 3′UTR cDNA was amplified by PCR with primers arranged on the 5′ end of exon 6 and the 3′ end of exon 8. As shown in Fig. 2, the agarose gel electrophoresis demonstrated that a short cDNA (355 bp) was detected in addition to the normal-sized cDNA (917 bp). The short cDNA seemed to predominate over the normal-sized cDNA in 11 of the 14 SLE patients (TaS, KaM, YaK, NoY, TaR, SeK, YoT, MaS, NaY, NiS, and HaE), and six of the SLE patients (KaM, SeK, MaS, NaY, NiS, and HaE) had only the short cDNA. On the other hand, the normal-sized cDNA was amplified dominantly in all five normal controls, the two SSc patients, and the two T cell lines.

Nucleotide sequence of the TCRζ 3′UTR cDNA in SLE patients

TCRζ 3′UTR cDNA was amplified by PCR of SLE patient TaS. After electrophoresis on 1.5% agarose gel, the normal-sized (917 bp) and short (355 bp) DNA bands were cut out of the gels and purified. The isolated DNA was then ligated into the polylinker regions of the pT7Blue T-vector to determine the nucleotide sequence. The nucleotide sequence of the normal-sized (917 bp) cDNA completely matched the sequence reported by Weissman et al.,[20] and this cDNA encoded exon 7 and exon 8, including the 3′UTR of TCRζ mRNA, whereas the short (355 bp) cDNA was 562-bp shorter than the normal-sized cDNA according to the nucleotide sequence (Fig. 3). Since the sequence of the 562-bp deleted portion included both the splicing donor and the acceptor sites ["GT" (+671 to +672) and "AG" (+1231 to +1232)] at the 3′ and 5′ end, respectively, the short (355 bp) cDNA may represent an alternatively spliced variant that appeared to result from splicing at these sites. On the other hand, no polymorphism or mutations were found in the

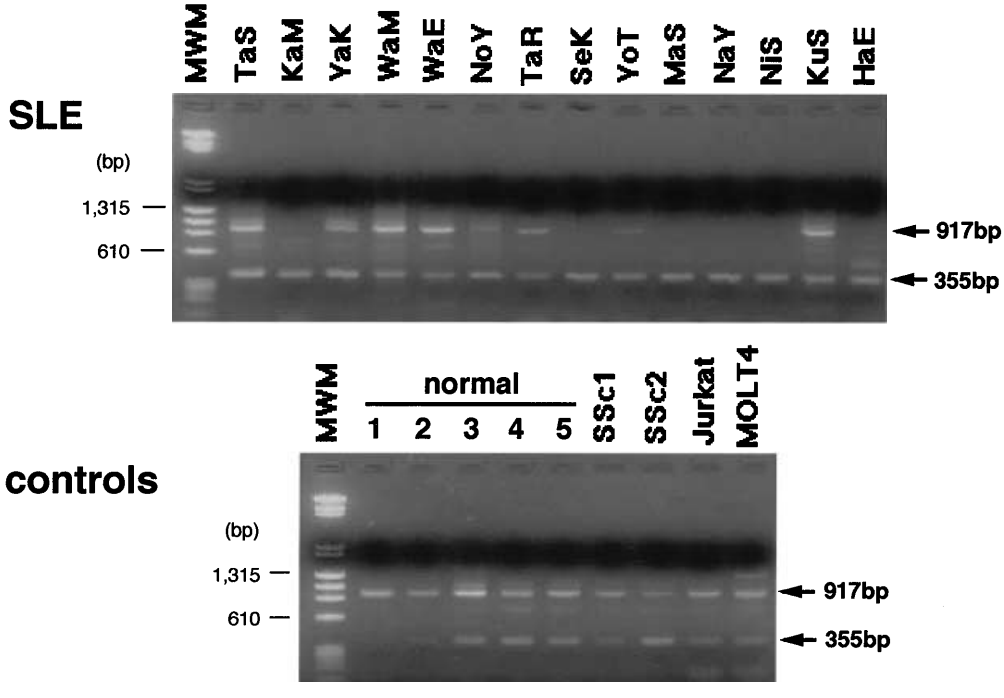

Fig. 2. PCR amplification of TCRζ 3′UTR cDNA. Whole mRNA was isolated from the peripheral blood T cells (PBTs) of 14 systemic lupus erythematosus (SLE) patients and 9 negative controls (two SSc patients (SSc1, SSc2), five normal controls, two leukemic T-cell lines (Jurkat and MOLT4)) and converted to the whole cDNA by reverse transcriptase. TCRζ 3′UTR cDNA was then amplified by PCR, and the PCR products were analyzed in the 1.5% agarose gels. *MWM* indicates the DNA molecular weight marker. As shown in this figure, a short-sized TCRζ 3′UTR cDNA (indicated as 355 bp) was detected as well as the normal-sized cDNA (indicated as 917 bp).

TCRζ genomic DNA around these splicing sites by analyzing the nucleotide sequence of genomic DNA.

Semiquantitative PCR for TCRζ 3′UTR cDNA

We then quantitatively investigated whether the TCRζ mRNA with alternatively spliced short 3′UTR is predominantly expressed in SLE patient T cells, and the TCRζ mRNA with wild-form 3′UTR is decreased by using semiquantitative PCR. TCRζ ORF cDNA, TCRζ 3′UTR cDNA, and G3PDH cDNA from seven SLE patients and four normal controls were then amplified by PCR and electrophoresed in 1.5% agarose gels. Figure 4 shows representative results of the semiquantitative PCR from a normal control and SLE patient NoY. The number of PCR cycles required for amplification of similar levels of cDNA coding for G3PDH among TCRζ ORF cDNA, TCRζ 3′UTR cDNA (917 bp), and TCRζ 3′UTR cDNA (355 bp) (Δ_1, Δ_2, Δ_3, respectively) was compared. The results showed that similar levels of TCRζ ORF cDNA were amplified in the normal control and in SLE patient NoY ($\Delta_1 = 4.4$ vs. $\Delta_1 = 4.1$) under these experimental conditions. TCRζ 3′UTR cDNA (917 bp) was expressed in the normal control ($\Delta_2 = 5$), whereas TCRζ 3′UTR cDNA (355 bp) was undetectable ($\Delta_3 > 33$). On the other hand, no TCRζ 3′UTR cDNA (917 bp) was expressed ($\Delta_2 > 33$) in SLE patient NoY, and TCRζ 3′UTR cDNA (355 bp) was predominantly expressed ($\Delta_3 = 7.1$). Since the levels of expression of TCRζ ORF cDNA, TCRζ 3′UTR cDNA (917 bp), and TCRζ 3′UTR cDNA (355 bp) correspond to that of TCRζ mRNA coding for ORF (ORF TCRζ mRNA), TCRζ mRNA with wild-form 3′UTR (TCRζ mRNA /w-3′UTR), and TCRζ mRNA with alternatively spliced 3′UTR (TCRζ mRNA/as-3′UTR), respectively, these results suggest that TCRζ mRNA/w-3′UTR was predominantly expressed in the normal control, whereas TCRζ mRNA/as-3′UTR was predominant in SLE patient NoY.

We also compared the levels of expression of each TCRζ mRNA in seven SLE patients and in four normal controls.

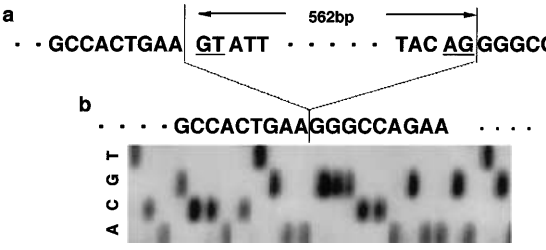

Fig. 3. Splicing donor and acceptor sites in the TCRζ mRNA 3′UTR. TCRζ 3′UTR cDNA from SLE patient TaS was amplified by PCR. After electrophoresis in a 1.5% agarose gel, the normal-sized (917 bp) and the short-sized (355 bp) DNA bands were cut out of the gels and purified. Isolated cDNA was ligated into the polylinker regions of pT7Blue T-vector, and the nucleotide sequences of the insert DNA were determined by using the dideoxychain termination method with T7 DNA polymerase. **a** The nucleotide sequence of the normal-sized (917 bp) TCRζ 3′UTR cDNA perfectly matched the sequence reported by Weissman et al.[20] and included both the splicing donor (GT) and the acceptor (AG) sites. **b** The short (355 bp) cDNA was 562-bp shorter than the normal-sized cDNA and encoded a part of the TCRζ mRNA 3′UTR which was expressed as a result of an alternative splicing at these splicing sites.

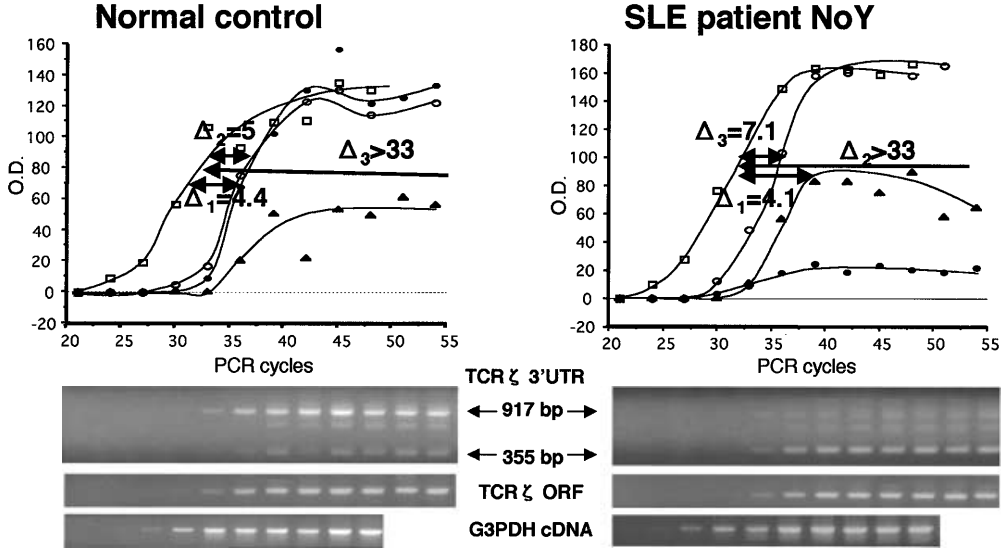

Fig. 4. Semiquantitative PCR for the TCRζ cDNA. TCRζ ORF cDNA, TCRζ 3′UTR cDNA, and G3PDH cDNA of PBTs from a normal control and from SLE patient NoY were amplified by PCR. Aliquots were taken from the reaction mixture every three cycles, beginning with cycle 21. After electrophoresing the aliquots in 1.5% agarose gels containing ethidium bromide, UV-induced fluorescence of specific bands [G3PDH cDNA (*open squares*), TCRζ ORF cDNA (*open circles*), 917 bp TCRζ 3′UTR cDNA (*solid circles*), 355 bp TCRζ 3′UTR cDNA (*solid triangles*)] were quantified as the relative OD value in relation to the DNA molecular weight marker. The relative OD values were graphically evaluated as shown in this figure. Differences in the numbers of cycles (Δn) needed to reach identical yields of the PCR products as compared with G3PDH cDNA were calculated according to the method described by Kinoshita et al.[21] (Δ_1, TCRζ ORF cDNA; Δ_2, TCRζ 3′UTR cDNA (917 bp); Δ_3, TCRζ 3′UTR cDNA (355 bp))

For cases needing more than 33 cycles, we calculated as 34 cycles in the statistical analysis. As shown in Table 1, expression of TCRζ mRNA/w-3′UTR in all of the normal controls ($\Delta_2 = 4.4 \pm 0.4$) was significantly ($P < 0.01$) higher than that estimated in the SLE patients ($\Delta_2 = 26.4 \pm 13.0$). In contrast, TCRζ mRNA/as-3′UTR was strongly detected ($P < 0.05$) in the SLE patients ($\Delta_3 = 6.0 \pm 1.9$) compared with the normal controls ($\Delta_3 = 20.2 \pm 15.9$). It is especially noteworthy that no TCRζ mRNA/w-3′UTR was detected ($\Delta_2 > 33$) in five SLE patients (NaY, NoY, YoT, KaM, and MaS). The level of expression of ORF TCRζ mRNA, on the other hand, did not differ between the normal controls ($\Delta_1 = 3.7 \pm 0.6$) and the SLE patients ($\Delta_1 = 3.5 \pm 0.6$).

Western blot analysis of TCRζ protein expression in SLE T cells

To investigate the relationship between the expression of TCRζ protein and mRNA, levels of expression of TCRζ protein were quantified by Western blot analysis in the PBTs of seven SLE patients and four normal controls by semiquantitative PCR analysis, as described above. As shown in Fig. 5, TCRζ was detected as an 18-kD protein band with a monoclonal antihuman TCRζ antibody TIA-2. The OD value of the TCRζ in the PBTs of seven SLE patients was significantly ($P < 0.001$) lower (range 5–60, mean ± SD 23 ± 21) than in the normal controls (range 70–99, mean ± SD 84 ± 12).

To confirm that TCRζ protein expression was affected by transcriptional or post-transcriptional regulation, we compared the density of TCRζ in Western blot analysis with the level of expression of each TCRζ mRNA (ORF TCRζ mRNA, TCRζ mRNA/w-3′UTR, and TCRζ mRNA/as-3′UTR) in the seven SLE patients and four normal controls. We evaluated the level of mRNA expression as $1/\Delta n$ by the semiquantitative PCR method described above. As shown in Fig. 6, the TCRζ protein expression level correlated well with that of TCRζ mRNA/w-3′UTR ($r = 0.931$) and reversibly with TCRζ mRNA/as-3′UTR ($r = -0.614$), whereas they were unassociated with that of ORF TCRζ mRNA ($r = -0.296$). These findings suggested that expression of the wild-form 3′UTR in the TCRζ mRNA, but not expression of the ORF region, correlated well with that of TCRζ protein. These results also appear to indicate that TCRζ expression in SLE patient T cells was downregulated because of a reduced expression of wild-form 3′UTR and the predominant expression of the alternatively spliced short 3′UTR. Furthermore, steroid doses and the SLE disease activity index (SLEDAI) of the SLE patients observed in this study were not associated with the expression of TCRζ protein, TCRζ mRNA/w-3′UTR, TCRζ mRNA/as-3′UTR, or that of ORF TCRζ mRNA (data not shown). Based on these observations, it can be concluded that expression of TCRζ protein in T cells is influenced by posttranscriptional regulation.

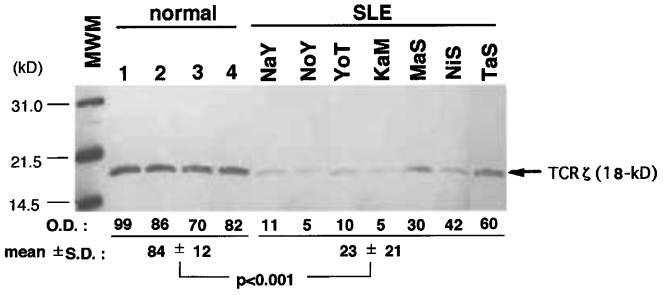

Fig. 5. Western blot analysis of TCRζ protein expression by SLE T cells. PBT lysates from four normal controls and seven SLE patients were electrophoresed in 15% sodium dodecyl sulfate (SDS)-polyacrylamide gels and blotted onto a PVDF membrane. The membranes were incubated with a mouse monoclonal antihuman TCRζ antibody TIA-2 and then with HRP-conjugated antimouse IgG. After treatment with chemiluminescence-enhancing reagents, the membranes were visualized on enhanced chemiluminescence (ECL) X-ray films, and the density of 18 kD TCRζ protein bands (*arrow*) was quantified as relative OD values

Table 1. Semiquantitative PCR for TCRζ cDNA

	Δ_1 TCRζ ORF cDNA		Δ_2 TCRζ 3′UTR cDNA (917 bp)		Δ_3 TCRζ 3′UTR cDNA (355 bp)	
Normal						
1	4.4		5		>33	
2	2		3.5		>33	
3	3.8		4		6.5	
4	4.5		5		6.3	
Mean ± SD	3.7 ± 0.6		4.4 ± 0.4		20.2 ± 15.9	
SLE						
NaY	3.3		>33		8.5	
NoY	4.1		>33		5	
YoT	1	ns	>33	$P < 0.01$	5	$P < 0.05$
KaM	4		>33		5	
MaS	1.5		>33		9	
NiS	5.3		8.8		4	
Tas	5		6		5.5	
Mean ± SD	3.5 ± 0.6		26.4 ± 13.0		6.0 ± 1.9	

PCR, polymerase chain reaction; TCRζ, T-cell receptor ζ; ns, not significant

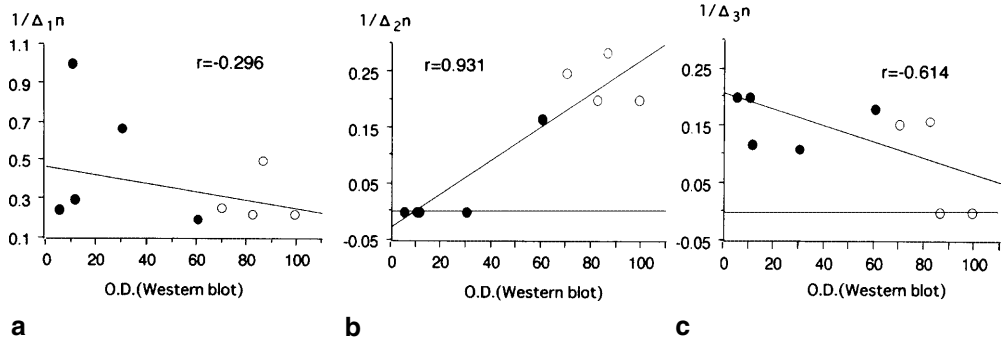

Fig. 6. The relationship between the expression of TCRζ protein and mRNA. Messenger RNA expression was estimated as $1/\Delta n$ by the semiquantitative PCR method described above. The OD values of TCRζ in Western blot analysis were compared with the level of expression of TCRζ mRNA (**a** TCRζ ORF mRNA; **b** TCRζ mRNA/w-3′UTR; **c** TCRζ mRNA/as-3′UTR) in seven SLE patients (*closed circles*) and four normal controls (*open circles*)

Discussion

In this study, RT-PCR demonstrated that the short TCRζ 3′UTR cDNA (355 bp) predominated in the T-cells of most of the SLE patients, whereas the normal-sized TCRζ 3′UTR cDNA (917 bp) predominated in all five normal controls, two SSc patients, and the two leukemic T-cell lines. TCRζ mRNA 3′UTR originally included "GT" and "AG," which correspond to a splicing donor site and an acceptor site.[24] Comparison of the nucleotide sequence of these two cDNAs suggested that the 355-bp cDNA fragment was expressed as an alternatively spliced TCRζ mRNA 3′UTR as a result of a splicing out of the 562-bp fragment at these sites (Fig. 7). Although no genomic DNA mutations involving these splicing sites were detected in this study, some other mechanisms might be responsible for the TCRζ mRNA/as-3′UTR expression in SLE T cells. Since the RT-PCR method applied at just one point in the cycle is unsuitable for quantification of mRNA expression, we analyzed the mRNA level by semiquantitative PCR. The results revealed the predominant expression of TCRζ mRNA/as-3′UTR in SLE T cells and the decreased expression of TCRζ mRNA/w-3′UTR. We previously reported that TCRζ expression was significantly decreased or absent in SLE patients' T cells,[16] and in this study Western blot analysis yielded the same results. However, the observation that expression of ORF TCRζ mRNA did not differ in the normal controls and the SLE patients suggested that a *cis*-acting element or *trans*-acting factor in the 5′UTR of the TCRζ gene is not associated with the downregulation of TCRζ protein in SLE patient T cells. Of even greater interest, TCRζ protein expression in the Western blot analysis correlated well with the expression of TCRζ mRNA/w-3′UTR and did so reversibly with TCRζ mRNA/as-3′UTR but not with ORF TCRζ mRNA. These findings might suggest that the 562-bp region in the 3′UTR of TCRζ mRNA is important for TCRζ protein expression, and that SLE patient T cells whose TCRζ mRNA 3′UTR lacks this region may have low levels of TCRζ protein. From our observations that SLE patients with low expression levels of TCRζ protein showed unusual clinical findings, including antiphospholipid antibodies, facial erythema, or possible vasculitic lesions (unpublished data), SLE patients with the alternatively spliced form of TCRζ mRNA 3′UTR could also present similar clinical findings.

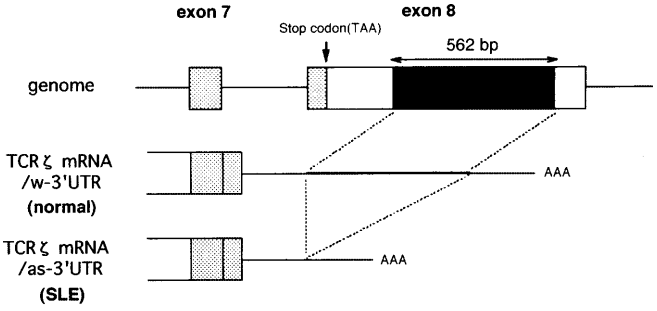

Fig. 7. Alternatively spliced 3′UTR of TCRζ mRNA in the T cells of SLE patients. Based on our observations, in the T cells of SLE patients, the dominant expression of TCRζ mRNA/as-3′UTR which is lacking 562 bp and the decreased expression of TCRζ mRNA/w-3′UTR may lead to a decrease or absence of expression of TCRζ protein

The 3′UTR in mRNA has generally been reported to play a pivotal role in post-transcriptional regulation by altering mRNA stability, and affecting the transportation and localization of mRNA. Messenger RNA 3′UTR contains *cis*-acting elements, i.e., adenosine–uridine (A–U)-rich elements, which bind to *trans*-acting proteins and participate in either the stabilization or destabilization of transcripts. Vascular endothelial growth factor (VEGF) and Na^+-independent GLUT1 glucose transporter transcripts have been reported to be stabilized in hypoxia through a mechanism that appears to involve A–U-rich elements located within 3′UTR and that bind to cytoplasmic proteins to form hypoxia-inducible RNA–protein complexes.[25,26] Boado and Pardridge[25] reported that a 10-nucleotide *cis*-acting element that is conserved among species is responsible for increased GLUT1 gene expression via enhanced GLUT1 mRNA stabilization. Ainger et al.,[27] on the other hand, reported that the 21-nucleotide RNA transport signal (RTS), which is also conserved among species, and the 344-nucleotide RNA localization region (RLR) are responsible for RNA transportation and localization.

The TCRζ mRNA 3′UTR also contains a 31-nucleotide sequence (5′-CTCCTGCTGTAAATTTGGCTTCTGTTG TCAC-3′) (from +973 to +1003) that is conserved only in the TCRζ mRNA 3′UTR of a few species (human, mouse, rat, and bovine), and this region was not included in the TCRζ mRNA/as-3′UTR in SLE patient T cells. If this conserved region is associated with RNA stabilization, transportation, or localization, its absence in 3′UTR is a plausible mechanism of TCRζ downregulation or absence in SLE T cells. Studies to determine how this region influences TCRζ protein expression are underway in our laboratory.

Acknowledgments This work was supported by grants from Grant-in-Aid for Scientific Research (C), the Ministry of Education, Science and Culture, and grants from the Ministry of Health and Welfare, Japan, and the Maruki Memorial Research Foundation.

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