

Stat3 promotes IL-10 expression in lupus T cells through *trans*-activation and chromatin remodeling

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The immune-regulatory cytokine IL-10 plays a central role during innate and adaptive immune responses. IL-10 is elevated in the serum and tissues of patients with systemic lupus erythematosus (SLE), an autoimmune disorder characterized by autoantibody production, immune-complex formation, and altered cytokine expression. Because of its B cell-promoting effects, IL-10 may contribute to autoantibody production and tissue damage in SLE. We aimed to determine molecular events governing T cell-derived IL-10 expression in health and disease. We link reduced DNA methylation of the *IL10* gene with increased recruitment of Stat family transcription factors. Stat3 and Stat5 recruitment to the *IL10* promoter and an intronic enhancer regulate gene expression. Both Stat3 and Stat5 mediate *trans*-activation and epigenetic remodeling of *IL10* through their interaction with the histone acetyltransferase p300. In T cells from SLE patients, activation of Stat3 is increased, resulting in enhanced recruitment to regulatory regions and competitive replacement of Stat5, subsequently promoting IL-10 expression. A complete understanding of the molecular events governing cytokine expression will provide new treatment options in autoimmune disorders, including SLE. The observation that altered activation of Stat3 influences IL-10 expression in T cells from SLE patients offers molecular targets in the search for novel target-directed treatment options.

IL-10 is an immune-regulatory cytokine that plays a central role in innate and adaptive immune responses (1, 2). Immune cells ubiquitously express IL-10 with T and B cells, natural killer (NK) cells, mast cells, eosinophils, dendritic cells, and monocytes/macrophages as major sources. IL-10 modulates T-cell responses through the inhibition of major histocompatibility complex class II expression, limited costimulation, and reduced proinflammatory cytokine expression from antigen-presenting cells. Conversely, IL-10 promotes B-cell differentiation, proliferation, survival, and antibody production. Thus, IL-10 has been implicated in the pathophysiology of autoimmune disorders (1–3).

Systemic lupus erythematosus (SLE) is an autoimmune disease of unknown etiology. In SLE, immune responses are directed against cells, tissues, and organs. Autoantibody production by B cells and plasma cells, the accumulation of immune complexes in tissues, and excessive cytokine production contribute to autoimmune pathology (4). A growing body of literature suggests increased IL-10/IL-10 receptor interactions contributing to SLE (1–3). Studies document increased IL-10 serum levels in SLE patients and lupus-prone mice correlating with disease activity, antibody production, and organ damage (2, 3, 5–9). The molecular mechanisms governing *IL10* are incompletely understood. IL-10 expression is controlled on the transcriptional and posttranscriptional levels. *IL10* is *trans*-regulated by a number of factors, including signal transducer and activator of transcription molecules Stat1, Stat3, Stat4, and Stat5 (1, 2). We and others demonstrated that, in addition to the *IL10* promoter, an enhancer in the fourth intron, referred to as intronic Stat-responsive element (I-SRE), regulates IL-10 in murine NK cells (through Stat4) and human T cells (through Stat5) (10, 11). Recruitment of Stat proteins to the I-SRE induces epigenetic remodeling. The molecular mechanisms, however, remained unknown (10, 11).

Here, we demonstrate that DNA methylation controls Stat recruitment to *IL10* regulatory elements. In SLE T cells, reduced DNA methylation allows for transcription-factor recruitment. Both Stat3 and Stat5 *trans*-activate the *IL10* promoter and the I-SRE. Furthermore, Stat3 and Stat5 interact with the histone acetyltransferase p300, instructing epigenetic remodeling. Increased Stat3 phosphorylation in SLE T cells enhances its recruitment to regulatory elements and the replacement of Stat5 at the I-SRE, resulting in *trans*-activation and epigenetic remodeling. Correction of increased Stat3 phosphorylation and the resulting imbalance between Stat3 and Stat5 could provide novel treatment strategies in SLE.

Results

IL-10 Expression Is Increased in SLE T Cells. IL-10 may contribute to SLE. The cellular sources, however, are incompletely understood. We asked whether IL-10 expression is increased in T cells from SLE patients. Indeed, we detected higher IL-10 mRNA expression in ex vivo isolated T cells from inactive [SLE disease activity index (SLEDAI) 0–4] and active SLE patients (SLEDAI >4) (Fig. 1A) compared with controls, reflecting disease activity in individual patients (Fig. S1A). Also, serum IL-10 protein levels were increased in active SLE patients (Fig. S1B) and correlated with disease activity (Fig. S1C). Our observations indicate that T cells are a source of IL-10 in SLE patients with T cell-derived IL-10 expression following disease activity.

Cytokine-Induced IL-10 Expression Is Regulated by Stat3 and Stat5. Stat transcription factors regulate *IL10*. The molecular mechanisms

Significance

IL-10 is an immune-regulatory cytokine with pro- and anti-inflammatory functions. Through its B cell-stimulating capacities, IL-10 contributes to the differentiation, activation and survival of B cells. Thus, it has been linked with autoimmune disorders, including systemic lupus erythematosus (SLE). Here, we demonstrate T cells as a source of increased IL-10 expression in SLE. Reduced DNA methylation of the *IL10* gene allows for transcription-factor recruitment. Increased phosphorylation of the transcription factor Stat3 in SLE T cells results in epigenetic remodeling and *trans*-activation of *IL10*, allowing for IL-10 expression. Thus, our observations offer molecular targets in the search for pathophysiologic mechanisms and target-directed treatment options in SLE.

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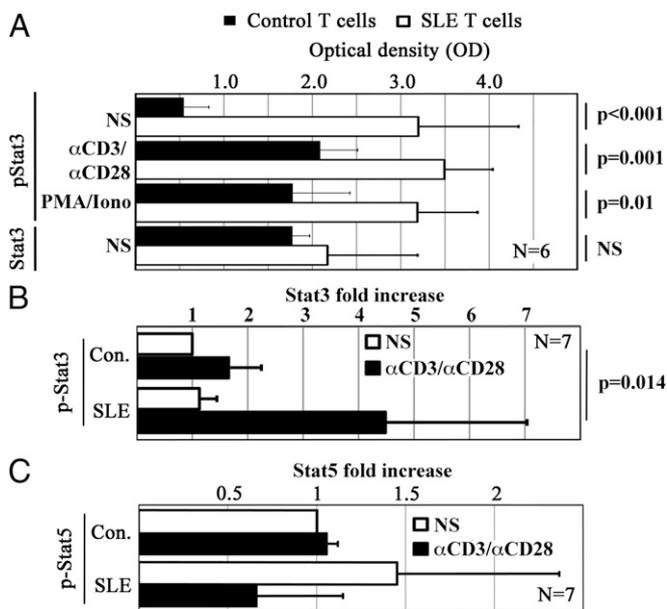


Fig. 3. T cells from SLE patients exhibit increased Stat3 phosphorylation. (A) Primary human T cells were cultured over 6 h in the absence (NS) or presence of anti-CD3 and anti-CD28 antibodies (α CD3/ α CD28) or phorbol 12-myristate 13-acetate and ionomycin as indicated. (A) Phospho-Stat3 (Tyr705) (pStat3) and total Stat3 levels were measured using intracellular ELISA kits. Although total Stat3 levels were comparable in T cells from SLE patients and controls, pStat3 was significantly increased in T cells from SLE patients under resting conditions and in response to T-cell stimulation. (B) Phospho-Stat3 (Tyr705) in response to stimulation with anti-CD3 and anti-CD28 antibodies was additionally assessed by intracellular staining, confirming the results from A. (C) Phospho-Stat5 (Tyr694) was monitored in resting and stimulated T cells from SLE patients and controls by intracellular staining. No significant differences were determined between the groups.

recruited to both promoter SREs (SRE-741 and SRE-149). Interestingly, Stat3 replaced Stat5 at the I-SRE in SLE T cells.

T Cells from SLE patients Exhibit Increased Stat3 Phosphorylation. Stat transcription factors undergo activation through phosphorylation. To determine whether increased Stat3 phosphorylation (pStat3) results in the replacement of Stat5 at the I-SRE of SLE T cells, we performed intracellular Stat3/pStat3 ELISAs (Pierce). SLE T cells exhibited comparable amounts of total Stat3 compared with controls. In contrast, pStat3 was significantly increased in resting SLE T cells, and after stimulation (Fig. 3A and B). Of interest, especially unstimulated SLE T cells had increased amounts of pStat3. Next, we aimed to determine whether potentially reduced Stat5 phosphorylation (pStat5) might partially be responsible for its replacement by pStat3 at the I-SRE. We performed intracellular transcription factor staining, followed by flow cytometry. No significant differences were detected between ex vivo isolated T cells from SLE patients and controls (Fig. 3C). Taken together, our findings indicate an imbalance between pStat3 and pStat5 in SLE T cells that may contribute to increased IL-10 expression.

Reduced DNA Methylation Promotes IL-10 Expression. Reduced DNA methylation of the *IL10* promoter and the fourth intron correlate with gene expression. Thus, we asked whether the *IL10* promoter and the I-SRE exhibit reduced DNA methylation, allowing for transcription factor/DNA interactions. Indeed, SLE T cells exhibited reduced DNA methylation within the proximal promoter and the I-SRE (Fig. 4A). To determine the potential influence of DNA methylation on IL-10 expression, we forced the expression of DNA methyltransferase (DNMT3A), inducing de novo DNA methylation. Indeed, methylation of *IL10* reduced gene expression (Fig. 4B). To target effects of DNA methylation on

IL10 regulation, we methylated our luciferase constructs with M.SssI (Zymo Research). DNA methylation resulted in reduced luciferase activity of both the promoter and the enhancer constructs (Fig. 4C), which was mediated by impaired Stat3 or Stat5 recruitment, as assessed by Reporter-ChIP (Fig. 4D and E). In agreement with the aforementioned ChIP experiments in human T cells, Stat5 was not recruited to the SRE-741.

Stat3 and Stat5 Mediate Histone Methylation Through Their Interaction with p300. T-cell activation results in histone acetylation around the *IL10* I-SRE. The exact molecular mechanisms, however, remained unclear. We aimed to investigate whether histone acetylation, as assessed by histone H3 acetylation at lysine K18 (H3K18ac), follows Stat recruitment. Indeed, in response to stimulation with anti-CD3 and anti-CD28 antibodies, T cells from controls and SLE patients exhibited increased H3K18ac of the *IL10* promoter. Of note, ex vivo isolated SLE T cells already exhibited H3K18ac levels that were achieved in control T cells only after stimulation (Fig. 5A). Interestingly, H3K18ac of the I-SRE was increased in control T cells only in response to stimulation. Ex vivo isolated T

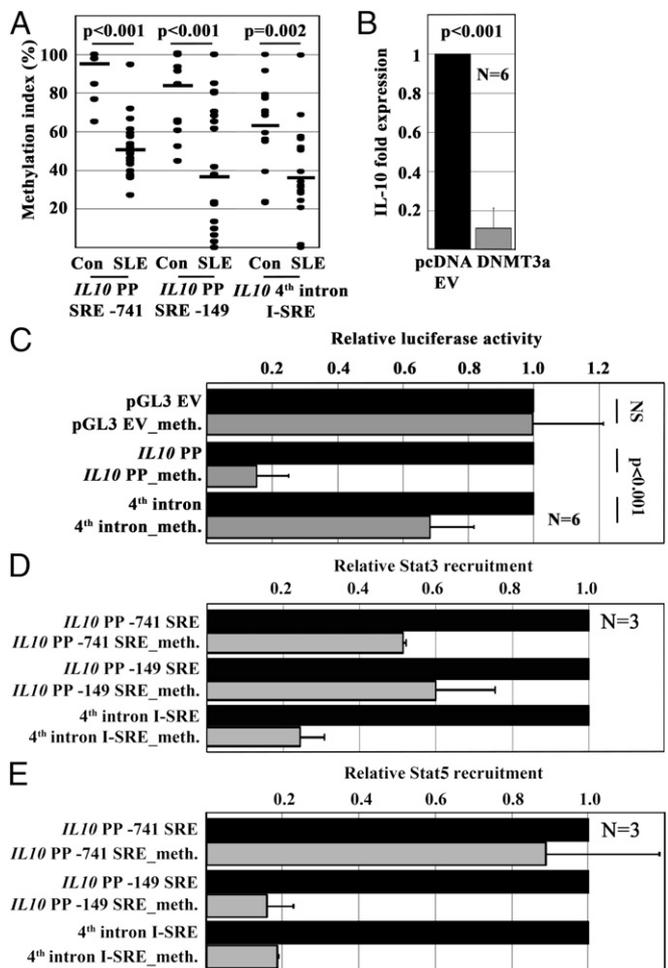


Fig. 4. Reduced DNA methylation allows for Stat recruitment in SLE T cells. (A) DNA methylation of regulatory regions within the *IL10* 5' proximal promoter (SRE-741 and SRE-149) and an intronic enhancer element (I-SRE) is significantly reduced in T cells from SLE patients compared with controls. (B) Increased DNA methylation through forced expression of DNMT3a results in significantly reduced IL-10 mRNA expression. (C) Enzymatic methylation of reporter constructs of *IL10* regulatory elements results in reduced luciferase activity. (D and E) Reduced luciferase activity is caused by impaired Stat3 (D) or Stat5 (E) recruitment to methylated SREs. Of note, in agreement with our findings from Fig. 2F, Stat5 is not recruited to the SRE-741 in the *IL10* promoter.

cells from SLE patients already exhibited H3K18ac, which was not further increased in response to stimulation.

Studies suggested Stat transcription factors interacting with the histone acetyltransferase p300. Thus, we mapped Stat3 and Stat5 recruitment with p300 (Fig. 5 B–D). Indeed, Stat3 in SLE T cells (Fig. 5C) and Stat5 in control T cells (Fig. 5D) colocalized with p300. To explore potentially synergistic effects between Stat3 or Stat5 and p300, we forced the expression of either Stat transcription factor in the absence or presence of p300 (Fig. 6A). Indeed, forced p300 expression significantly increased the transcription of IL-10. The combination of Stat3 or Stat5 with p300 had synergistic effects on IL-10 expression. Thus, we aimed to establish functional interactions between Stat transcription factors and p300. We forced the expression of p300, at the same time knocking down Stat3 or Stat5 with siRNAs (Fig. 6B). As before, p300 increased IL-10 expression. Interestingly, either Stat3 or Stat5 knockdown reduced the effects of p300. This finding was in agreement with our observations in response to forced Stat3 or Stat5 expression with or without p300 knockdown (Fig. 6C). Stat3 or Stat5 induced IL-10 expression that was reduced as a result of p300 knockdown, suggesting an interaction and functional synergy between Stat3 or Stat5 and p300. To investigate whether Stat3 or Stat5 mediates p300 recruitment to the *IL10* gene. Indeed, Stat3 or Stat5 expression in human T cells resulted in increased recruitment of p300 to SRE-149 and the I-SRE (Fig. S4A) that was reflected by H3K18ac (Fig. S4B). Interestingly, Stat3 did not recruit p300 to SRE-741. In agreement with these observations, inhibition of p300 with C646 resulted in impaired IL-10 expression in response to stimulation with Stat-inducing cytokines (Fig. S4C). Experiments in T cells from healthy controls, which were stimulated with anti-CD3 and anti-CD28 antibodies in the absence or presence of Stat3 or Stat5 inhibitors confirmed differential Stat-dependent corecruitment of p300 (Fig. S5). Although p300 corecruits with Stat3 to the *IL10* promoter (SRE-149), it corecruits to the I-SRE with Stat5. In

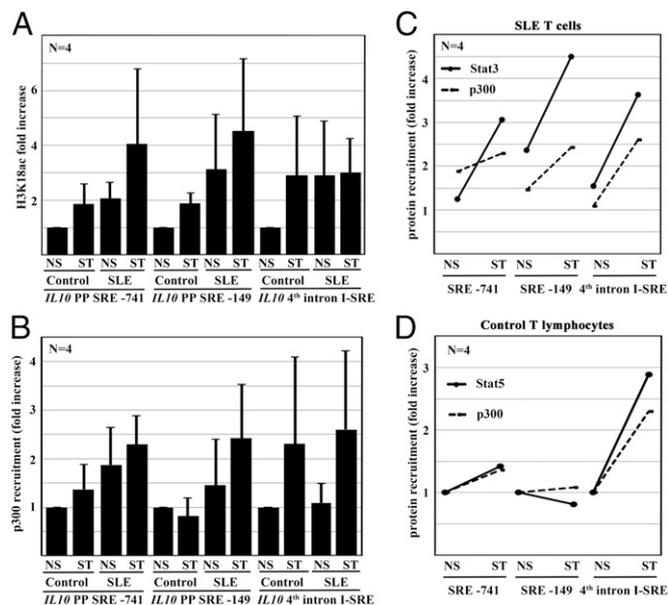


Fig. 5. *IL10* undergoes histone Hk18 acetylation through p300 in response to TCR stimulation. (A) *IL10* undergoes epigenetic modifications in response to TCR stimulation with anti-CD3 and anti-CD28 antibodies as assessed by ChIP. Both the proximal promoter and an intronic enhancer element exhibit increased H3K18ac. Of note, H3K18 in T cells from SLE patients is strikingly higher compared with controls. (B) The transcriptional coactivator p300 that has histone acetyltransferase activity recruits to the same regions. P300 recruitment is enhanced in T cells from SLE patients. (C and D) Stat3 (C, in SLE T cells) or Stat5 (D, in controls) recruitment maps p300 recruitment in response to TCR stimulation. NS, not stimulated; ST, stimulated.

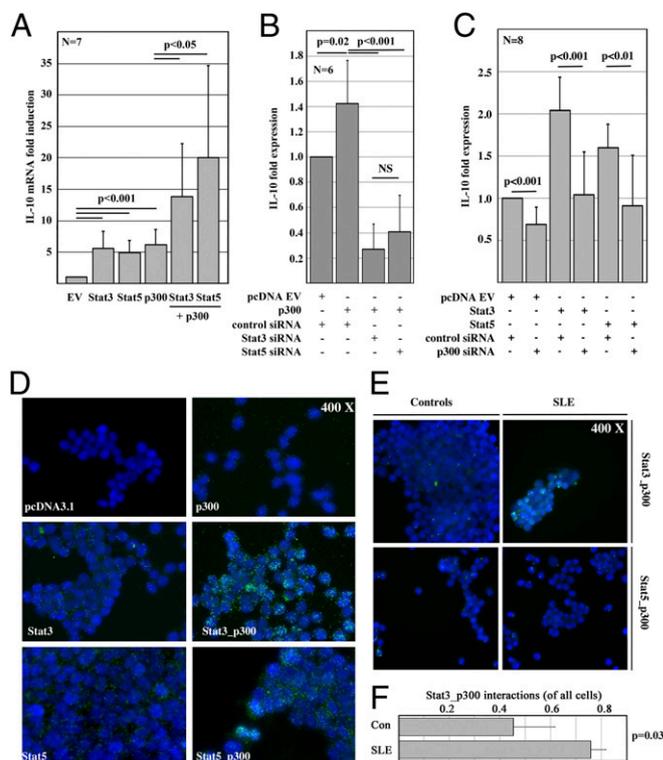


Fig. 6. Stat3 and Stat5 mediate chromatin remodeling through their interaction with p300. (A) Forced expression of Stat3, Stat5, or p300 induces IL-10 mRNA expression in Jurkat T cells. Stat3 or Stat5, in combination with p300, shows synergistic effects on IL-10 mRNA expression. (B) Effects of forced p300 expression are reversed by knockdown of Stat3 or Stat5 with siRNAs. (C) Effects of forced Stat3 or Stat5 expression are reversed by p300 knockdown with siRNAs. (D) Proximity ligation assays (PLAs) in HEK 293T cells indicate a physical interaction between Stat3 or Stat5 and p300. Although forced expression of p300 or Stat3/Stat5 alone results in a weak color reaction, indicating low levels of endogenous p300 and Stat3/Stat5 expression, the combination of forced p300 and Stat3/Stat5 expression results in a strong signal. Of note, the signal in response to the combination of forced p300 and Stat5 expression was weaker compared with Stat3 and p300. (E) PLAs in TCR-stimulated T cells from controls and SLE patients confirmed an interaction between Stat3 or Stat5 and p300. Furthermore, the interactions between Stat3 but not Stat5 and p300 were increased in SLE T cells. (F) The fraction of PLA signal-positive cells indicating Stat3/p300 interactions is given in T cells from five SLE patients and six controls. EV, empty vector.

both cases, p300 mediates H3K18ac. Of note, inhibition of Stat5 did not affect p300 corecruitment to the *IL10* promoter (SRE-149), which was occupied by Stat3. Comparably minor effects of Stat5 inhibition on p300 corecruitment to the I-SRE suggest a partial replacement with Stat3 secondary to an imbalance between Stat5 and Stat3 in analogy to SLE T cells (Fig. S5).

To test our hypothesis that T-cell receptor (TCR) stimulation mediates interactions between Stat3 or Stat5 with p300, we performed proximity ligation assays (PLAs; Olink) that indicate interactions between proteins. We transfected HEK293T cells with either an empty pcDNA3.1 vector, p300, Stat3, or Stat5 expression plasmids or their combination, monitoring for protein interactions (Fig. 6 D–F). Indeed, forced expression of p300, Stat3, or Stat5 resulted in a weak signal, reflecting endogenous Stat3/5 and p300 expression of HEK293T cells (Fig. 6D). The combination of forced Stat3 or Stat5 expression together with p300 resulted in a strong signal, indicating interactions between Stat transcription factors and p300. To investigate whether Stat3 or Stat5 also interact with p300 in human T cells, we performed PLA assays in T cells from controls or SLE patients. Indeed, TCR-stimulated cells exhibited interactions between Stat3 or Stat5 and p300 (Fig. 6 E and F). In agreement with

our colocalization (Figs. 2*F* and 5) and Stat activation (Fig. 2*B* and *C*) experiments, interactions between Stat3 and p300 were enhanced in SLE T cells. These observations indicate a replacement of Stat5 with Stat3 at the I-SRE, mediating *trans*-activation and chromatin remodeling through the histone acetyltransferase p300.

Discussion

The immune-regulatory cytokine IL-10 controls the growth, differentiation, and activity of immune cells. In various cells, including monocytes/macrophages and T lymphocytes, IL-10 reduces the expression of proinflammatory cytokines, thus inhibiting effector phenotypes (1, 2). Some effects of IL-10 on B cells, however, appear contradictory. IL-10 promotes B-cell survival, proliferation, differentiation, and antibody production (1, 2). Increased IL-10 expression in SLE patients correlates with disease activity and antibody production, and IL-10 blockade corrected dysregulated cytokine responses (12) and mediated clinical improvement in a subset of SLE patients (13). Given the immune-regulatory effects of IL-10 on T cells, enhanced IL-10 expression in SLE is seemingly conflicting with the well-established overexpression of IL-6 and IL-17A in SLE (14–16). Recently, however, this contradiction has been at least partially resolved. Reduced expression of the IL-10 receptor on SLE T cells alters the effects of IL-10 on activated or memory T cells that are major sources of proinflammatory cytokines. As a result, IL-10 fails to inhibit proinflammatory cytokine expression in SLE T cells (17).

Sources of IL-10 are manifold, including B and T cells, monocytes/macrophages, NK cells, and others. In SLE, monocytes and B cells have been reported as major sources of IL-10 (1, 2). Here, we demonstrate enhanced IL-10 expression in ex vivo isolated T cells from SLE patients. In individual patients, T cell-derived IL-10 expression followed disease activity, suggesting a role of T cell-derived IL-10 in disease pathology. This observation is of special interest, given the effects of IL-10 on differentiation, activity, and survival of B cells, promoting antibody production and immune complex generation (1, 18). Regardless of its role regulating the immune system and contributing to a number of autoimmune and infectious disorders, the molecular mechanism governing *IL10* remains poorly understood. Tsuji-Takayama et al. reported IL-2-dependent IL-10 expression in human regulatory T cells, which was mediated by Stat5 recruitment to a Stat-binding motif in the fourth intron of *IL10* (I-SRE) (10). At the same time, we demonstrated that IL-2 and IL-12 synergistically induce IL-10 in murine NK cells (11). IL-10 expression was dependent on Stat4 recruitment to the same region. In both reports, Stat recruitment to the I-SRE resulted in histone acetylation (10, 11). The molecular mechanisms mediating chromatin remodeling, however, remained elusive.

The involvement of Stat transcription factors in the regulation of *IL10* is of special interest because Stat3 has been demonstrated to be increased in SLE T cells (19, 20). Conflicting data exist regarding Stat5 in SLE (21, 22). Although attenuated IL-2 expression in SLE T cells suggests reduced Stat5 activation (23–25), one study demonstrated increased Stat5 phosphorylation in SLE (22). In T cells from SLE patients and lupus-prone MRL/lpr mice, pStat3 is increased (19, 26). Stat3 plays a central role in the regulation of proinflammatory effector cytokines, including IL-17A, promoting effector T-cell phenotypes (27, 28). Furthermore, Stat3 is involved in IL-10 induction, which resulted in the proposal of Stat3-blocking strategies in autoimmune disorders and cancer (1, 2, 12, 13). None of those studies, however, targeted the molecular events induced by Stat3 at the *IL10* gene. We aimed to determine the role of Stat3 and Stat5 in the regulation of *IL10* in T cells and their potential involvement in the molecular immune-pathology of SLE. Differentially inhibiting Stat3 or Stat5, we investigated their contribution to signal-specific IL-10 expression. Applying reporter constructs, we documented *trans*-regulatory effects of Stat3 and Stat5 on *IL10*. One intriguing finding in this context is that Stat3 and Stat5 transcription factors differentially recruit to select SREs: although Stat3 mainly recruits to the *IL10* promoter in control T cells (at SRE-149), Stat5 mainly recruits to the I-SRE. In SLE T cells, Stat3 recruitment to the promoter is increased, also involving

SRE-741. Furthermore, Stat3 replaces Stat5 at the I-SRE. Considering increased pStat3 in both resting and activated SLE T cells, the replacement of Stat5 is most likely caused by competition for the I-SRE. Because forced Stat3 or Stat5 expression in T cells had comparable effects on *IL10*, increased IL-10 expression in SLE T cells is most likely caused by increased pStat3 rather than increased activity of Stat3 over Stat5.

Although the majority of studies focused on transcriptional events, additional mechanisms govern gene expression. Epigenetic mechanisms govern gene expression without altering the DNA sequence (29, 30). DNA methylation regulates the recruitment of transcription factors to regulatory elements. Histone modifications control chromatin structure through nucleosome rearrangement. Usually, the histone code reflects DNA methylation with activating histone modifications in regions with low DNA methylation. The net result is a dynamic accessibility of DNA to transcriptional regulators, determining cell-, tissue-, and disease-specific expression of genes (29, 30). SLE T cells exhibit distinct epigenetic anomalies, including reduced DNA methylation and increased histone acetylation (8, 30–32). Thus, we wondered whether DNA methylation of *IL10* is reduced in SLE T cells, affecting transcription factor recruitment. Indeed, in agreement with recent findings in a Chinese cohort, DNA methylation of the *IL10* promoter was reduced in our study population (8). Furthermore, we for the first time, to our knowledge, demonstrate reduced DNA methylation of an intronic enhancer in SLE T cells, allowing for increased Stat transcription factor recruitment.

We and others previously documented that *IL10* undergoes epigenetic remodeling in response to stimulation of immune cells (10, 11, 33). The molecular events resulting in histone modifications, however, remained unknown. Reports indicated that Stat transcription factors might interact with the histone acetyltransferase p300 (34, 35). Indeed, we were able to detect H3K18ac in response to T-cell stimulation. Applying state-of-the-art technologies, we dissected functional interactions between Stat transcription factors and p300, mediating p300 recruitment to *IL10* regulatory regions in the *IL10* promoter (Stat3) and an intronic enhancer element (Stat3 and Stat5) instructing chromatin remodeling (H3K18ac). Thus, we for the first time, to our knowledge, have deciphered Stat3- and Stat5-mediated molecular events instructing epigenetic remodeling of *IL10* (Fig. S6). In SLE T cells, *trans*-activation and chromatin remodeling are enhanced as a result of increased Stat3 phosphorylation, resulting in a replacement of Stat5 at the I-SRE. Enhanced recruitment of Stat3 to the *IL10* promoter and the I-SRE mediates the recruitment of histone acetyltransferase p300 to those regions, instructing epigenetic remodeling (H3K18ac). Our findings provide previously unidentified molecular events, contributing to increased IL-10 expression in SLE T cells. Targeting the imbalance between phosphorylated Stat3 and Stat5 in SLE T cells may be a promising candidate in the search for novel, target-directed therapeutic approaches correcting dysregulated cytokine expression. The translational importance of our observations is underscored by promising results from the application of Stat3 inhibitors in select autoimmune disorders and cancers.

Materials and Methods

More detailed information on the applied methodology can be accessed in *SI Materials and Methods*.

Human Subjects. SLE patients included in our studies were diagnosed according to the American College of Rheumatology classification criteria (36) and gave written informed consent. Healthy age-, sex-, and ethnicity-matched individuals were chosen as controls (*SI Materials and Methods, Demographic Information*). Inactive patients were defined by an SLE disease activity index (SLEDAI) between 0 and 4.

Cytokine Stimulation and Stat Inhibition. Primary human T cells were cultured in RPMI 1640 with 10% (vol/vol) FCS in the absence or presence of Stat3 or Stat5 inhibitors (Stat3 inhibitor VI, CAS 501919–59-1, Stat5 CAS 285986–31-4; EMD Millipore) or vehicle control. As indicated, cytokines were added to some wells. Effects on IL-10 secretion were assessed after 24 h.

Semiquantitative Real-Time PCR. Semiquantitative real-time PCR (qRT-PCR) was performed using SybrGreen site-specific primers on an ABI StepOnePlus Real time PCR system. Results were normalized to 18S. Primer sequences for qRT-PCR are summarized in *SI Materials and Methods, Primers*.

Expression Plasmids and T-Cell Transfection. Expression plasmids for human DNMT3a have been described previously (24). Reporter constructs spanning the proximal 946 bp of the human *IL10* promoter or a 630 bp-spanning fragment of the fourth intron with enhancer activity were PCR-amplified and cloned into luciferase vector pGL3-Basic (Promega). Site-directed mutagenesis of SREs was performed (Stratagene). Human T cells were transfected with expression plasmids using the Amaxa transfection system (Lonza) or Lipofectamine (Life Technologies) as indicated.

Luciferase Assays. Ex vivo isolated primary human or unstimulated Jurkat T cells were transfected with the indicated plasmids, using the Amaxa transfection system (Lonza). Five hours after transfection, cells were collected and lysed, and luciferase activity was quantified using the Promega Dual Luciferase Assay System (Promega).

Methyl-CpG-DNA Immunoprecipitation. Methyl-CpG-DNA immunoprecipitation (MeDIP) assays were carried out following the manufacturer's instructions (Zymo Research). Equal amounts of methylated (100%) human CpG-DNA and demethylated human CpG-DNA (Zymo Research) were included as "input" and negative control.

Methylation of Reporter Plasmids. To investigate the effects of DNA methylation on *IL10* promoter activity, we methylated reporter constructs and the empty pGL3 plasmid using CpG-DNA methylase (Zymo Research).

Cotransfection of T cells with Stat3 or Stat5 Expression Plasmids and p300 siRNA. Jurkat T cells were transfected with Stat3 or Stat5 expression plasmids, control siRNA, or p300-specific siRNA (OriGene). Later, cells were

transfected with p300 expression plasmids and Stat3 or Stat5 siRNAs to reconfirm our findings following the same protocols.

ChIP. Anti-H3K18ac (Abcam), anti-p300 (Santa Cruz), anti-Stat3 (EMD Millipore), and anti-Stat5 (Abcam) antibodies and normal rabbit IgG were obtained from Upstate (EMD Millipore). ChIP was carried out according to the manufacturer's instructions (Invitrogen, Life Technologies). ChIP-DNA was subject to real-time qPCR. Where indicated, cells were pretreated with C646, a selective small-molecule inhibitor of the p300 histone acetyltransferase (Origene) according to the manufacturer's protocols.

Intracellular Stat Phosphorylation Assays. To determine intracellular Stat3 levels and Stat3 phosphorylation (Tyr705), Thermo Scientific Pierce STAT3 In-Cell ELISA Kits were applied following the manufacturer's protocols. Intracellular staining for phosphorylated Stat3 (Tyr705) (BD Biosciences) or phosphorylated Stat5 (Tyr694) (BD Biosciences) was carried out in resting or stimulated primary human T cells from controls and SLE patients with phycoerythrin (PE)-labeled antibodies (clones 4P-Stat3, and 47Stat5) as indicated.

Proximity Ligation Assay. HEK 293T cells were transfected with expression plasmids for Stat3, Stat5, p300, or the combination of Stat3 or Stat5 and p300. Cells were harvested and subjected to the Duolink proximity ligation assay following the manufacturer's instructions (Olink). Cells were mounted with DAPI-containing medium (Olink) and read on a fluorescence microscope (Zeiss). The number of PLA signals per cell was quantified using ImageJ software (<http://rsb.info.nih.gov/ij/disclaimer.html>).

Statistical Analysis. A paired two-tailed Student *t* test was used for statistical analysis. The Pearson product moment correlation coefficient (*r*) was used to determine the correlation between IL-10 mRNA levels and individual SLE disease activity indices (SLEDaIs).

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