Alternative Splicing Factor/Splicing Factor 2 Regulates the Expression of the ζ Subunit of the Human T Cell Receptor-associated CD3 Complex∗

Received for publication, December 11, 2009, and in revised form, January 7, 2010. Published, JBC Papers in Press, January 29, 2010, DOI 10.1074/jbc.M109.091660

Vaishali R. Moulton and George C. Tsokos†
From the Division of Rheumatology, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02115

T cells from patients with systemic lupus erythematosus express decreased levels of the T cell receptor-associated CD3ζ chain, a feature directly linked to their aberrant function. The decrease in CD3ζ protein expression is in part due to decreased levels of functional wild type isoform of the 3′-untranslated region (UTR) of CD3ζ mRNA with concomitant increased levels of an unstable alternatively spliced isoform. In order to identify factors involved in the post-transcriptional regulation of CD3ζ, we performed mass spectrometric analysis of Jurkat T cell nuclear proteins “pulled down” by a CD3ζ 3′-UTR oligonucleotide, which identified the splicing protein alternative splicing factor/splicing factor 2 (ASF/SF2). We show for the first time that ASF/SF2 binds specifically to the 3′-UTR of CD3ζ and regulates expression of CD3ζ protein by limiting the production of the alternatively spliced isoform. During activation of human T cells, an increase in the wild type CD3ζ mRNA is associated with increased expression of ASF/SF2. Finally, we show a significant correlation between ASF/SF2 and CD3ζ protein levels in T cells from systemic lupus erythematosus patients. Thus, our results identify ASF/SF2 as a novel factor in the regulation of alternative splicing of the 3′-UTR of CD3ζ and protein expression in human T cells.

Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune disease characterized by abnormal cellular and humoral immune responses and a disease course marked by relapses (flares) and remissions. A key signaling defect of T cells in SLE patients is their aberrant expression of the T cell receptor (TCR)-associated CD3ζ chain, the crucial signaling transducer of the TCR (1). CD3ζ protein expression is heterogeneous in SLE patients and tends to inversely correlate with severity of the disease. Consequently, many patients exhibit decreased (20–80% of normal) levels of CD3ζ protein, whereas others express normal levels of CD3ζ protein when compared with healthy individuals. The homologous Fc receptor γ chain replaces CD3ζ chain in the TCR CD3 complex in these CD3ζ-low T cells (2) and recruits the spleen tyrosine kinase (Syk) instead of the normally recruited ζ-associated protein (ZAP-70) (3). This “rewiring” of the TCR, along with clustered TCR-bearing lipid rafts, leads to aberrantly increased phosphorylation and calcium flux upon activation (4). Despite this overexcitable phenotype, SLE T cells fail to produce the vital cytokine interleukin-2. Replenishment of the CD3ζ in the SLE T cells in vitro reverts this phenotype and more importantly restores interleukin-2 production (5). Although these findings highlight a central role for the aberrant CD3ζ expression in the SLE T cell defect, the mechanisms regulating the expression of CD3ζ chain in physiology and in disease are not fully understood.

The CD3ζ gene spans 31 kb in the chromosome 1q23.1 locus, a region associated with SLE susceptibility (6), and bears eight exons separated by introns ranging from 700 bp to greater than 8 kb (Fig. 1A, top) (7, 8). The CD3ζ mRNA is a 1.472-kb spliced product of the eight exons with a coding region of 492 bp and an exon VIII-encoded long downstream 906-bp 3′-untranslated region (UTR) (Fig. 1A, middle). Activation of alternative splicing within the 3′-UTR due to usage of two internal (5′ and 3′) splice sites results in splice deletion of 562 bases (nucleotides 672–1233), leading to the generation of a 344-bp alternatively spliced (AS) variant. We have shown that T cells from healthy individuals express predominantly the wild type (WT) isoform, whereas SLE T cells exhibit variably increased levels of the AS isoform (9). Because this alternative splicing affects the UTR and not the coding region, the size of the protein generated from these two isoforms is identical. However, the 344-bp AS CD3ζ isoform lacks two critical regulatory adenosine/uridine-rich elements (ARE) and a translation regulatory sequence, the transcript stability and translation of this isoform are significantly lower than that of the 906-bp WT CD3ζ isoform; consequently, the relative amount of CD3ζ protein generated by the AS isoform is significantly lower than that from the WT isoform (10). Accordingly, the CD3ζ protein expression level directly correlates with the level of the WT isoform and inversely with the AS isoform (9). Although the differential expression of the CD3ζ 3′-UTR isoforms contributes to differential levels of CD3ζ protein expression, the factor(s) regulating the alternative splicing of CD3ζ 3′-UTR is unknown.

Alternative splicing is a powerful mechanism of gene regulation, which results in the generation of numerous transcripts.
and proteins from a single gene (11). Splice site selection is regulated by cis-acting elements, such as intronic and exonic splicing enhancer and silencer sequences, respectively, whereas trans-acting factors include the U small nuclear ribonucleoproteins, the heterogeneous nuclear ribonuclear proteins, and the serine-arginine (SR) family of splicing factors (12, 13).

ASF/SF2 (alternative splicing factor/splicing factor 2) is a prototype member of the SR family of splicing proteins and has an N-terminal domain bearing two RNA recognition motifs and a C-terminal arginine-serine (RS) domain, which is involved in protein-protein interactions. ASF/SF2 is a predominantly nuclear protein but is known to shuttle between the cytoplasm and nucleus (14). Phosphorylation and dephosphorylation are important in the splicing function of ASF/SF2 and affect its cellular localization and function (15). The serine/threonine-specific protein phosphatase PP1 is shown to affect its cellular localization and function (15). The serine/arginine (SR) family of splicing factors (12, 13).

In order to identify factors involved in the post transcriptional regulation of CD3ζ, we performed mass spectrometric analysis of Jurkat T cell nuclear proteins “pulled down” by a CD3ζ 3′-UTR-defined ARE-bearing oligonucleotide that identified the splicing factor ASF/SF2. We hypothesized that ASF/SF2 regulates the alternative splicing of the 3′-UTR of CD3ζ and thus regulates the expression of the CD3ζ protein. We show for the first time that ASF/SF2 binds to the 3′-UTR of CD3ζ and regulates the shift in alternative splicing from the AS to the WT isoform and regulates expression of CD3ζ protein. We also show that a preferential increase in the WT CD3ζ isoform occurs during T cell stimulation, which correlates with increased ASF/SF2 expression levels. Last, ASF/SF2 expression correlates directly with CD3ζ chain levels in T cells from SLE patients.

EXPERIMENTAL PROCEDURES

Patients and Controls—Twelve female SLE patients fulfilling the American College of Rheumatology classification criteria (22) and 10 healthy subjects (age-, race-, and gender-matched) as controls were chosen for this study. For some studies, other healthy volunteers were used. The study protocol was approved by the health use committees of Beth Israel Deaconess Medical Center. Written informed consent was obtained from all participating subjects.

Mass Spectrometry Protein Identification—Peptide sequencing and protein identification were performed by the core facility at Beth Israel Deaconess Medical Center (23).

Cells, Plasmids, and Antibodies—Peripheral blood was collected by venipuncture and T cells purified using the RosetteSep T cell kit (StemCell Technologies). The pcDNA3.1-ASF/SF2 vector was a kind gift from Dr. James Manley (Columbia University, New York). ASF/SF2 antibody was from Zymed (Invitrogen). CD3ζ, and goat anti-mouse horseradish peroxidase-conjugated antibody were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). β-Actin antibody was from Sigma, and β-tubulin antibody was from Upstate. d-erythro-C6- ceramide was from Matreya LLC (Pleasant Gap, PA).

RNA Electrophoretic Mobility Shift Assays—RNA oligonucleotides were end labeled with [32P]ATP (PerkinElmer Life Sciences) in a 10× T4 kinase buffer with T4 polynucleotide kinase for 1 h at 37 °C. The reaction was stopped with Tris-HCl, pH 8.0, buffer, and radiolabeled probes were separated by microspin columns (GE Healthcare). T cell nuclear extracts were incubated with antibody in a 5× high density TBE buffer with 1 N KCl, 500 μg of poly(dI-dC), and RNAse on ice for 15 min. 20 pmol of [32P]ATP-labeled probes were added, and reaction mixtures were kept at room temperature for 15 min and run on a 6% retardation gel. Gels were fixed in a 10% acetic acid and 10% methanol solution, dried, and exposed overnight to a phosphor screen and scanned with a phosphor imager scanner (GE Healthcare).

In Vitro Transcription and Translation—The pASF expression plasmid (1.5 μg) was used to transcribe and translate ASF/SF2 using the TNT T7 quick coupled transcription/translation kit (Promega) according to manufacturer’s instructions.

Plasmid Transfections—Transient transfections of peripheral blood T cells and were carried out using the Lonza Nucleofector system (Lonza, Walkersville, MD). Briefly, 5 × 10^6 cells were resuspended in 100 μl of Nucleofector solution, plasmid DNA (2 μg/10^6 cells) was added, and cells were transfected using the nuleofector U-014 program. Cells were rescued immediately after transfection in prewarmed RPMI medium supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin in 12-well culture plates. Cells were harvested, and total RNA and protein was extracted 24–48 h post-transfection.

Short Interfering RNA (siRNA) Knockdown—A pair of siRNAs against ASF/SF2 Hs_SFRS1_1 (target sequence CAG TAT TGA CCT TAT ACT AAA) and Hs_SFRS1_3 (target sequence ATC CAA CAA GAT AGA GTA TAA) and control non-silencing siRNA (target sequence AAT CTT CCG AAC GTG TCA CGT) were purchased from Qiagen (Valencia, CA). siRNA knockdown experiments were carried out using the Lonza Nucleofector system as described above.

Western Blotting—Cells were pelleted and lysed with radioimmunoprecipitation buffer (Boston Bioproducts). Lysates were resolved on 4–12% BisTris gels and transferred to polyvinylidene difluoride membrane. Membranes were blocked with 5% nonfat milk in Tris-buffered saline with Tween 20 (TBS-T) for 1 h, incubated with primary antibody (1:1000) for 1 h, washed three times with TBS-T, incubated with horseradish peroxidase-conjugated secondary antibody (1:2000) for 1 h, washed three times with TBS-T, developed with ECL reagents (GE Healthcare), and visualized by the Fujifilm LAS-4000 imager.

mRNA Expression Studies—Total RNA was isolated using the RNEasy minikit (Qiagen, CA). 100 ng of total RNA was reverse transcribed into single-stranded cDNA using the avian myeloblastosis virus reverse transcriptase kit (Promega, Madison, WI). CD3ζ 3′-UTR primers were as follows: forward,
Asf/Sf2 Regulates Cd3ζ Expression in Human T Cells

5′-CAGCCAGGGGATTTCCAACACTCAAAG-3′ (sense
bp 567–592); reverse, 5′-CCCTAGTACATGGACGGGT-
TTTCTCTG-3′ (antisense bp 1472–1443), according to the
numbering of Weisman et al. (24). GAPDH primers were
5′-CAACTACAGTTTACATGTGCC-3′ (forward) and
5′-GGACTGTTTACAGTCTCTG-3′ (reverse). Asf/Sf2 primers
were 5′-TCTCTGAGCTGCCACAGT-3′ (forward) and
5′-GGCTCTGCTACGAAGG-3′ (reverse). PCR amplifi-
cation was carried out in a Bio-Rad thermocycler as follows:
- denaturation at 94 °C for 1 min, annealing at 67 °C for 2 min;
- final extension at 72 °C for 2 min; and
- final cooling at 4 °C. PCR products were run on 1.2% aga-
rose gels in 1× Tris-acetate EDTA buffer, stained with ethidium
bromide, and scanned with a Bio-Rad gel viewer. Real time
PCR amplification of Asf/Sf2 was carried out in a LightCycler
480 (Roche Applied Science) as follows: initial denaturation
at 95 °C for 5 min, 40 cycles of amplification (denaturation at
95 °C for 15 s, annealing at 60 °C for 15 s, and
extension at 72 °C for 30 s); 1 cycle of melting curves at 95 °C for 15 s,
65 °C for 2 min, and 97 °C for 20 s; and a final cooling
step at 72 °C for 30 s. Threshold cycles (Ct) values were used
to calculate relative mRNA expression by the ΔΔCt relative
quantification method.

Cell Activation—T cells (2 × 10⁶ cells/ml) were resus-
pended in complete RPMI medium in 6-well plates. Soluble
α-Cd3 (10 μg/ml), α-Cd28 (5 μg/ml), and goat α-mouse IgG
cross-linker (10 μg/ml) antibodies were added for the indicated
time points.

Densitometry and Statistical Analysis—Densitometric anal-
ysis of the Western blots and agarose gels was performed with
the Quantity 1 software (Bio-Rad). Statistical analyses were per-
formed using Student’s t test (MS Excel) and Pearson’s R cor-
relation coefficient (GraphPad Prism software, version 5.0).

Results

Identification of Asf/Sf2 Binding to the Cd3ζ 3′-UTR—The
3′-UTR of Cd3ζ bears three AREs at positions 636, 705, and
985, designated ARE1, ARE2, and ARE3, respectively. We have
shown that ARE2 and ARE3 are critical in stabilizing the Cd3ζ
transcript (25), and using an ARE2-defined (nucleotides 693–
717) RNA oligonucleotide with Jurkat T cell nuclear proteins
“pulled down” several putative RNA-binding proteins in the
30–60 kDa range (23). Interestingly, mass spectrometry anal-
ysis of the ~30-kDa protein complex revealed peptides that
matched the amino acid sequence of the SR protein Asf/Sf2
(Fig. 1B).

To confirm specific Asf/Sf2 binding to the Cd3ζ 3′-UTR in
primary T cells, we used the RNA oligonucleotide (nucleotides
693–717) (Fig. 1C) in an RNA electrophoretic mobility shift
assay. Radiolabeled oligonucleotides were incubated with nu-
clear protein extract, and the RNA protein complexes were run
on a retardation gel (Fig. 1C, lane 1). Using an antibody specific
for Asf/Sf2, we showed that the binding complex was dimin-
ished (Fig. 1C, lane 2), whereas incubation with an irrelevant
control antibody did not affect the complex (Fig. 1C, lane 3).
Using in vitro translated Asf/Sf2 protein in the gel shift assay,
we observed the binding complexes diminish in the presence of
Asf/Sf2 antibody compared with in the presence of control
antibody (Fig. 1C, lanes 4–6). Specific binding of Asf/Sf2 was
observed with the addition of increasing amounts of Asf/Sf2
protein (Fig. 1C, lanes 7–11), and the binding complexes were
outcompeted in the presence of excess “cold” oligonucleotide
(Fig. 1C, lane 13) compared with that of a scrambled control
oligonucleotide (Fig. 1C, lane 14). These results indicate spe-
cific binding of Asf/Sf2 to the nucleotide 693–717 region of
the Cd3ζ 3′-UTR.

Asf/Sf2 Regulates the Expression of Cd3ζ Protein—The
finding that Asf/Sf2 binds to the 3′-UTR of Cd3ζ prompted us

FIGURE 1. A, schematic of Cd3ζ gene (top), and Cd3ζ mRNA bearing the full-
length WT (middle) and the AS (bottom) isoforms of the 3′-UTR. Arrows P1 (567
sense) and P2 (1472 antisense) indicate primer positions for PCR amplification
of 3′-UTR isoforms. B, Asf/Sf2 amino acid sequence showing peptides
boxed, in boldface and italic type) identified by mass spectrometry. C, gel shift
assay showing Asf/Sf2 binding to the Cd3ζ 3′-UTR oligonucleotide. T cell
nuclear extracts were incubated with 32P-labeled oligonucleotide (nucleo-
tides 693–717) in the absence (lane 1) or presence (lane 2) of Asf/Sf2 anti-
body or an unrelated control antibody (lane 3). 32P-labeled oligonucleotides
were incubated with in vitro translated Asf/Sf2 protein in the absence (lane 4)
or presence (lane 5) of Asf/Sf2 antibody or an unrelated control antibody
(lane 6); with increasing amounts of Asf/Sf2 protein (lanes 7–11); or with
excess cold oligonucleotides (lane 13) or excess cold scrambled control oligo-
nucleotides (lane 14).
to determine whether ASF/SF2 regulates the expression of CD3ξ protein. To address this question, we used a plasmid to overexpress ASF/SF2 and specific siRNA to knock down ASF/SF2 in primary T cells. Upon forced expression of ASF/SF2 (pASF), CD3ξ protein expression was significantly increased as compared with control (pcDNA) transfected cells (Fig. 2, A and B). Conversely, CD3ξ protein expression decreased by 36 ± 10% (p = 0.01) upon ASF/SF2 knockdown with siRNA (siASF) as compared with cells transfected with control (siCtrl) siRNA (Fig. 2, C and D). These results indicate that ASF/SF2 expression levels correlate directly with CD3ξ protein expression in human T cells.

**ASF/SF2 Represses Alternative Splicing of the Exon VIII-defined CD3ξ 3′-UTR**—The splicing regulatory activity of ASF/SF2 depends on its phosphorylation status, and the sphingolipid metabolite \( \text{\textit{d}-erythro-C}_6 \text{ceramide} \) is shown to modulate the splicing activity of ASF/SF2 through a protein phosphatase 1-mediated dephosphorylation of ASF/SF2 (19). We treated T cells with increasing concentrations of ceramide and assessed its effect on CD3ξ 3′-UTR alternative splicing by reverse transcription (RT)-PCR. A primer pair spanning the 5′- and 3′-ends of the 3′-UTR (arrows P1 and P2 in Fig. 1A, top) was used to amplify both isoforms simultaneously. In Fig. 3A, we show that the alternative splicing of the CD3ξ 3′-UTR is activated in a concentration-dependent manner, such that there is a gradual decline in the WT isoform and a concomitant increase in the AS isoform. At the 30 \( \mu \text{M} \) concentration, a clearly discernible effect on the alternative splicing is observed, and this ceramide dose was chosen for the next set of experiments. To specifically determine the role of ASF/SF2 in CD3ξ 3′-UTR alternative splicing, we overexpressed ASF/SF2 in T cells and analyzed the expression of the CD3ξ 3′-UTR splice isoforms by RT-PCR. As compared with control (pcDNA) transfected T cells (Fig. 3B, lanes 1–3), ASF/SF2 overexpression led to a repression of alternative splicing, causing an increase in the WT isoform and a parallel decrease in the AS isoform (Fig. 3B, lanes 4–6). The ratio of the WT to AS isoforms thus increased by 40 ± 20% (p = 0.01) in the ASF/SF2-transfected cells (Fig. 3C). Ceramide treatment activated 3′-UTR splicing to generate decreased levels of the WT isoform (Fig. 3B, lanes 7–9), whereas reconstitution of ASF/SF2 in the ceramide-treated cells led to a restoration of the WT isoform (Fig. 3B, lanes 10–12). These results show that ASF/SF2 represses the alternative splicing of CD3ξ 3′-UTR to favor generation of the functional WT isoform over the faulty AS isoform.

**Increased ASF/SF2 Levels Are Associated with Increased WT CD3ξ mRNA during T Cell Activation**—Because CD3ξ is a critical initiator of the TCR-mediated signaling, we asked whether its expression during T cell activation is regulated by the 3′-UTR alternative splicing. We activated T cells for 0, 3, 6, 24, 48, and 72 h and measured expression of the CD3ξ 3′-UTR splice variants. There was a rapid and prolonged increase in the WT CD3ξ isoform and a proportionate decrease in the AS isoform of CD3ξ (Fig. 4, A and B). Concomitantly, ASF/SF2 expression levels increased gradually over the 72 h of activation.

**FIGURE 2.** ASF/SF2 regulates positively the CD3ξ chain protein expression. A, T cells were transfected with pcDNA or pASF expression vectors. 24 h later, cells were harvested, and whole lysates were used in Western blots for ASF/SF2, CD3ξ, and β-actin. C, T cells were transfected with siRNA against ASF/SF2 (siASF) or control siRNA (siCtrl). 48 h later, cells were harvested, and whole lysates were used in Western blots for ASF/SF2, CD3ξ, and β-actin. B and D, quantitative analysis of average CD3ξ chain protein expression normalized to β-actin from three independent experiments. Error bars, S.D.

**FIGURE 3.** ASF/SF2 represses the alternative splicing of the CD3ξ 3′-UTR. A, T cells were treated with increasing concentrations (20, 30, and 40 \( \mu \text{M} \)) of ceramide or vehicle DMSO (0.4%) for 18 h. Cells were lysed, and total RNA was used in RT-PCR in triplicate for CD3ξ 3′-UTR and GAPDH. B, T cells were transfected with pcDNA or pASF/SF2 expression vectors without (lanes 1–6) or with (lanes 7–12) the addition of 30 \( \mu \text{M} \) ceramide. Cells were harvested 18 h later, total RNA was extracted and RT-PCR was performed in triplicate for CD3ξ 3′-UTR and GAPDH. C, quantitative analysis of the WT and AS CD3ξ 3′-UTR isoforms normalized to GAPDH from four independent experiments expressed as a ratio. Error bars, S.D.
ASF/SF2 Regulates CD3ξ Expression in Human T Cells

FIGURE 4. Increased ASF/SF2 levels are associated with increased WT CD3ξ mRNA during T cell activation. T cells were stimulated with α-CD3, α-CD28, and cross-linker antibodies for 0, 3, 6, 24, 48, and 72 h. Cells were harvested, and total protein and RNA were extracted. A, total RNA was used in RT-PCR for the CD3ξ 3′-UTR and GAPDH. Data represent one of three experiments. B, WT and AS CD3ξ isoform expressions were quantified, normalized to GAPDH, and expressed as a ratio. The graph shows mean ± S.D. from three independent experiments. C, total protein from activated cells was used in Western blots for ASF/SF2 and β-actin. D, graph shows ASF/SF2 protein levels normalized to β-actin from three independent experiments. Error bars, S.D.

FIGURE 5. ASF/SF2 expression directly correlates with CD3ξ chain expression in SLE patients. T cells from SLE patients and healthy individuals were lysed, and total protein was used in Western blots for ASF/SF2, CD3ξ chain, and β-actin. Quantification of ASF/SF2 and CD3ξ expression were normalized to β-actin. The ASF/SF2 and CD3ξ values from SLE patients were normalized to the corresponding values from healthy subjects. These relative values are plotted on an x/y axis such that each dot represents one SLE patient’s expression of ASF/SF2 on the x axis and CD3ξ on the y axis.

DISCUSSION

In this study, we present several novel findings. First, we have identified the splicing protein ASF/SF2 binding to the human T cell CD3ξ 3′-UTR (Fig. 1); second, we show that ASF/SF2 represses the alternative splicing of the CD3ξ 3′-UTR and promotes generation of the stable WT isoform (Figs. 2 and 3); third, we show that T cell activation leads to repression of the CD3ξ 3′-UTR alternative splicing associated with increased expression of ASF/SF2 levels (Fig. 4); and finally, we show a significant direct correlation between ASF/SF2 and CD3ξ protein expression in SLE patients (Fig. 5). During T cell activation, WT CD3ξ mRNA levels increase, whereas production of the AS isoform is repressed (Fig. 4, A and B) and ASF/SF2 levels increase concomitantly, showing that preferential splicing regulates CD3ξ expression levels. Additionally, increased transcription and mRNA stability following T cell activation in the presence of costimulatory signals may also contribute to increased WT CD3ξ mRNA expression, as
has been shown for cytokines interleukin-2 and TNFα mRNA (27, 28).

ASF/SF2 is known to regulate RNA stability of the PKCI-1-related mRNA (29) and is also involved in translation (30), and it is not known whether ASF/SF2 may also regulate stability and translation of CD3ζ. Our results from the oligonucleotide pull-down assay revealed that certain heterogeneous nuclear ribonuclear proteins (A1, A0, A2/B1, and G) also putatively bind to the ARE2-bearing region of the CD3ζ 3′-UTR, which may also be involved in the mRNA processing of CD3ζ. Further studies are needed to address whether these proteins may regulate CD3ζ expression.

We observed a significant direct correlation between ASF/SF2 levels and CD3ζ protein expression in SLE patients; CD3ζ-low patients were ASF/SF2-low, whereas the CD3ζ-normal patients were ASF/SF2-normal (Fig. 5). ASF/SF2 phosphorylation and dephosphorylation are important determinants of its splicing activity. At this point, we do not know whether phosphorylation status and other functions of ASF/SF2, such as RNA binding, nucleus to cytoplasm transport, and protein-protein interactions are also aberrant in SLE patients.

Aberrant expression of alternatively spliced isoforms of other genes is frequently observed in patients with SLE and includes the transcription factor cyclic AMP-response element modulator (CREM) (31) and the adhesion molecule CD44 (32). CREM alternative splicing produces the repressor (CREM-α) or the activator (CREM-tau2α) isoforms and is regulated by the SR family member SRp40 in human myometrial cells (33). It is not known whether ASF/SF2 is involved in the increased expression of the CREM-α isoform in SLE T cells. CD44 expression is increased in SLE patients (32) and is known to undergo extensive alternative splicing of variable (v) exons, and protein products of the CD44 v3 and CD44 v6 isoforms were observed in T cells infiltrating the kidneys of an SLE patient with nephritis (34). It is not known whether ASF/SF2 regulates CD44 alternative splicing.

Alternative splicing plays a key role in the generation of viral proteins during infection, and ASF/SF2 has been shown to regulate pre-mRNA splicing of HIV-1 (35). Such hijacking of host splicing factors by viruses may result in aberrant expression and/or modification of these proteins. Indeed, the human papilloma virus type 16 E2 protein has been shown to transactivate the promoter of ASF/SF2 (36). Aberrations may also include post-translational modifications of splicing proteins during viral infections, which may result in a break in immune tolerance to these self-proteins and trigger autoimmune cellular and antibody responses. Indeed, Epstein-Barr virus has been proposed to be involved in the expression of SLE (37), and sera from patients with SLE contain autoantibodies against SR splicing factors (38).

Because ASF/SF2 is important in the regulation of tumor suppressor, apoptosis-related, and cell-cycling genes, elevated levels have been reported in various cancers, and the encoding SFRS1 gene was recently confirmed to be a proto-oncogene (39). The dependence of viruses on ASF/SF2 and its oncogenic potential makes ASF/SF2 an attractive target for therapy in infections and cancer. Indeed, IDC-16, a small molecule inhibitor of ASF/SF2 activity, was shown to reduce human immuno-deficiency virus replication (40). Furthermore, ASF/SF2 was specifically up-regulated in a cell line model of cervical tumor progression (41), suggesting its potential as a biomarker for cancer. In our small cohort of patients, low levels of ASF/SF2 tended to correlate with increased disease activity (data not shown), suggesting that it may be exploited as a potential disease biomarker.

In conclusion, we have shown that ASF/SF2 is involved in the regulation of CD3ζ chain expression in human T cells and may represent a molecular mechanism for the defective expression of CD3ζ chain in SLE patients.

Acknowledgments—We thank Dr. Caroline Jefferies and Dr. Martin Flajnik for critical reading of the manuscript and Dr. Y. T. Juang for helpful discussions.

REFERENCES

1. Weiss, A., and Littman, D. R. (1994) Cell 76, 263–274
2. Enyedi, E. J., Nambiar, M. P., Lioussis, S. N., Dennis, G., Kammer, G. M., and Tsokos, G. C. (2001) Arthritis Rheum. 44, 1114–1121
3. Chan, A. C., Iwashima, M., Turck, C. W., and Weiss, A. (1992) Cell 71, 649–662
4. Krishnan, S., Farber, D. L., and Tsokos, G. C. (2003) J. Immunol. 171, 3325–3331
5. Nambiar, M. P., Fisher, C. U., Warke, V. G., Krishnan, S., Mitchell, J. P., Delaney, N., and Tsokos, G. C. (2003) Arthritis Rheum. 48, 1948–1955
6. Moser, K. L., Neas, B. R., Salmon, J. E., Yu, H., Gray-McGuire, C., Asundi, N., Bruner, G. R., Fox, J., Kelly, J., Henshall, S., Bacino, D., Dietz, M., Hogue, R., Koelsch, G., Nightingale, L., Shaver, T., Abdou, N. I., Albert, D. A., Carson, C., Petri, M., Treadwell, E. L., James, J. A., and Harley, J. B. (1998) Proc. Natl. Acad. Sci. U.S.A. 95, 14869–14874
7. Jensen, J. P., Hou, D., Ramburg, M., Taylor, A., Dean, M., and Weissman, A. M. (1992) J. Immunol. 148, 2563–2571
8. Pang, M., Abe, T., Fujihara, T., Mori, S., Tsuzaka, K., Amano, K., Koide, J., and Takeuchi, T. (1998) Arthritis Rheum. 41, 1456–1463
9. Nambiar, M. P., Enyedi, E. J., Warke, V. G., Krishnan, S., Dennis, G., Kammer, G. M., and Tsokos, G. C. (2001) J. Autoimmun. 16, 133–142
10. Chowdhury, B., Tsokos, C. G., Krishnan, S., Robertson, I., Fisher, C. U., Warke, R. G., Warke, V. G., Nambiar, M. P., and Tsokos, G. C. (2005) J. Biol. Chem. 280, 18959–18966
11. Johnson, J. M., Castle, J., Garrett-Engele, P., Kan, Z., Loecher, P. M., Armour, C. D., Santos, R., Schadt, E. E., Stoughton, R., and Shoemaker, D. D. (2003) Science 302, 2141–2144
12. Black, D. L. (2003) Annu. Rev. Biochem. 72, 291–336
13. Cáceres, J. F., Stamm, S., Helfman, D. M., and Krainer, A. R. (1994) Science 265, 1706–1709
14. Cáceres, J. F., Scereatt, G. R., and Krainer, A. R. (1998) Genes Dev. 12, 55–66
15. Xiao, S. H., and Manley, J. L. (1998) EMBO J. 17, 6359–6367
16. Chalfant, C. E., Ogretmen, B., Galadari, S., Kresse, B. J., Pettus, B. J., and Hannan, Y. A. (2001) J. Biol. Chem. 276, 44848–44855
17. Krainer, A. R., Conway, G. C., and Kozak, D. (1990) Cell 62, 35–42
18. Dauksaitė, V., and Akusjärvi, G. (2002) J. Biol. Chem. 277, 12579–12586
19. Massiello, A., and Chalfant, C. E. (2006) J. Lipid Res. 47, 892–897
20. Nowak, D. G., Wooldred, J., Amin, E. M., Konopatskaya, O., Saleem, M. A., Churchill, A. J., Ladomery, M. R., Harper, S. J., and Bates, D. O. (2008) J. Cell Sci. 121, 3487–3495
21. Lemaire, R., Winne, A., Sarkissian, M., and Lafayris, R. (1999) Eur. J. Immunol. 29, 823–837
22. Tan, E. M., Cohen, A. S., Fries, J. F., Masi, A. T., McShane, D. I., Rothfield, N. F., Schaller, J. G., Talal, N., and Winchester, R. J. (1982) Arthritis Rheum. 25, 1271–1277
23. Moulton, V. R., Kyttaris, V. C., Juang, Y. T., Chowdhury, B., and Tsokos, G. C. (2008) J. Biol. Chem. 283, 20037–20044
ASF/SF2 Regulates CD3ξ Expression in Human T Cells

24. Weissman, A. M., Hou, D., Orloff, D. G., Modi, W. S., Seuanez, H., O’Brien, S. J., and Klausner, R. D. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 9709–9713
25. Chowdhury, B., Krishnan, S., Tsokos, C. G., Robertson, J. W., Fisher, C. U., Nambiar, M. P., and Tsokos, G. C. (2006) J. Immunol. 177, 8248–8257
26. Nambiar, M. P., Mitchell, J. P., Cerutti, R. P., Malloy, M. A., and Tsokos, G. C. (2003) Lupus 12, 46–51
27. Sanchez-Lockhart, M., Marin, E., Graf, B., Abe, R., Harada, Y., Sedwick, C. E., and Miller, J. (2004) J. Immunol. 173, 7120–7124
28. Wang, J. G., Collinge, M., Ramgolam, V., Ayalon, O., Fan, X. C., Pardi, R., and Bender, J. R. (2006) J. Immunol. 176, 2105–2113
29. Lemaire, R., Prasad, J., Kashima, T., Gustafson, J., Manley, J. L., and Lafyatis, R. (2002) Genes Dev. 16, 594–607
30. Michlewski, G., Sanford, J. R., and Caceres, J. F. (2008) Mol. Cell 30, 179–189
31. Tenbrock, K., Juang, Y. T., Gourley, M. F., Nambiar, M. P., and Tsokos, G. C. (2002) J. Immunol. 169, 4147–4152
32. Li, Y., Harada, T., Juang, Y. T., Kyttaris, V. C., Wang, Y., Zidanic, M., Tung, K., and Tsokos, G. C. (2007) J. Immunol. 178, 1938–1947
33. Tyson-Capper, A. I., Bailey, I., Krainer, A. R., Robson, S. C., and Europe-Finnet, G. N. (2005) J. Biol. Chem. 280, 34521–34529
34. Cohen, R. A., Bayliss, G., Crispin, J. C., Kane-Wanger, G. F., Van Beek, C. A., Kyttaris, V. C., Avalos, I., Yu, C. Y., Tsokos, G. C., and Stillman, I. E. (2008) Clin. Immunol. 128, 1–7
35. Ropers, D., Ayadi, L., Gattoni, R., Jacquenet, S., Damier, L., Branlant, C., and Stévenin, J. (2004) J. Biol. Chem. 279, 29963–29973
36. Mole, S., Milligan, S. G., and Graham, S. V. (2009) J. Virol. 83, 357–367
37. Poole, B. D., Templeton, A. K., Guthridge, J. M., Brown, E. J., Harley, J. B., and James, J. A. (2009) Autoimmun. Rev. 8, 337–342
38. Neugebauer, K. M., Merrill, J. T., Wener, M. H., Lahita, R. G., and Roth, M. B. (2000) Arthritis Rheum. 43, 1768–1778
39. Karni, R., de Stanchina, E., Lowe, S. W., Sinha, R., Mu, D., and Krainer, A. R. (2007) Nat. Struct. Mol. Biol. 14, 185–193
40. Bakkour, N., Lin, Y. L., Maire, S., Ayadi, L., Mahuteau-Betzer, F., Nguyen, C. H., Mettling, C., Portales, P., Grierson, D., Chabot, B., Jeanteur, P., Branlant, C., Corbeau, P., and Tazi, J. (2007) PLoS Pathogens 3, 1530–1539
41. Mole, S., McFarlane, M., Chuen-Im, T., Milligan, S. G., Millan, D., and Graham, S. V. (2009) J. Pathol. 219, 383–391