Decreased Stability and Translation of T Cell Receptor \( \zeta \) mRNA with an Alternatively Spliced 3'-Untranslated Region Contribute to \( \zeta \) Chain Down-regulation in Patients with Systemic Lupus Erythematosus

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The molecular mechanisms involved in the aberrant expression of T cell receptor (TCR) \( \zeta \) chain of patients with systemic lupus erythematosus are not known. Previously we demonstrated that although normal T cells express high levels of TCR \( \zeta \) mRNA with wild-type (WT) 3’ untranslated region (3’ UTR), systemic lupus erythematosus T cells display significantly high levels of TCR \( \zeta \) mRNA with the alternatively spliced (AS) 3’ UTR form, which is derived by splice deletion of nucleotides 672–1233 of the TCR \( \zeta \) transcript. Here we report that the stability of TCR \( \zeta \) mRNA with an AS 3’ UTR is low compared with TCR \( \zeta \) mRNA with WT 3’ UTR. AS 3’ UTR, but not WT 3’ UTR, conferred similar instability to the luciferase gene. Immunoblotting of cell lysates derived from transfected COS-7 cells demonstrated that TCR \( \zeta \) with AS 3’ UTR produced low amounts of 16-kDa protein. In vitro transcription and translation also produced low amounts of protein from TCR \( \zeta \) with AS 3’ UTR. Together our findings suggest that nucleotides 672–1233 bp of TCR 3’ UTR play a critical role in its stability and also have elements required for the translational regulation of TCR \( \zeta \) chain expression in human T cells.

The TCR \( \zeta \) chain is a component of the T cell receptor (TCR)\(^3\) complex that plays a critical role in the assembly and transport of the TCR complex to the cell surface and signal transduction through the TCR that leads to T cell activation. The TCR \( \zeta \) gene is located in chromosome 1q23.1 (1–3), and genome-wide screening for systemic lupus erythematosus (SLE) susceptibility genes demonstrated a linkage between chromosome 1 and SLE susceptibility (4–6). SLE T cells express decreased amounts of TCR \( \zeta \) mRNA and protein (7–9). However, the TCR/CD3-initiated signaling events are increased in intensity compared with those in normal T cells (10). Specifically, cross-linking of the CD3-TCR complex in SLE T cells leads to increased free intracytoplasmic calcium levels (11) and tyrosine phosphorylation of cytosolic proteins (7). This “overexcitability” of the SLE T cell has been attributed to the fact that the missing TCR \( \zeta \) chain is replaced by the FcR\( \gamma \) chain (12) and the surface membrane lipid rafts are aggregated (13). Because replenishment of the TCR \( \zeta \) chain in SLE T cells corrects signaling aberrations and increases interleukin-2 production (14), it is clear that the decreased TCR \( \zeta \) chain expression is central in the disease process.

The molecular mechanisms of diminished expression of the TCR \( \zeta \) mRNA and protein in SLE T cells remain unknown. The TCR \( \zeta \) gene spans at least 31 kb, and the transcript is generated as a spliced product of 8 exons that are separated by distances of 0.7 to more than 8 kb (15, 16). Nucleotide sequence analysis of the TCR \( \zeta \) gene showed increased frequency of alternatively spliced (AS) forms missing various exons as well as splice insertion in SLE T cells (17). Analysis of the 3’ untranslated region (UTR) showed a novel 344-bp AS form with a deletion of nucleotides from 672 to 1233 of exon VIII of the TCR \( \zeta \) mRNA. Using a specific primer that spans either side of the newly identified alternatively spliced site, we recently reported that the AS form of TCR \( \zeta \) mRNA with 344 bp 3’ UTR was predominantly expressed in SLE T cells compared with normal T cells (14).

In SLE T cells, defective TCR \( \zeta \) protein expression inversely correlates with the level of TCR \( \zeta \) mRNA with AS 3’ UTR (18) and directly with WT 3’ UTR. Thus, a molecular switching of TCR \( \zeta \) mRNA with WT 3’ UTR to AS 3’ UTR in T cells contributes to the regulation of TCR \( \zeta \) chain expression. Several AS isoforms of the TCR \( \zeta \) mRNA with different nucleotide sequences of the 3' UTR have been recently identified in murine T cells (19). The expression of these TCR \( \zeta \) mRNA isoforms with alternatively spliced 3’ UTR can be modulated by exposure to pharmacologic agents (20, 21).

The regulation of mRNA decay rate is an important control point in determining the abundance of cellular transcripts (22). The 3’ UTRs of eukaryotic mRNAs are known to play a crucial role in post-transcriptional regulation of gene expression by modulating nucleocytoplasmic mRNA transport, polyadenylation status, subcellular targeting, translation efficiency, stability, and rates of degradation (23–25). Selective expression of TCR \( \zeta \) mRNA with very short AS 3’ UTR in SLE suggests that it plays an important role in the down-regulation of TCR \( \zeta \) chain expression in SLE T cells. Therefore, we conducted studies to determine the stability of TCR \( \zeta \) mRNA with an AS 3’ UTR in SLE T cells and the transport and subsequent trans-
lation of TCR \( \xi \) mRNA with AS 3′ UTR in normal and SLE T cells. In this report we demonstrate that AS 3′ UTR confers instability to the TCR \( \xi \) mRNA, as it does to the mRNA of other genes, and thus contributes to decreased levels of TCR \( \xi \) mRNA. In addition, we show that the TCR \( \xi \) mRNA with AS 3′ UTR displayed poor translation efficiency leading to the production of low amounts of protein. Therefore, the production of TCR \( \xi \) mRNA with short 3′ UTR represents a molecular mechanism that contributes to decreased expression of TCR \( \xi \) chain in SLE T cells.

MATERIALS AND METHODS

SLE Subjects and Controls—Patients fulfilling the American College of Rheumatology Classification criteria for SLE (26) were chosen for the study. Subjects who were on prednisone were asked not to take this medication at least 24 h before drawing the blood. Disease activity for the SLE patients was scored by the SLE disease activity index (SLEDAI) system. Our studies included patients with SLEDAI scores ranging from 0 to 20. The protocol of the study was approved by the Human Use Committees of the involved institutes. Written informed consent was obtained from all participating subjects.

Cell Lines—Purified peripheral blood mononuclear cells from heparinized venous blood were obtained by density gradient centrifugation over Ficoll. T cells were isolated by magnetic separation of non-T cells using a mixture of hapten-conjugated antibodies and MACS microbeads coupled to anti-hapten mAb as described earlier (17) (Miltenyi Biotech, Auburn, CA). In all cases, the percentage of T cells in the isolated subpopulation was >97% as determined by fluorescence-activated cell sorter analysis. The TCR \( \xi \) chain mAb 6B10.2 (Santa Cruz Biotechnology, Santa Cruz, CA) recognizing amino acids 31–45 of the polypeptide was purchased either from Sigma, Jackson ImmunoResearch Laboratories (West Grove, PA), or BD Biosciences. The C-terminal TCR \( \xi \) chain mAb recognizing amino acids 145–161 (28) and horseradish peroxidase-conjugated anti-phosphotyrosine mAb 4G10 were purchased from Immunotech (Coulter Corp., Miami, FL), CD16-PE was from BD Biosciences. Fluorescent isotype control mAbs were from Immunotech (Coulter Corp., Miami, FL). 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) was from Promega (Madison, WI) and oligo(dT) primer as instructed by the manufacturer. The 5′-UTR of TCR \( \xi \) mRNA was amplified by PCR using primers 5′-AGC CTC TGC CTC CGG CCT TCT TGA G-3′ (sense bp 34–62) and 5′-GCC TGG TAG TCT GCT GAG-3′ (antisense bp 1472–1446). The amplified cDNA was cloned into the high-fidelity cloning system of Roche Applied Science in a Biometra T-3 thermal cycler after initial denaturation of 94 °C for 6 min, 33 cycles at 94 °C, 1 min; 67 °C, 1 min; 72 °C for 2 min, and a final extension at 72 °C for 7 min. The PCR products containing 1438 bp WT and 916 bp AS TCR \( \xi \) were ligated to unidirectional pcDNA 3.1 His TOPO vector (Invitrogen). Minipreps were prepared from 12 recloning clones, and the direction of the insert was confirmed by sequencing. AS TCR \( \xi \) clones with proper orientation were subjected to DNA sequencing from both orientations on an ABI 377 sequencer using the ABI dye terminator cycle sequencing kit (ABI PRISM; Applied Biosystems Inc., Foster City, CA).

Transfection of TCR \( \xi \) with WT and AS 3′ UTR to COS-7 cells—The DNA expression construct, COS-7 cells were subcultured in RPMI 1640 containing 10% fetal bovine serum and penicillin streptomycin at 37 °C in a 5% CO2 incubator. For transfection, cells were trypsinnized, washed, and resuspended in 250 μl of Opti-MEM serum-free medium (Invitrogen). 15 μg of plasmid pcDNA 3.1 V5 HIS TOPO containing TCR \( \xi \) with WT or AS 3′ UTR was added and electroporated at 250 V, 960 μF in a 0.4-cm cuvette (Bio-Rad). Electroporated cells were immediately washed in medium and cultured. Transfected cells were lysed at different time points after incubation with actinomycin D (5 μg/ml), and the mRNA was isolated after lysis of the cell membrane by Nonidet P-40 (7).

In Vitro Transcription and Translation—The WT and AS TCR \( \xi \) mRNA were transcribed and translated using the TNT T7 quick-coupled rabbit reticulocyte lysate transcription/translation system as recommended by the manufacturer (Promega). 10 μg of plasmids containing WT or AS TCR \( \xi \) was incubated with the transcription/translation system in the presence of Transcend Botin-Lysyl-tRNA for 60 min at 30 °C. The translated product was electrophoresed and transferred to polyvinylidene difluoride membranes. The incorporated biotinylated lysine was detected non-radioactively by blotting with streptavidin-horseradish peroxidase and developed using an ECL chemiluminescent kit (Amersham Biosciences).

Reporter Gene Construction and Transient Transfection—The full-length 3′ UTRs of WT and AS TCR \( \xi \) mRNA were amplified by PCR and cloned into the XbaI site downstream of the luciferase reporter gene in the pG3-Lucase Basic and Enhancer vector(s) (Promega). Transcription from this construct is driven by an SV40 promoter. The correct orientations of the clones were verified by restriction mapping and sequencing. Plasmid DNA transfections of Jurkat cells, COS-7 cells, and T cells were performed using Lipofectamine (Gibco BRL, Gaithersburg, MD) according to the manufacturer's instructions.
were carried out in 24-well plates (Corning Inc., Corning, NY) using Lipofectamine™2000 reagent (Invitrogen) following the manufacturer’s protocol. The day before transfection, 6 × 10^6 COS-7 cells or 0.6 × 10^6 HeLa cells were plated in 0.5 ml of medium/well. For AS 3
TCR
/H9262
Lipofectamine™2000 reagent (2–3 μl) was mixed with plasmid DNA (1.5 μg) in serum-free Opti-MEM to allow DNA-Lipofectamine™ reagent complexes to form. The complexes were added to respective wells and mixed by gently rocking the plate back and forth. The transfected cells were washed with 1× phosphate-buffered saline three times.

Luciferase Activity Assays—Luciferase activity was determined using a luciferase assay system (Promega) following the manufacturer’s protocol. Briefly, the transfected cells were incubated in a CO_2 incubator at 37 °C for 18 h and then lysed with 60 μl of reporter lysis buffer (Promega). Cellular debris was removed by centrifugation for 2 min at 12,000 rpm. Luciferase activity was assayed with 20 μl of lyase and 80 μl of luciferase assay reagent (Promega) in a TD20/20 luminometer (Turner Designs) using a commercially available kit (Promega). Light output was measured over a 10-s time period in triplicate for each sample. Relative luciferase activity was calculated by averaging the readings. Transfection efficiency was established in all samples by cotransfection with 1 μg of a plasmid encoding the cytomegalovirus promoter-driven β-galactosidase gene. Luciferase activity was normalized for transfection efficiency using the corresponding β-galactosidase activity. Data are presented as a fold increase in luciferase activity compared with that obtained from cells transfected with vector alone.

Densitometry and Statistical Analysis—Densitometry analysis of the Western blot was performed with the software program GelPro (Media Cybernetics). Statistical analysis was done using the software Minitab Version 13 (Minitab Inc., State College, PA) by applying the Student’s t test.

RESULTS
Stability of TCR ζ mRNA with AS 3’ UTR in SLE T Cells—Cloning and sequence analysis of TCR ζ mRNA revealed the presence of a novel TCR ζ chain transcript with alternatively spliced 3’ UTR in human T lymphocytes (Fig. 1). Although the TCR ζ mRNA with AS 3’ UTR was detected at low levels in normal human T cells, it was found to be predominantly expressed in SLE T cells (18). Because a vast majority of lupus patients display decreased expression of TCR ζ mRNA (7, 17), we considered the TCR ζ mRNA with AS 3’ UTR to be more unstable than the TCR ζ mRNA with WT 3’ UTR. Accordingly, purified T cells were incubated with transcription inhibitor actinomycin D (5 μg/ml) for different periods of time (0, 1, 2, and 4 h), and the levels of expression of WT and AS 3’ UTR TCR ζ mRNA were quantitated by semiquantitative RT-PCR using specific primers as described under “Materials and Methods.” By calculating the quantity of mRNA as a percentage of the amount at 0 h, we determined that the levels of TCR ζ mRNA with AS 3’ UTR were reduced compared with the WT ζ mRNA in SLE T cells (Fig. 2, A and B). We also compared the stability of the TCR ζ mRNA with WT 3’ UTR to that with the AS 3’ UTR after calculating the ratio of the levels of each mRNA to β-actin mRNA. As shown in Fig. 2C, the stability of AS TCR ζ mRNA is decreased compared with the WT 3’ UTR in SLE T cells (Fig. 2C).

Stability of TCR ζ mRNA with AS 3’ UTR in Normal T Cells—To address the question whether the decreased half-life of TCR ζ mRNA with AS 3’ UTR is restricted to SLE T cells, we analyzed the stability of TCR ζ mRNA in normal T cells. Purified normal T cells were incubated with transcription inhibitor actinomycin D (5 μg/ml) for different periods of time, and the levels of TCR ζ mRNA expression with WT and AS 3’ UTR were measured in parallel. We observed that the mRNA stability of TCR ζ mRNA with AS 3’ UTR was also low in normal T cells (Fig. 3, A and B). Although the stability of TCR ζ mRNA with AS 3’ UTR was low in normal cells, it was not as prominent as it was recorded in SLE T cells. After 4 h of incubation with actinomycin D, the amount of TCR ζ mRNA with AS 3’ UTR was 17 and 33% in SLE and normal T cells, respectively (Figs. 2B and 3B). However, the rate of degradation of TCR ζ mRNA with WT 3’ UTR was not significantly different between SLE and normal T cells.

Real-time Quantitative PCR Analysis of TCR ζ mRNA with AS 3’ UTR in SLE and Normal T Cells—We used a quantitative real-time RT-PCR to measure the stability of TCR ζ mRNA with WT and AS 3’ UTR in SLE T cells and normal T cells treated with actinomycin D (5 μg/ml) for 0, 1, 2, or 4 h to confirm the observed differences in the stability of the TCR ζ mRNA with WT or AS 3’ UTR. The mRNA from the samples was converted to cDNA by reverse transcriptase. The reverse transcription product of cDNA was PCR amplified using TCR ζ WT- and AS 3’ UTR-specific primers in the presence of SYBR green in a Cepheid thermocycler as described under “Materials and Methods.” To validate the real-time quantitative PCR, the standard curves for TCR ζ mRNA with WT and AS 3’ UTR and GAPDH cDNA were constructed first (data not shown). The cycle threshold values for TCR ζ mRNA with WT/AS 3’ UTR and GAPDH mRNA in the sample were measured in triplicate by real-time quantitative PCR, and the amounts of these mRNA were determined from the standard curves. TCR ζ mRNA with WT or AS 3’ UTR was evaluated as the relative quantity against GAPDH mRNA in the cells before treatment with actinomycin D (Fig. 4). Real-time quantitative PCR demonstrated that WT 3’ UTR mRNA was more stable than AS 3’ UTR mRNA during the first 4 h. These results also show that TCR ζ mRNA with AS 3’ UTR (AS mRNA: GAPDH mRNA = 0 h, p = 0.037; 4 h, p = 0.005) is more stable than the WT 3’ UTR (WT mRNA: GAPDH mRNA = 0 h, p = 0.005) after 4 h of incubation with actinomycin D in SLE T cells (WT 3’ UTR, p = 0.042 and AS 3’ UTR, p = 0.007) (Fig. 4A). The stability of TCR ζ mRNA with WT 3’ UTR (WT mRNA: GAPDH mRNA = 0 h, p = 0.06; 4 h, p = 0.03) and AS 3’ UTR (AS mRNA: GAPDH mRNA = 0 h, p = 0.005; 4 h, p = 0.001) was also analyzed in normal T cell by
real-time quantitative PCR (WT 3′/H11032 UTR, p/H11005 0.05 and AS 3′/H11032 UTR, p/H11005 0.015) (Fig. 4B). The results show that TCR mRNA with WT 3′/H11032 UTR degraded to a lower extent compared with the TCR mRNA with AS 3′/H11032 UTR after 4 h of incubation with actinomycin D (Fig. 4).

**Stability of TCR mRNA with AS 3′ UTR in COS-7 Cells**—Next we studied the stability of TCR mRNA with WT and AS 3′ UTR transfected in COS-7 cells. TCR mRNA with WT or AS 3′ UTR was cloned into a eukaryotic expression vector, pcDNA3.1/V5-HIS-TOPO, and transfected to COS-7 cells. After 18 h of transfection, the cells were incubated with actinomycin D for 0 or 4 h, and the mRNA were measured by RT-PCR analysis. Although in COS-7 cells there was no significant degradation of TCR mRNA by 4 h, by 8 h we recorded a significant decrease in the level of TCR mRNA with AS 3′ UTR compared with WT 3′ UTR (Fig. 5). To rule out that the difference was due to differences in export from nucleus or transport of TCR mRNA with WT and AS 3′ UTR from nucleus to cytoplasm as a result of alternative splicing, the transfected cells were lysed at different time points after incubation with actinomycin D and mRNA was isolated after lysis of the cell membrane by Nonidet P-40. Levels of TCR mRNA between the cytoplasm and nucleus were measured by semiquantitative RT-PCR. The data showed that the ratio of TCR mRNA between the cytoplasm and nucleus was similar at different points, suggesting that alternative splicing of TCR 3′ UTR does not affect the transport of the TCR mRNA (data not shown).

**Translational Regulation of TCR Expression in COS-7 Cells Transfected with TCR Carrying WT and AS 3′ UTR**—Our previous data showed that the down-regulation of TCR mRNA with AS 3′ UTR inversely correlated with the level of TCR mRNA with AS 3′ UTR (18). This finding suggested that, although the coding region is intact in TCR mRNA with AS 3′ UTR, the translational regulation of TCR mRNA is defective. We analyzed the translational efficiency of TCR mRNA with
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**Fig. 4.** Stability of alternatively spliced TCR \( \zeta \) mRNA in SLE and normal T cells by real-time quantitative PCR analysis. SLE and normal T cells were isolated by magnetic separation and incubated with transcription inhibitor actinomycin D for 0, 1, 2, and 4 h. The cells were lysed, the total RNA was reverse transcribed, and the stability of TCR \( \zeta \) mRNA was determined by real-time quantitative PCR. Real-time quantitative PCR was carried out in a Cepheid Smart Thermocycler using PCR beads by adding SYBR green to the reaction mixture. A, real-time quantitative PCR analysis of TCR \( \zeta \) mRNA stability in SLE T cells. B, real-time quantitative PCR analysis of TCR \( \zeta \) mRNA stability in normal T cells. Figure is representative of three experiments with similar results. Vertical bars indicate S.E.

**Fig. 5.** Stability of alternatively spliced TCR \( \zeta \) mRNA in transfected COS-7 cells. TCR \( \zeta \) with WT or AS 3' UTR cloned in pCDNA3.1/V5-HIS-TOPO and transfected to COS-7 cells by electroporation. After 18 h of transfection, the cells were lysed and the total RNA was reverse transcribed and PCR amplified using a high fidelity PCR system as described for Fig. 2. Quantitation of the RT-PCR product was done using GEL-PRO software, and the data are represented as percentage of control. The data shown are representative of three experiments with similar results from three different transfections. Vertical bars indicate S.E.

WT and AS 3' UTR in transfected COS-7 cells by immunoblotting experiments. Eighteen hours after transfection with equal amounts of the constructs, the cells were lysed, electrophoresed, and immunoblotted with a TCR \( \zeta \) chain-specific mAb. As shown in Fig. 6A, TCR \( \zeta \) with WT 3' UTR expressed a single band with a molecular mass of 16 kDa as reported previously (14, 29). Cells transfected with TCR \( \zeta \) carrying AS 3' UTR produced very low amounts of the 16-kDa form of TCR \( \zeta \) protein. Densitometric analysis showed a 10-fold decrease in the protein level in COS-7 cells transfected with AS TCR \( \zeta \) (Fig. 6B). However, RT-PCR showed equal amounts of mRNA in WT- and AS TCR \( \zeta \)-transfected cells. This suggests that alternative splicing of TCR \( \zeta \) 3' UTR has a major impact on the translation of TCR \( \zeta \) mRNA. Indirectly the data also suggest that the splice-deleted 562-bp region contains some elements that play important roles in the translational regulation of TCR \( \zeta \) chain.

**In Vitro Transcription and Translation of TCR \( \zeta \) with WT and AS 3' UTR**—To provide further evidence that the TCR \( \zeta \) mRNA with AS 3' UTR results in lower production of protein and rule out the possibility of transfection variability in the above experiments, we transcribed and translated the TCR \( \zeta \) with AS 3' UTR in vitro using biotinylated lysine as a non-radioactive label. The in vitro transcribed and translated products were electrophoretically separated and blotted with streptavidin-horseradish peroxidase conjugate. Similar to the expression in COS-7 cells, in vitro transcription and translation of TCR \( \zeta \) with WT 3' UTR produced a protein of 16-kDa TCR \( \zeta \) chain (Fig. 7A). The TCR \( \zeta \) with WT 3' UTR produced high levels of 16-kDa protein, whereas the TCR \( \zeta \) with AS 3' UTR produced significantly low levels of the 16-kDa TCR \( \zeta \) protein. The results were also confirmed by stripping and reprobing the blots with TCR \( \zeta \) chain-specific antibody (Fig. 7B). Densitometric analysis showed that transfected COS-7 cells with TCR \( \zeta \) AS 3' UTR resulted in an 8-fold reduction in the protein level (Fig. 7C).
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8E) and T cells (data not shown). These data indicate that AS 3' UTR confers instability that is not gene- or cell type-specific.

DISCUSSION

One of the major outcomes of the present study is the finding that TCR ζ mRNA with AS 3' UTR that is selectively expressed in SLE T cells is less stable compared with the WT 3' UTR in T cells of SLE patients as well as normal subjects (Figs. 2 and 3). These results are consistent with the general notion that the shorter the 3' UTR, the less stable is the message (25, 30–32). However, compared with normal subjects, the level of degradation of the TCR ζ mRNA with AS 3' UTR is more severe in SLE T cells (Fig. 4), suggesting that the SLE T cells have additional factors that promote TCR ζ mRNA degradation. However, the stability of TCR ζ mRNA with WT 3' UTR is similar between SLE and normal T cells. These data, along with the fact that TCR ζ mRNA with AS 3' UTR is selectively expressed in SLE T cells and TCR ζ chain protein expression inversely correlates with TCR ζ mRNA with AS 3' UTR, suggest that SLE T cells preferentially produce TCR ζ mRNA with the AS 3' UTR form that is unstable. This process represents a new pathologic mechanism for the down-regulation of TCR ζ mRNA in SLE. The levels of AS 3' UTR TCR ζ mRNA are not increased in T cells from patients with other systemic inflammatory rheumatic diseases such as rheumatoid arthritis (18).

The 3' UTR of mRNAs play important roles in regulating gene expression at the post-transcriptional level (23, 24, 33–35). To date, the minimum and maximum lengths of 3' UTR observed in human mRNAs are 21 nucleotides and 8.5 kb, respectively, and average length is 0.3–0.7 kb (32, 36). The 3' UTR of TCR ζ is ~1 kb, which is considerably longer, suggesting that it may have one or more important roles in regulation of gene expression. The molecular mechanisms that lead to destabilization of the TCR ζ mRNA with AS 3' UTR are currently unknown. 3' UTR of mRNA contains cis-acting elements, for example, adenosine-uridine (AU)-rich elements that bind to trans-activating factors and either stabilize or destabilize the transcripts (37, 38). Sequence analysis indicates the presence of one AU-rich sequence in the splice-deleted 562 bp of TCR ζ mRNA with AS 3' UTR. The TCR ζ mRNA with AS 3' UTR also does not contain a 31-nucleotide sequence (from 973 to 1003) that is conserved across the TCR ζ mRNA of several species. If this conserved region is involved in the regulation of mRNA stability, then the absence of this region would explain the decreased stability of the TCR ζ mRNA with AS 3' UTR. Alternatively, the new splicing introduces exon-exon boundaries more than 50 base pairs from the stop codon and likely to be subjected to nonsense-mediated mRNA decay (39, 40). Nonsense-mediated mRNA decay has been implicated in the regulation of AU1 expression via conserved alternatively spliced elements in the 3' UTR (41, 42).

Regulation of mRNA stability is often mediated by elements within the 3' UTR (23, 25, 37). To facilitate identification of regulatory elements within 3' UTRs, reporter systems using constitutive promoters have been extensively used. We tested a constitutive luciferase reporter model to determine whether the WT 3' UTR and AS 3' UTR of the TCR ζ could affect mRNA stability. The luciferase activity in the transfected cells with the AS 3' UTR construct was significantly decreased compared with WT 3' UTR (Fig. 8). These data demonstrate that the destabilizing effect of the AS 3' UTR that was identified as part of the TCR ζ mRNA is not gene-specific and may confer instability to other genes. In addition, the effect was found not to be cell type-specific, suggesting that either trans factors are not required and the destabilizing effect is simply 3' UTR length-dependent or the required trans factors are non-cell type-specific and rather universal in nature.

**Stability and Expression of Luciferase Gene Fused with TCR ζ WT and AS 3' UTR**—To determine whether the AS 3' UTR conferred instability to genes other than that of TCR ζ, we introduced the WT and AS 3' UTRs downstream of the luciferase gene. Luciferase constructs containing TCR ζ WT and AS 3' UTR were made by PCR amplification by engineered primers containing the XbaI site (Fig. 8A). PCR-amplified products with the XbaI site (Fig. 8B) were cloned into pGL2 basic and enhancer vector(s) after digestion with XbaI, which is located immediately after the luciferase gene at the 3' region (Fig. 8A). The luciferase clones were confirmed by restriction mapping that resulted in appropriate sizes of inserts (Fig. 5C). Finally luciferase clones were reconfirmed by sequence analysis (data not shown). The resulting luciferase reporter constructs containing TCR ζ WT and AS 3' UTR were individually co-transfected with β-galactosidase to COS-7 cells, Jurkat cells, and normal T cells. Cells were harvested after 18 h of transfection with phosphate-buffered saline, and the luciferase activity of the transfected cells was measured. As shown in Fig. 8, D and E, luciferase activity is significantly decreased in cells transfected with the AS 3' UTR construct compared with WT 3' UTR in Jurkat, COS-7, and normal T cells (COS-7 cells, p = 0.001 and Jurkat cells, p = 0.049). Maximal decrease was found in COS-7 cells (2.8-fold) (Fig. 8D), followed by Jurkat cells (2.1-fold) (Fig. 8E) and T cells (data not shown).
We have previously reported that in SLE T cells the mutations/polymorphisms and splice variation of TCR mRNA including a single exon or combination of exons are significantly higher compared with normal subjects (17, 18). These abnormalities could provide an additive effect on the degradation of TCR mRNA with AS 3' UTR in SLE T cells. It has been reported that in patients with multiple sclerosis the regulation of major histocompatibility class II transactivator expression is associated with alternative splicing in the 3' UTR (43, 44). It is also possible that abnormalities in other cellular factors may contribute to increased degradation of TCR mRNA with 3' UTR in SLE T cells.

The second major finding is that the TCR mRNA with AS 3' UTR produces low amounts of 16-kDa TCR protein compared with the chain with WT 3' UTR (Figs. 5 and 6). This result strongly suggests that the presence of translational regulatory elements in the splice-deleted 562 bp region is required for the proper and efficient translation of TCR chain mRNA. Clearly, more experiments are needed to explain how the splice-deleted region influences TCR chain protein expression. There are many reports describing the regulation of protein expression by AS elements (45, 46). This regulation is mediated by the binding of protein factors to the 3' UTR, and splice deletion of TCR mRNA with AS 3' UTR may abate the binding of these factors. Identification of the proteins or factors that interact with the 562-bp splice-deleted region will support this hypothesis.
We and others have shown that T cells from SLE patients are deficient in TCR ζ chain and display abnormal TCR/CD3-induced early signaling events (7, 27, 47). The molecular basis of the TCR ζ chain decrease in SLE T cells, and its significance in the pathogenesis of the disease has not been defined. Our present findings suggest that selective expression of TCR ζ mRNA with AS 3’ UTR is associated with the abnormalities of TCR ζ chain expression in SLE patients. These results also infer an important pathway for the regulation of gene expression involved in the immune response by exploiting splicing processes that destabilize mRNAs or modulate its expression. In conclusion, we have reported herein that the instability and limited translation of the AS TCR ζ mRNA contribute to the decreased expression of TCR ζ chain in SLE T cells. We propose that the 562-bp splice-deleted region of the TCR ζ 3’ UTR contains crucial cis elements that bind proteins that confer stability and sufficient translation rate and when spliced out render the mRNA unstable and with limited translational efficiency.

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