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A Novel Polymorphic CAAT/Enhancer-Binding Protein β Element in the FasL Gene Promoter Alters Fas Ligand Expression: A Candidate Background Gene in African American Systemic Lupus Erythematosus Patients

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A single-nucleotide polymorphism (SNP), identified at nucleotide position −844 in the 5′ promoter of the FasL gene, lies within a putative binding motif for CAAT/ enhancer-binding protein β (C/EBPβ). Electrophoretic mobility shift and supershift assays confirmed that this element binds specifically to C/EBPβ and demonstrated that the two alleles of this element have different affinities for C/EBPβ. In luciferase reporter assays, the −844C genotype had twice the basal activity of the −844T construct, and basal expression of Fas ligand (Fasl) on peripheral blood fibrocytes was also significantly higher in −844C than in −844T homozygous donors. Fasl is located on human chromosome 1q23, a region that shows linkage to the systemic lupus autoimmunity phenotype. Analysis of 211 African American systemic lupus erythematosus patients revealed enrichment of the −844C homozygous genotype in these systemic lupus erythematosus patients compared with 150 ethnically matched normal controls (p = 0.024). The −844C homozygous genotype may lead to the increased expression of Fasl, to altered FasL-mediated signaling in lymphocytes, and to enhanced risk for autoimmunity. This functionally significant SNP demonstrates the potential importance of SNPs in regulatory regions and suggests that in the regulation of FasL expression may contribute to the development of the autoimmune phenotype. The Journal of Immunology, 2003, 170: 132–138.

Received for publication February 6, 2001. Accepted for publication November 1, 2001.

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1 This work was supported by National Institutes of Health Grants RO1 AR33062 (to R.P.K.), P01 AR45221 (to R.P.K.) and P01 AR40084 (Program Project in the Genetics of SLE). The Flow Cytometry Core Facility was supported by the Rheumatic Diseases Core Center (P30 AR48311). A.W.G. was supported in part by the National Institutes of Health Postdoctoral Training Program in Rheumatic Diseases (T32 AR07350).

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Abbreviations used in this paper: Fasl, Fas ligand; SLE, systemic lupus erythematosus; SNP, single-nucleotide polymorphism; C/EBPβ, CAAT/ enhancer-binding protein β.

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0022-1767/03/$02.00
cells and other cells. Therefore, we analyzed the promoter of FasL and have identified a naturally occurring, functionally significant single-nucleotide polymorphism (SNP) in a newly recognized CAAT/enhancer-binding protein (C/EBPβ) element in the enhancer region of FasL. This C/EBPβ element affects FasL protein expression in vitro and ex vivo and is associated with SLE in African American patients. These data suggest that genetic variation in promoter elements of key immunoregulatory molecules may contribute to an autoimmune diathesis.

### Materials and Methods

#### Donors

Anticoagulated peripheral blood was obtained from healthy normal volunteers, from rheumatoid arthritis patients fulfilling the criteria of the American College of Rheumatology for rheumatoid arthritis (27), and from SLE patients fulfilling the revised American College of Rheumatology criteria for SLE (28). SLE patients and controls were recruited as part of the University of Alabama-based DISCOVERY cohort and as part of the Carolina Lupus Study, a population-based case-control study of risk factors for SLE. The human studies were reviewed and approved by the Institution Review Board, and all donors provided written informed consent.

#### Reagents

- Anti-CD3 mAb was purified from the culture supernatant from OKT3 hybridoma (American Type Culture Collection, Manassas, VA). Mouse anti-human FasL mAb (NOK-1), anti-CD40 mAb, and FITC-conjugated antimouse IgG1 mAb were purchased from BD PharMingen (San Diego, CA).
- PMA, LPS, and recombinant human IL-6 (IL-6) were obtained from Sigma-Aldrich (St. Louis, MO). Ionomycin was purchased from Calbiochem (La Jolla, CA).
- Rabbit anti-C/EBPβ polyclonal IgG was obtained from Santa Cruz Biotechnology (Santa Cruz, CA).
- Transfection reagents (Gentra Systems, Minneapolis, MN).
- Brieﬂy, 300 µl of blood were lysed in 900 µl of 9M urea, 5 mM EDTA, and lysed by the addition of Nonidet P-40 to a concentration of 0.1%. Nuclei were pelleted and washed in buffer A, and nuclei protein was extracted in buffer C (20 mM HEPES, 25% glycerol, 1, 5 M DTT), and lysed by the addition of 1% sodium deoxycholate and 0.5% sodium dodecyl sulfate.
- Nucleic acid isolation

#### Nucleic acid isolation

Human genomic DNA was isolated using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN). Briefly, 300 µl of blood were lysed in 900 µl of the red blood lysis solution, and the leukocytes were pelleted and lysed with 300 µl of cell lysis solution. The RNase A solution was added to the cell lyses and incubated at 37°C for 15 min. Proteins were precipitated by the addition of 100 µl of protein precipitation solution. DNA in the supernatant was precipitated with 300 µl of 100% isopropanol and washed once with 70% ethanol.

#### RT-PCR and cDNA sequencing

To facilitate heterozygote detection, a dye primer strategy was adopted for fluorescence-based automated cycle sequencing of PCR products on an ABI 3700 (Applied Biosystems, Foster City, CA). For FasL RT-PCR, sense primer 5'-TGG AAA ACG ACG GCC AGT CCT GAC TCA CCA GCT GCC AT-3' with M13 forward sequence tag (underlined letters) anneals to nucleotide positions 68 to 87, and antisense primer 5'-CAG GAA ACA GCT ATG ACC GAA AAG AAT CCC AAA GTG-3' with M13 reverse sequence tag (underlined letters) anneals to positions 938 to 955 (GenBank accession number U08137). PCR was performed in a 9600 PCR System with 2 µl of cDNA synthesized with the SuperScript Preamplification System (Life Technologies), 300 nM concentrations of each primer, 200 µM concentrations of dNTPs, 1.5 mM MgCl2, and 2.5 U of Tag DNA polymerase in a 100-µl reaction volume starting with 95°C for 5 min, 35 cycles of denaturing at 94°C for 30 s, annealing at 58°C for 45 s, and extension at 72°C for 1 min, with a final extension at 72°C for 7 min. The PCR product was purified with the QiAquick Gel Extraction Kit (Qiagen, Chatsworth, CA). All PCR products were sequenced in both directions.

#### PCR amplification and sequencing of FasL promoter region

The FasL promoter region was amplified with the sense primer 5'-TTA TGC CTA TAA TCC CAG CTA CTC A-3' annealing to nucleotide positions from −1032 to −1008, and antisense primer 5'-CTG GGA ATA TGG GTA ATT GAA G-3' annealing to positions from +12 to +33 (Fig. 1, +1 site corresponds to the A of ATG translation start codon). The PCR was performed with 500 ng of DNA, 300 nM concentrations of each primer, 200 µM concentrations of dNTPs, 1.5 mM MgCl2, and 2.5 U of Tag DNA polymerase in a 100-µl reaction volume starting with 95°C for 5 min, 35 cycles of denaturing at 94°C for 30 s, annealing at 58°C for 45 s, and extension at 72°C for 1 min, with a final extension at 72°C for 7 min. All the PCR products were purified from a 2.5% agarose gel with the QiAquick Gel Extraction Kit. The purified PCR products were sequenced from both directions using the BigDye terminator sequence on an ABI 377.

#### Verification of FasL promoter region sequence

The characterization of the FasL promoter was verified by dye-primer sequencing with M13 sequence-tagged primers. The sense primer, 5'-TGG AAA ACG ACG GCC AGT CCT GAC TCA CCA GCT GCC AT-3', anneals from position −933 to −913; and the antisense primer, 5'-CAG GAA ACA GCT ATG ACC GAA AAG AAT CCC AAA GTG-3', anneals from position −416 and −495 (underlined nucleotides are M13 forward or reverse sequences). In addition, gel-purified 1 kb of PCR product was directly cloned into pGEM-T Easy Vector (Promega). At least 10 clones from each of 6 heterozygous donors were sequenced. All cloned sequences and dye-primer sequences were in agreement with cycle sequencing of genomic template using the BigDye terminator strategy.

#### Nuclear extract preparations

Jurkat cells (107/ml) were stimulated with 10 µg/ml LPS for 2 h or cultured on anti-CD3 mAb-coated plates for 3.5 h before nuclear extraction. Cells were washed with PBS (pH 7.4), resuspended in buffer (10 mM HEPES, 1.5 mM MgCl2, and 10 mM KCl in the presence of protease inhibitors and 100 µM DTT), and lysed by the addition of Nonidet P-40 to a final concentration of 0.1%. Nuclei were pelleted and washed in buffer A, and nuclear protein was extracted in buffer C (20 mM HEPES, 25% glycerol, 420 mM NaCl, 1.5 mM MgCl2, and 0.2 mM EDTA). After the nuclear debris was pelleted, the supernatant was removed and diluted with an equal volume of buffer D (20 mM HEPES, 20% glycerol, 50 mM KCl, and 0.2 mM EDTA). Protein concentration was determined by measuring OD280.

![FIGURE 1.](http://www.jimmunol.org/)

Sequence analysis of the FasL promoter. One kilobase of the FasL promoter region was amplified from positions −1032 to +33, and a C/EBPβ element was identified between positions −848 and −839. The underlined polymorphic site at position −844 is within the core of the C/EBP binding motif. The sequences of the sense strand, in the 5' to 3' orientation, of various oligonucleotides used for EMSA are provided and the potential binding sequences are included in the rectangular boxes. Egr-3, Early growth response gene-3.
Electrophoretic mobility shift and supershift assays

For each binding reaction, 8 μg of nuclear extract were incubated in 1× binding buffer (10 mM HEPES, pH 7.5, 500 μM EDTA, 50 mM NaCl, 10 mM Tris-HCl (pH 7.5), and 50 μg/ml poly(dI- dC)poly(dI-dC)) with 32P-labeled oligonucleotides in a volume of 10 μl. Binding reactions were incubated at room temperature for 30 min with 50,000 cpm (0.1–0.5 ng) of double-stranded oligonucleotides end-labeled with [γ-32P]ATP using T4 polynucleotide kinase. Unlabeled specific or nonspecific competitor oligonucleotides were used where indicated at a 200-fold molar excess. Protein/DNA complexes and unbound DNA probe were resolved on 5% nondenaturing polyacrylamide gel and visualized by autoradiography. The following double-stranded oligonucleotide probes were used in these experiments: C/EBPβ consensus, 5′-TGC AGA TTG CGC AAT CTG CA-3′ (Santa Cruz Biotechnology); nonspecific, 5′-GTG GGT TTT GTT AGA-3′; C/EBPβ–844T allele, 5′-AAA ACA TTG GA AAT ACA-3′; (polymorphic nucleotide position –844 is underlined) (Fig. 1). The C/EBPβ supershift assay was conducted with specific polyclonal Abs by following the manufacturer’s instruction (Santa Cruz Biotechnology).

FasL reporter constructs

The FasL luciferase reporter constructs of the –844C and –844T alleles of C/EBPβ were generated by cloning a KpnI/HindIII-flanked PCR product with 1026 nucleotides of FasL promoter region into pGL3-Basic vector (Promega). The KpnI/HindIII-flanked PCR product was obtained by amplifying from human genomic DNA with upper primer 5′-GCC GGA GGT ACC CCA TTA TAC CGA TTA CTC AG-3′ (underlined and bold nucleotides are KpnI cutting site) annealing at positions –1026 to –927 and lower primer 5′-GTT CCG AAG CTT GGC ACC TGG TGA GTG AGG C-3′ (underlined and bold nucleotides are HindIII cutting site) annealing at positions –919 to –916. The subsequent change at nucleotide position –844 was made using a QuickChanger, Site-Directed mutagenesis kit (Stratagene, La Jolla, CA). For –844T construct, sense primer 5′-AAA TAA CAT TGT GAA ATA CAA AGC AG-3′ and antisense primer 5′-CTG CTT TCT TTT CAT TCA CAA TTT CAT T-3′ were used for mutagenesis. All the constructs were sequenced from both directions.

Transient transfection, cell stimulation, and luciferase assays

The Jurkat human leukemic T cell line was maintained in RPMI with 10% FCS, penicillin (1000 U/ml), streptomycin (1000 μg/ml), and glutamine (2 mM). Each transient transfection experiment was conducted with 2 × 106 Jurkat cells and 2 μg of construct plasmid DNA plus 0.5 μg of pCMV. SPORT-β-galactosidase plasmid (Life Technologies) purified with the Wizard Purification Plasmid DNA Purification System (Promega) by using 4 μl of DMRIE-C reagent (Life Technologies). Transfected cells were cultured for 18 h without stimulation, with 2 μg/ml LPS, 20 ng/ml human TNF-α, and 10 ng/ml IL-2. The adherent and nonadherent cell populations were used for luciferase reporter assay using a Monolight 2010 luminometer (Promega). Luciferase light units, standardized to β-galactosidase activity, are reported as the mean of triplicate samples.

Fibrocyte isolation and detection of FasL expression

Human fibrocytes were purified from peripheral blood as previously described (29, 30). Briefly, heparinized blood was mixed with PBS (1:1), and 8 ml were layered over 4 ml of Ficoll-Hypaque and centrifuged at 400 × g for 30 min at room temperature. The buffy coat was isolated, washed with PBS, and spun at 500 × g and then washed with PBS. Human fibrocytes were maintained in DMEM supplemented with 20% FCS (HyClone Laboratories, Logan, UT), and 1% penicillin-streptomycin-glutamine. After 7 days in culture, fibrocytes were treated with anti-CD40 mAb (2.5 μg/ml) or untreated and incubated for an additional 2 days. The adherent fibrocytes were lifted by incubation in ice cold 0.05% EDTA (Sigma) in PBS and collected by analysis of FasL expression by flow cytometry. Cells (106 aliquots) were resuspended in PBS containing 3% BSA and 0.1% sodium azide and incubated with anti-human FasL mAb for 30 min at 4°C. After two washes, the cells were incubated with FITC-conjugated anti-mouse IgG1 for 30 min. The cells were washed and fixed (R&D (Minneapolis, MN) fixative), and fluorescence data were acquired on a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA) and analyzed using CellQuest (BD Biosciences) software.

Data analysis

TESS (Transcription Element Search Software; http://www.cbil.upenn.edu/tess/) and MatInspector (http://www.gsf.de/biodb/matinspector.html) were used to search for candidate transcription factors that might bind the regions adjacent to and including nucleotide position –844. Fisher’s exact test was used to analyze the distribution of FasL promoter genotypes and gene frequencies in SLE patients and normal controls. Differences in FasL promoter activities of various constructs were analyzed by Student’s t test. Results from FasL expression in paired samples of genotyped normal donors were analyzed by the Wilcoxon matched pairs signed rank test. The null hypothesis was rejected at the 95% confidence level (p < 0.05).

Results

Detection of a FasL promoter polymorphism

A search of SNP databases did not reveal any polymorphisms in the FasL coding region. Therefore, we sequenced the coding region of 51 donors (14 SLE patients, 30 rheumatoid arthritis patients, and 7 normal subjects) and found no polymorphic sites that predict amino acid changes within the FasL protein. Next, we amplified the proximal 1 kb of FasL promoter region and directly sequenced the purified PCR products (Fig. 1). As shown in Fig. 2, we observed a SNP at nt –844 within this fragment of the FasL promoter region. In addition to the C at position –844 found in GenBank (Z96050), we found a T allele in our normal populations with an allele frequency of 0.82 for African Americans (Table I). Homozygous C/C, homozygous T/T, and heterozygous C/T donors were identified (Fig. 2).

Identification of a C/EBPβ-binding region and allele-specific effects on the binding affinity for the C/EBPβ

Computer analysis indicated that sequences surrounding nt –844 might bind members of the CCAAT/enhancer binding protein family with a perfect match of the sequence, ATTCGGAATT (the underlined nucleotide is polymorphic), for the binding site of CCAAT/enhancer binding protein β (C/EBPβ, or NF-IL-6). C/EBPβ has the consensus recognition site as 5′-T(T/G)NN NAA(T/G)-3′, and the polymorphic nucleotide sits in the core of the C/EBPβ binding motif (Fig. 1).

EMSA analysis of the surrounding 18-bp genomic sequence confirmed that the putative C/EBPβ motif from position –848 to –839 is indeed a putative C/EBPβ binding site. Radiolabeled oligonucleotide probe containing the putative –848 to –839 C/EBPβ motif binds nuclear extract from activated Jurkat cells (Fig. 3, lane 2) and is inhibited both by the consensus CCAAT enhancer-binding oligonucleotide (lane 1) and by cold oligonucleotide containing the putative C/EBPβ motif (lane 3). Anti-C/EBPβ

FIGURE 2. Nucleotide position –844 polymorphism in the FasL promoter region. One kilobase of the FasL promoter region was amplified in three different donors. Donors homozygous for nt –844T (lower row), homozygous for nt –844C (upper row), and heterozygous for nt –844TC (middle row) are shown. Polymorphic sites were also identified at nucleotide positions –205, –478, and –756.
Table 1. Distribution of C/EBPβ (NF-IL-6) binding site polymorphism (nt −844) within the promoter region of the FasL gene in AA SLE patients, AA normal controls, Caucasian SLE patients, and Caucasian normal controls

<table>
<thead>
<tr>
<th>Genotype, no. of subjects (%) of group</th>
<th>African American</th>
<th>Caucasians</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 211)</td>
<td>(n = 88)</td>
</tr>
<tr>
<td>CC</td>
<td>19 (9)</td>
<td>77 (39)</td>
</tr>
<tr>
<td>CT</td>
<td>57 (27)</td>
<td>40 (45)</td>
</tr>
<tr>
<td>TT</td>
<td>135 (64)</td>
<td>9 (11)</td>
</tr>
<tr>
<td>Allelic frequency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.23</td>
<td>0.67</td>
</tr>
<tr>
<td>T</td>
<td>0.77</td>
<td>0.64</td>
</tr>
</tbody>
</table>

*The genotype distribution of CC homozygotes (high activity allele) in African American (AA) SLE patients is significantly enriched compared with that in AA normal controls (Fisher’s exact test, p = 0.024). The frequency of CC homozygotes in Caucasian SLE patients is also increased compared with that in Caucasian normal controls, but the difference is statistically not significant (Fisher’s exact test, p = 0.231).

Ab can supershift the labeled probe/nuclear extract complexes (lane 4), providing further evidence that C/EBPβ is indeed the transcription factor that binds the region containing position −844. Interestingly, labeled probe with the −844T allele (lane 6) bound but appeared to have much lower affinity for the transcription factor C/EBPβ than that of the −844C allele. Taken together, these data indicate that nt −844 and the adjacent sequences constitute a putative C/EBPβ element and that the two different alleles have significantly different affinities for the C/EBPβ.

**Influence of the SNP within the C/EBPβ element on the promoter activity in Jurkat cells**

FasL reporter constructs differing only by the single SNP at position −844 demonstrated significantly different promoter activities in Jurkat cells in both basal and stimulated conditions (Fig. 4A). Under basal conditions, the promoter activity incorporating the −844C allele was 2.4-fold greater than that of the −844T allele (means: 16,203 vs 6,706 relative light units for −844C vs −844T, respectively; p < 0.01). Neither human IL-6 nor LPS alone enhanced the activity of the FasL promoter (Fig. 4B), but after stimulation with anti-CD3, the promoter activity of the −844C allele was 2.3 greater than that of −844T allele (93,117 vs 40,187 relative light units, respectively; p < 0.01), a difference that was also maintained with the stimulation by PMA plus ionomycin (66,867 vs 29,594 relative light units, respectively; p < 0.01). Therefore, in support of the EMSA data, the luciferase reporter assay provided strong evidence that the polymorphism within the C/EBPβ-binding site of the FasL promoter enhancer region could significantly alter the promoter activity. The luciferase data also suggest that additional regulatory elements are involved in the response to stimulation.

**Effect of the SNP with the C/EBPβ element on the expression of Fasl by human fibrocytes**

Because attempts to demonstrate FasL protein expression on peripheral T cells were unsuccessful, we examined circulating fibrocytes as a model system for Fasl expression (31). The Fasl promoter of normal donors was sequenced and donors homozygous for either −844C or −844T were identified. Consistent with the results obtained from the luciferase promoter activity assay, the basal expression of FasL on fibrocytes derived from −844C homozygous donors were 2- to 3-fold higher than that on fibrocytes from −844T homozygous donors in eight of eight paired comparisons (Fig. 5, p < 0.01). Expression levels stimulated in vitro by anti-CD40 were less consistent, with four of six paired comparisons higher and the average level in −844C homozygous donors −15% above that for −844T donor values.

The association of FasL promoter genotypes with SLE

Because there is accelerated apoptosis of lymphocytes and monocytes from patients with systemic lupus erythematosus, we hypothesized that the −844C allele of the Fasl promoter might be associated with autoimmune disease. Therefore, we compared genotype frequencies in 211 African American SLE patients with those of 150 ethnically matched normal subjects (Table 1). Among African Americans, the percentage of −844C homozygotes was significantly enriched in SLE patients (9%) compared with those of corresponding normal controls (3%), suggesting a recessive effect (Fisher’s exact test, p < 0.024). Although the CC homozygotes in Caucasian SLE patients were also enriched compared with those of Caucasian normal controls, the difference was not statistically significant.

**Discussion**

C/EBPs belong to a family of leucine zipper transcription factors involved in the regulation of various aspects of cellular differentiation and function in multiple tissues. Six different members of the family have been isolated and characterized (C/EBPs to ζ), all sharing high homology in the C-terminal domain, which carries a basic DNA-binding domain and a leucine zipper motif. C/EBPβ was originally identified as a mediator of IL-6 signaling, binding to IL-6-responsive elements in the promoters of acute phase response genes such as TNF, IL-8, and G-CSF (32, 33). IL-1 and LPS also induce C/EBPβ transcription (32, 34). C/EBPβ plays crucial roles in the functional regulation and homeostatic control of the myeloid and lymphoid compartments. C/EBPβ-deficient mice have altered antibacterial and tumoricidal activities reflecting defective macrophage activation. C/EBPβ-deficient mice also develop a lymphoproliferative disorder reminiscent of Fas/Fasl mutations in mice. These mice have altered CD4+Th responses and an expansion of

**FIGURE 3.** EMSA targeting the C/EBPβ binding element. Radioactive labeled double stranded −844C (lanes 1-5) and −844T oligonucleotides (lane 6) were incubated for 30 min with 8 μg of Jurkat cell nuclear extract. Competition experiments were performed by preincubating with a 200-fold molar excess of the unlabeled C/EBPβ consensus oligonucleotides (lane 1), unlabeled specific oligonucleotides (oligos) (lane 3), or nonspecific oligonucleotides (lane 5) for 30 min at room temperature. Supershift experiments were conducted by following the manufacturer’s instruction and by incubating with anti-C/EBPβ Ab for 30 min at room temperature (lane 4). Arrows and bracket, Position of specific complex. Results are representative of four experiments.
the B cell compartment accompanied by high levels of IgG-bearing cells in the lymph nodes and spleens (35, 36). Gene expression arrays indicate that C/EBP\(^{H9252}\) is highly induced in tolerant B cells, strongly suggesting a relationship with immune tolerance (37). Given this context, our identification of a functionally important C/EBP\(^{H9252}\) enhancer element in the FasL promoter region suggests one mechanism for the crucial role of C/EBP\(^{H9252}\) in the peripheral tolerance and cytotoxicity in C/EBP\(^{H9252}\)-deficient mice.

Despite our understanding of the importance of FasL in immune homeostasis, little is known about its natural transcriptional regulation. Jurkat T cells have been widely used in the characterization of the FasL promoter elements (22–24, 26). The concordant results of FasL promoter activities of −844 SNPs both in fibrocytes and in Jurkat T cells indicate that they are suitable model cell systems for FasL promoter characterization. Our data indicate that the C/EBP\(^{\beta}\) element in the FasL promoter plays an important role in the regulation of expression of FasL gene both in vitro and in vivo. The polymorphism of C/EBP\(^{\beta}\) element significantly affects the biological activity of FasL, promoter and altered the basal FasL expression on fibrocytes. Human fibrocytes can serve as APCs and induce APC-dependent T cell proliferation when cultured with specific Ag. T cell-proliferative activity induced by fibrocytes is significantly higher than that induced by monocytes and may be nearly as high as that induced by purified dendritic cells (38). The expression of FasL indicates that fibrocytes may also be involved in immune tolerance, and the difference in FasL expression by fibrocytes could have significant effects on the apoptosis of Fas-expressing lymphocytes and monocytes. Together with the luciferase reporter assays

**FIGURE 4.** Effects of the −844 SNP on FasL promoter activity of in Jurkat cells. Jurkat cells were transiently transfected with the FasL 1026/pGL3 reporter containing either −844C allele or the −844T allele. A, Cells were left unstimulated or stimulated for 18 h with immobilized anti-CD3 mAb or PMA (50 ng/ml) plus ionomycin (1 μM). Luciferase light units, normalized to β-galactosidase activity, are reported as the mean of samples. The fold increases of stimulated over unstimulated baselines for each reporter are presented as numerical values above the bars. Data represent seven independent experiments. The relative luciferase light units are given as the means ± SEM. * Significant expression differences between two genotypes (\(p < 0.01\)). B, Cells were left unstimulated or stimulated for 18 h with PMA (50 ng/ml) plus ionomycin (1 μM), recombinant human IL-6 (20 ng/ml), or LPS (2 μg/ml). Normalized luciferase light units are reported as the mean of samples. Data represent four independent experiments. The relative luciferase light units are given as the means ± SEM. * Significant expression differences between two genotypes (\(p < 0.01\)).

**FIGURE 5.** Effects of the −844 SNP on promoter activity. A, Basal expression of FasL on human fibrocytes derived from −844C homozygous donors (□) and −844T homozygous donor (■). The −844C homozygous donors expressed significantly more FasL on the fibrocytes than the −844T homozygous donors (Wilcoxon matched pairs signed rank test, \(p < 0.01\)). The results indicated are averages of the normalized mean fluorescent intensity in eight paired comparisons. B, Representative histogram of FasL expression on human fibrocytes. FasL expression on fibrocytes derived from two −844C homozygous donors (dark line and dotted line on the right side) and one −844T homozygous donor (dark line on the left side) is shown. Shaded area, Isotype control.
which indicate that the C/EBPβ polymorphism has a persistent effect on the induction levels of the FasL expression in T cells, the polymorphism of the C/EBPβ element in the Fasl promoter could have significant implication in the pathogenesis of autoimmune diseases.

Predicting the net impact of FasL promoter SNPs on an autoimmune disease phenotype is complicated both by the capacity of FasL to deliver costimulatory as well as proapoptotic signals and by the observation that the Fas/FasL-related phenotype in mice is background dependent. Individuals carrying the high activity allele of the Fasl C/EBPβ element could have more apoptosis of the activated lymphocytes, monocytes, macrophages, and other cells, which in turn might contribute the increased levels of nucleosomes found in the circulation of SLE patients (39). Such an ongoing source of extracellular nuclear Ags to drive more profound immune response and to allow the formation of extra immune complexes in the patients and may overwhelm the clearance system of the human body. Indeed, Kovacs et al. (13) have reported the increased expression of functional FasL in activated T cells from SLE patients and have proposed that the elevated FasL expression could account for the high apoptotic rate of lymphocytes in SLE patients. Alternatively, the autoimmune interaction of Fas and Fasl on activated T cells contribute to the maintenance of peripheral T cell tolerance, and FasL expressing APCs can delete responding T cells in the periphery, leading to Ag-specific systemic T cell tolerance (40).

Our genotyping data suggest that the higher activity allele of the C/EBPβ element is associated with SLE in African Americans. This association must be confirmed in independent case-control and family-based association studies. Based on the established epistatic interaction of Fas/FasL mutations with background genes in mouse models, it will be interesting to assess the genetic associations in humans, conditioned on other genetic effects and stratified by different ethnic groups. These studies and studies to consider that the ~844 genotype may also be in linkage disequilibrium with other susceptibility gene(s) in this region are currently under way.

Like other spontaneous autoimmune disease, the lupus diathesis most likely represents the summation of multiple genetic effects, each contributing a small risk of disease. The C/EBPβ element polymorphism may play such a role in the pathogenesis of autoimmune diseases as a disease susceptibility gene. However, it is also important to recognize that Fasl participates in more than maintenance of tolerance and in the genesis of autoimmune disease. The integrity of sites of immune privilege and the elimination of interaction with iC3b-opsonized apoptotic cells.

**Acknowledgments**

We thank Dr. Richard Bucala for critical review of the manuscript and helpful suggestions.

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2. Singer, G. G., and A. K. Abbas. 1994. The Fas antigen is involved in peripheral tolerance and in the genesis of autoimmune disease. The integrity of sites of immune privilege and the efficiency of immune surveillance and evasion by cancer cells may also be affected by variation in Fasl expression. Indeed, these areas, although unexplored, may be among the most important physiological settings for the role(s) of this promoter variant.

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