To better understand the control of T helper (T<sub>H</sub>) 1-expressed genes, we compared and contrasted acetylation and expression for three key genes, IFNG, TBET, and IL18RAP and found them to be distinctly regulated. The TBET and the IFNG genes, but not the IL18RAP gene, showed preferential acetylation of histones H3 and H4 during T<sub>H1</sub> differentiation. Analysis of acetylation of specific histone residues revealed that H3(Lys-9), H4(Lys-8), and H4(Lys-12) were preferentially modified in T<sub>H1</sub> cells, suggesting a possible contribution of acetylation of these residues for induction of these genes. On the other hand, the acetylation of IL18RAP gene occurred both in T<sub>H1</sub> and T<sub>H2</sub> cells with the similar kinetics and on the same residues, demonstrating that selective histone acetylation was not universally the case for all T<sub>H1</sub>-expressed genes. H3(Lys-9) and H4 acetylation of IFNG and TBET genes occurred with different kinetics, however, and was distinctly regulated by cytokines. Interleukin (IL)-12 and IL-18 enhanced the histone acetylation of the IFNG gene. By contrast, histone acetylation of the TBET gene was markedly suppressed by IL-4, whereas IL-12 and IL-18 had only modest effects suggesting that histone acetylation during T<sub>H1</sub> differentiation is a process that is regulated by various factors at multiple levels. By treating Th2 cells with a histone deacetylase inhibitor, we restored histone acetylation of the IFNG and TBET genes, but it did not fully restore their expression in T<sub>H2</sub> cells, again suggesting that histone acetylation explains one but not all the aspects of T<sub>H1</sub>-specific gene expression.

A critical aspect of the adaptive immune response is that naive CD4<sup>+</sup> T cells can develop into distinct T helper (T<sub>H</sub>)<sup>1</sup> subsets depending on pathogens they encounter, and appropriate T<sub>H</sub> differentiation is essential for elimination of these pathogens (1). That is, T<sub>H1</sub> cells are involved in cell-mediated immunity and critical in protection against intracellular pathogens, whereas T<sub>H2</sub> cells are essential for a humoral immune response and elimination of extracellular microorganisms. T<sub>H1</sub> and T<sub>H2</sub> cells are principally characterized by the cytokines they secrete with T<sub>H1</sub> cells producing interferon (IFN)-γ, tumor necrosis factor-α, and lymphotixin, which activate macrophages and T<sub>H2</sub> cells producing interleukin (IL)-4, IL-5, IL-10, and IL-13, which promote B cell immunoglobulin class switching (2).

A number of factors, including signals emanating from the T cell receptor are involved in the regulation T<sub>H1</sub> and T<sub>H2</sub> differentiation, but cytokines themselves are particularly important (3–5). IL-12, produced by dendritic cells and other antigen-presenting cells, plays a central role in T<sub>H1</sub> differentiation (6). Additionally, other cytokines produced by dendritic cells, including type I IFNs, IL-23, IL-27, and even IFN-γ, also contribute to T<sub>H1</sub> differentiation (7–15). IL-18 acts synergistically with IL-12 to enhance IFN-γ production, and its receptor subunits are preferentially expressed on T<sub>H1</sub> cells (16–18). IL-12 activates signal transducer and activator of transcription 4 (Stat4), which is critical for T<sub>H1</sub> differentiation as evidenced by impaired T<sub>H1</sub> differentiation in Stat4<sup>−/−</sup> mice (19, 20). Among the defects present in Stat4<sup>−/−</sup> mice are impaired expression of IFN-γ production by CD8<sup>+</sup> cells. T-bet-deficient mice exhibit defective T<sub>H1</sub> differentiation and default T<sub>H2</sub> response and consequently develop an airway inflammation similar to asthma (22, 23). T-bet also induces expression of IL-12 receptor β2, thus making these cells responsive to IL-12 (24, 25). T-bet expression is regulated by T cell receptor occupancy and cytokines, especially IFN-γ and IL-27 (25–27).

Increasing evidence indicates that an important aspect of T<sub>H1</sub> and T<sub>H2</sub> differentiation is chromatin remodeling and the accessibility of the loci encoding IFN-γ and IL-4, respectively (28). For instance, naive CD4<sup>+</sup> T cells acquire distinctive patterns of DNase I hypersensitivity at the IFNG or IL4 loci during T<sub>H1</sub> differentiation according to the respective subsets (29). Overexpression of T-bet has been reported to enhance IFN-γ gene transcription and induce the DNase I hypersensitivity of the IFNG locus and in this manner is thought to promote T<sub>H1</sub> differentiation, although a direct role of chromatin remodeling has not been established (24, 30). Although sites of DNase I hypersensitivity are indicative of the accessibility of transcription factors, histone modification is also associated with chromosome structure accessibility and transcriptional activation of the locus (31). Recently, two groups (32, 33) have demonstrated increased histone acetylation in IFNG and IL4 loci during T<sub>H1</sub>/T<sub>H2</sub> differentiation, respectively, and involvement of Stat4, T-bet, and Stat6 using knock-out mice. Thus, the role of epigenetic mechanisms in lineage commitment and regulation of distinct patterns of gene expression in T<sub>H1</sub> and T<sub>H2</sub> cells is an important area of investigation.
Although Th1 differentiation is regulated by multiple cytokines and transcription factors (34), exactly how histone acetylation might play a role in the expression of Th1 genes has not been determined. In this study we set out to compare and contrast acetylation and expression for three Th1 genes, IFNG, TBET, and IL18RAP, focusing on the differentiation of human cells. Surprisingly, we found that the acetylation of these three genes is regulated quite differently. We showed that histone acetylation of the TBET gene, like the IFNG gene, occurs preferentially during Th1 differentiation. However the modifications of the genes occurred with different kinetics, and the principal cytokines that govern these modifications are distinct; IFNG gene acetylation is positively regulated by IL-12, IL-18, and Stat4, whereas the TBET gene is predominantly negatively regulated by IL-4. Selective histone acetylation, however, was not universally the case for all genes expressed in Th1 cells. Despite its preferential expression, the IL18RAP gene did not exhibit selective histone acetylation. IFN-γ gene expression correlated well with the extent of histone acetylation, however, was not universally the case for all genes expressed in Th1 cells. Although it is not sufficient to explain all aspects of preferential gene expression.

**EXPERIMENTAL PROCEDURES**

**Reagents**—Human IL-4, IL-12, and IL-18 were purchased from R&D Systems, Minneapolis, MN, and human IL-2 was from Dr. Reynolds, NC1, Frederick, MD. Anti-CD3, anti-CD28, anti-IL-4, anti-IL-12, and anti-IFN-γ antibodies were purchased from Pharmingen. Anti-acetylated H3, anti-acetylated H4, anti-acetylhistone H3(Lys-9), anti-acetylhistone H3(Lys-14), and normal rabbit IgG were from Upstate Biotechnology, Lake Placid, NY. Anti-acetylhistone H4(Lys-8) and anti-acetylhistone H4(Lys-12) were from Abcam, Inc. Cambridge MA. Anti-Stat4 was from Zymed Laboratory, San Francisco, CA. Sodium butyrate was purchased from Sigma.

**Cell Culture**—Human peripheral blood mononuclear cells were isolated from healthy volunteer cells over Ficoll-HyPaque, and naïve CD4+ T cells were purified with human CD4/45RO- naïve T cell subset column kit (R&D Systems) or human CD4+ T cell subset column kit (R&D Systems) followed by positive selection using CD45RA beads (Myteni Biotech Inc., Auburn, CA). Purity was usually >90%. Cells were stimulated with plate-bound anti-CD3 (10 μg/ml) plus anti-CD28 (5 μg/ml) antibodies (Abes) and IL-2 (50 units/ml) for 3 days and expanded in a new flask for an extra 3 days with exogenous IL-2. To induce Th1 cells, IL-12 (10 ng/ml) was added throughout the culture period, and anti-IL-4 Ab (10 μg/ml) was added for the first 3 days. To induce Th2 cells, IL-4 (25 ng/ml) was added throughout the culture period, and anti-IL-12 Ab (5 μg/ml) was added for the first 3 days. IL-18 (10 ng/ml) and anti-IFN-γ Ab (10 μg/ml) were added throughout the culture period when indicated.

**Gene Expression in Th1 and Th2 Cells**—Th1 and Th2 cells were generated as described above, harvested at day 6, and left unstimulated or stimulated with plate-bound CD3 mAb for 5 h, and mRNA was isolated. First strand cDNA synthesis and real time PCR were done as described previously (35). Commercial assay reagents for IFN-γ and glyceraldehyde-3-phosphate dehydrogenase were purchased from Applied Biosystems (Foster City, CA). The sequences of primer pairs and probes for T-bet were described previously, and those for IL18RAP were 5’-CCCCAGATCTTGGAGAGGCTT-3’, 5’-GGCAGTTGCTTGAGGCATTT-3’, and 6-carboxyfluorescein-5’-AGTTCTCCTCAATCTAGGT-TCTGGGCC-3’-6-carboxytetramethylrhodamine (26). Levels of gene expression are normalized to glyceraldehyde-3-phosphate dehydrogenase expression.

**Chromatin Immunoprecipitation (ChIP) Assay**—The ChIP assay was carried out essentially according to manufacturer’s instructions (Upstate Biotechnology). Briefly, 1–2 × 10^6 cells were fixed with 1% formaldehyde, washed with cold phosphate-buffered saline, and lysed in buffer containing 10 μg/ml protease (ICN Biomedicals, Aurora, OH), 10 μg/ml leupeptin (RACHEM, Torrance, CA), and 2.5 μg 4-nitrophenyl 4-guanidinobenzoate hydrochloride (Sigma). Nuclei were sonicated to shear DNA (Heat Systems, Farmingdale, NY), sedimented, and diluted supernatants were immunoprecipitated with antibodies. A proportion (2%) of the diluted supernatants were kept as “input.” The bead-bound protein-DNA complexes were eluted in 1% SDS, 0.1 M NaHCO_3, and cross-links were reversed at 65 °C. DNA was recovered by phenolchloroform extraction and ethanol precipitation and subjected to PCR or real time PCR analysis. PCR was carried out with ampliTaq Gold (Applied Biosystems) for 35 cycles (40 s at 95 °C, 40 s at 59 °C, and 40 s at 72 °C), and the products were visualized by ethidium bromide staining. The sequences of the primer pairs used are listed in Table I. In selected experiments, real time PCR was performed to quantify the ChIP assay. The sequences of primer pairs and probes used are listed in Table II. The amount of immunoprecipitated DNA samples was normalized against input DNA samples.

**RESULTS**

**Preferential Histone Acetylation of the IFNG Gene in Human Th1 Cells**—Previous reports in mouse cells have indicated that the proximal promoter region of the IFNG gene becomes hyperacetylated in Th1 cells compared with Th2 cells (32, 33). To compare the acetylation of both histone H3 and histone H4 in the human IFNG gene, we prepared a series of primer pairs throughout this locus (Fig. 1A). As shown in Fig. 1B (primer α), the proximal promoter of the human IFNG gene also exhibits histone acetylation in Th1 cells. Interestingly these modifications were observed throughout the IFNG gene, although the differential modification between Th1 and Th2 cells was more evident in the areas surrounding the coding regions than the promoter. Specifically, the proximal promoter region (primer α), the first and second introns (primers b, c, and d), and the 1.4-kb downstream region (primer e) were also hyperacetylated in Th1 cells. To confirm these data, we conducted quantitative ChIP assays using real time PCR in which the IFNG promoter region was amplified (Fig. 1A). Histone H3 and H4 acetylation of the IFNG promoter was higher in Th1 cells than in Th2 cells (Fig. 1C).

Stat4 is presumably important for IFNG acetylation, because Stat4-deficient mice show impaired histone acetylation of the IFNG gene (32, 33); however a direct role for Stat4 in IFNG gene regulation has not been established. Thus, we set out to determine whether Stat4 is recruited to the regions of the IFNG gene (proximal promoter and the first intron), which have been suggested to be Stat4 binding sites (36). Purified CD4+ T cells were stimulated with anti-CD3 and CD28 mAbs for 2 days, resteed overnight, and then restimulated with IL-12 for 1 h. Using the ChIP assay, we found that Stat4 binds to both sites on the IFNG gene in response to IL-12 (Fig. 1D). This then provides the first direct evidence of Stat4 binding to the IFNG gene in differentiating CD4+ T cells and thus raises the possi-
and H4 in Human TH1 Cells

For instance, the acetylation of H3(Lys-9) and H4(Lys-8) correlate well with overall H3 and H4 acetylation of the IFN-gamma (IFNG) promoter (25, 30). Thus, we next examined the modification of individual residues (Fig. 4, A–C). The histone modification of another TH1-expression gene, the IL18RAP gene, is presented below.

Histone Acetylation of Specific Lysine Residues of Histone H3 and H4 in Human TH1 Cells—Specific lysine residues on histone tails can be modified including acetylation, and it has been shown that the pattern of histone tail modification could determine the transcriptional activity of the gene (31). For instance, the acetylation of H3(Lys-9) and H4(Lys-8) correlates well with overall H3 and H4 acetylation of the IFN-beta (IFNB) promoter following virus stimulation (37). To confirm and extend the data presented above (Figs. 1 and 2), we next asked whether preferential acetylation of H3(Lys-9), H3(Lys-14), H4(Lys-8), and H4(Lys-12) occurred in the IFNG and TBET promoters in TH1 cells. As shown in Fig. 3, H3(Lys-9), H4(Lys-8), and H4(Lys-12) are preferentially acetylated in both IFNG and TBET genes in TH1 cells as determined by quantitative ChIP assay. On the other hand, H3(Lys-14) acetylation did not distinguish TH1 and TH2 cells. Therefore, acetylation of H3(Lys-9), H4(Lys-8), and H4(Lys-12) residues may contribute to the preferential induction of IFN-gamma and T-bet genes in TH1 cells, whereas a modification of H3(Lys-14) would presumably not.

Collectively, these results indicate that the regulation of acetylation of the IL18RAP gene is quite distinct from the IFNG and TBET genes. Preferential histone acetylation is likely important as a TH1-specific expression of IFNG and TBET genes. Although IL18Rap is a well known marker of TH1 cells, the IL18RAP gene does not undergo preferential histone modification in TH1 cells. This is important, because it establishes the fact that preferential histone acetylation is not the

### Table II

<table>
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<th>Gene</th>
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</tr>
<tr>
<td>hIFNG-p</td>
<td>5′-GGGCTGTGGGAGTAAAGTGCGTCA-3′ 5′-CGATGAAAGCCAGCATATGGC-3′</td>
</tr>
<tr>
<td>hIL18RAP-p</td>
<td>5′-AGGATCCCAAGCTGAAGGCGCAAGTGTTGCCTC-3′ 5′-TCCCTGGGACGCGCAGAC-3′</td>
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**Fig. 1.** Histone acetylation of IFNG gene in TH1 and TH2 cells. A, diagram of the human IFNG locus and the regions (not to scale) amplified for ChIP assay by PCR (a–e). B, ChIP assay for acetylated (ac) histone H3 and H4 between TH1 and TH2 cells throughout the IFNG gene. In, input; C, control antibody; a–e, PCR primers in A. C, quantitative ChIP assay for IFNG promoter (IFNG-P) region. The immunoprecipitated DNA were normalized against input DNA and expressed as a ratio to that of TH1 cells. D, Stat4 binding to the IFNG gene in response to IL-12. CD4+ T cells were activated with anti-CD3 mAb for 48 h and then stimulated with IL-12 for 1 h. ChIP assays using anti-Stat4 Ab were used to amplify the promoter and intron 1 regions.

**Fig. 2.** Histone acetylation of TBET genes in TH1 and TH2 cells. A, diagram of the human TBET locus and the regions (not to scale) amplified for the ChIP assay. B, ChIP assay for acetylated (ac) histone H3 and H4 between TH1 and TH2 cells throughout the TBET gene. C, quantitative ChIP assay for the TBET promoter (TBET-P) region. Results were expressed as a ratio to that of TH1 cells. Data are representative of more than three similar experiments. In, input; C, control antibody.

**Gene Sequences (forward and reverse primers and a probe)**

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</tr>
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<td>hIFNG-p</td>
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</tr>
<tr>
<td>hIL18RAP-p</td>
<td>5′-AGGATCCCAAGCTGAAGGCGCAAGTGTTGCCTC-3′ 5′-TCCCTGGGACGCGCAGAC-3′</td>
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Histone Acetylation in Human TH1 Cells

Differential Kinetics of Histone Acetylation of IFNG and TBET Genes

A quantitative PCR of ChIP samples was performed in triplicate using the IFNG promoter (IFNG-P) (A and B) and TBET promoter (TBET-P) (C and D). Antibodies against acetylated Lys-9 and Lys-14 of H3 or Lys-8 and Lys-12 of H4 were used for immunoprecipitation and anti-total-acetyl H3 was also included as a reference control. The immunoprecipitated DNA samples were normalized against input DNA samples and expressed as a ratio to that of total-acetyl H3 in TH1 cells. A Mvc, acetated lysine.

Differential Kinetics of Histone Acetylation of IFNG and TBET Promoters During TH1 Differentiation—We next asked how histone acetylation of the IFNG and TBET genes occurs during TH1 differentiation. IFN-γ induces T-bet expression and vice versa, suggesting a positive feedback effect between the two genes (25, 26, 30). Although mouse T-bet is preferentially expressed in TH1 cells, the expression of the human T-bet gene during TH1 differentiation has not been examined. Thus, we first analyzed T-bet and IFN-γ gene expression on day 2 of the differentiation of human CD4+ T cells. Interestingly, IFN-γ and T-bet mRNA levels in TH2 conditions were 1.28% and 50% of that in TH1 conditions on day 2, respectively, arguing that the preferential “TH1-like” pattern of IFN-γ expression precedes T-bet expression (Fig. 5, A and B). The expression of T-bet under TH2 conditions is presumably due to the presence of residual IFN-γ in the cultures. In this experiment, we purposely omitted the anti-IL-4 antibody thinking that this situation would more closely mimic in vivo conditions (IL-12 without anti-IL-4 antibodies); in fact, when anti-IFN-γ antibody was included, T-bet expression was abrogated (see below). To examine whether the gene expression levels are associated with histone acetylation, we determined the time course of the histone H3 acetylation of IFNG and TBET genes. IFNG promoter acetylation was significantly greater on day 2 and increased further by day 6 in TH1 cells, whereas it remained at low levels throughout this period in TH2 cells (Fig. 5C). TBET promoter acetylation was observed in both TH1 and TH2 cells on day 2 and remained high in TH1 cells but decreased in TH2 cells on day 6 (Fig. 5C). Thus, the results showed that selective IFNG gene acetylation in TH1 cells was evident by day 2, whereas TBET gene acetylation in TH1 cells (or deacetylation in TH2 cells) occurred at later time points. Finally, we tested whether IL-4 regulates T-bet gene expression. CD4+ T cells were pre-activated with CD3 and CD28 for 2 days, rested in medium overnight, and then cultured in the presence or absence of IL-4 or IFN-γ. T-bet expres-
addition of IL-18 to IL-12 (Fig. 6). Thus we compared mRNA levels and histone acetylation (Fig. 6). Hyperacetylated histones are associated with transcriptionally repressed genes whereas hypoacetylated histones are associated with transcriptionally active genes. Histone acetylation of the IFNG promoter was suppressed by the presence of IL-4 Ab only. Representative results of three separate experiments were shown.

**DISCUSSION**

Despite their importance in host defense, the molecular mechanisms governing a distinct gene expression pattern by TH1 cells from TH2 cells are not fully understood. In this study, a real time PCR ChIP assay allowed us to quantitatively analyze the histone acetylation of three canonical TH1 genes, IFNG, TBET, and IL18RAP in response to different cytokines. We demonstrated that increased histone acetylation of the
IFNG and TBET genes in human T\textsubscript{H}1 cells correlated well with gene expression levels indicating that an alteration of histone acetylation is one possible mechanism behind a T\textsubscript{H}1-specific gene expression pattern. We also have shown that IFNG and TBET genes are acetylated with different kinetics and by different cytokines. In contrast to these genes, IL18RAP is acetylated equivalently despite their selective mRNA expression meaning that histone modification does not explain the preferential gene expression of all T\textsubscript{H}1 genes. Thus histone acetylation explains one but not all aspects of T\textsubscript{H}1 gene expression.

The present study is the first to examine human TBET gene acetylation and its regulation. It has been shown previously that IL-4 inhibits and IFN-\gamma enhances T\textsubscript{H}1 gene expression (24, 26). In this study, we show that histone acetylation of TBET gene, as well as its gene expression level, are regulated more dramatically by IL-4 than IL-12 or IL-18 (Fig. 6). Precisely which mechanisms serve to repress TBET gene acetylation remain to be elucidated. IL-4 activates Stat6, which in turn regulates the transcription factor GATA-3. It will be interesting to determine whether Stat6, GATA-3, or both are involved in T\textsubscript{H}1 gene expression and acetylation. TBET gene acetylation was initially induced in both T\textsubscript{H}1 and T\textsubscript{H}2 cells, and the kinetics correlated with that of gene expression levels (Fig. 5).

Our results also demonstrated that histone acetylation of the IFNG gene is regulated by multiple cytokines and presumably multiple transcription factors. As with the TBET gene, a blockade of IL-4 alone was associated with substantial histone acetylation of the IFNG gene. However the addition of IL-12 and IL-18 increased the modification of the IFNG gene in contrast to the TBET gene (Fig. 6B). Although the histone acetylation of the IFNG gene in T\textsubscript{H}1 cells is likely to be regulated by both T-bet and IL-12, we suggest that the effects of IL-12 and IL-18 could be independent of T-bet. Our results showed that the commitment of the IFNG gene to a T\textsubscript{H}1 pattern precedes that of the TBET gene in terms of gene expression and histone acetylation (Fig. 5). Also the difference in T-bet mRNA levels between T\textsubscript{H}1 and T\textsubscript{H}2 cells was surprisingly modest, compared with the differences in IFN-\gamma mRNA levels (Fig. 5B). Therefore both the kinetics and the magnitude of induction of T-bet suggest that this nuclear factor might not be an early regulator of the IFNG gene as has been proposed (3, 24). T-bet induces the IL-12 receptor \(\beta_2\) and induces chromatin remodeling of the IFNG gene (24, 25), so it may be that T-bet principally affects T\textsubscript{H}1 commitment and IFNG gene regulation indirectly by regulating the expression of IL-12 receptor \(\beta_2\), rather than by directly remodeling IFNG locus. Alternatively, although T-bet may have direct effects on histone acetylation at later time points in T\textsubscript{H}1 differentiation, overexpression of this gene has been reported to induce chromatin remodeling of IFNG gene, although a T-bet binding site within the IFNG gene has not been documented (24).

Our data also argued that the effect of IL-12 is mediated by Stat4 as we have demonstrated Stat4 binding within both human (Fig. 1D) and mouse IFNG genes (43). IL-12 and IL-18 synergistically induce IFN-\gamma production presumably through the activation of Stat4 and AP-1 both of which bind sites in the IFNG promoter (44). The same mechanism may explain the cooperative induction of histone acetylation by IL-12 and IL-18. Thus multiple levels of regulation of IFNG histone acetylation might reflect multiple checkpoints for T\textsubscript{H}1 differentiation (34). It is also interesting that the entire IFNG locus showed increased histone acetylation, raising the possibility that an expanded region around this locus might be modified in T\textsubscript{H}1 cells. A quantitative measurement of histone acetylation throughout the IFNG locus and the surrounding regions is likely to be useful to understand the regulation of this gene. The modification of the \(\beta\)-globin locus, for instance, occurs in a tissue-specific manner with clearly defined boundaries (45, 46), and presumably the same scenario applies to the IFNG gene.

The histone code hypothesis proposes that a specific combination of modifications of individual histone residues may be critical in regulating gene expression. Acetylation of both H3(Lys-9) and H3(Lys-14) is considered to be a specific marker of active genes and critical for the recruitment of TFHID (31, 37). In contrast, acetylation of H4(Lys-8) has been shown to mediate recruitment of SWI/SNF proteins. It will be important to analyze these issues specifically with respect to the IFNG and TBET genes. We found preferential acetylation of H3(Lys-9), H4(Lys-8), and H4(Lys-12) but not of H3(Lys-14) in T\textsubscript{H}1 cells. It will be of interest to address why H3(Lys-14) was not preferentially acetylated, whereas other residues were hyperacetylated, and how the acetylation pattern contributes to the selective gene expression in T\textsubscript{H}1 cells.

Histone acetylation is regulated by histone acetyltransferases and HDACs. Various histone acetyltransferases and HDACs have been identified (41), and it is likely that some of them are recruited to form complexes with specific transcription factors such as Stat4 and T-bet to induce histone acetylation/deacetylation of the IFNG gene. Among them CBP/p300 is a reasonable candidate, because CBP+/− cells have relatively poor IFN-\gamma production (24). We used an HDAC inhibitor to examine whether HDACs play roles in T\textsubscript{H}1 cell differentiation and did not find that this reagent fully restored IFN-\gamma production, implying that other mechanisms are operative in silencing the IFNG locus in T\textsubscript{H}2 conditions. In fact, preliminary experiments showed that DNA methylation may be involved, because a combination of 5′-azacytidine and sodium butyrate induced higher IFN-\gamma production. Thus histone acetylation is one but not the only mechanism that determines T\textsubscript{H} lineage commitment.

In summary, T\textsubscript{H}1 differentiation is a process that is regulated by various factors at multiple levels including histone acetylation. Further studies will be required to obtain a comprehensive understanding of the epigenetic regulation of T\textsubscript{H}1 cell differentiation. We demonstrated that T\textsubscript{H}1 cell differentiation accompanies the dynamic change of histone acetylation. It will be interesting to examine whether histone methylation or phosphorylation is also involved in the process. It will also be important to understand how T cell receptor and cytokine signaling activate transcription factors and influence histone modification, remodeling, and CpG methylation to induce a fully open or silenced IFNG gene, which is characteristic of T\textsubscript{H}1 differentiation.

REFERENCES

Histone Acetylation in Human T_h1 Cells


Discrete Roles for Histone Acetylation in Human T Helper 1 Cell-specific Gene Expression

Akio Morinobu, Yuka Kanno and John J. O'Shea


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