cAMP-responsive Element Modulator \(\alpha\) (CREM\(\alpha\)) Contributes to Decreased Notch-1 Expression in T Cells from Patients with Active Systemic Lupus Erythematosus (SLE)*

Received for publication, October 6, 2012, and in revised form, October 26, 2012. Published, JBC Papers in Press, November 2, 2012, DOI 10.1074/jbc.M112.425371

Thomas Rauen‡, Alexandros P. Grammatikos‡, Christian M. Hedrich‡, Jürgen Floege¶, Klaus Tenbrock‡, Kim Ohl‡, Vasileios C. Kytaridis‡, and George C. Tsokos‡

From the ‡Division of Rheumatology, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02115 and the ¶Department of Nephrology and Clinical Immunology and the ‡Department of Pediatrics, Division of Allergology and Immunology, RWTH University of Aachen, 52074 Aachen, Germany

Background: T cells from SLE patients display multiple signaling aberrations, many of which are attributed to increased presence of transcription factor CREM\(\alpha\).

Results: Notch-1 expression is significantly reduced in T cells from active SLE patients. Both epigenetic and transcriptional effects mediated through CREM\(\alpha\) contribute to dysregulated Notch-1 expression in SLE T cells.

Conclusion: Notch-1 levels inversely correlate with SLE disease activity.

Significance: Boosting endogenous Notch-1 levels may redirect T cell function in SLE patients.

Notch signaling constitutes an evolutionarily conserved pathway that transduces signals between neighboring cells and determines major decisions in cell proliferation, survival, and differentiation. Notch signaling has been shown to play a pivotal role during T cell lineage determination. T lymphocytes from patients with systemic lupus erythematosus (SLE) display a severely altered phenotype with several molecular and functional aberrations, including defective capacities to up-regulate Notch-1 receptor expression upon T cell receptor activation. Here, we demonstrate that basal Notch-1 expression is decreased in T cells from active SLE patients at the mRNA and protein levels in various T cell subpopulations. Notch-1 transcript numbers inversely correlate with disease activity in SLE patients. We provide evidence that both enhanced histone H3 methylation and CpG DNA methylation of the human Notch-1 promoter contribute to decreased Notch-1 expression in SLE T cells. Previous data from our group have highlighted the pivotal role of the transcription factor cAMP-responsive element modulator \(\alpha\) (CREM\(\alpha\)) in the epigenetic and transcriptional regulation of several SLE-relevant target genes in T cells (3–10). The CREM protein family comprises >20 isoforms in humans, and there is broad evidence that the CREM\(\alpha\) isoform is robustly overexpressed in SLE T cells. CREM\(\alpha\) gene transcription itself is regulated through different promoters (denoted P1 and P2), and activity levels of P1 correlate with disease activity in SLE patients (11, 12). CREM\(\alpha\) binds DNA motifs denoted cAMP-responsive elements (CREs), which are defined by the palindromic nucleotide sequence TGACGTCA or its 5’-half-site TGAC. Within the dysregulated cytokine profile of SLE T cells, CREM\(\alpha\) suppresses IL-2 and increases proinflammatory IL-17A production by virtue of its propensities as a transcription factor and “epigenetic modifier” of histones and cytosine-phosphoguanosine (CpG) DNA sequences (5–7).

T cell activation through the canonical T cell receptor complex can be significantly influenced by various co-stimulatory signals, including CD28, CTLA-4, and signaling lymphocyte activation molecules. Coactivation of these surface proteins may transmit inhibitory or stimulatory messages for T cell function (13–16). The Notch receptors constitute an evolutionarily conserved family of transmembrane molecules (in mammals, Notch-1–4) that transduce signals between neighboring cells. They are involved in short-distance cell-cell communica-

Systemic lupus erythematosus (SLE)³ was initially described as a B cell-dependent disease with an excessive production of autoantibodies; however, it has become evident over the last decades that severe signaling aberrations in T cells also play a major role in SLE pathogenesis (1, 2). This may include defective T cell activation and proliferation, dysregulated T helper cell differentiation, and impaired cytokine production. Previous studies from our group have highlighted the pivotal role of the transcription factor cAMP-responsive element modulator \(\alpha\) (CREM\(\alpha\)) in the epigenetic and transcriptional regulation of several SLE-relevant target genes in T cells (3–10). The CREM protein family comprises >20 isoforms in humans, and there is broad evidence that the CREM\(\alpha\) isoform is robustly overexpressed in SLE T cells. CREM\(\alpha\) gene transcription itself is regulated through different promoters (denoted P1 and P2), and activity levels of P1 correlate with disease activity in SLE patients (11, 12). CREM\(\alpha\) binds DNA motifs denoted cAMP-responsive elements (CREs), which are defined by the palindromic nucleotide sequence TGACGTCA or its 5’-half-site TGAC. Within the dysregulated cytokine profile of SLE T cells, CREM\(\alpha\) suppresses IL-2 and increases proinflammatory IL-17A production by virtue of its propensities as a transcription factor and “epigenetic modifier” of histones and cytosine-phosphoguanosine (CpG) DNA sequences (5–7).

³The abbreviations used are: SLE, systemic lupus erythematosus; CREM\(\alpha\), cAMP-responsive element modulator \(\alpha\); CRE, cAMP-responsive element; qRT-PCR, quantitative RT-PCR; SLEDAI, SLE disease activity index.

This is an Open Access article under the CC BY license.
Notch-1 Gene Regulation in SLE T Cells

Notch signaling is involved in various peripheral T cell responses, e.g. polarization of T helper (Th) cells. Both receptors and ligands can be regulated by post-translational protein modifications, proteolytic processing, endocytosis, and membrane trafficking. Overall, the cytokine milieu, expression pattern of the receptors and their ligands on peripheral T and B cells, and the specificity of the ligand-receptor interaction may determine specific cell fate decisions (23). Notably, chemical inhibition of all four Notch receptors by nonspecific γ-secretase inhibitors inhibits Th1- and Th17-type differentiation and ameliorates signs of autoimmunity and renal damage in lupus-prone MRL-lpr mice (24). γ-Secretase inhibitors have also been shown to be beneficial in experimental autoimmune encephalomyelitis, which is another Th17-dependent disease model and has features that resemble multiple sclerosis in humans (25). γ-Secretase inhibitors have already entered the stage of clinical trials in humans; however, they are associated with major side effects (mainly gut toxicity), which have been attributed to the pan-Notch blockade (26). More specific interventions targeting individual Notch receptors, e.g. by application of monoclonal antibodies, appear to be more tolerable (27). In the experimental autoimmune encephalomyelitis model, selective inhibition of individual Notch receptors using specific antibodies abrogated Th1- and Th17-type responses (25).

In this study, we observed significantly decreased amounts of Notch-1 in T cells from clinically active SLE patients at both the mRNA and protein levels. We provide evidence that Notch-1 gene expression is highly controlled through changes in the epigenetic conformation of the Notch-1 promoter, including histone and CpG DNA methylation and transcriptional repression mediated by CREMα. Eventually, reduced Notch-1 levels in human T cells are associated with increased IL-17A production, as observed in SLE patients.

EXPERIMENTAL PROCEDURES

Primary Human T Cells and Human T Cell Line—The SLE patients included in our analyses were female and diagnosed according to the American College of Rheumatology classification criteria (28). They were recruited from the Division of Rheumatology at the Beth Israel Deaconess Medical Center after written informed consent under protocol 2006-P-0298. Healthy individuals were chosen as controls. Peripheral venous blood was collected in heparin lithium tubes, and total human T cells were purified as described previously (18). All primary human T cells and human Jurkat T cells were kept in RPMI 1640 medium supplemented with 10% fetal bovine serum.

mRNA Extraction and Quantitative RT-PCR—Total RNA was isolated from purified human T cells using a RNeasy mini kit (Qiagen). Residual genomic DNA contamination was removed by DNase I (Qiagen). RNA was reverse-transcribed into cDNA using a reverse transcription system (Promega). Sequences for real-time quantitative RT-PCR (qRT-PCR) primers were as follows: Notch-1, 5′-ctgctgtgctaggtcagc-3′ (forward) and 5′-taagctgcttaccc-3′ (reverse); IL17A, 5′-gaaatccagtagcttcc-3′ (forward) and 5′-gacccagatcctc-3′ (reverse); and 18S rRNA, 5′-actcagggagacca-3′ (forward) and 5′-aaccagagaactgctac-3′ (reverse). Real-time qPCR was performed on an ABI OneStepPlus real-time PCR system.

Flow Cytometry—Cells (0.5 million) from each blood donor were stained ex vivo with FITC-labeled anti-CD3, Pacific Blue-labeled anti-CD4, phycoerythrin-labeled anti-CD8, and allophycocyanin-labeled anti-Notch-1 antibodies. Samples were incubated at room temperature for 30 min, washed twice with PBS, and fixed in a 4% formaldehyde solution. Expression of cell surface markers was assessed on a BD Biosciences LSRII flow cytometer, and data were gated and displayed on FlowJo Version 7.6.5 (TreeStar Inc., San Carlos, CA).

Plasmids and Luciferase Assays—An expression plasmid for human CREMα (in the pcDNA3.1/V5-His-TOPO vector, Invitrogen) was kindly provided by G. N. Europe-Finner (Faculty of Medical Sciences, Newcastle upon Tyne, United Kingdom) (29). A 2.1-bp spanning Notch-1 reporter construct (in pGL3-Basic vector, Promega) was generated and kindly donated by M. Ruppert (University of Alabama at Birmingham) (30). An expression plasmid encoding the constitutively active intracellular Notch-1 domains was kindly provided by K. Sakamoto (Tokyo Medical and Dental University, Tokyo, Japan) (31). All plasmid DNA preparations were carried out with DNA purification kits (Qiagen) and sequence-verified (GENEWIZ, Inc., Cambridge, MA). Jurkat T cells (3 million) were transfected with a total amount of 3 μg of plasmid DNA at a molar effector:reporter transfection ratio of 3:1 using an Amaza human T cell Nucleofector kit (Lonza) and an Amaza Nucleofector II device (program U014, Lonza). Each reporter experiment included 10 ng of Renilla luciferase construct as an internal control. Five hours after transfection, cells were collected and lysed, and luciferase activity was quantified using the Promega Dual-Luciferase assay system according to the manufacturer’s instructions. Luciferase experiments were repeated three times, and values in the bar diagrams are given as mean ± S.D.

ChIP Assays—Anti-H3K27me3 antibody and nonspecific normal rabbit IgG were obtained from Upstate Biotechnology. Polyclonal anti-CREMα antibody detecting human CREMα was designed in our group and has been described previously.
ChIP-grade ChIP assay was carried out according to the manufacturer’s instructions (Upstate Biotechnology). Briefly, 1–2 million total T cells were cross-linked with 1% formaldehyde, washed with cold PBS, and lysed in buffer containing protease inhibitors (Roche Applied Science). Cell lysates were sonicated to shear DNA and sedimented, and diluted supernatants were immunoprecipitated with the indicated antibodies and ChIP-grade Protein A/G Plus-agarose (Thermo Scientific). A proportion (20%) of the diluted supernatants were kept as “input” (input represents PCR amplification of the total sample). Protein-DNA complexes were eluted in 1% SDS and reverse-cross-linked at 65 °C. DNA was recovered using a QIAamp DNA mini kit (Qiagen) and subjected to PCR analysis using an ABI OneStepPlus real-time PCR system. The real-time qPCR primer sequences used to detect the CRE site within the human Notch-1 promoter were as follows: 3’-aaatcaggagggacagc-5’ (forward) and 3’-tggattctcgcggcttttc-5’ (reverse). The amount of immunoprecipitated DNA was subtracted by the amplified DNA that was bound by the nonspecific normal IgG and subsequently calculated relative to the respective input DNA.

Methylated CpG DNA Immunoprecipitation—A methylated CpG DNA immunoprecipitation assay was carried out according to the manufacturer’s instructions (Zymo Research). Briefly, genomic DNA from T cells obtained from SLE patients and healthy control individuals was purified using the AllPrep RNA/DNA/protein mini kit (Qiagen) and sheared to fragments of ~200 bp with DNA Shearase (Zymo Research). Subsequently, 100 ng of sheared genomic DNA were used for methylated CpG DNA immunoprecipitation. Methylated DNA was recovered and subjected to PCR analysis with an ABI OneStepPlus real-time PCR system using the same Notch-1 promoter primers used in the ChIP experiments (see sequences above). Equal amounts (100 ng) of completely (100%) methylated human DNA and demethylated human DNA (Zymo Research) were included as the input and negative control.

CREMa Transgenic Mice—Transgenic mice on a FVB background with T cell-specific CREMa overexpression (under the control of the CD2 promoter) have recently been described (33). Naïve T cells from these mice were obtained from spleens by negative isolation using magnetic cell separation with CD4 (33). Naïve T cells from these mice were obtained from spleens by negative isolation using magnetic cell separation with CD4 (33). Naïve T cells from these mice were obtained from spleens by negative isolation using magnetic cell separation with CD4 (33). Naïve T cells from these mice were obtained from spleens by negative isolation using magnetic cell separation with CD4 (33).

Statistical Analyses—Student’s paired two-tailed t test was used for statistical analysis. The Pearson product moment correlation coefficient (r) was used to determine the correlation between Notch-1 mRNA levels and individual SLE disease activity indices (SLEDAIs).

RESULTS

Notch-1 mRNA Expression Is Decreased in T Cells from Active SLE Patients—Notch-1 mRNA expression was analyzed by real-time qRT-PCR in total T cells obtained from a cohort of 61 SLE patients, with 32 of them being classified as active patients and 24 as healthy control individuals (Fig. 1). A composite SLEDAI, which reflects clinical symptoms and the degree of laboratory alterations in SLE patients, was used to define active patients (SLEDAI > 3). Notably, active patients displayed significantly lower Notch-1 mRNA levels (as assessed by normalized Ct values using the second derivative maximum method) than both inactive SLE patients (SLEDAI = 1–3) and healthy controls. Expression levels were not significantly different between healthy controls and inactive patients. mRNA expression analyses for another member of the Notch receptor family, i.e. Notch-2, did not yield major differences between these groups (data not shown). To further prove that Notch-1 mRNA expression varies with SLE disease activity levels, we performed longitudinal analyses in four SLE individuals and compared Notch-1 mRNA expression with the corresponding SLEDAIs over time (Fig. 2). Indeed, we observed strong negative correlations between Notch-1 mRNA magnitudes and SLEDAIs in the patients analyzed (Pearson’s r between −0.56 and −0.90). Thus, the extent of Notch-1 mRNA expression mirrors disease activity in SLE patients.
Notch-1 Gene Regulation in SLE T Cells

Surface Expression of Notch-1 Protein Is Also Reduced in Active SLE Individuals—Next, we determined Notch-1 protein expression at the membrane surface of primary T cells purified from 12 SLE patients and 6 healthy controls by flow cytometry using an allophycocyanin-labeled anti-Notch-1 antibody (Fig. 3). These studies were performed in total T cells; however, co-staining for CD4 and CD8 surface markers allowed for more specific conclusions with regard to T cell subpopulations. Notch-1 protein expression was markedly decreased in active SLE individuals compared with inactive patients and healthy individuals. This difference reached the level of statistical significance in total T cells (Fig. 3, A and B) and CD4+ T helper cells (Fig. 3C), whereas the difference was almost significant in the CD8+ cytotoxic T cell subset (Fig. 3D).

Histone Methylation and CpG DNA Methylation Are Involved in Notch-1 Gene Regulation—Given the observed differences in Notch-1 expression between active SLE patients and inactive patients as well as healthy controls, we next investigated the underlying molecular mechanisms that mediate decreased Notch-1 expression in active SLE patients. We have previously demonstrated the prominent role of CREMγ and histone and CpG DNA methylation in T cell-dependent target gene regulation (5–7, 32). Thus, we hypothesized that Notch-1 gene expression may be regulated by an aberrant methylation pattern of the Notch-1 gene promoter, which is highly conserved throughout evolution and comprises several putative CREs. To this end, we performed ChIP experiments in total T cells obtained from three age-, gender-, and ethnicity-matched pairs of active SLE patients (all SLEDAI > 3) and healthy controls with an antibody that specifically detects trimethylated Lys-27 of histone H3 (H3K27me3). Immunoprecipitated DNA was PCR-amplified, covering a region within the Notch-1 gene promoter that spans a CRE half-site, −991 to −988 bp upstream of the start codon (TGAC) (see Fig. 5A). Histone H3K27 trimethylation was significantly higher in SLE patients compared with healthy controls (Fig. 4, A and B). Furthermore, CpG DNA methylation was examined using methylated DNA immunoprecipitation assays in a cohort of 15 healthy individuals and 18 SLE patients (with 12 of them classified as active patients). Notably, all (active and inactive) SLE patients displayed an elevated CpG DNA methylation status compared with healthy individuals (Fig. 4C). Taken together, our findings argue for a concordant histone and CpG DNA hypermethylation at the Notch-1 gene promoter in T cells from active SLE patients.

CREMγ Affects Notch-1 Gene Transcription—The transcription factor CREMγ has been demonstrated to be crucially involved in the trans-regulation and epigenetic “remodeling” of several gene loci that contribute to SLE pathogenesis (4, 5, 7). As the human Notch-1 promoter defines a putative CRE half-site within the region that we identified to be sensitive to methylation (Fig. 5A), we investigated whether CREMγ indeed may bind to this element. Thus, we performed ChIP experiments in total T cells from four matched SLE/control pairs using a polyclonal anti-CREMγ antibody. CREMγ binding to the CRE (−991/−988) was significantly increased in T cells from SLE patients (all of them being active patients) compared with that in healthy control individuals (Fig. 5, B and C). To test for the functional relevance of CREMγ binding to the Notch-1 promoter, we transiently overexpressed CREMγ in a human T cell line (Jurkat). This approach was chosen because T cells from SLE patients display increased CREMγ levels. Five hours after transfection, mRNA was collected and analyzed for Notch-1 transcript numbers by real-time qRT-PCR. An increased presence of CREMγ in T cells led to significantly decreased Notch-1 mRNA expression (Fig. 5D). Most likely, this effect is mediated by direct trans-repression of the Notch-1 gene promoter.

FIGURE 2. Longitudinal analyses of Notch-1 mRNA expression and corresponding SLEDAIs. Total T cell mRNAs from four SLE patients (SLE 1 to SLE 4) were collected every other month, and both relative Notch-1 mRNA expression in total T cells (normalized Ct values; left y axis, ▲) and SLEDAIs (right y axis, ◆) were determined at each time point. Individual Pearson’s correlation coefficients (r) are given in the upper right corner of each panel.
through CREM as evidenced by luciferase experiments in human T cells. A reporter construct harboring 2.1 bp of the human Notch-1 gene promoter showed significantly decreased activity when CREM was co-introduced into these cells (Fig. 5E). Taken together, these findings support the hypothesis that CREM physically binds to the Notch-1 promoter and acts as a potent repressor of Notch-1 gene transcription. Next, we analyzed splenic T cells from FVB mice that were transgenically engineered to express increased CREM levels in their T lymphocytes (33). These mice have recently been shown to produce increased amounts of proinflammatory IL-17A and to be more prone to develop signs of autoimmunity that resemble the SLE phenotype. We examined Notch-1 mRNA expression in CD4+ T cells from these mice. Interestingly, we observed decreased

FIGURE 3. Notch-1 protein expression at the surface of T cells from SLE patients and healthy controls. A, CD3+ T cells from healthy control individuals (CON) and SLE patients were analyzed for Notch-1 protein expression by flow cytometry. Percentages of Notch-1+ cells are given in the diagram. B, a representative staining pattern is shown. T helper (CD3+ CD4+) and cytotoxic (CD3+ CD8+) T cells were analyzed for Notch-1 protein expression by flow cytometry. Horizontal lines indicate the mean ± S.D. ns, not significant.

FIGURE 4. Histone methylation and CpG DNA methylation of the Notch-1 promoter are increased in T cells from SLE patients. A, histone H3K27 methylation was analyzed in total T cells from three age-, gender-, and ethnicity-matched control (CON)/SLE pairs by ChIP assays. Dotted lines associate matched samples. A region of interest within the human Notch-1 promoter (harboring a putative CRE) was amplified by qPCR, and the proportion of immunoprecipitated DNA was calculated as relative to the non-immunoprecipitated input DNA in each sample. Subsequently, the ratio of relative expression was calculated between each SLE patient and the corresponding control individual. Horizontal lines represent mean values. B, the dotted line represents the H3K27 methylation status in control T cells, for each of which was set to 1.0. Changes in the methylation status in the matched SLE patient are given in the bar diagram (mean ± S.D.). C, total T cells from 15 healthy controls (light gray bar), 6 inactive SLE patients (dark gray bar), and 12 active SLE patients (black bar) were subjected to methylated DNA immunoprecipitation. The percentage of methylated DNA is given as mean ± S.D.
CREMα has been shown to act as a strong inducer of IL17A gene transcription and synthesis in human and murine T cells (5, 33). As Notch-1 expression is down-regulated in T cells from SLE patients, we wondered whether this might be of functional relevance for IL17A production. To this end, we mimicked the “SLE phenotype” by silencing endogenous Notch-1 levels in SLE T cells from active SLE patients, which was associated with increased IL17A transcript numbers (Fig. 6A). Vice versa, we overexpressed a constitutively active Notch-1 construct that spans only the intracellular receptor domains in this T cell line. This approach yielded robustly decreased IL17A mRNA expression (Fig. 6B).

DISCUSSION

In this study, we have presented evidence that T cells from active SLE patients display significantly decreased basal levels of the transmembrane receptor Notch-1. This is in line with the previous report by Sodai et al. (34) that SLE T cells fail to up-regulate Notch-1 expression after T cell receptor-mediated cell activation. However, the authors did not examine basal Notch-1 expression levels. Thus, our findings constitute the first description of Notch-1 expression in unstimulated T cells in a large cohort of active and non-active SLE patients and healthy control individuals. Furthermore, it has not been shown before that decreased Notch-1 transcript numbers are transduced into reduced Notch-1 protein levels.

Aberrant gene expression in immune cells from SLE patients has largely been attributed to specific epigenetic modifications, including histone and CpG DNA methylation, as well as aberrant transcriptional activities (32, 35). DNA hypomethylation is a well recognized key determinant in SLE pathogenesis, and several gene loci have been identified that are hypomethylated in SLE T cells, contributing to increased gene expression, e.g., IL4, IL6, IL10, IL13, IL17A, IFNγ, and protein phosphatase-2A (5, 36–39). Recently, it has become clear that there are also
genes that may be hypermethylated in T cells from SLE patients, including IL2 (6, 7). We have now provided evidence that Notch-1 marks another gene with a markedly hypermethylated promoter region in T cells from SLE patients. This involves concordant histone H3 and CpG DNA hypermethylation. The epigenetic pattern under pathophysiological conditions is governed by specialized enzymes that are recruited to promoter regions and/or additional cis-regulatory regions. These enzymes alter histone tail or DNA modifications and comprise histone deacetylases and DNA methyltransferases (32). Usually, histone methylation and CpG DNA methylation follow the same pattern, i.e. either hypo- or hypermethylation (40). The mechanisms by which these modifications are induced during the development of autoimmune diseases remain poorly understood. We have previously reported that CREMα, which is the dominant CREM isoform in T cells from SLE patients, may regulate gene expression not only by virtue of its transcription factor capacities but also because it may directly interact with HDAC1 and DNMT3a and thus affect epigenetic modifications (7, 41). CREMα differentially induces or represses cytokine expression in SLE T cells.

In this study, we have demonstrated that CREMα binds to and trans-represses a CRE site within the Notch-1 promoter and thereby contributes to decreased Notch-1 gene expression. Furthermore, lowering endogenous Notch-1 levels using siRNA is associated with increased amounts of IL-17A, which is a hallmark of SLE T cells. Whether the reduced presence of Notch-1 at the surface of T cells from active SLE patients is associated with additional alterations observed in this autoimmune disease, including aberrant synthesis of other cytokines and/or impaired differentiation and proliferation capacities, remains to be elucidated in future studies.

Our findings provide evidence that (i) the gene and protein expression of Notch-1 receptors is markedly decreased in T cells from active SLE patients, (ii) transcript numbers inversely mirror disease activity in these patients, and (iii) epigenetic and CREMα-induced transcriptional effects are important upstream mechanisms to explain this phenomenon. Thus, Notch-1 constitutes a novel molecule within the growing network of T cell-relevant genes that are tightly controlled by the transcription factor CREMα.

The observed epigenetic patterns and transcriptional CREMα effects at the Notch-1 promoter are very similar to those observed at the IL2 gene locus (i.e. histone H3K27 and CpG DNA hypermethylation, increased CREMα binding, and direct trans-repression of the IL2 promoter through CREMα). This suggests congenerous epigenetic and CREMα mechanisms at multiple gene loci in SLE T cells. CREMα expression in SLE T cells strongly correlates with SLE disease activity at the promoter and mRNA and protein expression levels (9, 12). Thus, we hypothesize that, among the observed regulatory mechanisms that control Notch-1 expression in SLE T cells, transcriptional repression through CREMα is the most decisive one. This idea is also supported by our observation that the overall CpG DNA methylation of the Notch-1 promoter is significantly increased in inactive SLE patients, whereas Notch-1 expression levels do not differ between controls and inactive patients. We conclude that the impaired epigenetic pattern of an increased histone and CpG DNA methylation in SLE T cells (inactive and active) constitutes the “background condition” of the Notch-1 promoter, but it is only the repressive effects exerted by CREMα that really make the significant differences in active patients.

Taken together, the results indicate that the CREMα/Notch-1/IL-17A axis appears to be part of the impaired cytokine network in T cells from SLE patients. Corrections along these lines, e.g. by the development of strategies to increase or reactivate Notch-1 signaling in T cells from active SLE patients and/or target the outlined upstream molecules, might well be of therapeutic importance. It has been shown in murine lupus models that a pan-Notch blockade using γ-secretase inhibitors results in decreased autoantibody production and kidney pathology (24). However, application of these agents is not feasible in humans (27). More specific interventions targeting individual Notch receptors in autoimmune diseases appear to be more promising but demand a systematic expression analysis of the involved immune and resident tissue-specific cells. In this context, this study contributes novel data on reduced Notch-1 expression in T cells from active SLE patients (whereas Notch-2 is not regulated) and the underlying molecular mechanisms. Once more, the transcription factor CREMα arises as a promising target to correct cytokine and disease expression in patients with SLE and other autoimmune diseases.

REFERENCES

1. Tsokos, G. C. (2011) Systemic lupus erythematosus. N. Engl. J. Med. 365, 2110–2121
2. Grammatikos, A. P., and Tsokos, G. C. (2012) Immunodeficiency and autoimmunity: lessons from systemic lupus erythematosus. Trends Mol. Med. 18, 101–108
3. Ahlmann, M., Varga, G., Sturm, K., Lippe, R., Benedyk, K., Viemann, D., Scholzen, T., Ehrchen, J., Müller, F. U., Seidl, M., Matus, M., Tsokos, G. C., Roth, J., and Tenbrock, K. (2009) The cyclic AMP response element modulator α suppresses CD86 expression and APC function. J. Immunol. 182, 4167–4174
4. Ghosh, D., Kis-Toth, K., Jiang, Y. T., and Tsokos, G. C. (2012) CREMα suppresses spleen tyrosine kinase expression in normal but not systemic lupus erythematosus T cells. Arthritis Rheum. 64, 799–807
5. Rauen, T., Hedrich, C. M., Jiang, Y. T., Tenbrock, K., and Tsokos, G. C. (2011) cAMP-responsive element modulator (CREM) α protein induces interleukin-17A expression and mediates epigenetic alterations at the interleukin-17A gene locus in patients with systemic lupus erythematosus. J. Biol. Chem. 286, 43437–43446
6. Hedrich, C. M., Rauen, T., Kis-Toth, K., Kyttarlis, V. C., and Tsokos, G. C. (2012) cAMP-responsive element modulator α (CREMα) suppresses IL-17F protein expression in T lymphocytes from patients with systemic lupus erythematosus (SLE). J. Biol. Chem. 287, 4715–4725
7. Hedrich, C. M., Rauen, T., and Tsokos, G. C. (2011) cAMP-responsive element modulator (CREM) α protein signaling mediates epigenetic remodeling of the human interleukin-2 gene: implications in systemic lupus erythematosus. J. Biol. Chem. 286, 43429–43436
8. Kyttarlis, V. C., Jiang, Y. T., Tenbrock, K., Weinstein, A., and Tsokos, G. C. (2004) Cyclic adenosine 5’-monophosphate response element modulator is responsible for the decreased expression of c-fos and activator protein-1 binding in T cells from patients with systemic lupus erythematosus. J. Immunol. 173, 3557–3563
9. Solomou, E. E., Jiang, Y. T., Gourley, M. F., Kammer, G. M., and Tsokos, G. C. (2001) Molecular basis of deficient IL-2 production in T cells from patients with systemic lupus erythematosus. J. Immunol. 166, 4216–4222
10. Tenbrock, K., and Tsokos, G. C. (2004) Transcriptional regulation of interleukin-2 in SLE T cells. Int. Rev. Immunol. 23, 333–345

Notch-1 Gene Regulation in SLE T Cells

JOURNAL OF BIOLOGICAL CHEMISTRY

DECEMBER 14, 2012 • VOLUME 287 • NUMBER 51

42531
Notch-1 Gene Regulation in SLE T Cells

11. Rauen, T., Benedyk, K., Jiang, Y. T., Kerkhoff, C., Kyttariss, V. C., Roth, J., Tsokos, G. C., and Tenbrock, K. (2011) A novel intrinsic cAMP response element modulator (CREM) promoter is regulated by activator protein-1 (AP-1) and accounts for altered activation-induced CREM expression in T cells from patients with systemic lupus erythematosus. J. Biol. Chem. 286, 32366 – 32372

12. Jiang, Y. T., Rauen, T., Wang, Y., Ichinose, K., Benedyk, K., Tenbrock, K., and Tsokos, G. C. (2011) Transcriptional activation of the cAMP-responsive modulator promoter in human T cells is regulated by protein phosphatase 2A-mediated dephosphorylation of SP-1 and reflects disease activity in patients with systemic lupus erythematosus. J. Biol. Chem. 286, 1795 – 1801

13. Bour-Jordan, H., Esensten, J. H., Martinez-Llordella, M., Penaranda, C., Stumpf, M., and Bluestone, J. A. (2011) Intrinsic and extrinsic control of peripheral T-cell tolerance by costimulatory molecules of the CD28/B7 family. Immunol. Rev. 241, 180 – 205

14. Veillette, A., Latour, S., and Davidson, D. (2002) Negative regulation of immunoreceptor signaling. Annu. Rev. Immunol. 20, 669 – 702

15. Scalapino, K. J., and Daikh, D. I. (2008) CTLA-4: a key regulatory point in the control of autoimmune disease. Immunol. Rev. 223, 143 – 155

16. Chatterjee, M., Rauen, T., Kis-Toth, K., Kyttariss, V. C., Hedrich, C. M., Terhorst, C., and Tsokos, G. C. (2012) Increased expression of SLAM receptors SLAMF3 and SLAMF6 in systemic lupus erythematosus T lymphocytes promotes Th17 differentiation. J. Immunol. 188, 1206 – 1212

17. Bray, S. J. (2006) Notch signalling: a simple pathway becomes complex. Nat. Rev. Mol. Cell Biol. 7, 678 – 689

18. D’Souza, B., Miyamoto, A., and Weinmaster, G. (2008) The many facets of Notch ligands. Oncogene 27, 5148 – 5167

19. Rauen, T., Raffetseder, U., Frye, B. C., Djudjaj, S., Mühlenberg, P. J., Eitner, F., Lendahl, U., Bernhagen, J., Dooley, S., and Mertens, P. R. (2009) YB-1 acts as a ligand for Notch-3 receptors and modulates receptor activation. J. Biol. Chem. 284, 26928 – 26940

20. De Strooper, B., Annaert, W., Cupers, P., Saftig, P., Craessaerts, K., Mumma, J. S., Schroeter, E. H., Schrijvers, V., Wolfe, M. S., Ray, W. I., Goate, A., and Kopsan, R. (1999) A presenilin-1-dependent γ-secretase-like protease mediates release of Notch intracellular domain. Nature 398, 518 – 522

21. Rizzo, P., Miao, H., D’Souza, G., Osipo, C., Song, L. L., Yun, J., Zhao, H., Mascarenhas, J., Wyatt, D., Antico, G., Hao, L., Yao, K., Rajan, P., Hicks, C., Siziopikou, K., Selvaggi, S., Bashir, A., Bhandari, D., Marchese, A., Lendahl, U., Qin, J. Z., Tonetti, D. A., Albain, K., Nickoloff, B. J., and Miele, L. (2008) Cross-talk between Notch and the estrogen receptor in breast cancer suggests novel therapeutic approaches. Cancer Res. 68, 5226 – 5235

22. Iso, T., Kedes, L., and Hamamori, Y. (2003) HES and HERP families: multiple effectors of the Notch signaling pathway. J. Biol. Chem. 278, 25366 – 25372

23. Riccio, O., van Gijn, M. E., Bezdek, A. C., Pellegrinet, L., van Es, J. H., Zimber-Strobl, U., Strobl, L. I., Honjo, T., Clevers, H., and Radcliffe, F. (2008) Loss of intestinal crypt progenitor cells owing to inactivation of both Notch1 and Notch2 is accompanied by derepression of CDK inhibitors p27Kip1 and p57Kip2. EMBO Rep. 9, 377 – 383

24. Wu, Y., Cain-Hom, C., Choy, L., Hagenbeck, T. J., de Leon, G. P., Chen, Y., Finkle, D., Venook, R., Wu, X., Ridgway, J., Schahn-Reed, D., Dow, G. J., Shelton, A., Stawicki, S., Watts, R. J., Zhang, J., Choy, R., Howard, P., Kadyk, L., Yan, M., Zha, J., Callahan, C. A., Hymowitz, S. G., and Siebel, C. W. (2010) Therapeutic antibody targeting of individual Notch receptors. Nature 464, 1052 – 1057

25. Hochberg, M. C. (1997) Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 40, 1275

26. Bailey, J., Tyson-Capper, A. J., Gilmore, K., Robson, S. C., and Europe-Finner, G. N. (2005) Identification of human myometrial target genes of the cAMP pathway: the role of cAMP-response element binding (CREB) and modulator (CREMα and CREMβ) proteins. J. Mol. Endocrinol. 34, 1 – 17

27. Liu, Z., Teng, L., Bailey, S. K., Frost, A. R., Bland, K. I., LoBuglio, A. F., Ruppert, J. M., and Lobo-Ruppert, S. M. (2009) Epithelial transformation by KLF4 requires Notch1 but not canonical Notch1 signaling. Cancer Biol. Ther. 8, 1840 – 1851

28. Sakamoto, K., Yamaguchi, S., Ando, R., Miyawaki, A., Kasaway, S., Takagi, M., Li, C. L., Perbal, B., and Katsube, K. (2002) The nephroblastoma overexpressed gene (NOV/ccn3) protein associates with Notch1 extracellular domain and inhibits myoblast differentiation via Notch signaling pathway. J. Biol. Chem. 277, 29399 – 29405

29. Hedrich, C. M., and Tsokos, G. C. (2011) Epigenetic mechanisms in systemic lupus erythematosus and other autoimmune diseases. Trends Mol. Med. 17, 714 – 724

30. Lippe, R., Ohl, K., Varga, G., Rauen, T., Crispin, J. C., Jiang, Y. T., Kuertzen, S., Tacke, F., Wolf, M., Roebrock, K., Vogl, T., Verjans, E., Honke, N., Ehren, J., Foeld, D., Skryabin, B., Wagner, N., Tsokos, G. C., Roth, J., and Tenbrock, K. (2012) CREMs overexpression decreases IL-2 production, induces a Th17 phenotype and accelerates autoimmunity. J. Mol. Cell Biol. 4, 121 – 123

31. Sodai, P., Hirankarn, N., Avihingsanon, Y., and Palaga, T. (2008) Defects in Notch1 upregulation upon activation of T cells from patients with systemic lupus erythematosus are related to lupus disease activity. Lupus 17, 645 – 653

32. Tenbrock, K., Jiang, Y. T., Kyttariss, V. C., and Tsokos, G. C. (2007) Altered signal transduction in SLE T cells. Rheumatol. Arthritis 46, 1525 – 1530

33. Mi, X. B., and Zeng, F. Q. (2008) Hypomethylation of interleukin-4 and -6 promoters in T cells from systemic lupus erythematosus patients. Acta Pharmacol. Sin. 29, 105 – 112

34. Janson, P. C., Marits, P., Thörn, M., Ohlsson, R., and Winqvist, O. (2008) CpG methylation of the IFNG gene as a mechanism to induce immunosuppression in tumor-infiltrating lymphocytes. J. Immunol. 181, 2878 – 2886

35. Sunahori, K., Jiang, Y. T., and Tsokos, G. C. (2009) Methylation status of CpG islands flanking a CAMP response element motif on the protein phosphatase 2A promoter determines CREB binding and activity. J. Immunol. 182, 1500 – 1508

36. Zhao, M., Tang, J., Gao, F., Wu, X., Liang, Y., Yin, H., and Lu, Q. (2010) Hypomethylation of IL10 and IL13 promoters in CD4+ T cells of patients with systemic lupus erythematosus. J. Biomed. Biotechnol. 2010, 931018

37. Brenner, C., and Fuchs, F. (2007) A methylation rendezvous: reader meets writer. Dev. Cell 12, 829 – 844

38. Tenbrock, K., Jiang, Y. T., Leukert, N., Roth, J., and Tsokos, G. C. (2006) The transcriptional repressor cAMP response element modulator α interacts with histone deacetylase 1 to repress promoter activity. J. Immunol. 177, 6159 – 6164