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Increased Levels of NF-ATc2 Differentially Regulate CD154 and IL-2 Genes in T Cells from Patients with Systemic Lupus Erythematosus

Vasileios C. Kyttariss,† Ying Wang,†† Yuang-Taung Juang,† Arthur Weinstein,‡ and George C. Tsokos2††

T cells from patients with systemic lupus erythematosus (SLE) are characterized by heightened TCR-initiated free intracytoplasmic calcium responses. We demonstrate that activated T cells from SLE patients, but not from rheumatoid arthritis patients, displayed higher levels of the calcineurin-dependent transcription factor NF-ATc2 in the nucleus compared with control T cells. DNA NF-AT-binding activity was also increased, as was the amount of NF-ATc2 bound to the promoters of CD154 (CD40L) and IL-2 genes. Nevertheless, although high NF-ATc2 levels translated into higher CD154 transcription in SLE, IL-2 transcription was decreased. The presence of important transcriptional activators (AP-1, NF-κB) and the absence of transcriptional repressors (cAMP response element modulator) on the IL-2 promoter explain this dichotomous effect. The Journal of Immunology, 2007, 178: 1960–1966.

Systemic lupus erythematosus (SLE) is characterized by significant T and B cell signaling aberrancies that play a central role in the expression of disease pathology (1). In particular, T cells once activated through the TCR show earlier and higher than normal mobilization of calcium in the cytoplasm in part due to the preaggregation of lipid rafts and the substitution of TCR-ζ-chain by the Fcγ chain (2). This heightened calcium influx, however, does not lead to a uniform up-regulation of all calcium-dependent genes (such as IL-2 and CD154 (CD40L, CD40L) in SLE T cells for reasons that are not fully understood to date (3); in particular, whereas IL-2 production is decreased, CD154 is increased (4, 5).

In normal T cells, calcium influx leads to activation of the phosphatase calcineurin (6), which in turn dephosphorylates the transcription factor NF-AT (7). The dephosphorylated NF-AT molecule enters the nucleus and binds to promoters of many genes often in conjunction with other molecules, such as AP-1 (8) initiating gene transcription. Gene transcription ceases once NF-AT is dephosphorylated by the export kinases casein kinase-1 and glycogen synthase kinase-3 and is transported back to the cytoplasm (9).

Of the five known NF-AT isoforms, NF-ATc2 (also called NF-AT-1 or NF-ATp) accounts for 80–90% of total NF-AT in resting T cells and is rapidly dephosphorylated by calcineurin soon after TCR-Ag-MHC engagement, thus playing a vital role in the transcription of calcium-dependent early response genes (9). NF-ATc1 (NF-AT-2/NF-ATc) is expressed in immune cells following activation, and therefore plays a role in later stages of cellular activation (9, 10). NF-ATc3 is expressed mainly in thymocytes (9), NF-ATc4 plays a role in cardiac tissue hypertrophy, and NF-AT5 is important in immune cell osmotic stress response (9).

We hypothesized that the heightened calcium influx in SLE T cells (11) is associated with increased translocation of NF-ATc2 to the nucleus in the early stages of cell activation. In this study, we present evidence that NF-ATc2 is increased in the nuclei of activated SLE T cells and that it binds to the promoters of CD154 and IL-2 genes. Nevertheless, although it results in enhanced transcription of the CD154 gene, it fails to regulate the expression of the IL-2 gene.

Materials and Methods

Study subjects

Twenty-five female patients diagnosed with SLE and fulfilling the American College of Rheumatology criteria diagnosed 30–50 ml of blood for our studies. The mean age of the SLE patients was 35 (20–61) years old, and the mean SLE disease activity index (SLEDAI) was 2.7 (0–8) (12). One patient donated blood on three separate occasions. A total of 62.9% of the patients was taking oral prednisone at a mean dose of 13.3 mg (2.5–40). Other immunosuppressive medications that the patients were on at the time of the study were as follows: hydroxychloroquine (81.4% of the patients), azathioprine (three patients), mycophenolate mofetil (five patients), methotrexate (three patients), leflunomide (one patient), and dapsone (one patient). One patient was receiving monthly i.v. Ig, with the last dose given 1 mo before the blood draw for the study. Prednisone was held for at least 12 h before the blood draw. Each patient was matched for age with a healthy female volunteer. In addition, eight patients with rheumatoid arthritis (RA) and matched controls were also analyzed as part of the study. The institutional review boards of all involved institutions approved the study protocol, and informed consents were obtained from all of the study subjects.

T lymphocyte isolation and stimulation

Peripheral venous blood was obtained from each study subject in heparin-lithium tubes. The specimens were processed between 6 and 20 h after...
collection. The blood was incubated for 30 min with a tetrameric Ab mixture against CD14, CD16, CD19, CD56, and glycA that attaches non-T cells to erythrocytes. Ficoll containing Lymphoprep gradient (Nycomed) was subsequently used to separate these complexes from T cells. Using flow cytometry, we established that the purified cells were >98% positive for CD3. Where mentioned, the T cells were stimulated with either PMA (10 ng/ml) and calcium ionophore A23187 (ionophore) (0.5 μM/μl) or anti-CD3 Ab (10 μg/ml) and goat anti-mouse cross-linking Ab (25 μg/μl). For the cyclosporin treatment experiments, purified T cells were incubated at 37°C for 1 h with cyclosporin A (EMD Biosciences) at a concentration of 100 ng/μl before stimulation.

Antibodies
Anti-NF-ATc2, anti-NF-ATc1, anti-actin, anti-c-fos, anti-GKLF (gift-enriched Kruppel-like factor), anti-rabbit HRP, anti-goat HRP, and anti-mouse HRP Abs were purchased from Santa Cruz Biotechnology. Rabbit anti-heterogeneous nuclear ribonucleoprotein (hnRNP) serum was purchased from Ortho-McNeil Pharmaceuticals, and the goat anti-mouse cross-link Ab was purchased from Sigma Genosys.

Protein purification and Western blotting
Blood from 18 patients and 18 controls was used for protein extraction. The cells after treatment were treated initially on ice with a 200 μl lysis buffer (10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA supplemented with freshly added 1 mM DTT, 0.5 mM PMSF, 2 mM aprotinin, 1 mM leupeptin, 10 mM NaF, and 2 mM Na3VO4) for 15 min. At the end of the incubation, Nonidet P-40 was added to the reaction mixture at a concentration of 0.6%. The reaction mixture was vortexed for 10 s and then subjected to centrifugation at 13,000 rpm for 15 s. The supernatant was saved as cytoplasmic extract. The pellet was resuspended in 25 μl of buffer (20 mM HEPES (pH 7.9), 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 10 mM NaF, 1 mM Na3VO4, 1 mM PMSF, 2 mM aprotinin, and 1 mM leupeptin) and then shaken for 15 min at 4°C. After centrifugation for 5 min at 13,000 rpm, the supernatant was stored as nuclear extract. We followed the manufacturer’s instructions (ECL; Amersham) for the Western blotting. The film was scanned, and the density of each band was calculated with QuantityOne software (Bio-Rad).

EMSAs
Nuclear extracts (2 μg) were incubated with a radiolabeled dsDNA probe, 1 μg of poly(dI:dC), and potassium chloride in a binding buffer for 15 min at room temperature. The reaction mixture was then subjected to separation in 6% polyacrylamide gel (Invitrogen Life Technologies). The dried gel was then autoradiographed. For supershift assays, the nuclear proteins were incubated with specific Abs at 4°C for 10 min before the probe, poly(dI:dC), and binding buffer were added. The reaction was further conducted for another 15 min at room temperature. The sequences of the probes used were as follows: NF-AT consensus-binding oligonucleotide, forward, 5'-GGGACACCCAGGAGGGCCCTCCCGGTGGGC-3' and reverse, 5'-TATGAGATTTTGAATGACCTTGGGCG-3'. Mutated NF-AT oligonucleotide was as follows: forward, 5'-GGCGGGACCCAGGAGGGCCCTCCCGGTGGGC-3' and reverse, 5'-TATGAGATTTTGAATGACCTTGGGCG-3'. Elf-1-binding oligonucleotide was as follows: forward, 5'-TCGAGAACCCTCCAGGAGGGCCCTCCCGGTGGGC-3' and reverse, 5'-TTGGTTCACAGGGAGGAGGGCCCTCCCGGTGGGC-3'.

Chromatin immunoprecipitation (ChIP)
Four million T cells were used per investigation. The cells were fixed with 1% formalin for 10 min, washed, lyzed, and sonicated. The DNA-protein complexes were immunoprecipitated with the desired Ab and extracted by protein A/G-Sepharose beads (Santa Cruz Biotechnology). After several washing steps, the cross-link between DNA and protein was reversed with 1% formalin for 10 min, washed, lysed, and sonicated. The DNA was amplified with conventional PCR and real-time PCR, as described below, with primers flanking the CD154 and IL-2 promoters. The sequences used were as follows: CD154, forward, 5'-CCAGGCATCGCCTTGGGAGTGGTC-3' and reverse, 5'-GCCTTGGGCATTGCTCGTG-3'; IL-2, forward, 5'-CGGATGACTGGTCTTTATCCCTGCGC-3' and reverse, 5'-GGTTTGTCACAGGAACCTCCGGGTGG-3'; NFAT cold competitor, 5'-ATGTGCCGGCGAGTTGACTGGTCTCTGGC-3'; NFAT cold competitor, 5'-ATGTGCCGGCGAGTTGACTGGTCTCTGGC-3'; elf-1-binding oligonucleotide in excess (lane 10) as well as the presence of an unrelated elf-1-binding oligonucleotide in excess (lane 3). The same was performed (lanes 4–7) in the presence of anti-NF-ATc2, anti-NF-ATc1, anti-NF-ATc3, or anti-c-fos Abs, as described in Materials and Methods. In the last three lanes, a mutated NF-AT-binding oligonucleotide is incubated with the nuclear protein from PMA/ionophore-stimulated T cells in the absence (lane 8) or presence of the same oligonucleotide in excess (lane 9) as well as the presence of an unrelated elf-1-binding oligonucleotide in excess (lane 10).

250 ng of total RNA was used for cDNA synthesis by reverse transcription (RT-PCR kit; Promega). Sigma Genosys synthesized the PCR primers. PCR beads were used for amplification (Pharmacia). PCR was conducted on a conventional thermocycler. Real-time PCR was conducted with a Cepheid Smart Thermocycler (Cepheid) by adding SYBR green to the reaction mixtures. Each real-time PCR was repeated twice, and the mean threshold cycle was calculated. Primers used for PCR and real-time PCR were as follows: GAPDH, forward, 5'-CAACAATCTGTTGTATCGTCCCTG-3' and reverse, 5'-GAACTGGTGGATGATGCTCT-3'; CD154, forward, 5'-ATTTCGCGCAGACATCGTCAAA-3' and reverse, 5'-CTCCCGGTTGGACAGAGAAG-3'; IL-2, forward, 5'-CACTTCAAGATAACCCACCTTCCGGTGCAGT-3' and reverse, 5'-GTTGGGGAACCTTACCTTATAAACCC-3'. CDNA specimens were used to create serially diluted samples (standard samples) that were subsequently amplified using different primers. A standard curve was generated for each set of primers, by plotting the threshold cycles of the real-time PCR against the logarithm of the relative concentrations of the standard samples. Thereafter, the relative mRNA copies of the experimental samples were calculated after real-time amplification, using the standard curve.

Statistical analysis
The nonparametric Wilcoxon matched-pairs signed-ranks test (two-tailed unless stated) was used. For correlations, the Spearman’s test was used. Statistical significance was defined as p < 0.05.

Results
Activated T cells from SLE patients have higher levels of NF-ATc2 than controls
Following T cell activation, NF-ATc2, the main isoform found in resting T cells, is dephosphorylated and translocated to the nucleus. We initially established the time course of NF-ATc2 translocation to the T cell nucleus by stimulating normal T cells with
sus-binding oligonucleotide and nuclear protein from PMA/ionophore.

After stimulation, we performed EMSA using an NF-AT consensus binding oligonucleotide and nuclear protein from PMA/ionophore-stimulated T cells, as described in Materials and Methods. As shown in Fig. 1A, NF-ATc2 protein levels in the nucleus of T cells peaked 1 h after stimulation.

To confirm that NF-ATc2 was the main NF-AT molecule that contributes to NF-AT-binding activity in T cells in the early stages after stimulation, we performed EMSA using an NF-AT consensus binding oligonucleotide and nuclear protein from PMA/ionophore-stimulated T cells. As shown in Fig. 1B, NF-AT DNA binding was specific and the binding activity was lost when key nucleotides were mutated. The oligonucleotide-protein complex was shifted with an anti-NF-ATc2 mAb. Although the addition of anti-NF-ATc1 Ab caused partial shift, the addition of either anti-NF-ATc3 or anti-c-fos Ab failed to shift the complex.

The data corroborate previous observations (9) that activation of T cells causes early translocation of NF-ATc2 to the nucleus, where it constitutes the major determinant of NF-AT DNA-binding activity.

We subsequently measured the levels of NF-ATc2 in the nuclei of T cells that had been stimulated with PMA/ionophore for 1 h, from 14 patients with SLE and matched controls. We found that stimulated SLE T cells had a 2-fold increase in the levels of NF-ATc2 in their nucleus compared with control T cells (SLE NF-ATc2 levels ± SEM: 2.1 ± 0.6-fold increase over control, p = 0.012; Fig. 2, A and B). As can be seen in Fig. 2A, specimens from stimulated T cells exhibit two bands, one of the same m.w. as the one from unstimulated T cells and one of lower m.w. The lower band is considered to represent the dephosphorylated form of NF-ATc2 (that is the form that binds to DNA) in the nucleus because it disappears in the presence of calcineurin (phosphatase) inhibitors in a dose-specific manner. Less likely, it may represent a variant of NF-ATc2 that is induced upon stimulation and reacts with the anti-NF-ATc2 Ab (14–16). As shown in Fig. 2, A and C, the intensity of the lower m.w. band was higher in SLE T cell nuclear extracts than controls. We analyzed the density of the lower m.w. band in eight pairs of SLE and controls in which this lower m.w. band was observed. We found that seven of eight SLE patients had significantly higher density of this band NF-ATc2 than controls (SLE lower m.w. NF-ATc2 band density ± SEM: 2.8 ± 0.7-fold increase over control, p = 0.0156).

As expected, most resting SLE as well as control T cells had undetectable NF-ATc2 protein in their nuclei, as determined by Western blotting. Interestingly though, in 4 of 14 SLE-control pairs that had detectable NF-ATc2, there was a trend for higher NF-ATc2 expression in resting SLE T cells than controls (SLE lower m.w. NF-ATc2 band density ± SEM: 2.0 ± 0.7-fold increase over control, p = 0.09).

FIGURE 2. After stimulation, SLE T cells show higher translocation of NF-ATc2 to the nucleus than control T cells. T cells from SLE patients, RA, and matched controls (Control) were stimulated with PMA/ionophore or anti-CD3 and goat anti-mouse cross-linking Ab for 1 h. The nuclear protein was extracted and used for Western blot analysis, as described in Materials and Methods. A, Representative experiment of PMA/ionophore-treated T cell nuclear protein extracts from SLE patients and matched controls that were immunoblotted using anti-NF-ATc2 (upper panel) and anti-hnRNP (lower panel) Ab. The intensity of the bands was measured by densitometry and the background was subtracted; the ratio of NF-ATc2 to hnRNP nuclear protein was calculated for each SLE-control pair. The normalized NF-ATc2:hnRNP ratio for each SLE patient was subsequently calculated by dividing the NF-ATc2:hnRNP ratio of the patient by the NF-ATc2:hnRNP ratio of its matched control. This normalized NF-ATc2:hnRNP ratio represents the fold increase of NF-ATc2 in SLE T cells over the controls. B, Means and SEM of normalized NF-ATc2:hnRNP ratios from PMA/ionophore-treated T cells from 14 SLE-control pairs are shown here. C, Nuclear extracts from anti-CD3- or PMA/ionophore-treated T cells from a patient with SLE and matched control were immunoblotted, as described in A. D, Means and SEM of normalized NF-ATc2:hnRNP ratios from anti-CD3-treated T cells from 5 SLE-control pairs are shown here. E, Nuclear extracts from PMA/ionophore-treated T cells from patients with RA and normal control were immunoblotted, as described in A.

FIGURE 3. NF-ATc2 levels are higher in patients with active SLE. T cells from SLE patients and matched controls (Control) were stimulated with PMA/ionophore for 1 h. The nuclear extracts were immunoblotted, and the normalized NF-ATc2:hnRNP ratios were calculated, as described in Fig. 2A. Here are shown the cumulative data (means and SEM) from 14 SLE-control pairs.

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NF-ATc2 levels ± SEM: 3.4 ± 1.1-fold increase over control, \( p = 0.12 \). There was no detectable difference in the cytoplasmic levels of NF-ATc2 between SLE and control T cells (data not shown).

Subsequently, we asked whether direct stimulation of T cells with an anti-CD3 Ab would also lead to higher NF-ATc2 levels in SLE. Indeed, as shown in Fig. 2, C and D, anti-CD3-stimulated SLE T cells displayed significantly higher NF-ATc2 levels than controls (SLE NF-ATc2 levels ± SEM: 3.2 ± 0.6-fold increase over control, \( p = 0.031 \)). In addition, stimulation of T cells from eight patients with mild to moderately active RA and eight controls showed no significant differences between the two groups (RA NF-ATc2:hnRNP ratio ± SEM: 0.47 ± 0.08; control NF-ATc2:hnRNP ratio ± SEM: 0.53 ± 0.07, Mann-Whitney \( U \) test, \( p = 0.79 \)) (Fig. 2E). These experiments demonstrate that activation of SLE T cells leads to a significantly higher translocation of NF-ATc2 to the nucleus than activation of normal T cells.

**Increased nuclear T cell content of NF-ATc2 is a feature of SLE disease activity**

To examine the effect of disease activity, disease manifestations, and medications used on NF-ATc2 nuclear translocation, we correlated the levels of NF-ATc2 with the disease activity of the patients, as determined by SLEDAI, as well as with the type and dose of immunosuppressive medications. As shown in Fig. 3, NF-ATc2 levels in the PMA/ionophore-activated T cells of non-active (SLEDAI: 0) patients were comparable to controls, whereas patients that had even minimally active disease (SLEDAI: 2–8) had a 2.8 ± 1.0-fold increase over controls (\( p = 0.0078 \)). On the contrary, there was no correlation between NF-ATc2 levels and immunosuppressive medications or specific disease manifestations (such as skin disease, neuropsychiatric lupus, nephritis, and arthritis). In particular, corticosteroid dose did not correlate with levels...
of NF-ATc2 (Spearman’s \( r = 0.2747 \)). From these data we conclude that high NF-ATc2 translocation to the nuclei of SLE T cells is seen in patients with active disease; this phenomenon is not a medication effect, and therefore it may represent a significant signaling aberration contributing potentially to the clinical abnormalities seen in patients with active SLE.

**NF-AT-binding activity and NF-ATc2 binding to CD154 and IL-2 promoters are increased in SLE T cells compared with controls**

Because NF-ATc2 translocates to the nucleus of SLE T cells in higher quantities than in control T cells, we evaluated the DNA-binding activity of nuclear protein from T cells stimulated for 1 h with PMA/ionophore to the NF-AT-specific oligonucleotide described above and shown in Fig. 1B. Nuclear protein from SLE T cells had significantly higher binding activity to the NF-AT-specific binding oligonucleotide than nuclear protein from control T cells (SLE NF-AT-binding activity ± SEM: 2.58 ± 0.47-fold increase over control, \( p = 0.0117 \); Fig. 4, representative experiment and cumulative data). Similar results were obtained when anti-CD3 Ab was used to stimulate the T cells for 1 h (Fig. 4A).

Subsequently, we assessed the physiologic effect of the observed higher NF-ATc2 levels and the associated higher NF-AT-binding activity in SLE T cells. After stimulating T cells from patients with SLE and controls for 1 h, the cells were fixed with formalin, lysed, and sonicated, and the DNA fragments that bound NF-ATc2 were extracted by immune precipitation. In Fig. 5, A and D (upper row), we show that using specific primers for the promoter of the CD154 gene, SLE T cells had higher levels of NF-ATc2-bound DNA following stimulation than controls. This trend was not observed when an unrelated anti-GKLF Ab was used (data not shown). Similarly, in Fig. 5, B and D (middle row), we show that SLE T cells have higher than controls NF-ATc2-bound DNA when using specific primers for the IL-2 promoter. In Fig. 5, C and D (lower row), we show that if no Ab was used, the amount of extracted DNA (input DNA) was similar between SLE and controls. All SLE patients that were included in this experiment had SLEDAI score >2 and all displayed higher binding of NF-ATc2 to both CD154 and IL-2 promoters (Fig. 5E, cumulative results) than the matched controls.

**Pathophysiologic effects of heightened NF-ATc2 recruitment in the nucleus of SLE T cells**

Because NF-ATc2 binding to CD154 and IL-2 promoters is higher in stimulated SLE T cells than controls, we evaluated its effect on the transcription of CD154 and IL-2 genes. To this end, we incubated T cells from both SLE patients and normal controls with either cyclosporin A (a small peptide that blocks the calcineurin-induced NF-AT dephosphorylation) (17) or control (ethanol). After 1 h of incubation, the cells were stimulated with PMA/ionophore for 3 h. Subsequently, the cells were lysed, and the mRNA was collected and reverse transcribed to cDNA, which was in turn amplified with primers specific for the CD154, IL-2, and GAPDH genes. In Fig. 6A (representative of three independent experiments), we show that after stimulation, SLE T cells up-regulated CD154 transcription significantly more than controls (in all three experiments) in accordance with previous publications (5). In contrast, the expression of GAPDH was similar between SLE and controls (Fig. 6C).

Incubation of both SLE and control T cells with cyclosporin A led to a significant decrease in the transcription of CD154 following stimulation (Fig. 6A, one of three independent experiments). Furthermore, the levels of CD154 transcription in stimulated, cyclosporin-treated T cells did not differ between SLE patients and controls. These data point to the fact that the higher NF-ATc2
nuclear translocation is responsible for the higher early transcription rate of CD154 in SLE T cells when compared with controls.

We observed a different effect of NF-AT on the expression of IL-2. Although NF-ATc2 binding to the IL-2 promoter was higher in SLE T cells than controls, IL-2 transcription (Fig. 6B, one of three independent experiments) was lower in T cells from patients with SLE, as demonstrated previously (18). Similar to CD154, treatment of T cells with cyclosporin A leads to a significant decrease in IL-2 transcription. Therefore, although NF-AT is responsible to a significant level for the transcription of IL-2, just as it is for the transcription of CD154, other factors present (increased binding of the repressor cAMP-responsive element modulator (CREM) (19)), or absent (decreased binding of AP-1 (20) and NF-κB (21)) in SLE T cells interfere with the proper NF-AT-mediated IL-2 transcription in SLE (Fig. 7).

We conclude from this set of experiments that although NF-AT translocation to the nucleus is necessary (as demonstrated by cyclosporin inhibition) for the transcription of both CD154 and IL-2 genes, it is not sufficient for the transcription of IL-2 in SLE in which other transcription factors play a significant role.

Discussion

We present evidence that the main T cell isoform of NF-AT, NF-ATc2, translocates to the nuclei of SLE T cells at higher levels than controls following T cell activation. This results in higher NF-ATc2 binding to the promoters of both CD154 and IL-2 genes. In turn, SLE T cells show higher rates of NF-AT-mediated transcription of the CD154, but not of the IL-2 gene. Blocking the calcium-dependent phosphatase calcineurin leads to a significant decrease in the transcription of both genes. These observations establish a link between the higher TCR-mediated free intracytoplasmic calcium response that is observed in SLE T cells following activation (2) and the expression of molecules that are involved in the pathogenesis of SLE.

Our results are consistent with multiple studies, which have established that SLE T cells have higher and more sustained surface expression of CD154 following stimulation when compared with controls (5, 22), which is partially reversed by cyclosporin especially if the drug is added during the early stages of stimulation (23, 24). Transcriptional, posttranscriptional, and posttranslational mechanisms may all contribute to the increased expression of CD154 by SLE T cells. In this study, we focused on the early transcriptional regulation of the CD154 gene and we show that early increased CD154 mRNA production is linked to the establishment of higher CD3-mediated free intracytoplasmic calcium response in SLE T cells. Because cyclosporin A cannot effectively abrogate the prolonged expression of CD154 if added in activated SLE T cells, other mechanisms and not just increased early nuclear translocation of NF-ATc2, such as phosphorylated ERK (23), also contribute to the abnormally high and prolonged expression of CD154 by SLE T cells. CD154 is a costimulatory molecule on the surface of T cells that is important for B cell activation and proliferation as well as Ig isotype switch. Enhanced CD40-CD154 interaction in SLE has been demonstrated to contribute to increased Ig and autoantibody production (5), and treatment with anti-CD154 Ab has been successfully attempted (25, 26). Due to significant expression of CD154 on other cells such as platelets, direct blocking of this molecule, although proving useful, also caused significant thromboembolic side effects. For this reason, it is important that CD154 blockade be attempted by specifically targeting the expression of CD154.

Consistent with previous data, and contrary to CD154 transcription, IL-2 production following in vitro activation is decreased in SLE T cells (4). The relative deficiency in IL-2 production in SLE contributes to the increased infection rate in these patients (27), decreased activation-induced cell death (28), and regulatory T cell numbers (29).

The dichotomous effect of NF-AT on these two genes can be explained by the fact that other transcription factors that are aberrantly regulated in SLE bind to the IL-2, but not to the CD154 gene promoter. Specifically, the transcriptional repressor CREM is expressed in increased amounts in SLE T cells, binds to the IL-2 promoter, and limits directly its transcription (18). CREM also binds to the promoter of c-fos and suppresses its transcription (20). Under normal conditions, c-fos forms together with c-jun, the dimeric transcription factor AP-1, a transcriptional activator of IL-2. AP-1 combines with NF-AT on the promoter of the IL-2 gene, leading to the initiation of transcription. Lack of AP-1 leads to inability of NF-AT to act as a transcriptional activator of IL-2 (30). Therefore, the CREM-mediated AP-1 deficiency in SLE T cells helps explain the null effect of up-regulated NF-AT on the transcription of IL-2 in SLE T cells. On the contrary, other transcription factors such as Egr-1 (31) and not AP-1 may act as molecular coactivators for the NF-AT-mediated CD154 transcription.
While searching for the underlying factors of the signaling abnormalities that have been described in SLE, our laboratory has shown that CREM recruitment to the promoter of IL-2 is caused by anti-TCR/CD3 autoantibodies present in the serum of patients with SLE (32). We have not been able to show such a serum-mediated effect on either calcium response (G. Tsokos, unpublished data) or NF-AT translocation (data not shown). These observations lead us to hypothesize that both exogenous factors (autoantibodies and/or cytokines) and intracellular mechanisms play a critical role in the expression of aberrant cell signaling observed in SLE T cells. We propose that chronic exposure of T cells to serologic factors leads to the recruitment of CREM to the IL-2 and c-fos promoters of resting T cells. Once these T cells are further activated through their rewired TCR, calcium enters at much higher speed and quantity leading to increased calcineurin-mediated NF-AT translocation to the nuclei. In turn, this (coupled with other signaling abnormalities) leads to up-regulation of CD154 transcription and increased B cell activity. At the same time, the presence of CREM prevents the up-regulation of AP-1 (the molecular part-