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IFN-α Suppresses GATA3 Transcription from a Distal Exon and Promotes H3K27 Trimethylation of the CNS-1 Enhancer in Human Th2 Cells

Jonathan P. Huber,*1,2 Sarah R. Gonzales-van Horn,*1 Kole T. Roybal,* Michelle A. Gill,† and J. David Farrar*

CD4+ Th2 development is regulated by the zinc finger transcription factor GATA3. Once induced by acute priming signals, such as IL-4, GATA3 poises the Th2 cytokine locus for rapid activation and establishes a positive-feedback loop that maintains elevated GATA3 expression. Type 1 IFN (IFN-α/β) inhibits Th2 cells by blocking the expression of GATA3 during Th2 development and in fully committed Th2 cells. In this study, we uncovered a unique mechanism by which IFN-α/β signaling represses the GATA3 gene in human Th2 cells. IFN-α/β suppressed expression of GATA3 mRNA that was transcribed from an alternative distal upstream exon (1A). This suppression was not mediated through DNA methylation, but rather by histone modifications localized to a conserved noncoding sequence (CNS-1) upstream of exon 1A. IFN-α/β treatment led to a closed conformation of CNS-1, as assessed by DNase I hypersensitivity, along with enhanced accumulation of H3K27me3 mark at this CNS region, which correlated with increased density of total nucleosomes at this putative enhancer. Consequently, accessibility of CNS-1 to GATA3 DNA binding activity was reduced in response to IFN-α/β signaling, even in the presence of IL-4. Thus, IFN-α/β disrupts the GATA3-autoactivation loop and promotes epigenetic silencing of a Th2-specific regulatory region within the GATA3 gene. The Journal of Immunology, 2014, 192: 5687–5694.

A critical transcriptional regulator, GATA3, is involved in a variety of cellular-differentiation pathways. In the immune system, GATA3 is required for hematopoiesis, thymic development, and peripheral T cell effector functions (1). GATA3 is a critical regulator of the Th2 phenotype, and its elevated expression in T cells is required for both Th2 development and for maintaining the stability of Th2 memory cells (2–4). Although GATA3 is expressed at basal levels in naïve T cells, modest increases in GATA3 protein levels can promote Th2 commitment, even under a variety of conditions that drive other phenotypes (5). Moreover, early studies by Murphy and colleagues (3) demonstrated that ectopic expression of GATA3 via retroviral transduction led to the induction of GATA3 mRNA encoded by the endogenous gene. These data suggested a mechanism whereby GATA3 autoactivation could not only drive Th2 development but also maintain the Th2 phenotype in the absence of further acute developmental signals, such as IL-4 (6). Formal proof for the requirement of GATA3 in maintaining the Th2 program was demonstrated by deleting GATA3 in fully committed mouse and human Th2 cells (7, 8). Thus, GATA3 plays a dominant role in maintaining the stability of Th2 cells, and any pathway that suppresses its expression would be predicted to inhibit Th2 functions.

Recently, we (9) and other investigators (10) demonstrated that, unlike IL-12 or other innate cytokines, type 1 IFN (IFN-α/β) blocked IL-4–mediated Th2 development in human T cells and destabilized the Th2 phenotype by suppressing IL-4, IL-5, and IL-13 secretion. However, this effect was not observed in murine T cells (9, 11). Further, we found that the inhibition was mediated by suppressing GATA3 expression during Th2 development and in committed Th2 cells. In this study, we found that IFN-α/β suppressed GATA3 by selectively targeting the expression of the GATA3 gene at an alternative upstream exon (1A) used in response to IL-4 during Th2 commitment. The repression of exon 1A correlated with a condensed chromatin conformation of a conserved noncoding sequence 1 (CNS-1) region located 5 kb upstream of the alternative exon. Thus, epigenetic silencing of a putative enhancer of the Th2-specific GATA-3 exon 1A promoter is a potential target for the induction of tolerance in atopic Th2 cells.

Material and Methods

Human subjects

Peripheral blood was obtained from healthy adults by venipuncture. Informed consent was obtained from each donor in accordance with guidelines established by the Institutional Review Board at UT Southwestern Medical Center.

Cell culture and reagents

Human naive T cells (CD4+CD45RA+) were purified (≥90%) from buffy coats either by flow cytometric sorting or by magnetic bead separation. Cells were activated with plate-bound anti-CD3 (OKT3, 3 μg/ml), anti-CD28 (3 μg/ml), and IL-2 (50 U/ml) in complete IMDM supplemented...
with 10% FBS under the following polarizing conditions: neutralized (anti–IL-4, 2 μg/ml; anti–IL-12, 5 μg/ml; anti–IFN-γ, 5 μg/ml; anti–IFNAR2, 2 μg/ml), IL-4 (IL-4, 20 ng/ml; anti–IL-12, 5 μg/ml; anti–IFN-γ, 5 μg/ml; anti–IFN-α, 5 μg/ml; anti–IFNAR2, 2 μg/ml), IFN-α (IFN-α, 1000 U/ml; anti–IL-4, 2 μg/ml; anti–IL-12, 5 μg/ml; anti–IFN-γ, 5 μg/ml), and IL-4 + IFN-α (IL-4, 20 ng/ml; IFN-α, 1000 U/ml; anti–IL-12, 5 μg/ml; anti–IFN-γ, 5 μg/ml). In some experiments, MG132 (50 μM) and 5-Aza-2′-deoxycytidine (1 μM) were used as inhibitors. Cells were cultured for 3, 5, or 7 d prior to being used for analysis.

**Flow cytometry**

Intracellular cytokine staining was performed as previously described (12). Cell proliferation was assessed using CFSE at day 5. For live cell sorting of CFSE-labeled cells for mRNA analysis on day 5, CFSE-labeled cells were washed and sorted on a MoFlo or a FACSAria cell sorter. RNA was harvested and used for quantitative PCR (qPCR) analysis.

**Fluorescence microscopy**

Cells were adhered to coverslips, fixed with 4% paraformaldehyde, and stained with anti-human CD4-PER and anti-human GATA3–Alexa Fluor 647. Coverslips were mounted onto slides with Prolong Gold + DAPI (Invitrogen). Fluorescence microscopy was performed on a DeltaVision deconvolution microscope, and images were prepared using ImageJ software (National Institutes of Health).

**Quantitative PCR**

qPCR was performed as described (13). Human PPIA was used as a reference gene. Relative changes in mRNA expression were calculated by the 2^(-ΔΔCt) method. All treatments were referenced to the neutralized control. Primers can be found in Supplemental Table I.

**EMSA**

Clarified nuclear lysate was incubated with 3′-biotin–labeled dsDNA probes, resolved on a 4.5% nondenaturing polyacrylamide gel, and transferred to nylon membranes. Complexes were detected with streptavidin–HRP with chemiluminescence. Oligo sequences can be found in Supplemental Table I.

**Chromatin immunoprecipitation**

Cells were fixed with 1% formaldehyde, and nuclear DNA was sheared by sonication. Precleared lysates were incubated overnight with 1–4 μg Ab, followed by incubation with protein A/G beads. Cross-links were reversed from eluted protein/DNA complexes, and remaining protein was degraded with Proteinase K. Purified DNA was used for qPCR analysis; primer sequences can be found in Supplemental Table I. Chromatin immunoprecipitation (ChIP) efficiency was calculated using the following formula: 2^(-ΔΔCt) × dilution factor. All primer and probe sequences are listed in Supplemental Table I.

**DNase I hypersensitivity analysis**

In vitro–primed human CD4+ T cells were harvested on day 5 of culture and washed in cold PBS. Nuclei were permeabilized with 0.3% Nonidet P-40 in Buffer A (15 mM Tris-HCl [pH 8], 15 mM NaCl, 60 mM KCl, 1 mM EDTA, 0.5 mM EGTA, 0.5 mM spermidine, and 0.15 mM spermine) for 10 min on ice, rinsed once, and incubated with DNase I (0–120 U/ml; Worthington) at 37°C for 5 min. The reactions were stopped with the addition of Stop Buffer (50 mM Tris-HCl [pH 8], 100 mM NaCl, 1% SDS, 100 mM EDTA) supplemented with RNase A. Nuclei were digested with proteinase K at 55°C for 1 h, followed by DNA extraction. Semi-quantitative PCR analysis of incrementally tiled segments spanning across GATA3 CNS-1 through exon 1A were performed; the primer sequences for each segment are listed in Supplemental Table I. DNA band intensities were quantified by ImageJ software, and relative DNase hypersensitivity was calculated by comparing the slope of DNA decay between cytokine-treated and neutralized samples. qPCR of specific regions was performed as described above with the primers listed in Supplemental Table I. The slope of the line formed by the reduction in Ct value as a function of DNase I concentration was calculated, and relative DNase I hypersensitivity was calculated as a ratio of slopes. Data for each sample were referenced to the neutralized control.

**Statistical analysis**

All data are shown as mean ± SEM. Statistical analysis was performed by one-way or two-way ANOVA with GraphPad Prism software. The p values < 0.05 were considered significant.

**Results**

**Inhibition of GATA-3 expression by IFN-α/β**

The GATA3 transcription factor is expressed during all stages of thymic development and remains expressed constitutively in resting peripheral naive CD4+ T cells. Cells are prevented from committing to the Th2 lineage as a result of the stoichiometric expression of the ROG-1/FOG-1 repressors (14, 15), which prevent GATA3 from driving differentiation. However, in response to IL-4, GATA3 levels are elevated sufficiently to overcome this repression. We recently demonstrated that IFN-α/β blocked the induction of GATA3 in response to IL-4 (9). This effect was confirmed in human CD4+CD45RA+ T cells differentiated in response to IL-4 in the absence or presence of IFN-α (Fig. 1A,
IL-4 increased GATA3 protein levels <2-fold, which was completely suppressed by treatment with IFN-α (Fig. 1A). Furthermore, this suppression was seen at the RNA level, where as little as 100 U/ml of IFN-α significantly reduced IL-4-driven GATA3 induction (Fig. 1B).

During the early phases of innate priming, T cells divide rapidly in response to TCR and costimulatory signals. Innate cytokines drive their differentiation into effector cells, which occurs progressively at each cell division (4, 16–19). During Th2 development, the ability to secrete IL-4 increases incrementally in daughter cells as they divide in the presence of IL-4. Because IFN-α inhibits the Th2 differentiation process by suppressing GATA3 (9), we wished to determine whether IL-4–driven GATA3 expression was inhibited by IFN-α by slowing the progression of cell division or by direct repression of GATA3 in daughter cells. To test this, human CD4+/CD45RA+ cells were activated with anti-CD3/anti-CD28 in the presence or absence of IL-4, IFN-α, or IL-4 + IFN-α, and GATA3 protein levels were quantified as a function of CFSE dilution (Fig. 1C). At day 5 of activation, a similar proportion of divided cells at each division was observed, regardless of the innate cytokine priming condition imposed at the beginning of the culture. GATA3 expression was enhanced incrementally at each cell division (Fig. 1D), and IFN-α completely inhibited the induction of GATA3 protein by IL-4. The inhibition of GATA3 by IFN-α correlated with a reduction in the percentage of cells that secreted IL-4 upon restimulation (Fig. 1E). Thus, IFN-α inhibited the induction of GATA3 by IL-4 without significantly altering cellular expansion in response to TCR stimulation.

Because IL-4 signaling establishes a positive-feedback loop of GATA3 autoactivation (3, 4), there are several steps at which IFN-α could block this pathway. For example, IFN-α could suppress the initial induction of GATA3 by downregulating IL-4R or by interfering with STAT6 activation (20, 21). However, our recent studies ruled out this possibility (9), and our observations that IFN-α can repress GATA3 expression in fully committed Th2 cells make this scenario unlikely. Further downstream, IFN-α could disrupt GATA3 autoactivation through a variety of posttranslational mechanisms. First, GATA3 nuclear localization was examined by confocal microscopy in cells differentiated in vitro with IL-4 or IFN-α (Fig. 2A). Although IFN-α decreased the total staining intensity of GATA3, all of the detectable GATA3 was colocalized with DAPI staining under all cytokine conditions, suggesting that IFN-α did not block GATA3 autoactivation by excluding GATA3 from the nucleus. Second, IFN-α could lead to proteasomal degradation of GATA3 protein. However, treatment of cells with the general proteasome inhibitor, MG132, failed to reverse the suppressive effects of IFN-α (Fig. 2B). Other posttranslational modifications could disrupt the ability of GATA3 to bind DNA and regulate transcription. We tested the ability of IFN-α to alter GATA3 DNA-binding activity by both EMSA and ChIP. First, IL-4 significantly enhanced binding of GATA3 to a consensus GATA3 target sequence, which was completely inhibited by IFN-α (Fig. 2C, 2D). Of note, the enhancement of GATA3 DNA-binding activity by IL-4 was greater in magnitude (>3-fold) than what can be accounted for by a modest (<1.5-fold) induction of total GATA3 protein, perhaps suggesting mechanisms of regulation in addition to simple increases in protein content. Further, the inhibition of GATA3 binding activity by IFN-α was paralleled by a reduction in GATA3 bound to the canonical GATA3 site found within the first intron of the IL-4 gene (Fig. 2E). Collectively, these data rule out the possibility that IFN-α blocks GATA3 expression either by preventing nuclear import or by proteasomal degradation. However, the reduction in GATA3 DNA-binding activity could be due to overall reductions in GATA3 protein content, which may be regulated transcriptionally rather than at the posttranslational level.
**FIGURE 3.** Progressive expression of GATA3 exon 1A and inhibition by IFN-α/β during cell division. (A) GATA3 mRNA transcription can proceed from two alternative first exons, 1A and 1B, each controlled by separate promoters. Arrows indicate the direction of primers to distinguish 1A from 1B by qPCR. The CNS-1 site is positioned 5 kb upstream of exon 1A. (B) Purified human CD4+/CD45RA+ cells were activated for 3 d with plate-bound anti-CD3/anti-CD28 with the indicated cytokine conditions. GATA3 mRNA transcripts were quantified by qPCR with primers that measured total GATA3 spanning exons 5–6 (top panel) and with primers that distinguished exons 1A (middle panel) from 1B (bottom panel). (C) CFSE-labeled human CD4+/CD45RA+ cells were activated as above for 5 d, and individual cell divisions were sorted based on the gates shown (top panel). GATA3 mRNA transcripts derived from exon 1A (middle panel) and exon 1B (bottom panel) were quantified by qPCR and expressed relative to the neutralized Div 0 population. (D) Purified human CD4+/CD45RA+ cells were activated with plate-bound anti-CD3/anti-CD28 for 7 d under the indicated cytokine conditions for primary activation (1°). Cells were then washed and restimulated for an additional 7 d with the cytokine conditions indicated for secondary activation (2°). GATA3 exon 1A and 1B transcripts were quantified by qPCR.

**IFN-α/β–mediated transcriptional repression of an alternative GATA-3 distal exon**

The GATA3 gene contains two independently regulated first exons (22, 23), denoted 1A and 1B (Fig. 3A). These exons are separated by ∼10 kb; when transcribed, each first exon is spliced to exon 2, which contains the initiator codon. Splicing of exon 1A with 1B has not been detected in any cell type, which may indicate that the two exons operate as distinct transcriptional units. In support of this, previous studies identified exon 1A to be selectively induced by IL-4 in murine peripheral CD4+ T cells, leading to Th2 development (22). We tested whether induction of GATA3 exon 1A was selectively induced by IL-4 in human T cells. Indeed, IL-4 increased total GATA3 mRNA content, as assessed by qPCR with primers that spanned across exons 5 and 6 (Fig. 3B, top panel). As previously demonstrated, IFN-α inhibited total GATA3 mRNA, which correlated with the decrease in GATA3 protein content (Fig. 3B, top panel). Further, IL-4 selectively induced exon 1A, but not 1B, and this induction was also blocked by IFN-α (Fig. 3B, middle and bottom panels). In contrast, exon 1B was not affected by any cytokine condition and may be the main exon that contributes to constitutive low expression of GATA3 in naive T cells.

We further assessed GATA3 mRNA expression in dividing populations of cells undergoing differentiation in response to IL-4 or IFN-α (19, 24). Cells were labeled with CFSE, activated with anti-CD3/anti-CD28 for 5 d, and then purified by sorting based on CFSE dilution, as indicated by the gates in Fig. 3C. In parallel with the expression of GATA3 protein (Fig. 1A, 1C, 1D), we found that GATA3 exon 1A was selectively induced by IL-4 at each incremental cell division. Exon 1B was not altered by cell division or by cytokines. In addition, we analyzed exon 1A and 1B expression in committed Th2 cells. Once induced by IL-4, GATA3 autoactivation stabilizes Th2 cells by uncoupling their phenotype from the initial signals that drove their development. Further, our previous studies demonstrated that IFN-α could suppress GATA3 expression in fully committed Th2 cells, thus disrupting the overall Th2 program (9). As shown in Fig. 3D, IL-4 induced the expression of GATA3 exon 1A, which remained elevated in subsequent rounds of stimulation regardless of whether IL-4 was neutralized in the second week. However, in agreement with our previous findings, IFN-α suppressed GATA3 exon 1A expression in committed Th2 cells down to levels observed in cells that were activated under neutralizing conditions. In summary, we found that the induction of GATA3 mRNA by IL-4 was regulated exclusively at exon 1A, increased as a function of cell division, and was stabilized in differentiated Th2 cells. Importantly, this regulation was blocked by IFN-α, which even suppressed GATA3 exon 1A expression in committed Th2 cells.

**Epigenetic modification of GATA-3 CNS-1 in human Th2 cells**

Located upstream of exon 1A are several conserved noncoding sequences that have been identified previously by VISTA analysis (23). The most proximal CNS-1 region is positioned 5 kb upstream of exon 1A (Fig. 4A) and is of particular interest because it contains multiple GATA3 consensus binding sites, which may be targets for GATA3 autoactivation. We wished to determine how this genomic region was being regulated both positively by IL-4 and negatively by IFN-α/β. We assessed DNase I hypersensitivity, as well as various primary chromatin modifications that directly impact transcription, including DNA methylation and histone acetylation/methylation. First, given that local increases in DNA methylation patterns often correspond with reductions in transcriptional activity, we proposed that CNS-1 could be a target of IFN-α–mediated recruitment of DNA methyltransferases (25). However, the general DNA methylation inhibitor 5-Azacytidine did not prevent IFN-α from markedly suppressing GATA-3 exon 1A expression (Supplemental Fig. 1), suggesting that IFN-α was able to suppress GATA-3 mRNA expression independently of the DNA methylation status of the cells or the GATA-3 locus specifically.

Local chromatin compaction at both promoters and enhancers can significantly alter transcription rates. Such regions can be
FIGURE 4. IFN-α/β signaling selectively decreases DNase I relative hypersensitivity at the GATA3 CNS-1 region and the exon 1A transcriptional start site. (A) Purified human CD4+/CD45RA+ cells were activated with plate-bound anti-CD3/anti-CD28 for 5 d under the indicated cytokine conditions. Cells were permeabilized and incubated with increasing concentrations of DNase I. DNA was purified, and semiquantitative PCR analyses were performed with primers spanning 10 overlapping tiled intervals across the CNS-1/exon 1A region (gray bars) (Supplemental Table I). Amplicons were resolved by gel electrophoresis (Supplemental Fig. 2), and band intensities were quantified by densitometry using ImageJ software. The slope of the line formed by the reduction in amplon as a function of DNase I concentration was calculated, and relative DNase I hypersensitivity was calculated as a ratio of slopes of neutralized versus cytokine treatment. (B) Quantitative DNase I hypersensitivity analysis of CNS-1 region was performed as described above from four healthy adult donors. Samples 4a and 4b are separate experiments performed on cells from the same donor. Relative hypersensitivity is referenced to the neutralized control of each donor/experiment. (C) Averaged data from (B) are expressed relative to the neutralized control. *p = 0.05.

qualitatively compared by their accessibility to digestion with DNase I. As a first approach, we assessed relative DNase I hypersensitivity across the entire CNS-1/exon 1A region of the GATA3 gene as a function of cytokine treatment. Primary naive human CD4+ T cells were differentiated in vitro in the presence of IL-4 or IL-4 + IFN-α and compared with cells differentiated under neutralizing conditions. DNase I hypersensitivity analysis was performed on these cells by interrogating tiled intervals across the CNS-1/exon 1A region by semiquantitative PCR analysis (Fig. 4A, Supplemental Fig. 2). We identified two regions in which relative DNase I hypersensitivity was reduced in the presence of IFN-γ. The first region mapped across tiling interval 1, which spanned the entirety of CNS-1. The second region spanned intervals 9–10, corresponding to the transcriptional start site and first intron of exon 1A (Fig. 1A). We did not observe any cytokine-mediated changes in DNase I hypersensitivity within the 5′ untranslated region of exon 1A spanning from the putative promoter (interval 8) through the most distal region adjacent to CNS-1 (interval 2). Because CNS-1 has been identified as a critical regulatory element of GATA3 expression in murine Th2 cells, we confirmed the DNase I hypersensitivity of CNS-1 in T cells isolated from multiple donors by qPCR assay. IL-4 enhanced the relative DNase I hypersensitivity of CNS-1 in T cells from four donors, whereas IFN-α suppressed this activity to levels comparable to the neutralized control (Fig. 4B, 4C). Thus, IFN-α promoted a closed chromatin configuration of CNS-1 and potentially at the exon 1A transcriptional start site. These data suggest a role for IFN-α/β signaling in suppressing the transcriptional accessibility of this region during human Th2 commitment.

Changes in specific histone modifications, such as acetylation and methylation, can often distinguish or predict regions of enhanced or suppressed transcriptional activity. We quantified various histone modifications by ChIP at the GATA-3 CNS-1 region, as well as local segments spanning the putative promoter region of exon 1A (Fig. 5A). For these experiments, ChIP of total histone H3, along with H3K27me3, H3K9me2, and H4Ac, was performed in naive CD4+ T cells isolated from four or five donors, depending upon the modification. The relative ChIP efficiencies for each donor were calculated and expressed as a percentage of the neutralized control. For H3K9me2 (Fig. 5A), none of the cytokine conditions led to a significant alteration in the density of these marks at any of the sites interrogated. The H4Ac modification was significantly increased at proximal promoter sites by IL-4, regardless of whether IFN-α was present during the priming, whereas this modification was not significantly altered at CNS-1 (Fig. 5A, bottom graph). However, the H4Ac modification pattern at the exon 1A promoter cannot explain the dominant effect of IFN-α in suppressing GATA3 expression in the presence of IL-4. In assessing repressive marks, we found that H3K27me3 was not significantly altered by cytokine activation at the exon 1A promoter. In contrast, we found that the H3K27me3 modification was significantly enriched at the CNS-1 site in response to IFN-α in both the absence and presence of IL-4, which correlates with the inhibition of exon 1A expression by IFN-α. The increased H3K27me3 modification was reflected by a significant increase in the total density of H3 in response to IFN-α at CNS-1 but not the exon 1A promoter (Fig. 5A, upper graph). Thus, the repressive marks at CNS-1 correlate with the reduction in DNase I hypersensitivity at this region, implicating IFN-α/β signaling in blocking this potential enhancer element.

The CNS-1 region contains at least eight conserved GATA3 consensus binding sites (Fig. 5B, upper diagram), which are potential targets for GATA3 autoactivation. Because IFN-α/β led to a closed confirmation of this region (shown above), we wished to determine whether this site was bound by GATA3 and, if so, whether this binding was blocked by IFN-α/β treatment. To test this, GATA3 ChIP was performed at CNS-1 in human naive CD4+ T cells differentiated in the presence of IL-4 or IFN-α. IL-4 significantly increased GATA3 binding to both segments of the CNS-1 region spanning 650 bp, both of which contained consensus GATA3-binding sequences (Fig. 5B). Further, IFN-α inhibited IL-4-driven binding of GATA3 to CNS-1 in T cells from both donors. We also assessed GATA-3 binding at the exon 1A proximal promoter but were unable to detect any significant GATA-3 binding activity under any condition. Thus, the CNS-1 site may contribute to GATA3 exon 1A regulation by acting as an enhancer for GATA3 autoactivation. Further, IFN-α/β represses this activity, which correlates with epigenetic silencing of CNS-1, even in the presence of IL-4.

In summary, we found that DNA binding by GATA3 at the CNS-1 region increased in response to IL-4 but was inhibited by IFN-α. This activity coincided with enrichment of the suppressive H3K27me3 mark selectively at the CNS-1 site but not the exon 1A promoter. The reduction in DNase I hypersensitivity, the increase in the H3K27me3 mark, and, consequently, the increase in total H3 content selectively at CNS-1 demonstrate that this area becomes more compact and inaccessible in response to IFN-α.
signaling. Collectively, these data highlight the selective repressive activity of IFN-α on GATA3 exon 1A transcription in human CD4+ T cells (Fig. 6).

Discussion

In mouse CD4+ T cells, various external stimuli, such as IL-4 and Notch, can promote elevated expression of GATA3, which is selectively encoded from the alternative exon 1A within the GATA3 locus (22, 23, 26). To our knowledge, the present study is the first to demonstrate that IL-4 preferentially induces GATA3 exon 1A expression during human Th2 development. Furthermore, we show that the exon 1A transcript, but not the exon 1B transcript, remains elevated in Th2 cells in the absence of further IL-4 signaling, suggesting that GATA3 feedback in human Th2 cells preferentially maintains expression of the exon 1A transcript. Moreover, we identified the CNS-1 region as a potential GATA3 autoregulatory enhancer element that was bound by GATA3 in response to IL-4. Finally, we uncovered an IFN-α/β-dependent mechanism that suppressed Th2 development and stability by disrupting the GATA3-autoactivation loop. We systematically ruled out defects in nuclear localization or proteasome-mediated degradation while finding that overall GATA3 DNA binding activity was reduced. However, a reduction in GATA3 DNA binding activity could be due to reduced levels of GATA3 present in the nucleus of IFN-α-treated cells. Alternatively, IFN-α signaling could alter the function of the GATA3 protein by inhibiting the ability of GATA3 to regulate its own expression. However, this possibility is somewhat unlikely, because IFN-α also blocks the induction of GATA3 expression by IL-4 prior to the establishment of the autoactivation loop. Finally, IFN-α could inhibit transcriptional activation of the GATA3 gene, which is supported by our data demonstrating a selective block in GATA3 exon 1A expression both in response to IL-4 and in fully committed Th2 cells.

IFN-α/β is a potent inducer of hundreds of IFN-sensitive genes that regulate the antiviral response, but very few genes are actually suppressed by IFN-α/β signaling. Of the few select genes that are
repressed by IFN-α/β, some are involved in cell cycle regulation, such as cyclins (27, 28). As such, we showed previously that IFN-α slows the progression of cell division in human CD8+ T cells, thus preventing some cells from terminally differentiating into effectors (19, 24). However, in this study, we found that IFN-α did not have this effect on cells in the naive CD4+ T cell compartment. Rather, IFN-α suppressed GATA3 expression without significantly altering TCR-mediated proliferation, thus blocking IL-4–driven terminal differentiation of Th2 cells. Although cell division is not a “clock” that strictly controls cytokine production (18), S phase offers the best opportunity to modify local chromatin architecture and alter its accessibility. In this regard, we observed a marked loss in DNase I hypersensitivity, which correlated with a significant increase in the total density of histone H3 specifically at CNS-1 in response to IFN-α. There are three forms of histone H3 (H3.1, H3.2, and H3.3), which are encoded by distinct genes. Although our study design did not distinguish between these H3 variants, recent reports suggested that H3.3, in particular, can be deposited at nucleosome-depleted gaps in chromatin (29). H3.3 is usually associated with areas of active transcription, but it also has been found in repressed and poised regions (30, 31). Furthermore, changes in nucleosome density have been reported as a mechanism that controls epigenetic modifications of the histones (32), suggesting that nucleosome density could be regulated through a mechanism distinct from direct modification of the histones. In most cases, IFN-α/β signaling generally promotes transcription rather than repressing it, which is accounted for by specific deposition of histones H3.3 at IFN-sensitive genes (33). However, we find that IFN-α/β selectively repressed expression of GATA3 in a manner that may be distinct from the modes of transcriptional regulation of IFN-sensitive genes.

Based on our current findings, we propose a unique model of GATA3 regulation that accounts for both the induction by IL-4 and repression by IFN-α/β (Fig. 6). The region encompassing CNS-1 displays different epigenetic patterns in response to cytokine activation than do the regions more proximal to the exon 1A promoter. The permissive mark H4Ac is increased by IL-4 but reduced by IFN-α in the absence of IL-4. Although IL-4 increases H4Ac even in the presence of IFN-α at the exon 1A promoter, this does not occur at CNS-1. Furthermore, IFN-α reduced H4Ac levels below baseline near exon 1A but not at CNS-1. This is in stark contrast to the repressive H3K27 mark, in which IFN-α treatment, even in the presence of IL-4, increased H3K27me3 at CNS-1 but induced no significant changes at the exon 1A promoter. That IL-4 signaling does not result in significant changes in permissive marks at CNS-1 is even more surprising considering that this region is bound by GATA3. GATA3 can complex with trithorax group proteins that normally increase chromatin accessibility (34, 35). As such, GATA3 binding to CNS-1 would be predicted to increase permissive marks on the histones and reduce nucleosomal density. However, IL-4 treatment only marginally increased DNase I hypersensitivity and did not reduce H3 density. Thus, the cause–effect relationship between GATA3 binding and H3K27me3 marking is still unclear. In summary, the IL-4–driven permissive H4Ac mark at the exon 1A promoter does not obviate the repressive effects of IFN-α that are imposed by the H3K27me3-silencing mark at CNS-1.

IFN-α/β signaling could inhibit exon 1A expression and promote chromatin modifications within the CNS-1 region by either direct or indirect mechanisms. Perhaps the most linear pathway would involve direct recruitment of STAT2 to the GATA3 locus, which would place STAT2 in the position of a transcriptional repressor. A recent report found that STAT2 could recruit the histone methyl transferase, Ezh2, which is responsible for catalyzing the H3K27 trimethyl modification (36). Alternatively, IFN-α signaling, either through STAT2 or other signaling intermediates, may induce the expression of a downstream repressor. Achieving a complete understanding of GATA3 regulation is difficult because of the complexity of the GATA3 locus. Our data suggest that epigenetic modifications to the CNS-1 region may play an important role in the suppression of GATA3 by IFN-α, and our data suggest that these repressive modifications may block GATA3 binding and autoactivation at this site. Furthermore, there are likely additional regulatory regions involved in the induction of GATA3 by IL-4 and perhaps in the counter-regulation by IFN-α. It is noteworthy that murine CD4+ T cells are completely resistant to the counter-regulatory effects of IFN-α/β to block IL-4–driven Th2 development. If the CNS-1 region regulates the suppression of GATA3 in human, but not mouse, CD4+ T cells, it is likely that small sequence differences within this region may confer species-specific specification. Alternatively, there may be unique IFN-induced genes that target CNS-1 for silencing in human T cells that are not expressed in mouse. Nonetheless, our data suggest that accessibility of CNS-1 in human CD4+ T cells is integral to IFN-α’s inhibitory effect on exon 1A, which would play an important role in driving permanent suppression of Th2 function in response to IFN-α/β.

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Disclosures

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