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Demethylation of *ITGAL* (CD11a) Regulatory Sequences in Systemic Lupus Erythematosus

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Objective. **Inhibition of T cell DNA methylation causes autoreactivity in vitro and a lupus-like disease in vivo, suggesting that T cell DNA hypomethylation may contribute to autoimmunity. The hypomethylation effects are due, in part, to overexpression of lymphocyte function–associated antigen 1 (LFA-1) (CD11a/CD18). Importantly, T cells from patients with active lupus have hypomethylated DNA and overexpress LFA-1 on an autoreactive subset, suggesting that the same mechanism could contribute to human lupus. The present study investigated the nature of the methylation change that affects LFA-1 expression in vitro and in human lupus.**

Methods. **Bisulfite sequencing was used to determine the methylation status of the *ITGAL* promoter and flanking regions in T cells from lupus patients and healthy subjects, and in T cells treated with DNA methylation inhibitors. "Patch" methylation of promoter sequences in reporter constructs was used to determine the functional significance of the methylation changes.**

Results. **Hypomethylation of specific sequences flanking the *ITGAL* promoter was seen in T cells from patients with active lupus and in T cells treated with 5-azacytidine and procainamide. Patch methylation of this region suppressed *ITGAL* promoter function.**

Conclusion. **DNA methylation changes occur in specific sequences that regulate LFA-1 expression in lupus T cells and in the hypomethylation model, indicating that altered methylation of specific genes may play a role in the pathogenesis of lupus.**

The mechanisms initiating human systemic lupus erythematosus (SLE) remain unknown. The finding that exposure to certain drugs can induce a lupus-like disease has provided leads into the nature of biochemical alterations associated with lupus. The 2 drugs most frequently associated with lupus, procainamide and hydralazine, can cause a lupus-like disease through effects on T cell DNA methylation. Treating T cells with procainamide, hydralazine, or 5-azacytidine (5-azaC; the prototypic DNA methylation inhibitor) demethylates DNA, alters gene expression, and induces major histocompatibility complex–specific T cell autoreactivity (1–5). Adoptive transfer of the autoreactive cells causes a lupus-like disease in animal models (5–7). The autoimmune effects of the methylation inhibitors are due, in part, to overexpression of lymphocyte function–associated antigen 1 (LFA-1) (CD11a/CD18), because increasing T cell LFA-1 by transfection causes an identical autoreactivity in vitro, and a similar autoimmune disease in vivo (4,8).

Altered DNA methylation has also been implicated in human lupus. T cells from patients with active lupus have an ~17% decrease in genomic deoxymethylcytosine content and overexpress LFA-1 on an autoreactive subset (9–11). However, whether the DNA hypomethylation occurring in lupus affects transcriptionally relevant regions is not known. It is similarly unknown if the DNA hypomethylation induced by 5-azaC or procainamide affects the same sequences as those affected in SLE.

DNA methylation inhibitors increase LFA-1 through their effects on the gene encoding CD11a (9),

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Table 1. Clinical characteristics of the lupus patients*

Patient/age/sex	SLEDAI	Medication
1/47/F	2	AZA, HCQ, low pred
2/37/M	2	HCQ
3/46/F	4	HCQ
4/40/F	4	HCQ, low pred
5/36/F	5	HCQ, moderate pred
6/53/F	7	HCQ
7/46/F	9	HCQ, high pred
8/48/F	10	AZA, HCQ, moderate pred
9/30/F	12	CTX, HCQ, low pred
10/21/F	24	HCQ, high pred

* SLEDAI = Systemic Lupus Erythematosus Disease Activity Index; AZA = azathioprine; HCQ = hydroxychloroquine; low pred = <0.5 mg/kg/day prednisone; moderate pred = 0.5–1.0 mg/kg/day prednisone; high pred = >1.0 mg/kg/day prednisone; CTX = cyclophosphamide.

termed *ITGAL* (derived from Integrin Alpha L). The promoter of this gene contains PU.1 and Sp1 binding elements within 120 bp of the transcription start site, and the 40-bp fragment located 5′ to the transcription start site is necessary for most of the promoter activity in T cells (12).

To determine if altered *ITGAL* methylation could cause autoimmunity in the DNA hypomethylation model of lupus and in idiopathic lupus, and to extend the previous association of genome-wide demethylation and autoimmunity to specific, transcriptionally relevant sequences, we determined the methylation status of sequences in or near the *ITGAL* promoter in 5-azaC–treated and procainamide-treated cells and in lupus T cells. We also determined the effect of altered methylation of specific sequences on CD11a expression.

PATIENTS AND METHODS

Human subjects. Patients with SLE and patients with rheumatoid arthritis (RA) were recruited from the outpatient clinics and inpatient rheumatology service at the University of Michigan. Patients with SLE or RA met the criteria for their respective diseases (13,14), and SLE disease activity was assessed using the SLE Disease Activity Index (SLEDAI) (15). Inactive disease was defined as a SLEDAI score ≤4, and active disease as a SLEDAI score ≥5. The clinical characteristics of the patients and controls are listed in Table 1.

T cell isolation and culture. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation and T cells were purified by e-rosetting as described previously (9), and the CD4+ subset was isolated by magnetic cell sorting using CD4 microbeads (Miltenyi Biotec, Auburn, CA). Where indicated, the cells were stimulated with phytohemagglutinin (PHA) (9), treated with 1 μM 5-azaC (Fluka, Milwaukee, WI), 50 μM procainamide (Sigma, St. Louis, MO), or 10 μM dexamethasone (Aldritch, Milwaukee, WI), and cultured for 3 days as previously described (1). Jurkat T cells were cultured as previously described (1). Proliferation assays were performed as described (3), using 2×10^4 T cells and irradiated autologous PBMCs as antigen-presenting cells.

Flow cytometric analysis. T lymphocytes were analyzed for CD2 and CD11a expression using 2-color flow cytometry and fluorescein isothiocyanate– or phycoerythrin-conjugated monoclonal antibodies from PharMingen, as previously described (9). Each lupus patient was analyzed in parallel with at least 1 healthy control. The CD11a mean channel fluorescence (MCF) was calculated for each patient and subject, and results were tabulated as the patient:subject MCF ratio.

Bisulfite sequencing. One to five micrograms of purified T cell DNA was treated with sodium bisulfite (16), and then the 2.3-kb CD11a promoter fragment (12) was amplified in 5 overlapping fragments. The fragments were cloned into PBS+ (Stratagene, La Jolla, CA), and 5 independent clones were sequenced by the University of Michigan Sequencing Core for each of the amplified fragments.

Patch methylation. A 1.9-kb fragment containing the human *ITGAL* promoter (accession number M87662; kindly provided by Dr. Dennis Hickstein, NIH, Bethesda, MD) was cloned into the luciferase-containing reporter vector pGL3-Basic (Promega, Madison, WI). An *Nde* 1 site was engineered into the promoter at bp −382 using the QuikChange site-directed mutagenesis kit (Stratagene), and intact function was confirmed by transfection into Jurkat cells. The region from the beginning of the fragment to the *Nde* 1 site was excised, methylated with *Sss* 1 and S-adenosylmethionine (both from New England Biolabs, Beverly, MA) using instructions provided by the manufacturer, and then ligated back into the reporter construct and purified by gel electrophoresis. *Sss* 1 catalyzes the transfer of methyl groups from S-adenosylmethionine to unmethylated dC residues only in CpG pairs (17). Completeness of methylation was tested by digestion with the methylation-sensitive restriction endonuclease (*Aci* 1) (New England Biolabs). Controls included a mock methylated construct, prepared by omitting the *Sss* 1.

Transient transfection. Plasmid DNA was introduced into Jurkat cells by electroporation using a modification of previously described protocols (4,8). Twenty-four hours later, the cells were washed twice, suspended in 400 μl reporter lysis buffer (Promega), and lysed by freeze–thaw. Insoluble material was removed by centrifugation and luciferase assays were performed using 100 μl as described previously (18). Similarly, 20 μl was used for β-galactosidase determinations, which were performed using the Galacto-Light system as per the manufacturer's protocol (Tropix, Bedford, MA).

Quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) of CD11a messenger RNA (mRNA). Real-time quantitative RT-PCR was performed using a LightCycler (Roche, Indianapolis, IN) and previously published protocols (19). A series of 5 dilutions of 1 RNA sample was also included to generate a standard curve, and this was used to obtain relative concentrations of the transcript of interest in each of the RNA samples. Relative concentrations were then determined by the second derivative method using the LightCycler computer software. Amplification of β-actin was performed to confirm that equal amounts of total RNA were added for each sample and that the RNA was intact and equally amplifiable

among all samples. The CD11a primers were as follows: forward 5′-AAATGGAAGGACCCTGATGCTC-3′, backward 5′-TGTAGCGGATGATGTCTTTGGC-3′. The β-actin primers were as follows: forward 5′-GCACCACACCTTCTACAATGAGC-3′, backward 5′-GGATAGCACAGCCTGGATAGCAAC-3′.

Statistical analysis. Data were analyzed using Student's *t*-test, regression analysis, or analysis of variance (ANOVA) as appropriate, calculated with SyStat software (Evanston, IL).

RESULTS

LFA-1 expression and disease activity. Previous studies demonstrated that T cells from patients with active lupus typically overexpress CD11a, with an overall average increase of ~40% (10). Figure 1A shows a representative histogram of CD11a staining on peripheral blood T lymphocytes from a patient with a SLEDAI of 12 and from a normal healthy control. Figure 1B shows the ratio of the CD11a MCF of T cells from 6 of the lupus patients, who exhibited a range of disease activity (Table 1), and from 6 paired, healthy controls, plotted against the SLEDAI score. T cell CD11a expression increased a mean ± SEM of 60 ± 17% (range 41–105%) with increasing disease activity in the 4 subjects with active disease ($P = 0.026$ by regression analysis).

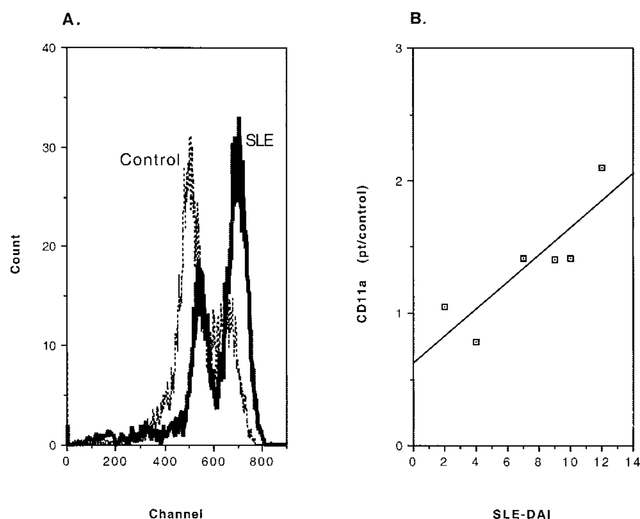

Figure 1. Overexpression of lymphocyte function–associated antigen 1 (LFA-1) by lupus T cells. Peripheral blood mononuclear cells were stained with anti–CD11a–fluorescein isothiocyanate and anti–CD2–phycoerythrin and then analyzed by flow cytometry. **A,** Histogram of LFA-1 expression on the CD2+ cells from a patient with systemic lupus erythematosus (SLE) and a healthy control. **B,** The CD11a mean channel fluorescence (MCF) was determined for each 6 lupus patients (pt) and 6 paired controls, and the ratio of patient to control MCF is plotted against the SLE Disease Activity Index (SLE-DAI).

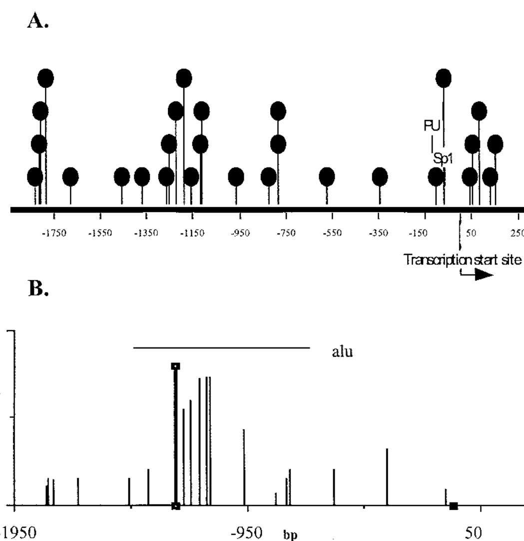

Figure 2. *ITGAL* promoter methylation in healthy controls. **A,** The 27 potentially methylated CpG pairs in the *ITGAL* promoter fragment are identified by the "lollipops." The transcriptionally relevant PU.1 and Sp1 sites and the transcription start site are also shown. **B,** T cell DNA was isolated from heathy donors and treated with sodium bisulfite, and then the fragment (shown in **A**) was amplified in overlapping fragments. Five cloned fragments from each section were then sequenced from each donor, and the fraction methylated for each CpG pair was averaged across the 5 cloned and sequenced fragments. The region from the beginning (−1950 bp) to bp −1262 represents the mean of 5 fragments from each of 4 donors, the region from −1261 to −68 (identified by the solid squares) shows the mean of 5 fragments from each of 6 donors, and the region from −68 to the end shows the mean of 5 fragments from each of 4 donors. The region containing *Alu* elements is denoted by the horizontal line.

***ITGAL* promoter methylation.** We next determined the methylation pattern of the *ITGAL* promoter and flanking sequences in 4–6 healthy controls. Figure 2A shows the 22 potentially methylatable CpG pairs located 5′ to the transcription start site, and the 5 pairs following the start site. For reference, the transcriptionally important PU.1 and Sp1 sites and the transcription start site are also shown (12,20). Figure 2B shows the methylation pattern of this region in T cells from healthy controls. For each CpG pair, the methylation status was assessed in 5 cloned fragments from each individual, and the results are presented as the average (mean) methylation of the dC residue from the donors. The transcribed region (3′ to bp 0) was completely demethylated in all fragments from the 4 healthy subjects examined, while the majority of the sequences 5′ to the start site were

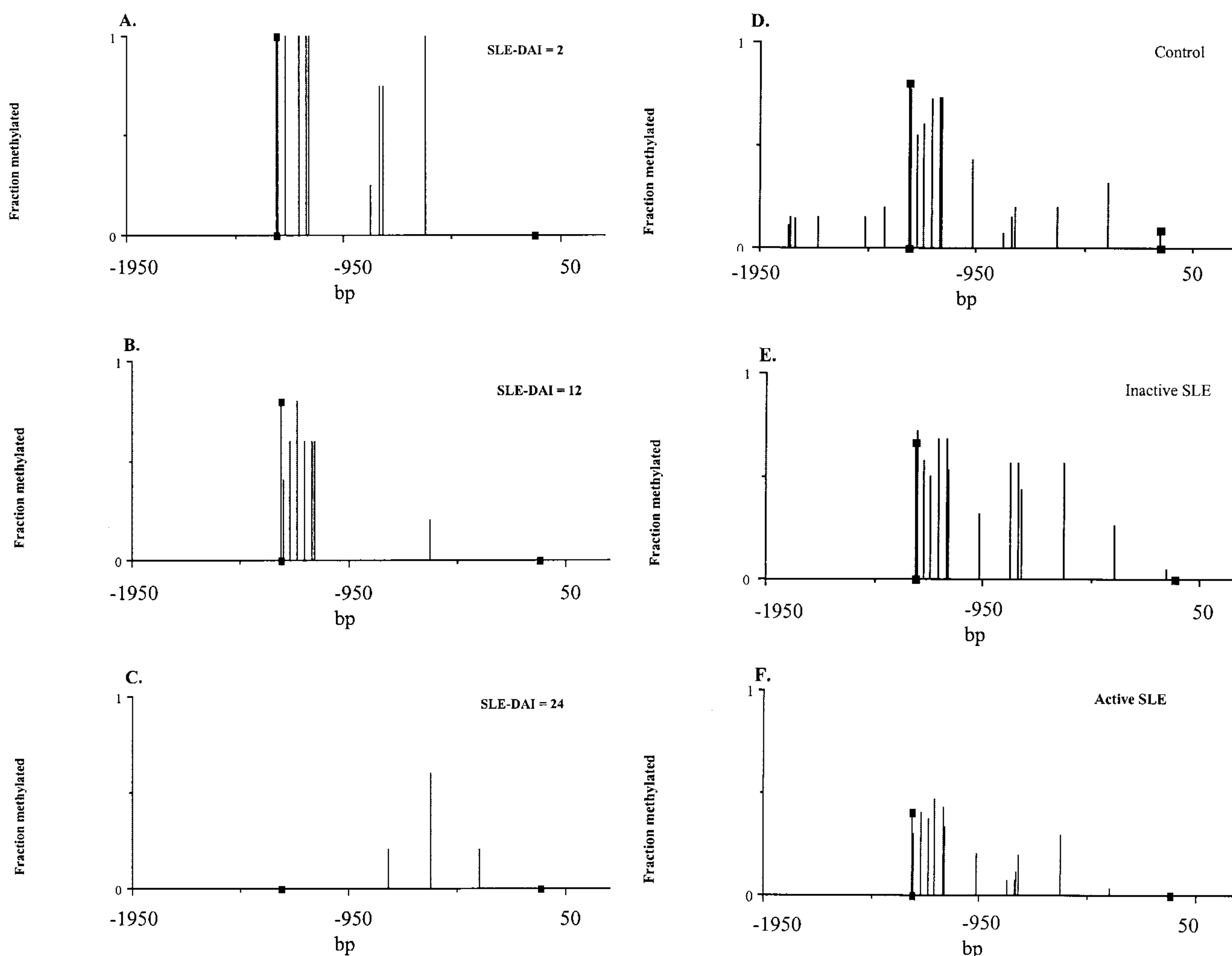

Figure 3. *ITGAL* promoter methylation in patients with systemic lupus erythematosus (SLE). The region from −1261 to −68 (identified by the solid squares) was amplified and 5 fragments sequenced from a patient with **A,** inactive lupus (SLE Disease Activity Index [SLE-DAI] of 2), **B,** active lupus (SLE-DAI of 12), and **C,** very active lupus (SLE-DAI of 24). Results are presented as averages, as shown in Figure 2. **D,** For comparison, the average methylation at each CpG pair across the promoter fragment from 4–6 healthy donors is shown (as in Figure 2) versus **E,** the average methylation of the CpG pairs in the −1261 to −68 fragment from 4 patients with inactive lupus (SLE-DAI of ≤4) and **F,** the average methylation of the same region shown in **E,** using T cell DNA from 6 patients with active lupus (SLE-DAI of ≥5).

partially methylated in all 6 controls. Of note is a region containing *Alu* elements, which was more heavily methylated in all controls, consistent with previous reports demonstrating that repetitive DNA sequences are usually heavily methylated (21).

ITGAL **promoter methylation in lupus.** Figure 3 compares the methylation pattern of the region from the beginning of the *Alu* elements to the transcription start site (bp −1261 to −68) in 3 lupus patients with varying levels of disease activity. Ten of the 16 CpG pairs in this region were relatively heavily methylated in a patient with inactive lupus (SLEDAI of 2) (Figure 3A). In contrast, the patient with very active lupus (SLEDAI of 24) (Figure 3C) had partial methylation of only 3 CpG pairs in this region, while the patient with less active lupus (SLEDAI of 12) showed an intermediate pattern (Figure 3B). The 5 CpG pairs distal to the transcription start site were also examined in 1 lupus patient and were found to be unmethylated, similar to the findings in controls. Methylation of this region would not be expected to affect transcription (22), and therefore this region was not studied further.

Figure 3 also compares the average methylation pattern across this region in T cells from 6 healthy controls

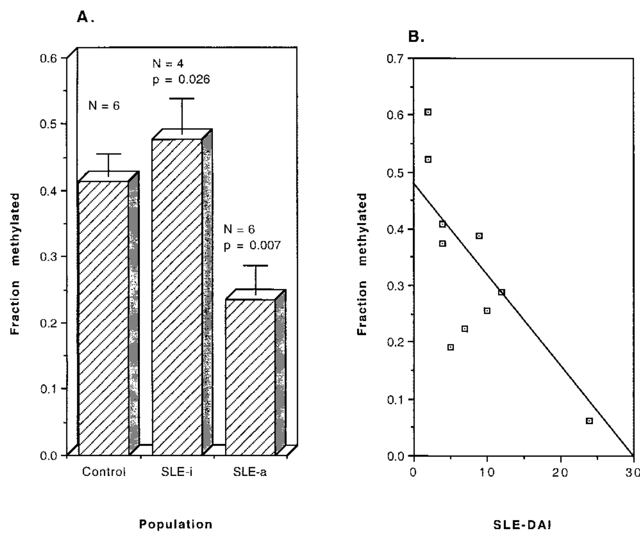

Figure 4. Relationship of *ITGAL* methylation to disease activity. **A,** The methylation status of the CpG pairs in the −1261 to −68 fragment was averaged among the 6 healthy control subjects, 4 subjects with inactive systemic lupus erythematosus (SLE-i), and 6 subjects with active SLE (SLE-a). Bars show the mean and SEM. $P = 0.026$ versus active SLE; $P = 0.007$ versus controls. **B,** For each of the 10 lupus patients (dotted squares), the fraction methylated from −1261 to −68 is plotted against the SLE Disease Activity Index (SLE-DAI).

(Figure 3D), 4 patients with inactive lupus (SLEDAI of ≤4) (Figure 3E), and 6 patients with active lupus (SLEDAI of ≥5) (Figure 3F). The pattern in the patients with inactive lupus resembled that in the healthy controls, although the region between the *Alu* elements and the transcription start site (bp −794 to −68) appeared somewhat more heavily methylated. In contrast, the patients with active lupus had less methylation overall.

Figure 4A compares the average methylation of the promoter between these 3 groups. For each subject, the methylation status of all 16 CpG pairs in the region from bp −1261 to −68 was averaged over the 4–5 sequenced fragments, and this number was then averaged among the subjects in each group. This region was significantly hypomethylated in the lupus T cells, relative to that in both the controls and the patients with inactive lupus ($P = 0.010$ between groups, by ANOVA; $P = 0.026$ for active versus inactive lupus; $P = 0.007$ for active lupus versus controls, all by post hoc analysis). Figure 4B plots the average methylation for each lupus patient against the SLEDAI. Patients with more active disease were more hypomethylated than were those with inactive disease ($P = 0.010$, by regression analysis). Interestingly, the region between −794 and −68 (3′ to

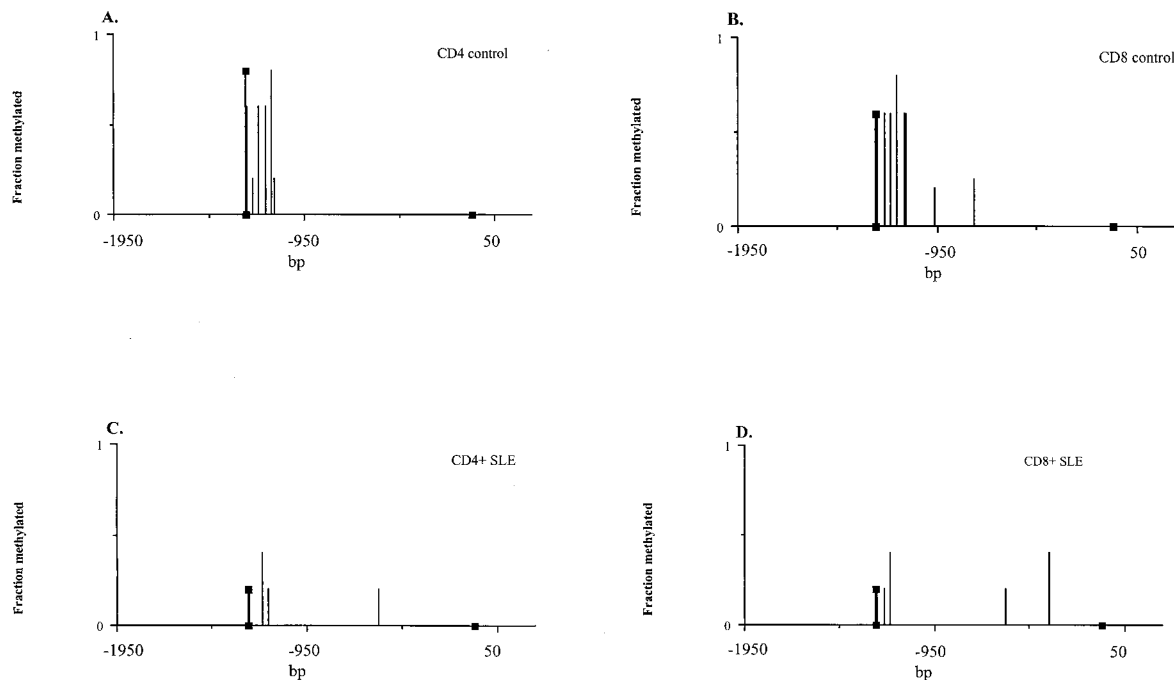

Figure 5. *ITGAL* methylation in T cell subsets. DNA was isolated from **A,** CD4+ T cells and **B,** CD8+ T cells from a healthy donor, **C,** CD4+ T cells from a patient with active systemic lupus erythematosus (SLE) (SLE Disease Activity Index of 6), and **D,** CD8+ T cells from the same lupus patient. The region from −1261 to −68 (identified by the solid squares) is shown, and represents the average methylation of 5 cloned and sequenced fragments.

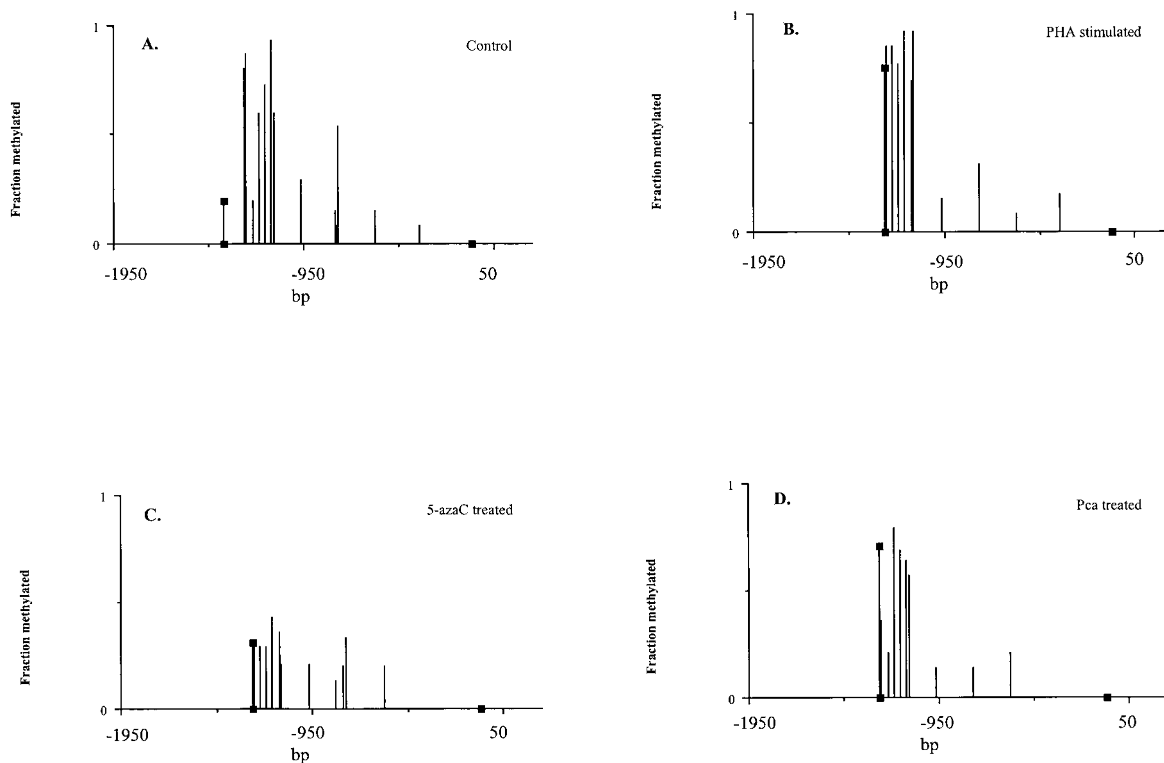

Figure 6. Effect of T cell activation and DNA methylation inhibitors on *ITGAL* promoter methylation. The average methylation from −1261 to −68 (identified by the solid squares) using **A,** unstimulated T cells, **B,** phytohemagglutinin (PHA)–stimulated T cells, **C,** PHA-stimulated, 5-azacytidine (5-azaC)–treated T cells, and **D,** PHA-stimulated, procainamide (Pca)–treated T cells is shown. In each case, the results represent the average methylation of 5 fragments from 3 individuals.

the *Alu* elements) was significantly more heavily methylated in the patients with inactive lupus relative to that in the controls and that in the patients with active lupus ($P = 0.010$ and $P = 0.004$, respectively, by ANOVA with post hoc testing), suggesting the possibility of compensatory mechanisms (19,23).

The changes in methylation status in the lupus patients were unlikely to be due to the types of medications being taken or the patient's age (Table 1). Patient 6 was significantly hypomethylated but was only receiving hydroxychloroquine, similar to patients 2 and 3, who also had greater methylation, arguing against an effect of hydroxychloroquine. Similarly, patient 7 was receiving high doses of corticosteroids but was more methylated than patient 6. An effect of corticosteroids was further excluded by treating normal T cells for 24 hours with 10 μM dexamethasone. This did not significantly affect the methylation status of the 13 CpG pairs between bp −1261 and −574 of the *ITGAL* promoter (mean ± SEM fraction methylated 0.42 ± 0.12 versus 0.39 ± 0.13 for untreated versus treated groups, 10 fragments/group). Similarly, there was no correlation of hypomethylation with the use of immunosuppressives, since patients 6 and 10 had hypomethylated DNA but were not receiving antimetabolites. Finally, regression analysis of the degree of methylation versus age, which has also been associated with T cell DNA hypomethylation (19,24), did not indicate a statistically significant association ($P = 0.211$) in this group of patients, although the power to detect an association was small.

The differences in methylation could reflect differences in the methylation patterns of normal T cell subsets. However, CD4+ and CD8+ T cells from a healthy donor showed no differences, arguing against this possibility (Figures 5A and B). Since the CD4+ subset has been implicated in the pathogenesis of SLE (6–10,25), the methylation pattern in the CD4 subset was examined in a patient with active SLE (SLEDAI of 6) (Figure 5C). The *ITGAL* promoter was significantly ($P = 0.027$, by paired *t*-test) hypomethylated, indicating that the changes can occur in this subset. The *ITGAL* promoter was similarly hypomethylated in the CD8+

subset from the same patient (Figure 5D), indicating that both subsets are affected.

Since T cells from patients with active lupus are frequently activated in vivo, it is possible that activation contributed to the differences in methylation patterns. To directly test this, T cells from 3 healthy donors were stimulated for 3 days with PHA, and then methylation patterns were compared. Figure 6A shows the methylation pattern across the same region in unstimulated T cells, and 6B depicts the same region in the stimulated cells. No differences were apparent, thus arguing against a significant role for T cell activation.

Effect of DNA methylation inhibitors. Since the DNA methylation inhibitors 5-azaC and procainamide increase CD11a expression, and T cells treated with these drugs cause a lupus-like disease (5–8), we examined the effect of these agents to determine whether they demethylated the *ITGAL* promoter. Treating PHA-stimulated T cells with 5-azaC caused significant hypomethylation of this region (mean ± SEM fraction methylated 0.44 ± 0.05 versus 0.26 ± 0.04 for PHA-treated versus 5-azaC–treated cells; $P = 0.048$) (Figure 6C). Similar results were observed in PHA-stimulated T cells treated with procainamide (fraction methylated 0.30 ± 0.02 versus 0.38 ± 0.03 for procainamide-treated cells versus control cells; $P = 0.01$) (Figure 6D), although procainamide appeared to be less potent than 5-azaC.

We determined the significance of the procainamide-induced methylation changes by determining its effects on CD11a expression and autoreactivity. Figure 7A compares CD11a expression on procainamide-treated and untreated T cells. High- and low-expressing subsets were seen within the untreated cell population, which differed in fluorescence intensity by ~3-fold (peak fluorescence intensity 30 in the low-expressing subset and 100 in the high-expressing subset). It should be noted that the data shown in Figure 7A are plotted as the channel in arbitrary units, representing the log of the fluorescence intensity. There was a shift of cells from the low-expressing subset to the high-expressing subset in the treated cells, resembling that seen in T cells from patients with active lupus (Figure 1A). Procainamide similarly increased CD11a mRNA 3.4-fold relative to β-actin at the same time point, as measured by quantitative real-time RT-PCR (CD11a: β-actin 0.54 in untreated cells versus 1.84 in procainamide-treated cells, determined relative to standard curves that consisted of 5 serial dilutions of a reference RNA preparation). Culturing the treated and untreated cells with irradiated autologous PBMC demonstrated that the treated cells were autoreactive (Figure 7B).

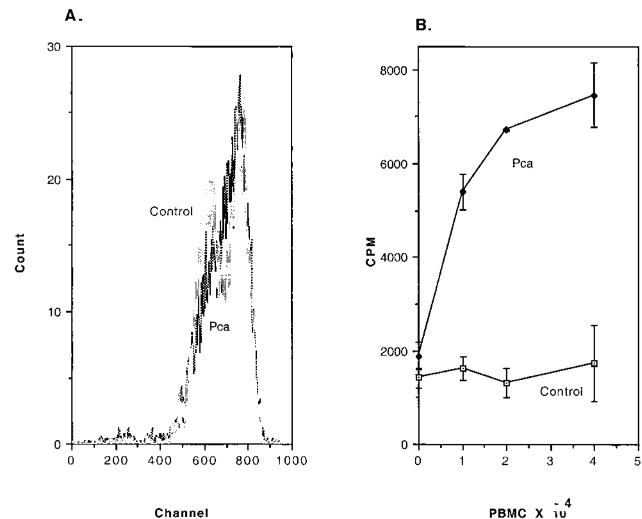

Figure 7. Effect of procainamide (Pca) on T cell CD11a expression and autoreactivity. Phytohemagglutinin (PHA)–stimulated T cells were treated with procainamide as in Figure 6. **A,** CD11a expression was compared on untreated (control) and Pca-treated cells. **B,** Untreated (control) and Pca-treated T cells were cultured with irradiated autologous peripheral blood mononuclear cells (PBMCs) and proliferation was measured 4 days later. Results are presented as the mean ± SEM of quadruplicate determinations. Positive controls included stimulating untreated and treated T cells with 40,000 PBMCs and 5 ng/ml PHA, resulting in proliferation of the untreated cells of 7,467 ± 684 counts per minute (CPM), and of the treated cells of 8,820 ± 1,149 CPM.

Effect of methylation on *ITGAL* promoter function. To determine the functional importance of methylation of the region variably methylated in the lupus and drug-treated T cells, the region between the start of the promoter (−1950) and −382 was excised, methylated in vitro, ligated back into the luciferase reporter construct, and transfected into Jurkat cells, using co-transfection with β-galactosidase as a control. Methylation decreased promoter expression by 40% (mean ± SEM of 4 experiments luciferase:β-galactosidase ratio 9.5 ± 0.9 versus 5.7 ± 0.5 for mock methylated versus methylated cells; $P = 0.02$, by paired *t*-test). The results speak for the functional significance of the methylation changes in this region.

***ITGAL* promoter methylation in RA.** Peripheral blood T cells from patients with RA can also have hypomethylated DNA (11), although there is considerable interpatient variability, and relatively few of the cells, if any, overexpress LFA-1 (9,26). To determine if RA patients also have hypomethylated *ITGAL* promoters, we analyzed methylation in T cells from 4 patients

with RA, of whom 3 had active disease and 1 had inactive disease. The overall methylation was modestly decreased (mean ± SEM 0.34 ± 0.07), but this was not significantly different from that in controls, and there was no evidence for LFA-1 overexpression (patient: control MCF 0.91 ± 0.32).

DISCUSSION

Although much of the interest in lupus has focused on B cells since autoantibodies are a hallmark of the disease, recent evidence suggests that T cells are driving the autoantibody response. Nucleosome-reactive T cells promote autoantibody synthesis in SLE (25), and modification of T cells by genetic manipulation or DNA hypomethylation can cause a lupus-like disease (6–8). T cells from lupus patients demonstrate multiple biochemical and functional abnormalities, including relative anergy, altered signaling, and impaired protein synthesis (27,28), suggesting profound biochemical abnormalities in these cells. Included in these biochemical abnormalities are decreased expression of DNA methyltransferase enzyme activity (11), decreased expression of DNA methyltransferase 1 (Dnmt1) mRNA (17), and globally hypomethylated DNA (11). Interestingly, non–T peripheral blood mononuclear cells do not have hypomethylated DNA (11), suggesting T cell specificity for this abnormality. Since the DNA methyltransferases mediate DNA methylation (22), it is likely that the decreased enzyme activity contributes to the DNA hypomethylation. The decreased Dnmt1 expression may be due to decreased ERK pathway signaling, since inhibition of this signaling pathway leads to diminished Dnmt1 expression and DNA hypomethylation (17). Thus, signaling abnormalities may contribute to DNA hypomethylation in SLE.

In this study, we have determined that DNA hypomethylation in lupus affects sequences flanking the *ITGAL* promoter. This gene was selected because it is overexpressed following treatment with DNA methylation inhibitors (6–9), because LFA-1 overexpression causes T cell autoreactivity in vitro and a lupus-like disease in vivo (4,8), and because a similar LFA-1 overexpression occurs on an autoreactive T cell subset in lupus (9,10). It is likely that DNA hypomethylation affects other T cell genes in addition to *ITGAL*, and recent studies using oligonucleotide arrays have demonstrated that transcription of >100 known genes increases following treatment with 5-azaC, including genes of possible relevance to lupus, such as perforin and CD70 (Richardson B: unpublished observations). Whether the same methylation-sensitive genes are affected in SLE and whether they contribute to disease pathogenesis is at present unknown, but these issues are addressable with the use of approaches similar to those described in this report.

The present study demonstrates that the 1,200-bp region 5′ to the transcription start site demethylates in T cells from patients with active SLE, and that the degree of hypomethylation is directly proportional to the severity of the flare as determined by the SLEDAI. The same sequences also demethylate following treatment of normal T cells with 5-azaC or procainamide. Whereas the 200 bp immediately 5′ to the start site, recognized by transcription factors, is constitutively demethylated in both lupus and control T cells, the demethylation of the further 5′ regions could be functionally significant. Methylation suppresses transcription by targeting methylcytosine-binding proteins to the relevant regions of the DNA, and some methylcytosine-binding proteins, such as MeCP2, suppress gene expression from a distance by promoting chromatin inactivation. MeCP2 binding to methylated cytosine bases attracts a chromatin-inactivation complex containing histone deacetylases as well as other proteins, which condense chromatin into an inactive configuration (22). The patch methylation studies indicate that methylation of these sequences can, in fact, modify *ITGAL* promoter function, indicating that the demethylation occurring in SLE can contribute to the LFA-1 overexpression.

Whether *ITGAL* promoter methylation patterns are affected by mature T cell differentiation is unknown. The data presented herein demonstrate that the decrease in *ITGAL* promoter methylation occurs in the majority of T cells from patients with active lupus, and analysis of the CD4+ population confirms that hypomethylation occurs in these cells. This is relevant because autoreactive CD4+ T cells are responsible for the autoantibody response in this disorder (7,9,29). In lupus, the most consistent finding in this subset is in vivo activation (30). The present data indicate that the patterns are not affected by PHA stimulation, arguing against activation as a mechanism for the hypomethylation, although it is possible that PHA stimulation does not exactly mimic the in vivo conditions encountered by lupus T cells.

LFA-1 expression is also increased on memory T cells relative to naive T cells. However, this increase is associated with a parallel increase in CD2 expression (31), and the increase in LFA-1 on lupus T cells is independent of increased CD2 expression (9,10). This indicates that different mechanisms contribute to LFA-1

overexpression in lupus and in differentiation. Whether the methylation pattern is different in a minor CD4+ subset is uncertain, but the observation that class II–restricted, LFA-1–overexpressing cells contain the autoreactive subset (9) supports the concept that the *ITGAL* promoter hypomethylation observed may contribute to the overexpression in autoreactive CD4+ cells.

Other conditions, including RA and the processes of aging, are associated with T cell DNA hypomethylation (11,24). The present study demonstrates that the *ITGAL* promoter is not significantly hypomethylated in T cells from RA patients, and that LFA-1 is not overexpressed. This suggests that the methylation changes may affect different sequences in RA T cells. However, LFA-1 expression increases with age, as do antinuclear antibodies (32,33). This raises the possibility that progressive *ITGAL* demethylation may also contribute to some forms of autoimmunity in the elderly.

In summary, we have shown that regions flanking the *ITGAL* promoter demethylate in T cells from patients with active SLE. We have also shown that demethylation of these sequences can contribute to increased *ITGAL* promoter activity, and thus could lead to increased LFA-1 expression, similar to that observed in T cells treated with DNA methylation inhibitors, including procainamide (5–9). Since LFA-1 overexpression is sufficient to cause a lupus-like disease (8), these methylation changes could contribute to the development of idiopathic and perhaps procainamide-induced SLE.

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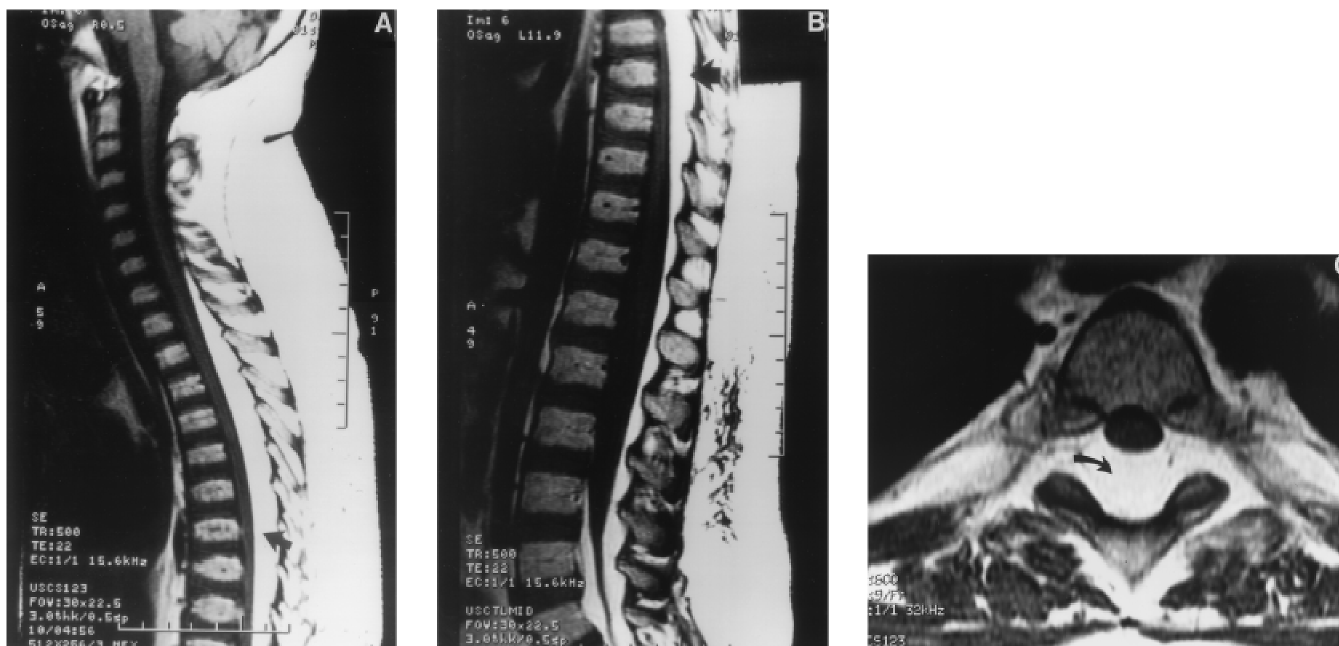
Clinical Images: Epidural lipomatosis in a 14-year-old boy with systemic lupus erythematosus



The patient, a 14-year-old boy with systemic lupus erythematosus that had been treated with high-dose prednisone for 8 weeks, presented with severe thoracolumbar back pain. Results of a neurologic examination were normal. Plain radiographs demonstrated decreased bone mineralization but no fracture. T1-weighted sagittal (TR 500 msec/TE 22 msec) (**A** and **B**) and axial (TR 600 msec/TE 9 msec) (**C**) images of the spine revealed epidural lipomatosis, recognized as a marked increase in the amount of dorsal epidural fat posterior to the cord **(arrows).** Note that the cerebrospinal fluid column is effaced in this region, with compression and ventral displacement of the thecal sac and cord. Epidural lipomatosis may cause myelopathy, radiculopathy, back pain, and syrinx.

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