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Molecular Basis of Deficient IL-2 Production in T Cells from Patients with Systemic Lupus Erythematosus

Elena E. Solomou,*† Yuang-Taung Juang,*† Mark F. Gourley,‡ Gary M. Kammer,§ and George C. Tsokos2*†

Systemic lupus erythematosus (SLE) is a multifactorial autoimmune disease characterized by diverse cellular and biochemical aberrations, including decreased production of IL-2. Here we show that nuclear extracts from unstimulated SLE T cells, unlike extracts from normal T cells, express increased amounts of phosphorylated cAMP-responsive element modulator (p-CREM) that binds the −180 site of the IL-2 promoter. Nuclear extracts from stimulated normal T cells display increased binding of phosphorylated cAMP-responsive element binding protein (p-CREB) to the −180 site of the IL-2 promoter, whereas nuclear extracts from stimulated SLE T cells display primarily p-CREM and decreased p-CREB binding. In SLE T cells, p-CREM bound to the transcriptional coactivators, CREB binding protein and p300. Increased expression of p-CREM correlated with decreased production of IL-2. The transcription of a reporter gene driven by the −180 site was enhanced in normal T cells, but was suppressed in SLE T cells. These experiments demonstrate that transcriptional repression is responsible for the decreased production of IL-2 by SLE T cells. The Journal of Immunology, 2001, 166: 4216–4222.

S
ystemic lupus erythematosus (SLE) is a multifactorial human autoimmune disease that afflicts approximately 1 million Americans. The disease has high morbidity and mortality that results from involvement of vital organs, such as the kidney and the CNS, and an increased rate of infections. The fundamental disorder is an impaired immune response to Ag. Presentation of autoantigenic peptides by APCs to autoreactive T cells results in dysregulated production of autoantibodies by B cells. One outcome of T cell dysfunction is skewed cytokine production, particularly deficient production of IL-2 (1–3). In turn, defective T cell regulation of B cell Ig production leads to polyclonal hypergammaglobulinemia, inappropriate production of multiple autoantibodies, formation and deposition of immune complexes in tissues, inflammation, and organ failure (4, 5). Life-threatening consequences are the infection of this altered immune response (6, 7).

IL-2 is a growth factor for both T and B lymphocytes that is exclusively produced by T cells. IL-2 gene expression is regulated by the cooperative binding of discrete transcription factors on the IL-2 promoter/enhancer and is predominantly controlled at the transcriptional level. AP-1, NF-AT, and NF-κB bind at distinct sites on the IL-2 promoter and interact to regulate IL-2 transcriptional initiation in normal T cells (8–10). There is evidence that transcriptional repression of the IL-2 gene is associated with T cell anergy (9–11), and recently, it was reported that in anergic cells the −180 site of the IL-2 promoter binds cAMP-responsive element binding protein (CREB)/cAMP-responsive element modulator (CREM) and not AP-1 (12).

CREB, CREM, inducible cAMP early repressor, and activating transcription factor-1 are members of the cAMP-responsive NFs and exhibit a high degree of sequence homology. One common feature is the basic domain/leucine zipper motifs, which bind an 8-bp regulatory palindromic DNA sequence (cAMP-responsive elements (CREs)). These NFs are activated following phosphorylation by several protein kinases in response to different signaling routes, including protein kinase A, protein kinase C, ribosomal S6 kinase pp90rsk, mitogen- and stress-activated kinase, mitogen-activated protein kinase-activated protein kinase-2, and Ca2+/calmodulin-dependent kinase IV. Phosphorylation of Ser133 in CREB and Ser147 in CREM acts as a molecular switch, because it regulates the ability of these factors to interact with the ubiquitously expressed coactivators CREB binding protein (CBP) and p300 that form a bridge with the basal transcriptional machinery (13, 14).

The structure of CREB and CREM consists of the transcriptional activation domain (Q1, P box, Q2) and the DNA binding/dimerization domain (bZip region). Both the CREB and CREM genes encode multiple isoforms. For CREM, these mechanisms include alternative splicing, alternative initiation codon, and the presence of an intronic alternative promoter. CREM isoforms act as transcription activators, whereas CREM isoforms can act as either activators or repressors. CREM isoforms containing only the P box or the Q2 domain act as repressors (13, 14).

SLE T cells produce decreased amounts of IL-2 following antigenic stimulation in vitro (1, 2, 5, 15). Here, we show that the decreased production of IL-2 is the result of active IL-2 gene transcription repression mediated by the binding of phosphorylated CREM (p-CREM) to the −180 site of the IL-2 promoter.

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Materials and Methods

Patients and controls

Thirty SLE patients (29 women and 1 man) were studied. All subjects fulfilled at least 4 of the 11 revised criteria of the American College of Rheumatology for the Classification of SLE (16). The age of the patients ranged from 21–74 yr (mean ± SD, 42.9 ± 13.4). Six of the patients were Caucasian, and 24 were African-Americans. Disease activity (17) was quantified by the SLE disease activity index (SLEDAI) score (mean ± SD, 5.4 ± 4.5). Ten patients had been treated with prednisone, but they had taken no steroid for 24 h before venipuncture. Ten subjects were treated with hydroxychloroquine, three patients with prednisone and azathioprine, and three with cyclophosphamide and dexamethasone. The remainder were receiving no treatment. Seven additional patients (six women and one man), five with rheumatoid arthritis, one with Sjögren’s syndrome, and one with dermatomyositis, served as the disease control group. The age range of the subjects was 18–78 yr (mean ± SD, 45 ± 3.5). Twenty normal volunteers (normal control group; mean age ± SD, 32 ± 4.2) served as controls.

Lymphocytosis isolation and stimulation conditions

Heparinized peripheral venous blood was obtained from the study subjects. PBMC were separated from RBC on Lymphoprep gradient (Nycomed Pharma, Oslo, Norway), and T cells were separated subsequently by magnetic depletion of non-T cells, as recommended by the manufacturer (MACS Pan T cell isolation kit, Miltenyi Biotec, Auburn, CA). Briefly, non-T cells (B cells, monocytes, NK cells, dendritic cells, early erythroid cells, platelets, and basophils) from PBMC were indirectly magnetically labeled using a cocktail of hapten-conjugated CD11b, CD16, CD19, CD36, and CD56 Abs and MACS microbeads coupled to an anti-hapten mAb. The magnetically labeled cells were depleted by retaining them on a MACS column in the magnetic field of MidiMACS. The purified T cells were >95% positive for CD3 as tested using flow cytometry. Where mentioned, stimulation of T cells was performed using 4 μg/ml anti-CD3 (OKT3) and 10 μg/ml anti-CD28 or 10 μg/ml anti-CD28, or 10 ng/ml PMA and 0.5 μg/ml ionomycin.

Antibodies

Anti-phospho-CREB mAb, anti-p300 mAb, and anti-mouse CBP were purchased from Upstate Biotechnology (Lake Placid, NY). Anti-phospho-CREB and anti-CREB as well as the goat anti-rabbit and goat anti-mouse HRP-conjugated mAbs were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Preparation of nuclear extracts, EMSA, immunoblotting, and immunoprecipitation

At least 5 million T cells were used for preparation of extracts as previously described (16). The dsDNA probe of the −180 site (−164 to −189 bp) on the IL-2 promoter used was 5′-catccacgatcagtcagtctttgggggt-3′ in shift and supershift assays as previously described (18). Nuclear and cytoplasmic extracts were separated electrophoretically and used in immunoblotting and immunoprecipitation studies as previously described (19). Ten micrograms of cytoplasmic and nuclear extracts were used for the immunoblotting experiments.

Transfection and luciferase assays

Freshly isolated normal T cells were rested overnight in medium containing 10% FCS and PHA (1 μg/ml). Plasmids encoding two tandem −180 sites on the IL-2 promoter or the IL-2 promoter (from −575 to +57 bp; a gift from Dr. A. Rao), and CREMα (a gift from Dr. Sassone-Corsi) were used for the transfection. T cells (5 × 10^6) were transiently transected by electroporation at 250 mV and 950 μF in 0.25 ml of complete medium. After 20 h, T cells were stimulated as described above, and cytoplasmic extracts were prepared using a luciferase assay kit (Promega, Madison, WI). Briefly, cells were resuspended in lysis buffer with 0.01 M DTT and incubated at room temperature for 15 min. A brief centrifugation 30 μl of the supernatant was used with 100 μl of luciferase assay reagent. Luminescence was measured immediately for 30 s using a Luminometer (Sunnyvale, CA). Transfection efficiency was established in all samples by cotransfection with a plasmid encoding β-galactosidase. The luciferase activity was normalized using the β-galactosidase readings.

Quantitative determination of IL-2

PBMC from SLE patients (n = 12) and control subjects (n = 9) were incubated for 24 h in the presence of 1 μg/ml PHA. IL-2 secretion was measured in culture supernatants by ELISA (R&D Systems, Minneapolis, MN).

Data analysis

Analysis of the OD of the CREB/CREM band was performed using QuantityOne software (Bio-Rad, Hercules, CA) after background subtraction from each band. Data were evaluated for statistical significance by Student’s t test. IL-2 levels were treated geometrically and geometric means (x+y/2)−log of the SD of the log values) are given.

Results

In SLE T cells p-CREM binds to the −180 site of the IL-2 promoter

SLE T cells display diverse cellular and cytokine aberrations (1, 5), including decreased production of IL-2 following antigenic stimulation in vitro (1, 2, 3, 15). The reported presence of p50-p50 homodimers and the reduction or the absence of p50-p65 heterodimers of NF-κB in the nuclear extracts of stimulated SLE T cells suggested that decreased production of IL-2 is the result of defective transcriptional regulation (20). To determine whether other factors directly contribute to impaired IL-2 production by SLE T cells, we studied the nuclear proteins that bind to the −180 site (−164 to −189 bp) of the IL-2 promoter (12).

First, we performed EMSAs using an oligonucleotide that spans from −164 to −189 bp (−180 site) on the IL-2 promoter using nuclear extracts from unstimulated T cells from patients with SLE (n = 30), the disease control group (n = 7), (rheumatoid arthritis, n = 5; dermatomyositis, n = 1; Sjögren’s syndrome, n = 1), and normal individuals (n = 20). We observed significantly increased binding of nuclear extracts from unstimulated SLE T cells to the −180 site. By contrast, binding from normal individuals and disease control patients was minimal or undetectable. To determine the composition of the shifted bands, we used Abs directed against phosphorylated CREB (p-CREB) and p-CREM in EMSA. Unexpectedly, nuclear extracts from 28 of 30 (93%) SLE patients displayed increased binding of p-CREM. In some extracts (10 of 30, 33%), p-CREM binding was also detected (Fig. 1a). Minimal p-CREB and no p-CREM binding were detected in extracts from unstimulated disease control and normal T cells (Fig. 1b). While unlabeled oligonucleotides completely inhibited the binding of SLE T cell nuclear extracts (Fig. 1c), irrelevant oligonucleotides failed to do so (data not shown), indicating binding specificity.

Subsequently, we determined whether stimulation of control and SLE T cells with anti-CD3 and anti-CD28 Abs or with PMA and ionomycin to bypass known membrane-mediated signaling defects (21) modifies p-CREM or p-CREM binding on the IL-2 promoter. Stimulation of normal T cells induced a 10-fold increase in p-CREM binding, but only minor p-CREM binding (10% of the band density; Fig. 1d). Maximal intensity was reached by 6 h in both normal subjects and SLE patients and decreased thereafter (Fig. 1e). Unlabeled −180 oligonucleotide inhibited p-CREB and p-CREM binding, demonstrating binding specificity (Fig. 1f). In contrast, activated SLE T cells displayed two patterns of binding: either p-CREM alone or a mixture of p-CREM (30% of the band density) and p-CREM (60% of the band density). On the average, binding by nuclear extracts of activated SLE T cells was 5-fold less than that in unstimulated nuclear extracts (Fig. 1, d and g). Moreover, using anti-AP-1 Abs in EMSA, we failed to detect any AP-1 binding (10) (Fig. 1f). Finally, we examined binding of nuclear extracts from SLE and normal T cells that had been previously rested for 18 h and subsequently stimulated as described above. Although in stimulated and unstimulated normal T cells we detected binding patterns similar to those in the unrested cells, in unstimulated SLE T cells we observed decreased p-CREM binding, which was still higher than that in normal T cells. p-CREM binding of nuclear extracts from rested stimulated SLE T cells was still decreased (data not shown).
Together, these experiments clearly demonstrate that p-CREM alone or p-CREM and p-CREB (in which p-CREM contributes the most) bind the −180 site of the IL-2 promoter in SLE T cells, whereas in control T cells this represents almost exclusively a p-CREB binding site. p-CREM and p-CREB binding in all studied SLE subjects and normal subjects is shown in Table I.

Increased protein levels of p-CREM in SLE T cells

To determine whether increased binding of p-CREM or p-CREM/p-CREB identified in EMSA correlates with protein expression, we quantified immunoblots of nuclear extracts from SLE, rheumatoid arthritis, and normal T cells. SLE nuclear extracts immunoblotted with anti-p-CREM and anti-p-CREB Abs revealed the presence of p-CREM and p-CREB in all SLE subjects studied (Fig. 2a; n = 10 of 10, 100% for p-CREM; n = 9 of 10, 90% for p-CREB). By contrast, nuclear extracts form unstimulated normal T cells expressed minimal, but detectable, amounts of only p-CREB. When cells were activated by PMA and ionomycin, p-CREB, but not p-CREM, was detected in the nuclear extracts (Fig. 2a). Similar results were obtained following stimulation with anti-CD3 and anti-CD28 Abs (data not shown). Cytoplasmic extracts from unstimulated SLE T cells (9 of 10) displayed minimal amounts of CREB and p-CREB, but no p-CREM (Fig. 2b). The cytoplasmic extracts from T cells from rheumatoid arthritis patients (n = 2) and normal subjects (n = 7) expressed CREB (9, 10), but no p-CREB and p-CREM (Fig. 2a). Nuclear extracts from stimulated cells from SLE patients displayed decreased amounts of p-CREB and p-CREM compared with unstimulated cells (Fig. 2c).

Thus, elevated protein levels of nuclear p-CREM protein are associated with the increased p-CREM DNA binding observed in EMSA.

p-CREM in SLE T cells forms complexes with CBP and p300

CREB binding protein (CBP) and p300 are known to bind p-CREB, resulting in the formation of heteromeric activator complexes that contribute to efficient and specific initiation of transcription (13, 14). To determine whether the p-CREM detected in SLE T cells is functional by virtue of its binding to CBP and/or p300, we immunoprecipitated nuclear extracts from SLE and normal T cells with anti-p300 and anti-CBP Abs. Immunoprecipitation of p300 in nuclear extracts from unstimulated SLE T cells (n = 3) revealed a p-CREM-p300 complex, whereas in nuclear extracts from unstimulated normal T cells (n = 4) such complexes were barely detectable in all samples examined (Fig. 3). In the same SLE samples, p-CREB coprecipitated with p300 (Fig. 3). Also, immunoprecipitation of CBP from nuclear extracts from unstimulated SLE T cells (n = 2) revealed p-CREM-CBP complexes in both samples and p-CREB-CBP complexes in one of two samples, whereas normal unstimulated T cells did not reveal any p-CREB or p-CREM-CBP complexes (data not shown). Thus, p-CREM in SLE T cells interacts with both CBP and p300.

Table I. Comparison of p-CREM/p-CREB binding in SLE and normal T cells

<table>
<thead>
<tr>
<th>Condition</th>
<th>p-CREM (mean OD ± SEM)</th>
<th>p-CREB (mean OD ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstimulated SLE T cells</td>
<td>120 ± 10</td>
<td>30 ± 14</td>
</tr>
<tr>
<td>Unstimulated normal T cells</td>
<td>3 ± 1</td>
<td>19 ± 2</td>
</tr>
<tr>
<td>Stimulated SLE T cells</td>
<td>35 ± 8</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>Stimulated normal T cells</td>
<td>30 ± 6</td>
<td>113 ± 7</td>
</tr>
</tbody>
</table>

* T cells stimulated with PMA and ionomycin for 6 h.
coactivators, forming complexes that would be expected to modulate transcription.

**p-CREM binding to the −180 site is responsible for the decreased production of IL-2**

To determine whether p-CREM binding to the −180 site modifies the transcriptional regulation of the IL-2 promoter, we initially quantified IL-2 production following mitogenic stimulation in vitro. PBMC from SLE subjects and normal controls were stimulated with PHA (1 μg/ml) for 24 h, and secreted IL-2 in supernatants was quantified by ELISA. Compared with controls, the amount of IL-2 secreted by SLE cells was 4-fold lower ($p < 0.001$; Fig. 4a). Analysis of T cell nuclear extracts from these SLE cells displayed decreased amounts of p-CREB and p-CREM compared with unstimulated cells from the same patients. Left margins: molecular size marker migration. cyt, Cytoplasmic extracts; nuc, nuclear extracts; s, stimulation; P1I, PMA and ionomycin.

### FIGURE 2.

SLE T cells display increased protein levels of p-CREM. *a,* Cytoplasmic and nuclear extracts from unstimulated normal T cells were examined in immunoblots. Cytoplasmic extracts revealed only minimal amounts of CREB, but no p-CREB or p-CREM was detected. In nuclear extracts minimal amounts of p-CREB and p-CREM were also detected. When normal T cells were stimulated with PMA and ionomycin, a 4-fold (average) increase in p-CREB protein levels was observed in nuclear extracts. *b,* Cytoplasmic extracts from unstimulated SLE T cells displayed minimal amounts of CREB and p-CREB. In nuclear extracts from unstimulated SLE T cells, CREB and p-CREB levels were comparable with the levels detected in stimulated normal T cells. In contrast, p-CREM was abundant in SLE subjects, and it was barely detected in normal individuals. The patterns shown in this figure represent the observations of all normal, SLE, and disease controls examined. *c,* Stimulated cells from SLE patients displayed decreased amounts of p-CREB and p-CREM compared with unstimulated cells from the same patients.

### FIGURE 3.

p-CREM in SLE forms complexes with the coactivators CBP and p300. Cytoplasmic and nuclear extracts from normal and SLE T cells were immunoprecipitated with Ab against p300 and analyzed by immunoblots with specific Ab against p-CREM. p-CREB-p300 complexes could be detected only in nuclear extracts from stimulated normal T cells. In nuclear extracts from unstimulated SLE T cells, the formation of p-CREM-p300 and p-CREB-p300 complexes was detected. These complexes could be detected in minimal amounts in cytoplasmic extracts from unstimulated SLE T cells.

### FIGURE 4.

Decreased IL-2 production by SLE T cells. *a,* PBMC were cultured in the presence or the absence of PHA, and IL-2 production was measured by ELISA. SLE subjects displayed statistically significant decreased amounts of IL-2 production compared with normal individuals following stimulation with PHA for 18 h (unstimulated, 15.8 ×/+/− 0.03 vs 20.0 ×/+/− 0.08, geometric means ($p = 0.35$); stimulated, 3162 ×/+/− 0.03 vs 10232 ×/+/− 0.7 ($p < 0.001$)). The y-axis represents picograms per milliliter. The lower four dots in the SLE-IL-2 group represent patients L1, L2, L3, and L4 in Figs. 2b and 1a. *b,* Side-by-side comparison of p-CREB/p-CREM binding from a normal subject, an SLE normal IL-2 producer, and an SLE low IL-2 producer.
subjects by EMSA revealed increased amounts of p-CREM and p-CREB in 12 (100%) and five (42%) specimens, respectively. Particularly notable was the observation that nuclei with only p-CREM binding by EMSA were derived from the four SLE subjects whose cells produced the lowest amounts of IL-2 (Fig. 4b). When analyzed by immunoblotting and immunoprecipitation, these nuclear extracts possessed abundant amounts of p-CREM that formed heteromeric complexes with CBP and p300.

To prove directly that the −180 IL-2 promoter site represses gene transcription in SLE T cells, we transiently transfected freshly isolated normal and SLE T cells with a luciferase reporter construct driven by two tandem −180 sites. As shown in Fig. 5a, stimulation of transfected normal T cells with PMA and ionomycin for 6 h induced a mean 2.8-fold increase in luciferase activity compared with that in stimulated normal T cells transfected with pGL2 alone (n = 4; range, 1.8–3.7). By contrast, stimulation of SLE T cells resulted in a mean 1.1-fold increase in luciferase activity (n = 4; range, 0.2–1.2; p < 0.05 compared with normal stimulated T cells). To establish that CREM indeed suppresses the transcriptional activity driven by the −180 site, we cotransfected normal T cells with a construct encoding CREMα and then added PMA and ionomycin for 6 h. Indeed, cotransfection of CREMα

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**FIGURE 5.** The CREM binding −180 site of the IL-2 promoter suppresses transcription in SLE T cells. a. T cells from normal controls and SLE patients were transfected with the reporter construct driven by two tandem −180 sites and cultured in the presence or the absence of PMA and ionomycin. Normal cells displayed a 2.8-fold (mean) increase in luciferase activity following stimulation compared with a 1.1-fold seen in SLE T cells. When normal T cells were cotransfected with a plasmid encoding CREMα, a 1.8-fold decrease in luciferase activity was observed compared with that in cells that were transfected with the construct driven by two tandem −180 sites alone. b. When normal T cells were transfected with the construct driven by the IL-2 promoter (−575 to +57 bp), a 23.5-fold increase in luciferase activity was observed compared with that in unstimulated normal T cells that were transfected with the same construct (p < 0.001). When unstimulated normal T cells were cotransfected with the CREMα construct, a 50% decrease in luciferase activity was observed compared with that in unstimulated cells that were transfected with the IL-2 construct alone.
resulted in a 1.8-fold decrease (65% decrease) in luciferase activity compared with that in stimulated T cells that were transfected with the construct driven by the two −180 sites alone (p < 0.004). The luciferase activity in unstimulated normal and SLE T cells followed a similar pattern (Fig. 5a).

Finally, we examined whether longer segments of the IL-2 promoter, representing closer physiologic conditions, would yield the same results. We transfected normal T cells with a construct driven by the −575/+57 region of the IL-2 promoter alone or in the presence of a plasmid encoding CREMα. As shown in Fig. 5b, normal T cells stimulated with PMA and ionomycin induced a mean 23.5-fold increase in luciferase activity compared with that in unstimulated cells. Cotransfection of the cells with CREMα resulted in a mean 50% decrease in luciferase activity (n = 4; p < 0.001 compared with that in stimulated normal T cells transfected with IL-2 alone). Unstimulated normal T cells that were transfected with both the IL-2 promoter and CREMα constructs also displayed a mean 50% decrease in luciferase activity compared with that in normal unstimulated T cells that were transfected with the IL-2 promoter construct alone.

p-CREM DNA binding and protein levels are not affected by disease activity or medication and are persistent after 3 mo of follow-up

We analyzed the relationship among SLE disease activity, treatment modalities, and p-CREM binding by EMSA or p-CREM protein content by immunoblotting. We failed to detect any significant differences in p-CREM binding (Table II) or protein levels among patients with active (SLEDAI > 4; n = 12) or inactive disease (SLEDAI < 4; n = 11). Moreover, treatment did not significantly alter p-CREM binding. When T cells from the same SLE subjects (n = 7) were analyzed 3–4 mo later, neither p-CREM binding nor nuclear p-CREM protein levels were significantly different. Taken together, p-CREM binding to the −180 site on the IL-2 promoter as well as its nuclear protein content appear to be significantly increased compared with control values and are independent of disease activity (Table II) and mode of therapy.

Discussion

It has long been appreciated that mice (22) and subjects with SLE often have impaired delayed hypersensitivity responses to Ags due to anergy (5, 15). In vitro analyses have conclusively demonstrated that SLE T cells respond suboptimally to Ags, mitogens, and cell-associated Ags by proliferation and cytokine production (1, 5, 15). Because SLE T cells proliferate poorly and underproduce both IL-2 and IFN-γ in response to antigenic challenge in vitro, this has raised the possibility that SLE T cells may be anergic (1, 22, 23). This impaired cellular immunity contributes to the higher frequency of severe, often life-threatening infections in this disorder (6, 7).

Recently, it has been learned that T cell anergy is characterized in part by enhanced binding of p-CREM/p-CREB to the −180 site of the IL-2 promoter/enhancer (12). To establish whether diminished IL-2 production by SLE T cells is the result of reduced IL-2 transcriptional activation due to increased p-CREM binding to the −180 site in SLE T cells, we studied the T cells of a cross-section of SLE subjects with a spectrum of disease activity. Here, we demonstrate that unstimulated SLE T cells exhibit markedly increased nuclear binding to the −180 site of the IL-2 promoter compared with either normal or disease controls by EMSA. Importantly, this enhanced binding activity is the result of the transcriptional repressor, p-CREM, or of p-CREM/p-CREB complexes. Increased p-CREM binding is associated with accumulation of nuclear p-CREM, which can form heteromeric complexes with both CBP and p300 coactivators (12, 14). By contrast, p-CREB binding to the −180 site of the IL-2 promoter is under-represented, particularly when one compares p-CREB binding in activated T cells from normal or disease controls. In part, this is likely to reflect the disproportionate increase in nuclear p-CREM, although other, as yet unidentified mechanisms may also be operative that limit p-CREM content. Nevertheless, like p-CREM, p-CREB can form heteromeric complexes with both CBP and p300 coactivators. p-CREM may mediate transcriptional repression of IL-2-luciferase activity, possibly through its formation of heteromeric complexes with CBP and p300 (24, 25), resulting in diminished production of IL-2.

Certain CREM isoforms suppress the transcription activity of particular genes (13). At this point we do not know whether the CREM that we have observed to be increased in SLE T cells belongs to one of these isoforms, but because cotransfection in normal T cells with an inhibitory isoform (CREMα) resulted in decreased luciferase activity, we suspect that CREM in SLE represents an inhibitory isoform. It is possible, though, that the increased levels of CREM sequester the transcription coactivators CBP and p300 (Fig. 3), thereby making them unavailable to CREB or other transcription factors. Sequestration of the coactivators would prevent the formation of bridging complexes necessary for recruitment of the transcriptional machinery and could lead indirectly to decreased transactivation of the IL-2 promoter. Indeed, it has been shown that overexpression of CREB inhibits AP-1- and NF-κB-mediated gene transcription by competing for the limited amounts of the coactivators CBP and p300 (25, 26). Finally, it is possible that p-CREM exhibits higher DNA binding affinity than p-CREB, resulting in occupation of the −180 site in SLE and subsequent repression of the IL-2 transcription. Regulation of IL-2 transcription is complex, and its decreased transcription in SLE T cells is probably multifactorial. Previously, we reported decreased NF-κB (20) and AP-1 (27) activity in SLE T cells, two more factors that are also involved in transcription of the IL-2 gene. It is possible that decreased IL-2 production by SLE T cells reflects an integrated effect of diminished activators (e.g., NF-κB) and excessive expression of repressors (e.g., p-CREM).

The mechanism responsible for increased expression of CREM in SLE T cells remains unknown. It is fascinating that the same cells display decreased levels of certain molecules (e.g., TCR ζ-chain and p65 Rel A protein), but increased levels of others (e.g., CD40 ligand and c-myc proto-oncogene) (4). The pathways responsible for the phosphorylation of CREM in SLE T cells are also not known. The phosphorylation pattern of various cytosolic SLE T cell proteins is aberrant (19), and the activities of protein kinase A isozymes I and II (28, 29) and C (30) that phosphorylate CREB on Ser133 are decreased. The complete characterization of pathways involved in the phosphorylation of CREM may decipher its role in the transcriptional regulation of SLE T cell genes. Also, it is unclear at this point why the levels of p-CREM decrease in SLE T cells following stimulation.

Table II. p-CREM binding in SLE is independent of disease activity

<table>
<thead>
<tr>
<th>SLEDAI</th>
<th>No. of Patients</th>
<th>p-CREM Binding (OD ± SEM)</th>
<th>p-CREM Binding (OD ± SEM)</th>
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<tr>
<td>0–4</td>
<td>11</td>
<td>110 ± 11</td>
<td>24 ± 8</td>
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<td>5–10</td>
<td>5</td>
<td>123 ± 15</td>
<td>18 ± 10</td>
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<td>11–15</td>
<td>5</td>
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<td>17 ± 9</td>
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<tr>
<td>16–20</td>
<td>3</td>
<td>118 ± 9</td>
<td>19 ± 7</td>
</tr>
</tbody>
</table>
Genome-wide scans of SLE patients have revealed multiple disease susceptibility loci, some of which are shared by various cohorts of patients (31, 32). The CREB gene maps in the 2q33–35 region of the human genome, whereas CREM appears to localize to the 10p11.2 band (33). Although these loci have not been identified in these genome scans as disease susceptibility loci for SLE in the studied cohorts, it is possible that genes located in the identified SLE susceptibility loci could affect the expression and function of CREM and CREB. Transgenic mice expressing a dominant negative form of CREB display markedly decreased IL-2 production, G1 cell-cycle arrest, and subsequent apoptotic death (34).

Taken together, our data demonstrate that p-CREM acts as a repressor of the IL-2 promoter in SLE T cells. Additional studies are needed to understand the mechanisms that lead to the increased levels of p-CREM in SLE and whether it interacts with other positive transcription factors that bind to the IL-2 promoter.

Acknowledgments
We thank all patients and normal donors for kindly donating blood samples. We are grateful to Drs. A. Rao and P. Sassone-Corsi for providing the luciferase constructs. We also thank Lee Collins for help with the preparation of the figures. The help from all our colleagues is appreciated.

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References