SATB1 dictates expression of multiple genes including IL-5 involved in human T helper cell differentiation

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Special AT-rich binding protein 1 (SATB1) is a global chromatin organizer and a transcription factor regulated by interleukin-4 (IL-4) during the early T helper 2 (Th2) cell differentiation. Here we show that SATB1 controls multiple IL-4 target genes involved in human Th cell polarization or function. Among the genes regulated by SATB1 is that encoding the cytokine IL-5, which is predominantly produced by Th2 cells and plays a key role in the development of eosinophilia in asthma. We demonstrate that, during the early Th2 cell differentiation, IL-5 expression is repressed through direct binding of SATB1 to the *IL-5* promoter. Furthermore, SATB1 knockdown-induced up-regulation of IL-5 is partly counteracted by down-regulating GATA3 expression using RNAi in polarizing Th2 cells. Our results suggest that a competitive mechanism involving SATB1 and GATA3 regulates *IL-5* transcription, and provide new mechanistic insights into the stringent regulation of IL-5 expression during human Th2 cell differentiation. (*Blood*. 2010;116(9):1443-1453)

Introduction

Special AT-rich binding protein 1 (SATB1) is a T-cell-enriched transcription factor and chromatin organizer essential for controlling a large number of genes participating in T-cell development and activation.[1] SATB1 regulates gene expression by periodically anchoring matrix attachment regions to the nuclear matrix[2] and directly recruiting chromatin-modifying factors.[3,4] Depending on its post-translational modifications, SATB1 activates or represses multiple genes.[4] SATB1 expression is regulated by interleukin-4 (IL-4) in human differentiating Th2 cells.[5,6] On activation of mouse Th2 cells, SATB1 orchestrates the expression of Th2 cytokine genes.[7]

Naive $CD4^+$ T helper (Th) cells can differentiate into functionally distinct subsets defined by their characteristic cytokine profiles. Th1 cells produce proinflammatory cytokines IL-2 and interferon-γ and contribute to cell-mediated immunity, whereas Th2 cells secrete IL-4, IL-5, and IL-13 and are responsible for humoral responses (reviewed by Zhu and Paul[8]). Their task is complemented by Th17 cells producing IL-17A, IL-17F, and IL-22, which provide immunity against extracellular bacterial and fungal pathogens.[9-11] The key drivers for Th1 and Th2 cell differentiation are the cytokines IL-12 and IL-4 acting through STAT4 and STAT6, respectively. Th17 differentiation is initiated by IL-6 and transforming growth factor-β, which activate transcription factors STAT3 and RORγt.[12] Th subsets are implicated in pathologic responses; Th1 and Th17 cells contribute to autoimmune diseases, whereas Th2 cells are involved in the pathogenesis of asthma and allergy.[13-16]

IL-5, predominantly secreted by activated Th2 cells, is involved in control of growth, differentiation, and activation of eosinophils.[17-19] Dysregulated expression of IL-5 causes an accumulation of eosinophils in blood and tissues (eosinophilia), which is strongly linked with the pathogenesis of many allergic and inflammatory diseases.[20,21]

In this study, we investigated the role of SATB1 in T helper cell differentiation by performing gene expression profiling of polarizing human $CD4^+$ T cells in which expression of SATB1 was down-regulated by RNA interference (RNAi). Our results indicate that, during early Th1/Th2 differentiation, SATB1 is involved in the regulation of more than 300 genes, including several IL-4 and/or IL-12 regulated factors, suggesting a role in the development or function of Th subtypes. Furthermore, we show that SATB1 represses the expression of IL-5 by directly binding to its promoter and recruiting the HDAC1 corepressor, thereby possibly blocking the reciprocal regulation of *IL-5* transcription by GATA3.

Methods

Plasmid constructs and siRNA oligonucleotides

SATB1 shRNA oligonucleotides (DNA Technology) were cloned into the *Eco*RI and *Xho*I sites of pSUPER-H-$2K^k$ plasmid[22] to generate pSUPER-H-$2K^k$-SATB1-shRNA construct. pSUPER-H-$2K^k$-STAT6-shRNA, pSUPER-H-$2K^k$-Scramble-shRNA, and pSUPER-H-$2K^k$-Scramble2-shRNA were cloned previously.[22] siRNA oligonucleotides were used to knockdown SATB1, STAT6, or GATA3 (Sigma/Proligo; Table 1). The *IL-5* promoter sequence (−581 bp to +34 bp) was amplified from gDNA isolated from human cord blood $CD4^+$ cells with polymerase chain reaction (PCR) using IL5p-F and IL-5p-R primers and cloned into *Kpn*I and *Hin*dIII sites of pGL3-basic vector (Promega). Predicted SATB1-binding sites (SBSs) were removed from the *IL-5* promoter sequence using various combinations of

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Table 1. Sequences of shRNA and siRNA oligonucleotides

Oligonucleotide name	Sequence (5′-3′)
Scramble 1	5′-GCGCGCTTTGTAGGATTCG-3′
Scramble 2	5′-AATTCTCCGAACGTGTCACGT-3′
SATB1-shRNA 3	5′-CCAGCAGTATGCAGTGAATAG-3′
SATB1-shRNA 6	5′-GCATTATACCTTCTGTGATTA-3′
SATB1-siRNA 1	5′-ACCAGCAGTATGCAGTGAATAGA-3′
SATB1-siRNA 4	5′-GCTTCAAGATGTGTATCAT-3′
STAT6-shRNA 2	5′-CAGTTCCGCCACTTGCCAAT-3′
STAT6-shRNA 3	5′-GAATCAGTCAACGTGTTGTCAG-3′
STAT6-siRNA 3	5′-GAATCAGTCAACGTGTTGTCA-3′
STAT6-siRNA new	5′-AAGCAGGAAGAACTCAAGTTT-3′
GATA3-siRNA 1	5′-GAGTACAGCTCCGGACTCTTC-3′
GATA3-siRNA 2	5′-CTCTGGAGGAGGAATGCCA-3′

primers (Table 2). Cloning of GST:CD + HD has been described elsewhere.[23]

Polarization of primary CD4+ T cells

CD4+ T cells were purified from umbilical cord blood of healthy neonates (Turku University Central Hospital, Turku, Finland) or peripheral blood (buffy coats) from healthy blood donors (Finnish Red Cross) and cultured in Th1/Th2-polarizing or nonpolarizing Th0 conditions as previously described.[6] The cord blood cell cultures were generated from 4 to 9 persons and buffy coat cell cultures from 1 to 4 persons. Cord blood cell cultures may contain few memory CD4+ T cells. The culture of nucleofected cells was started 24 hours or 48 hours after nucleofection for siRNA or plasmid transfected cells, respectively. The usage of blood of unknown donors was approved by the Finnish Ethics Committee.

Nucleofection and enrichment of transfected cells

Nucleofection of CD4+ cells and enrichment of transfected cells for Illumina bead array experiment were described elsewhere.[22] A total of 4 million cord blood cells were nucleofected with 1.5 μg siRNA oligonucleotides targeting STAT6, GATA3, or SATB1 or with scrambled control siRNAs. Alternatively, 5×10^6 buffy coat cells were nucleofected with 10 μg of shRNA plasmid DNA or, for transactivation assay, with 2 μg reporter vector and 8 μg shRNA plasmid DNA. Cells nucleofected with plasmids were stained with H-2K^k-FITC (Miltenyi Biotec) antibody 20 hours after nucleofection to measure the transfection efficiency. Dead Cell Removal Kit and MACSelect Kk MicroBeads coated with the H-2K^k antibody were used according to the manufacturer's instructions (Miltenyi Biotec). Samples were analyzed with FACScan and CellQuest Software (BD Biosciences).

Table 2. Sequences of primers used in PCR amplifications

Oligonucleotide name	Sequence (5′-3′)
IL5p-F	5′-CGCGCGGGTACCGTTCTATGAGCCAATACCTTC-3′
IL5p-F1	5′-CGCGCGGGTACCGTTTTTAAAGGGGGG-3′
IL5p-F2	5′-CGCGCGGGTACCGATATAAGGCATTGGA-3′
IL5p-F3	5′-GCGGGTACCCAATCACTGTCTTCCCAC-3′
IL5p-F4	5′-GCGGGTACCCTGTTTAGTGGTCTACC-3′
IL5p-R	5′-CGCGCGAAGCTTCGTTCTGCGTTTGCCTTTGG-3′
IL5p-R1	5′-CGCGCGAAGCTTTTTCCCCCCTTTAAAAA-3′
IL5p-R2	5′-GCGAAGCTTGTGGGAAGACAGTGATTG-3′
IL5p-R3	5′-GCGAAGCTTGGTAGACCACTAAACAG-3′
IL5p-SBS1del-F	5′-GCGGAGCTCGATAAAAGTAAATTTATTTTT-3′
IL5p-SBS1del-R	5′-GCGGAGCTCAAAATCCCTGTTTCCC-3′
IL5p-SBS2del-F	5′-GCGCTCGAGCTGTTTAGTGGTCTACC-3′
IL5p-SBS2del-R	5′-GCGCTCGAGGGGAATGTTTTTTTCAG-3′
IL5p-SBS3del-F	5′-GCGGAATTCGTTTTTAAAGGGGGG-3′
IL5p-SBS3del-R	5′-GCGGAATTCCGAAGAATCTTTGGG-3′
IL5p-SBS4del-F	5′-GCGAGATCTGCAAATGTGGGGCAATG-3′
IL5p-SBS4del-R	5′-GCGAGATCTCAGAAAATTAACTTCCTC-3′

PCR indicates polymerase chain reaction.

Transactivation assay

Buffy coat CD4+ cells were cotransfected with wild-type (WT) or mutated pGL3-IL-5 luciferase reporter constructs and SATB1-shRNA or control shRNA plasmids. Luciferase activity was analyzed using BriteLite reagent and Victor 1420 Multilabel Counter (both from PerkinElmer Life and Analytical Sciences). Relative luciferase activity was calculated by normalizing the measurements of WT or mutated *IL-5* reporter vector to the empty pGL3-basic control measurement.

Gene expression profiling

Illumina sample preparations were started with 50 to 200 ng total RNA, pooled from several persons. The sample preparation was performed according to the instructions and recommendations provided by the manufacturer. The samples were hybridized by the Finnish DNA Microarray Center to Illumina Sentrix Human-6 Expression BeadChip arrays. The data were quantile normalized[24] and log-transformed using the R package limma[25] (http://www.bioconductor.org). Illumina BeadChip Versions V1 and V2 were combined using annotations (Ensembl BioMart database; hsapiens dataset) from biomaRt.[26] RankProd[27] was used to identify SATB1 target genes across the 5 biologic repeats (Table 3). For each biologic replicate, the maximum or the minimum signal log ratios (SLRs) over all time points were used as an input for the RP function. Genes having the estimated percentage of false-positive predictions of *P* less than .05 were considered to be differentially expressed. Fold changes were calculated as 2^{SLR} if SLR more than 0 and -2^{SLR} if SLR less than 0, where SLR is the average maximum (for up-regulated genes) or minimum (for down-regulated genes) SLR over the biologic replicates. The gene annotations were obtained from Illumina. Genes were categorized according to their Gene Ontology annotations.[28]

Quantitative RT-PCR analyses

cDNA was prepared as previously described[29] and used as a template for quantitative reverse-transcription (RT)–PCR analysis performed using TaqMan ABI Prism 7700 or 7900HT (Applied Biosystems). The housekeeping gene *EF1α* was used as a reference transcript. Primers and probes (Table 4; Oligomer Oy or Roche Applied Science) were designed using Primer Express (Applied Biosystems) and Universal ProbeLibrary Assay Design Center (Roche Applied Science) software Version 2.45. Quantitative chromatin immunoprecipitation (ChIP)–PCRs were performed using iCycler (Bio-Rad) and iQ SYBR Green mix (Bio-Rad). Change in threshold (ΔCt) values were calculated using the formula: $\Delta Ct = (Ct_{Target} - Ct_{Input})$ and $\Delta(\Delta Ct) = (\Delta Ct_{Target} - \Delta Ct_{IgG})$. Fold difference in occupancy was calculated as follows: Fold difference $= 2^{-\Delta(\Delta Ct)}$.

Western blotting

Cell lysates were prepared essentially as described.[29] Proteins were resolved on 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels, transferred onto polyvinylidene difluoride membranes (Millipore), and detected using the primary antibodies: mouse anti-GATA3, goat anti-SATB1, mouse anti-STAT6 (all from Santa Cruz Biotechnology) or mouse anti–β-actin (Sigma-Aldrich) and secondary antibodies: horseradish peroxidase-conjugated ant–mouse or anti–goat

Table 3. Details of SATB1 RNAi cultures for Illumina gene expression analysis

Culture no.	Cell type	RNAi	Polarization	Time points, h
1	Buffy coat CD4+	shRNA	Th1, Th2	0, 24, 48
2	Buffy coat CD4+	shRNA	Th1, Th2	0, 24, 48
3	Cord blood CD4+	siRNA	Th0, Th1, Th2	0, 12, 24, 48
4	Cord blood CD4+	siRNA	Th0, Th1, Th2	24
5	Cord blood CD4+	siRNA	Th0, Th1, Th2	0, 12, 24, 48

Table 4. Primers and probes used in quantitative RT-PCR

GeneBank ID	Gene	
		1) 5′-6(FAM)-PROBE-(TAMRA)-3′
		2) 5′-PRIMER 1–3′
		3) 5′-PRIMER 2–3′
NM_002971	SATB1	1) 5′-AACGAGCAGGAATCTCCCAGGCG-3′
		2) 5′-ACCAGTGGGTACGCGATGA-3′
		3) 5′-TGTTAAAAGCCACACGTGCAA-3′
NM_003153	STAT6	1) 5′-CAGGACACCATCAAACCACTGCCAAA-3′
		2) 5′-TGGGCCGTGGCTTCAC-3′
		3) 5′-CCGGAGACAGCGTTTGGT-3′
NM_001002295	GATA3	1) 5′-TGCCGGAGGAGGTGGATGTGCT-3′
		2) 5′-GGACGCGGCGCAGTAC-3′
		3) 5′-TGCCTTGACCGTCGATGTTA-3′
NM_000879	IL-5	1) 5′-TTGACTCTCCAGTGTGCCTATTCCCTGAA-3′
		2) 5′-AAATCACCAACTGTGCACTGAA-3′
		3) 5′-CGTCAATGTATTTCTTTATTAAGGACAA-3′
NM_001402	EF1α	1) 5′-AGCGCCGGCTATGCCCCTG-3′
		2) 5′-CTGAACCATCCAGGCCAAAT-3′
		3) 5′-GCCGTGTGGCAATCCAAT-3′

RT indicates reverse-transcribed polymerase chain reaction.

(Santa Cruz Biotechnology). The bound antibodies were visualized with ECL reaction (GE Healthcare).

Cytokine secretion assay

Secreted IL-5 was measured from the culture supernatants using Bio-Plex Cytokine Assay kit (Bio-Rad) according to the manufacturer's instructions. Measurements and data analysis were performed with the Bio-Plex system in combination with the Bio-Plex Manager software (Bio-Rad).

EMSA

For electrophoretic mobility shift assay (EMSA) probe preparation, the *IL-5* promoter and its truncations were amplified with PCR using WT or mutated pGL3-IL-5 reporter constructs and in the presence of α-^{32}P dATP and α-^{32}P dCTP (PerkinElmer Life and Analytical Sciences). Recombinant GST: CD + HD fusion protein containing the DNA-binding domain of SATB1[30] was expressed in BL21 strain of *Escherichia coli* and purified according to standard procedures. Cell extracts from Th1 or Th2 cells were prepared essentially as described.[31] The protein content of lysate was estimated using Bio-Rad DC Protein Assay. EMSA-binding reactions were performed as previously described.[31] For antibody-mediated supershifts, reaction mixtures were supplemented with anti-SATB1[4] or normal rabbit IgG (Santa Cruz Biotechnology). The reactions were loaded on 6% native polyacrylamide gels to resolve the binding products. Dried gels were subjected to autoradiography. The purified GST proteins were used in EMSA with various probes to determine the dissociation constants, the concentration (Molar) of protein required to bind 50% of the substrate DNA.

ChIP and ChIP-on-chip

Cord blood CD4$^+$ cells were crosslinked for 10 minutes at 22°C using formaldehyde at a final concentration of 1% in the culture medium. ChIP was performed as previously described.[32] Chromatin, sonicated into 300- to 1000-bp fragments using Bioruptor XL (Diagenode), was immunoprecipitated with anti-SATB1,[4] GATA3 or HDAC1 antibodies (Santa Cruz Biotechnology), or control normal rabbit IgG (Upstate Biotechnology). Part of the sheared chromatin was saved as input sample (whole cell extract), which was treated similarly excluding the IP step. DNA was PCR amplified using primers IL5p-F1 and IL5p-R (Table 2). PCR products were resolved by agarose gel electrophoresis, stained with ethidium bromide EtBr, and visualized under ultraviolet light.

A total of 200 ng of whole cell extract and anti-SATB1 immunoprecipitated DNA pooled from several persons were processed and

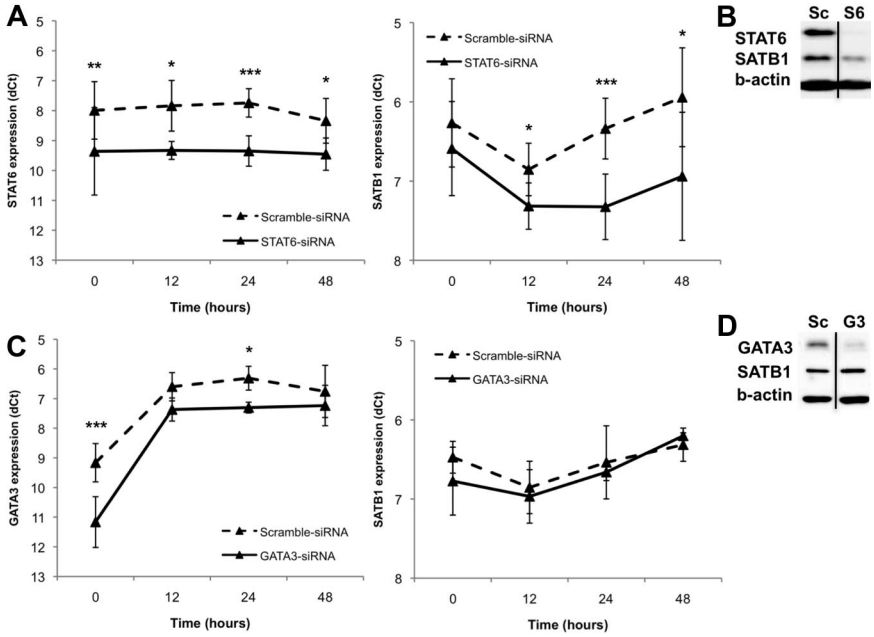

Figure 1. SATB1 expression is down-regulated by knockdown of STAT6 but not by knockdown of GATA3. (A) Cord blood and buffy coat CD4$^+$ T cells were nucleofected with STAT6-siRNA or scrambled control siRNA, and cells were cultured in Th2-polarizing conditions. Cells from 3 to 7 independent experiments were harvested at indicated time points and analyzed using quantitative RT-PCR. The normalized expression (dCt) of *STAT6* (left panel) and *SATB1* (right panel) mRNA of STAT6-siRNA (solid line) and control siRNA (dashed line) nucleofected cells is presented. (B) Effect of STAT6 knockdown on SATB1 expression at 3-day time point in Th2 condition analyzed with Western blotting. Sc indicates scrambled control siRNA; and S6, STAT6-siRNA. Representative value of 3 independent experiments. Vertical lines have been inserted to indicate a repositioned gel lane. (C) Cord blood CD4$^+$ T cells were nucleofected with GATA3-siRNA or scrambled control siRNA, and cells were cultured in Th2-polarizing conditions. Cells from 3 independent experiments were harvested at indicated time points and analyzed using quantitative RT-PCR. The normalized expression (dCt) of *GATA3* (left panel) and *SATB1* (right panel) mRNA of GATA3-siRNA (solid line) and control siRNA (dashed line) nucleofected cells is presented. (D) Effect of GATA3 knockdown on SATB1 expression at 1-day time point in Th2 condition analyzed with Western blotting. Sc indicates scrambled control siRNA; and G3, GATA3-siRNA. Representative value of 3 independent experiments. Vertical lines have been inserted to indicate a repositioned gel lane. *$P < .05$. **$P < .01$. ***$P < .005$.

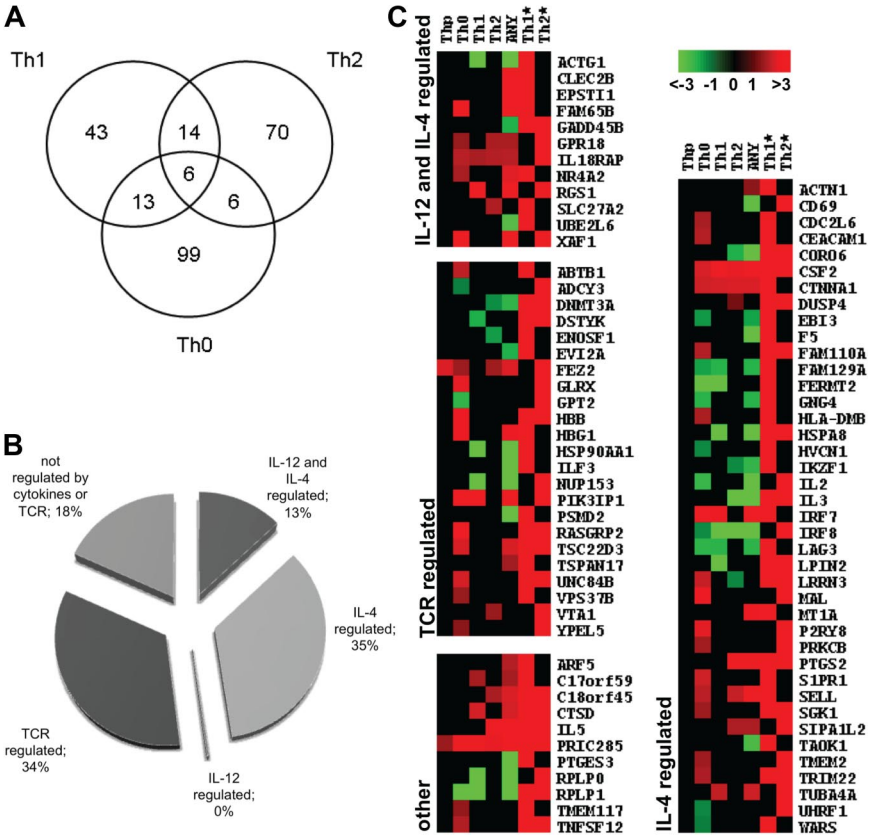

Figure 2. SATB1 target genes during Th1 and Th2 cell differentiation. (A) Expression profiles of SATB1-siRNA/shRNA and scrambled control siRNA/shRNA-treated cells were studied using Illumina bead arrays. Venn diagram shows the number of genes of which the expression was altered on SATB1 knockdown in each Th subtype. (B) The regulation of SATB1 RNAi target genes by cytokines (IL-12 and/or IL-4) and TCR was determined as described in "SATB1 regulates more than 300 genes in developing Th1 and Th2 cells." (C) Direct SATB1 target genes are common hits of 2 independent approaches: (1) gene expression profiling of SATB1-siRNA/shRNA-treated cells analyzed using Illumina bead arrays and (2) ChIP-on-chip analysis using SATB1-enriched chromatin from cord blood CD4$^+$ T cells polarized to Th1 and Th2 directions for 24 hours. Heat map visualization of direct SATB1 target genes grouped according to their regulation by IL-4, IL-12, and IL-4, T-cell activation (TCR), or none of the above (other). Green represents decreased; and red, increased gene expression (fold change) on SATB1 down-regulation in indicated Th subtypes (Thp, Th0, Th1, or Th2) or across all Th subtypes (Any). *The 2 last columns indicated represent the Th1 or Th2 specific binding of SATB1 detected with ChIP-on-chip approach.

hybridized by Genotypic Technology Ltd with human genome promoter microarrays (Agilent Technologies Inc). The arrays were custom designed at Genotypic Technologies to contain more than 244 000 probes covering −5.5 kb upstream to 2.5 kb downstream of approximately 15 000 known transcripts start site and known ENCODE regions. The text output file generated from the images using Agilent feature extraction software Version 9.3 was used for the analysis. The normalization, including Median Blanks substraction, interarray median normalization, and dye-bias median normalization, was done using Agilent DNA Analytics software Version 9.3. Genes having the normalized log ratio more than 2 and enriched with at least 3 probes were considered as specifically enriched. Visualization was performed using Eisen Treeview.[33]

Linear modeling

The association between the SBSs and the luciferase activity was investigated in terms of a multivariable linear model, assuming that the SBSs have an additive effect on the activity. More specifically, we considered the linear regression model $y = \beta_0 + S^4_{j=1}\,\beta_j x_j + \varepsilon$, where y is the relative activity of a reporter construct, x_j is the binary indicator for the presence of the individual SBS j in the construct, and ε is an error term. The model was fitted with the least squares method using the function lm in the statistical software R Version 2.8.1. For each coefficient $\beta_j, j = 0,...,4$, the null hypothesis $H_0{:}\beta_j = 0$ was tested under the assumption of normally distributed errors.

Statistical analysis

The statistical significance between means in the quantification of mRNA and secreted cytokines was calculated with paired 2-tailed Student t test. A P value less than .05 was considered statistically significant.

Accession numbers

The SATB1 RNAi gene expression and SATB1 ChIP-on-chip data can be found at the NCBI Gene Expression Omnibus with accession numbers GSE17241 and GSE17380, respectively.

Results

STAT6-dependent SATB1 expression in Th2 cells

The expression of SATB1 is induced by T-cell receptor (TCR) stimulation in human CD4$^+$ cells cultured in Th1 (anti-CD3 + anti-CD28 + IL-12), Th2 (anti-CD3 + anti-CD28 + IL-4), and Th0 (anti-CD3 + anti-CD28) conditions compared with naive Thp cells and further increased by IL-4 during Th2 differentiation.[6] We studied whether this IL-4-dependent regulation of SATB1 is mediated via STAT6, a transcription factor activated by stimulation through IL-4R. Inhibition of the expression of STAT6 using RNAi in CD4$^+$ cells isolated from cord blood or buffy coat and cultured in Th2 conditions led to a markedly diminished expression of SATB1 at mRNA and protein level analyzed by quantitative RT-PCR and Western blotting, respectively (Figure 1A-B). The positive regulation of SATB1 expression by STAT6 is not mediated via GATA3, a key transcription factor of Th2 cells regulated by STAT6, as knockdown of GATA3 using specific siRNA oligonucleotides in differentiating cord blood CD4$^+$ cells did not affect SATB1 expression (Figure 1C-D).

SATB1 regulates more than 300 genes in developing Th1 and Th2 cells

To investigate the role of SATB1 in Th cell differentiation, we examined gene expression in differentiating human CD4$^+$ cells in which expression of SATB1 had been down-regulated with shRNAs/siRNAs. Cells were cultured under Th0, Th1, or Th2 conditions for up to 48 hours, total RNA was extracted, and gene expression profiling was performed using the Illumina bead array platform. Comparing SATB1 knockdown CD4$^+$ cells with cells treated with the corresponding scrambled siRNA/shRNA controls, we found

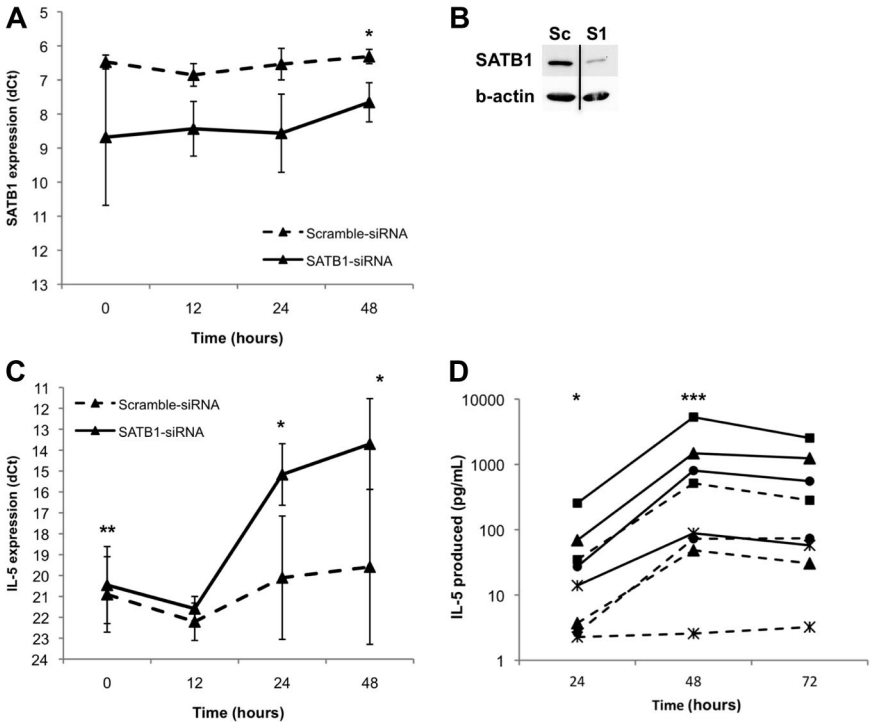

Figure 3. Knockdown of SATB1 greatly induces IL-5 expression in polarizing Th2 cells. Cord blood CD4$^+$ T cells were nucleofected with SATB1-siRNA or scrambled control siRNA and cultured in Th2-polarizing conditions. The cells and the culture media were collected at indicated time points. (A) Samples from 3 independent experiments were analyzed using quantitative RT-PCR. The normalized expression (dCt) of *SATB1* mRNA of SATB1-siRNA (solid line) and control siRNA (dashed line) nucleofected cells is presented. (B) Effect of SATB1-siRNA on SATB1 expression at the 24-hour time point in the Th2 condition analyzed with Western blotting. Sc indicates scrambled control siRNA; and S1, SATB1-siRNA. Representative value of 3 independent experiments. Vertical lines have been inserted to indicate a repositioned gel lane. (C) Samples from 3 independent experiments were analyzed using quantitative RT-PCR. The normalized expression (dCt) of *IL-5* mRNA of SATB1-siRNA (solid line) and control siRNA (dashed line) nucleofected cells is presented. (D) The culture media of 4 independent experiments was measured for secreted IL-5 using Bio-Plex assay and is expressed as picograms per milliliter. Each symbol type represents an independent experiment. Solid and dashed lines indicate SATB1-siRNA and scrambled control-treated cells, respectively. *$P < .05$. *$P < .01$. ***$P < .005$.

that 319 genes were directly or indirectly regulated by SATB1 (supplemental Tables 1-2, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Approximately 30% of these genes (99 genes) showed altered expression in Th0 conditions. SATB1 down-regulation selectively changed the expression of 43 genes in Th1 polarizing conditions and 70 genes in Th2-polarizing conditions (Figure 2A). In addition, 14 genes were regulated by SATB1 in both Th1 and Th2-polarizing conditions but not in Th0 conditions; thus, altogether, expression of 40% (43 + 70 + 14 = 127 genes) of SATB1-regulated genes was altered only in the presence of a polarizing cytokine (IL-12 or IL-4). Thus, SATB1 target genes were partly Th subtype specific.

We next studied whether SATB1-regulated genes are involved in Th cell differentiation. To determine genes regulated by TCR (Th0 vs Thp), IL-12 (Th1 vs Th0), or IL-4 (Th2 vs Th0), the gene expression profiles of Thp, Th0, Th1, and Th2 cells treated with the control siRNA were compared with each other. In addition, IL-12, IL-4, or TCR-regulated genes were determined by a detailed gene expression kinetics study on differentiating human Th1 and Th2 cells on whole genome level. The information of cytokine or TCR-specific regulation of SATB1 target genes was added to their annotation. Notably, 35% (111 genes) of the SATB1 target genes were specifically regulated by IL-4 and an additional 13% (41 genes) were regulated by both IL-4 and IL-12, revealing that altogether 48% of SATB1-regulated genes are IL-4 targets (Figure 2B). Furthermore, TCR stimulation alone regulated one-third (108 genes) of SATB1 targets and only 18% (= 100 − 35 − 13 − 34) of SATB1 target genes were not regulated by TCR or Th1/Th2-polarizing cytokines. Thus, SATB1 probably plays an essential role in the development or function of Th subtypes.

Next we investigated using ChIP-on-chip approach, whether the promoters of SATB1 target genes are bound by SATB1 in differentiating Th cells. SATB1-enriched chromatin from cord blood CD4$^+$ cells cultured in Th1 and Th2-polarizing conditions for 24 hours was hybridized on Agilent Human Promoter 244k arrays along with corresponding input control. SATB1 was bound to promoters of 3279 and 2729 genes in Th1 and Th2 cells, respectively (data not shown). A total of 27% (86 genes) of SATB1 target genes identified using the RNAi approach were also bound in vivo by SATB1 (Figure 2C; supplemental Table 3). In addition, direct SATB1 target genes were further enriched with IL-4-regulated genes as 60% (40 + 12 = 52 genes) of SATB1 ChIP-on-chip and siRNA targets were regulated by IL-4. These results suggest that SATB1 might have a role in IL-4-mediated signaling and thereby in Th2 cell differentiation or function.

IL-5 is greatly induced in SATB1-down-regulated Th2-polarizing cells

One of the most strikingly up-regulated genes on SATB1 knockdown was *IL-5* (supplemental table). To further analyze this result, we performed quantitative RT-PCR analysis using additional cultures generated from cord blood CD4$^+$ cells nucleofected with SATB1-siRNA or control oligonucleotides. SATB1 expression was markedly down-regulated by SATB1-siRNA (Figure 3A-B), and suppression of SATB1 expression greatly induced *IL-5* expression (Figure 3C). We measured using Bio-Plex assay the secreted IL-5 from the culture media of SATB1-siRNA or control siRNA-treated cells. At the 24- and 48-hour time points, the production of IL-5 was significantly increased on SATB1 knockdown (Figure 3D). The induction of IL-5 was at least 6-fold. These results indicate that SATB1 participates in a negative feedback loop in which it is induced during Th2 differentiation and represses *IL-5* expression in polarizing Th2 cells.

SATB1 binds to the *IL-5* promoter in vitro at multiple sites

Next we studied whether SATB1 inhibits IL-5 expression by directly binding to its promoter as in the case of many other SATB1-regulated genes.[32] Using a 615-bp fragment (−581 bp to 34 bp) of the *IL-5* proximal promoter as a probe (Figure 4A) in EMSA, SATB1 formed a characteristic complex of increasing size in a dose-dependent manner (Figure 4B). Such binding pattern

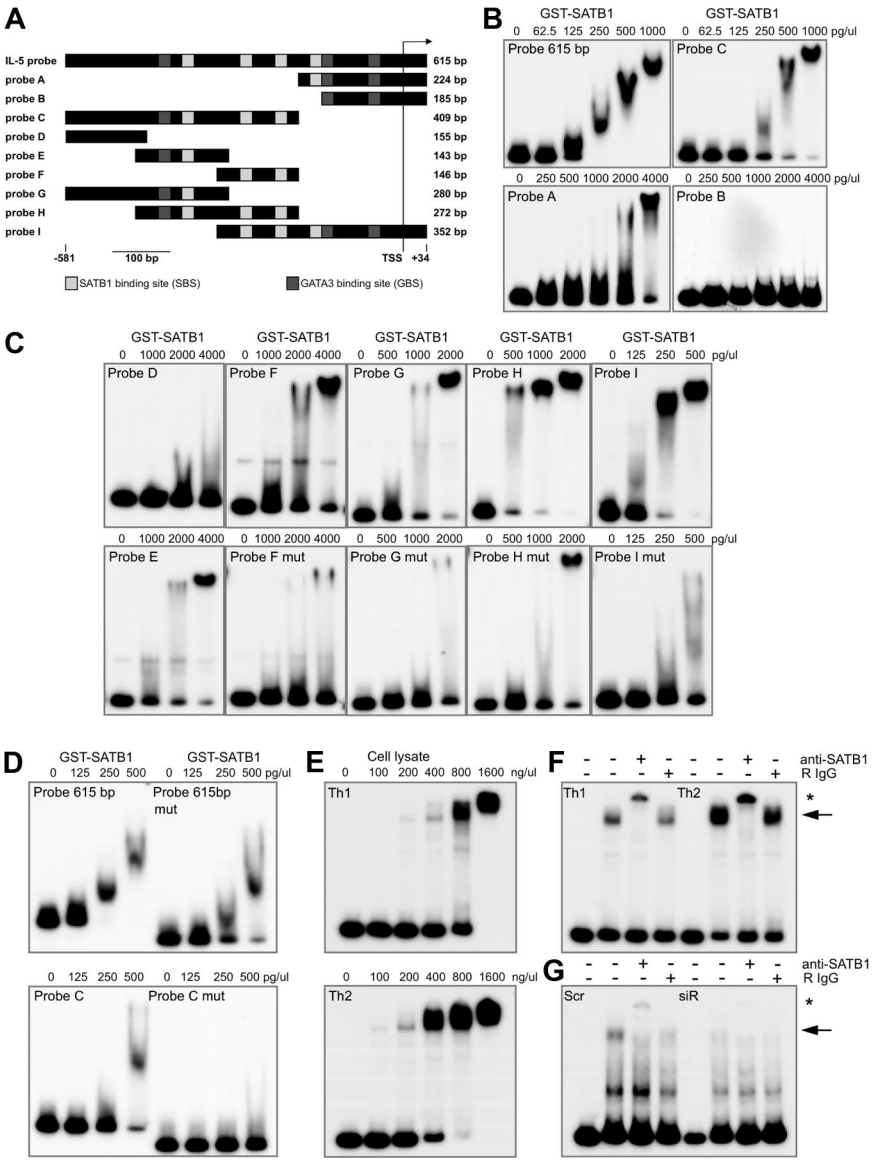

Figure 4. Human IL-5 promoter harbors several SBSs. (A) A schematic of *IL-5* promoter sequence and its truncations used in EMSAs as well as location of identified SBSs and previously published GATA3-binding sites. (B) Increasing concentrations of recombinant SATB1 was incubated with the full-length (615-bp) IL-5 probe and probes A, B, and C. (C) Increasing concentrations of recombinant SATB1 were incubated with probes D, E, F, G, H, and I or with the mutated (without SBSs) probes F, G, H, and I. (D) Increasing concentrations of recombinant SATB1 were incubated with the full-length IL-5 probe and probe C as well as with the corresponding mutated (without SBSs) probes. (E) Increasing concentrations of nuclear lysates from cord blood $CD4^+$ T cells polarized to Th1 and Th2 directions for 24 hours were incubated with probe A. (F) Probe A was incubated with 4.0 μg Th1 or Th2 nuclear extract and additionally with anti-SATB1 or normal rabbit IgG. (G) Probe A was incubated with 16.0 μg nuclear extract from cord blood $CD4^+$ T cells nucleofected with scrambled (Scr) control or SATB1-siRNA (siR) and cultured in Th2-polarizing conditions for 24 hours and additionally with anti-SATB1 or normal rabbit IgG. The arrow indicates the lysate-probe complex. *Band shift of the complex. The protein concentrations used with each probe are marked in the figure. Data are representative of 3 independent experiments.

suggested the presence of several binding sites for SATB1; therefore, the nucleotide sequence was screened for consensus SBSs as defined by Purbey et al.[30] We identified 4 putative SBSs and prepared suitable deletions and truncations of the *IL-5* promoter (Figure 4A; Table 5). Binding of SATB1 to the probe A (−190 bp to 34 bp) but not to the probe B (−151 bp to 34 bp) confirmed the presence of an SBS in the 5′ end of probe A (Figure 4B bottom panels). However, the high-affinity and progressively bigger dose-dependent complex formed by SATB1 with probe C (−581 bp to −172 bp) suggested the presence of multiple SBSs in this region of the promoter. The ultimate 5′ end of the promoter region used in this study is devoid of any SBSs as SATB1 failed to bind with probe D (−581 bp to −426 bp; Figure 4C). In contrast, SATB1 formed specific complexes with probe E (−444 bp to −301 bp) and probe F (−318 bp to −172 bp), suggesting that they harbor SBSs (Figure 4C). Probe F contains 2 putative SBSs, and deleting both of them in probe F substantially decreased the affinity of SATB1 (Figure 4C). SATB1 also formed specific complexes with probes G (−556 bp to −301 bp), H (−444 bp to −172 bp), and I (−318 bp to 34 bp; Figure 4C), and the binding of SATB1 to these probes was severely affected by removing the newly identified SBSs (Figure 4C-D bottom panels), confirming the absence of any further consensus SBSs in the *IL-5* promoter used in this study. Similarly, removing all 4 SBSs from the full-length *IL-5* probe resulted in a substantially weaker binding of SATB1 (Figure 4D). We determined the relative binding affinities of SATB1 to WT and mutated full-length and truncated *IL-5* probes (summarized in Table 6). Binding of SATB1 was strongest with WT *IL-5* probe and probe I, whereas deleting the putative SBSs abrogated the binding activity drastically. However, the mutated probes H and I have the same affinity than the full-length *IL-5* probe and probes A and C. Therefore, although our bioinformatics analyses could not find any potential SBSs within this region, it is feasible that another SBS that contains the context preferred by SATB1 may exist within

Table 5. Predicted SBSs and DNA sequences deleted from SBS mutated constructs

SBS	Location of SBS	Deleted DNA sequence (5′-3′)	Size, bp
S1	−162 bp to −152 bp	**TATTATTAAAA**	11
S2	−333 bp to −322 bp	AATATTTATGTA**TTTTAGCATAAA**ATT	27
S3	−201 bp to −190 bp	**TAATAGAAAAT**	11
S4	−254 bp to −243 bp	**ATTTTAAGAAAT**	12

Table 6. Summary of relative binding affinities of *IL-5* probes

Probe name	No. of SBSs	Binding affinity of SATB1, K_d/M
IL-5 probe (wt)	4	1×10^{-9}
Probe A	1	1×10^{-8}
Probe B	0	$> 2 \times 10^{-5}$
Probe C	3	3×10^{-9}
Probe D	0	5×10^{-8}
Probe E	1	1×10^{-8}
Probe F	2	1×10^{-8}
Probe F mut	0	2×10^{-7}
Probe G	1	7×10^{-9}
Probe G mut	0	4×10^{-8}
Probe H	3	4×10^{-10}
Probe H mut	0	1×10^{-8}
Probe I	3	1×10^{-9}
Probe I mut	0	4×10^{-9}

SBS indicates SATB1-binding site.

this region and give rise to the observed binding pattern. Thus, using in vitro binding analysis, 4 novel SBSs were identified in the human *IL-5* promoter.

To investigate whether these SBSs are occupied by SATB1 from cell extracts of $CD4^+$ cells, we performed EMSA using nuclear extracts from polarizing Th1 and Th2 cells. Such analysis showed that the proteins of Th2 cells give rise to substantially stronger complex with probe A than those of Th1 cells (Figure 4E), corroborating the earlier finding that Th2 cells express more SATB1 than Th1 cells during their early differentiation.[6] Both complexes were supershifted in the presence of anti-SATB1, confirming the presence of SATB1 both in Th1 and Th2 nuclear extracts (Figure 4F). The complex is SATB1-dependent as nuclear extracts from SATB1-siRNA nucleofected cells cultured in Th2 conditions for 24 hours failed to form complex in EMSA in contrast to control siRNA-treated cells (Figure 4G).

The functional role of the identified SBSs on the expression of *IL-5* was elucidated by performing luciferase assay using the pGL3 reporter vector containing the 615-bp fragment of the *IL-5* proximal promoter. Mutant reporter constructs were created from the

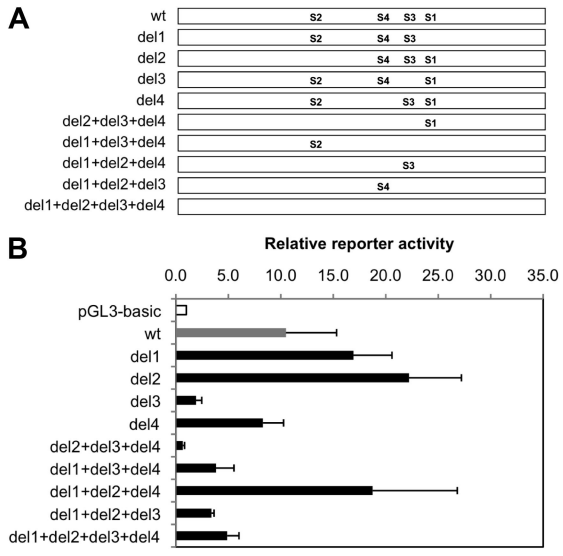

Figure 5. Differential role of SBSs on *IL-5* expression. (A) A schematic of IL-5 promoter constructs with different combinations of SBSs deleted used in the reporter assay. (B) Buffy coat $CD4^+$ T cells were nucleofected with pSUPER-H-$2K^k$-Scramble2-shRNA construct and WT *IL-5* luciferase reporter vector or its SBS deleted version. Data represent mean ± SD of 3 independent experiments.

Table 7. Summary of statistics for Figure 5B

SBS	Estimate	SE	T value	Pr(> t)
S1	−3.165	1.661	−1.906	.06824
S2	−4.232	1.661	−2.549	.01734
S3	13.374	1.661	8.053	2.08E-08
S4	2.512	1.661	1.513	.14291

Pr(> t) indicates probability that "t" is smaller than a specific value.

WT *IL-5* reporter construct by deleting all individual SBSs, deleting SBSs one by one, or deleting 3 SBSs at a time leaving 1 SBS intact (Figure 5A). Buffy coat $CD4^+$ cells were nucleofected with either WT or mutated reporter construct and pSUPER-scramble-H-$2K^k$ vector that enabled measuring the transfection efficiency (27%–45%). Cells were cultured under Th2-polarizing conditions for 24 hours and harvested for the transactivation assay. The luciferase activity was increased compared with WT *IL-5* promoter construct when SBSs S1 or S2 were deleted separately (del1, del2), or they were both deleted together with S4 (del1 + del2 + del4; Figure 5B). In contrast, the luciferase activity was markedly decreased when SBS S3 was deleted alone (del3) or in combination with SBSs S2 and S4 (del2 + del3 + del4), S1 and S4 (del1 + del3 + del4), or S1 and S2 (del1 + del2 + del3). Furthermore, the reporter activity was also decreased compared with WT construct by deleting all SBSs. These results indicate that SBSs S1 and S2 are repressive sites whereas S3 is a strongly activating site. This was further supported by the estimated coefficients of the individual SBSs in the linear model: S1, 3.165 ($P = .068$); S2, 4.232 ($P = .017$); S3, 13.374 ($P = .000$); and S4, 2.512 ($P = .142$; Table 7).

Occupancy of *IL-5* promoter by SATB1 is required for suppression of IL-5 expression during Th2 cell differentiation

We monitored the occupancy of SATB1, GATA3, and HDAC1 at the *IL-5* promoter during early Th cell differentiation. GATA3 mediates positive regulation of *IL-5* transcription,[35,36] whereas overexpression of HDAC1 augments the repression of *IL-5*.[37] Furthermore, phosphorylated SATB1 has been demonstrated to recruit HDAC1 to its targets, which leads to down-regulation of gene expression.[4] Cord blood $CD4^+$ cells were cultured in Th1 and Th2 conditions for 24 hours and subjected to ChIP assay. ChIP-PCR analysis revealed that SATB1 and HDAC1 were bound to *IL-5* proximal promoter both in Th1 and Th2 conditions, whereas GATA3 was bound specifically in Th2-polarizing cells (Figure 6A-B). Quantitative PCR revealed more than 2-fold increase in the occupancy of SATB1 in Th2 cells (Figure 6A) correlating with its higher expression in Th2 cells.[6] Interestingly, the occupancy of HDAC1 was also increased by approximately 2-fold in Th2 cells, suggesting a possible role of SATB1 in its recruitment. GATA3 occupancy was highest in Th2 cells, approximately 7-fold higher than the minimal occupancy observed in Th1 cells in concordance with its preferential expression in Th2 cells.[38] A similar occupancy profile was observed at the distal region of the *IL-5* promoter corresponding to probe C that also contains SBSs and GATA3-binding sites (data not shown). Next, we monitored the occupancy of these factors during the up-regulation of IL-5 on siRNA-mediated knockdown of SATB1 in Th2 cells. Quantitative ChIP-PCR analysis revealed that on SATB1 knockdown HDAC1 occupancy was also proportionately decreased at the *IL-5* proximal promoter in Th2 conditions (Figure 6C). Interestingly, GATA3 occupancy was slightly, but significantly, increased under these conditions, indicating that loss of SATB1 may favor occupancy of

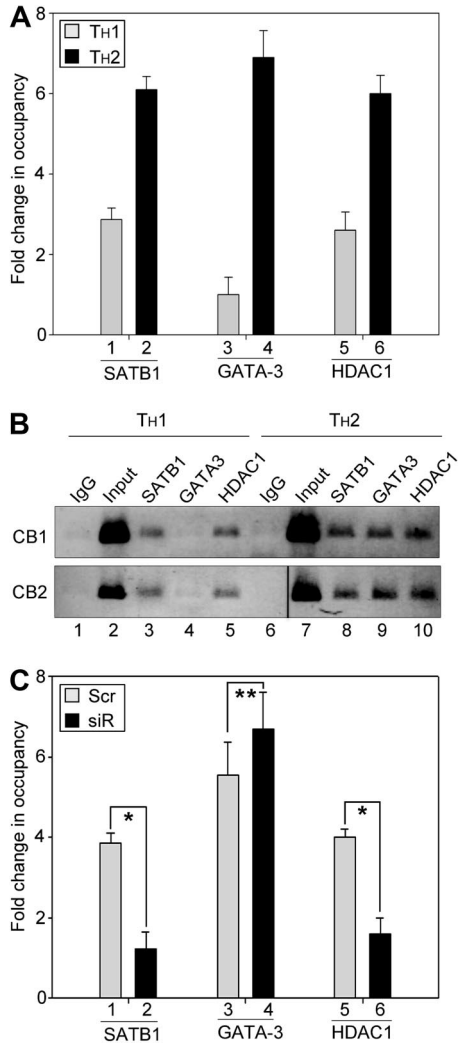

Figure 6. Differential occupancy of SATB1, GATA3, and HDAC1 on IL-5 proximal promoter in Th1 and Th2 cells during T-cell differentiation. (A) Naive CD4$^+$ cells were polarized for 24 hours and subjected to ChIP assay. Occupancy of *IL-5* promoter by SATB1, GATA3, and HDAC1 during Th1 (▭) and Th2 (■) differentiation was monitored by quantitative RT-PCR using primers corresponding to fragment A of the *IL-5* promoter. Data represent fold change in occupancy of indicated proteins compared with their corresponding IgG controls, after normalizing for the input chromatin. Each error bar represents SD calculated from triplicates. (B) ChIP-PCR analysis for occupancy of SATB1, GATA3, and HDAC1 on proximal IL-5 promoter in Th1 and Th2 cells was performed as described in "ChIP and ChIP-on-chip." ChIP-PCR products (~ 200 bp) from 2 representative cord blood (CB) samples are depicted. Vertical lines have been inserted to indicate a repositioned gel lane. (C) Naive CD4$^+$ T cells nucleofected with scrambled (Scr) control or SATB1-siRNA (siR) were cultured in Th2-polarizing conditions for 24 hours and subjected to ChIP assay. Occupancy of *IL-5* promoter by SATB1, GATA3, and HDAC1 during Th2 differentiation in the presence of Scr (▭) or siR (■) was monitored by quantitative RT-PCR using primers corresponding to fragment A of *IL-5* promoter as described in "Quantitative RT-PCR analyses." Error bar represents SD calculated from triplicates. $^*P < .005$. $^{**}P < .01$.

GATA3 (Figure 6C). In conclusion, our ChIP data suggest that SATB1 could repress IL-5 expression by recruiting the HDAC1 corepressor to *IL-5* promoter both in Th1 and Th2 cells during their early differentiation. In addition, binding of GATA3 to *IL-5* promoter in cells cultured in Th2 conditions presumably poises the *IL-5* gene for transcription later on activation of Th2 cells.

GATA3 has been shown to bind at least 3 sites (−70, −152, and −400 bp) in the *IL-5* promoter and to regulate *IL-5* transcription.[35,36] As knockdown of SATB1 does not induce GATA3 expression in developing Th2 cells (Figure 7B), which could explain the increased expression of IL-5, we hypothesized that, on SATB1 down-regulation, GATA3 could aberrantly bind to the *IL-5* promoter and induce *IL-5* expression. Cord blood isolated CD4$^+$ cells were nucleofected with siRNAs for SATB1, GATA3, or both, and cells were induced to polarize to Th2 direction. Each specific siRNA inhibited substantially their target gene expression (Figure 7A-B). SATB1 knockdown strongly induced *IL-5*, whereas down-regulation of GATA3 did not decrease *IL-5* expression presumably as the basal expression of *IL-5* is negligible during early Th2 differentiation (Figure 7C). Intriguingly, the induction of *IL-5* expression in cells where both SATB1 and GATA3 had been knocked down was only one-fourth of the induction resulting from knocking down SATB1 alone. Similarly, measuring the secreted IL-5 from the culture media of SATB1-siRNA, GATA3-siRNA, and/or scrambled control siRNA-treated cells using Bio-Plex assay confirmed that the production of IL-5 was significantly increased on SATB1 knockdown compared with cells in which both SATB1 and GATA3 were simultaneously knocked down (Figure 7D; Table 8). These results suggest that GATA3 mediates the up-regulation of IL-5 in the absence of SATB1; thus, SATB1 is presumably required to block GATA3-induced expression of IL-5 during the early Th2 cell differentiation.

Discussion

Transcription factors play a key role in driving cell differentiation, although the regulation of chromatin structure is required to maintain these changes. Here we have provided evidence that SATB1, a T lineage-enriched transcriptional regulator and chromatin-modifying factor, is involved in the regulation of more than 300 genes in primary human CD4$^+$ cells, including several factors regulated by IL-4 and/or IL-12 during the early Th cell differentiation. We have shown that SATB1 is positively regulated in Th2 lineage by STAT6, which is crucial for many IL-4–mediated effects, including Th2 differentiation and IgE response.[39-41] Furthermore, we have demonstrated that SATB1 represses the canonical Th2 cytokine gene *IL-5* by directly binding to its promoter and thereby possibly blocking the reciprocal regulation mediated by GATA3.

SATB1-regulated genes have previously been studied using SATB1 null mouse, human cancer cell lines, as well as normal and immortalized human mammary epithelial cells.[1,4,42] However, this study is the first report on SATB1 target genes in primary human CD4$^+$ cells on the whole genome level. Our results indicate an importance of SATB1, especially in Th2 differentiating cells, because SATB1 is regulated by STAT6, and more than half of direct SATB1 target genes are regulated by IL-4 during Th2 polarization. However, SATB1 also regulates genes in cells cultured under Th0 and Th1 conditions. The post-translational modification status of SATB1 may vary in different Th subtypes affecting the transcriptional role of SATB1.[4] The regulation of SATB1 by TCR and Th1/Th2-polarizing cytokines requires further investigation.

One of the most highly induced genes in Th2-polarizing cells on SATB1 knockdown is the Th2 hallmark cytokine, *IL-5*. IL-5 is not expressed during Th2 polarization but induced in fully differentiated effector Th2 cells on restimulation.[43] Notably, in vivo up-regulation of IL-5 during the priming of CD4$^+$ cells in lymph nodes or soon after that could lead to aberrant recruitment and activation of eosinophils and development of allergic disease or asthma.[20,21,44] The positive regulation of *IL-5* by SATB1 on activation in a mouse Th2 cell clone has been previously reported.[7] The contrasting

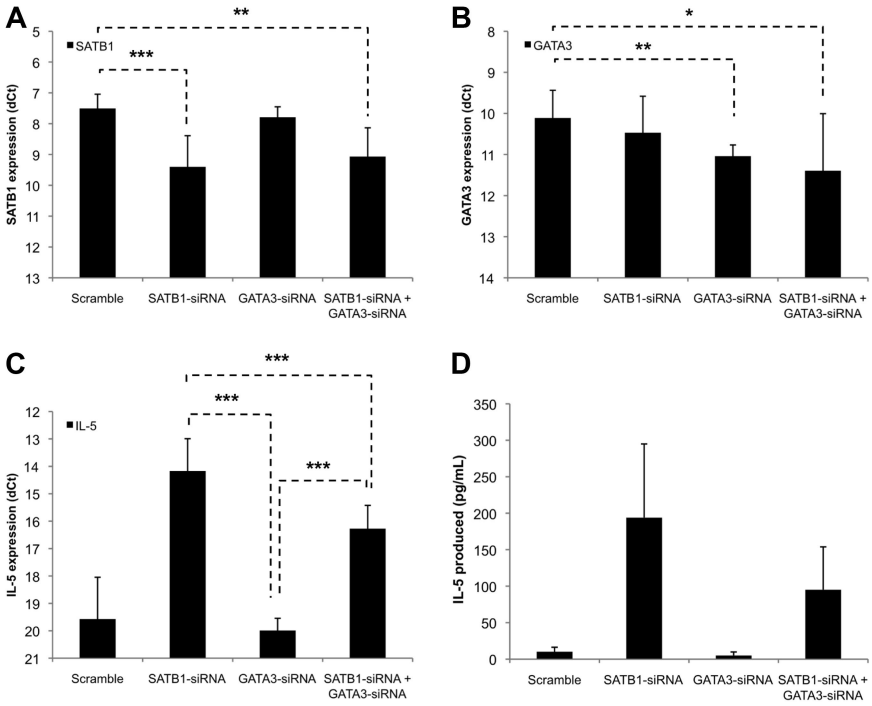

Figure 7. Induction of IL-5 in SATB1-silenced Th2-polarizing cells is GATA3-dependent. Cord blood $CD4^+$ T cells were nucleofected with siRNA oligonucleotides targeting SATB1 or GATA3 (1.5 μg specific siRNA and 1.5 μg scrambled control siRNA) or both (1.5 μg each siRNA) or only scrambled control siRNA (3 μg) and cells were cultured in Th2-polarizing conditions. (A-C) Samples of the 24-hour time point from 6 independent experiments were analyzed using quantitative RT-PCR. The normalized expression (dCt) of SATB1 (A), GATA3 (B), and IL-5 (C) mRNA of specific siRNA-treated and scrambled control siRNA nucleofected cells is presented. (D) The culture media of the 48-hour time point was measured for secreted IL-5 using Bio-Plex assay and is expressed in picograms per milliliter. Data are mean ± SD of 3 independent experiments. $^*P < .05$. $^{**}P < .01$. $^{***}P < .005$.

results regarding the regulation of *IL-5* in the Cai et al[7] study compared with ours may be explained by the difference in the polarization status of the cells (naive/early differentiating vs effector/memory $CD4^+$ cells) and by the origin of the cells (human vs mouse). Notably, similar dual function in cytokine gene control has recently been shown for IRF4, which regulated Th2 cytokine production, especially IL-4, differentially in naive $CD4^+$ cells compared with effector/memory $CD4^+$ cells.[45]

The 5′ flanking region of the human *IL-5* gene has been reported to interact with many transcription factors, including GATA3, NFAT, YY1, and glucocorticoid receptor, which play important roles in *IL-5* expression.[35,37,46] Our in vitro binding assay demonstrated that SATB1 binds 4 sites on the human *IL-5* promoter. The SBSs S1 and S2 map to previously identified negative regulatory elements,[47,48] whereas S4 is located in a region shown to function as a positive element.[47] S3 does not have a previous annotation. Our data indicate that S1 and S2 are repressive sites, which is concurrent with the previous observations, whereas S3 is a strongly activating site providing new information on the regulatory elements in *IL-5* promoter. However, the specific use of SBSs at various developmental or functional cell states needs further investigation. In addition, the *IL-5* promoter might harbor few other SBSs upstream of the region we have studied as SATB1-siRNA greatly increases *IL-5* mRNA expression, whereas deletion of all 4 SBSs did not increase IL-5 reporter activity by the same amount. Nevertheless, this study demonstrates a direct role of SATB1 in IL-5 expression.

Table 8. Summary of statistics for Figure 7D

Comparison	Fold change	SD	*P*
siSATB1 vs Scr	19.7	1.5	.002
siGATA3 vs Scr	0.4	0.2	.028
siSATB1 + siGATA3 vs Scr	9.3	1.8	.015
siSATB1 vs siGATA3	52.2	20.1	.003
siSATB1 + siGATA3 vs siGATA3	24.2	10.0	.013
siSATB1 + siGATA3 vs siSATB1	2.2	0.5	.017

Interestingly, 1 of the repressive SBSs (S1) juxtaposes an activating GATA3 site (−152 bp) in the *IL-5* promoter, which led us to study the reciprocal role of SATB1 and GATA3 in the regulation of *IL-5*. SATB1 and GATA3-binding sites are colocalized at the 3′ end of the human *CD8B* gene, a region suggested to regulate CD8 expression,[49] and in activated mouse Th2 clone cells SATB1 and GATA3 proteins colocalize.[7] However, any competition between SATB1 and GATA3 has not been previously observed. Our results indicated that the *IL-5* transcription was markedly less induced in cells where both SATB1 and GATA3 were knocked down than in cells where only SATB1 had been down-regulated. These findings led us to propose a competitive SATB1/GATA3-mediated regulatory mechanism for the control of *IL-5* transcription where SATB1 blocks binding or function of GATA3 and thereby represses *IL-5* expression during normal Th2 differentiation, whereas in SATB1-siRNA cells GATA3 induces aberrant IL-5 expression. A complete knockdown or overexpression system that does not induce apoptosis over a prolonged time period would enable further dissection of the specific roles of GATA3 and SATB1.

The mechanism how SATB1 represses *IL-5* expression may also involve recruitment of histone-modifying factors and change(s) in the histone modification status of the locus. Histone hyperacetylation of the *IL-5* gene in $CD4^+$ cells is Th2-specific and occurs in a STAT6- and GATA3-dependent manner,[50] whereas the overexpression of HDAC1 has been demonstrated to repress *IL-5*.[37] Because SATB1 has been shown to recruit HDAC1 in vivo to the regulatory sites causing a suppression of gene expression,[4,32] it is plausible that a similar mechanism may operate during repression of *IL-5* by SATB1 during early Th2 differentiation as supported by the occupancy of *IL-5* promoter by both SATB1 and HDAC1. Indeed, on SATB1 knockdown, the occupancy of HDAC1 at the *IL-5* promoter also decreased, suggesting an acetylation-dependent mechanism for up-regulation of *IL-5*. Moreover, a recent study has demonstrated that SATB1 regulates multiple Wnt target genes in thymocytes and differentiating Th2 cells by recruiting histone-modifying factors to its binding sites on upstream regulatory regions of these genes.[51]

In conclusion, in light of our findings, we propose that the transcriptional regulator and chromatin organizer SATB1 plays an important role in Th cell lineage decision because it coordinately regulates several IL-4 target genes involved in Th2 differentiation and function. Our results suggest that a competitive mechanism involving SATB1 and GATA3 regulates *IL-5* transcription, and provide new mechanistic insights into the stringent regulation of IL-5 expression during human Th2 differentiation.

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Authorship

Contribution: H.A., S.G., and R.L. designed the research and wrote the paper; H.A., A.L., S.T., M.B., K.G., and D.N. performed experiments; H.A. and S.G. analyzed results and made figures; H.A., A.L., S.T., M.B., S.G., and R.L. interpreted data; L.L.E., O.R., and S.G. provided expertise and guidance; and S.G. and R.L. contributed reagents.

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