

Rapid publications

Histone deacetylation inhibits *IL4* gene expression in T cells

Maryam Valapour, MD,^{a*} Jia Guo, MD,^{a*} John T. Schroeder, PhD,^b
Judith Keen, PhD,^a Antonella Cianferoni, MD,^b Vincenzo Casolaro, MD, PhD,^b
and Steve N. Georas, MD^{b*} *Baltimore, Md*

Background: Dysregulated expression of IL-4 has been linked with allergic diseases. IL-4 expression is controlled at the level of gene transcription by the coordinated action of multiple factors that bind regulatory promoter elements. In addition, alterations in chromatin structure are thought to play a role in regulating the expression of cytokines in the T_H2 gene cluster, although the biochemical basis for these alterations in human T cells is not well understood.

Objective: We sought to define the role of histone acetylation in the regulation of *IL4* gene expression in human T cells.

Methods: IL-4 protein production was measured by means of ELISA. IL-4 promoter activity was measured with luciferase-based reporter constructs transiently transfected into Jurkat T cells. The acetylation status of histones associated with the *IL4* gene was analyzed with chromatin immunoprecipitation assays.

Results: IL-4 production from activated peripheral blood T cells was enhanced by the histone deacetylase inhibitor trichostatin A. Overexpression of the type 1 histone deacetylases 1, 2, and 3 inhibited transcription driven by the IL-4 promoter in Jurkat T cells, whereas cotransfection of the histone acetyltransferase CREB-binding protein potentiated IL-4 promoter activity. Using chromatin immunoprecipitation assays, we show that nucleosomes in the proximal IL-4 promoter are acetylated on T-cell activation.

Conclusion: Our results demonstrate that the acetylation state of histones associated with the IL-4 promoter is a key regulator of *IL4* gene expression. (*J Allergy Clin Immunol* 2002;109:238-45.)

Key words: Gene expression, T lymphocyte, IL-4, histone deacetylase, gene transcription

Abbreviations used

CBP: CREB-binding protein
ChIP: chromatin immunoprecipitation
HDAC: Histone deacetylase
NFAT: Nuclear factor of activated T cells
PBT: Peripheral blood T
TSA: Trichostatin A

The packaging of DNA into chromatin has a generally repressive effect on gene expression. Tight associations between DNA and histones (as well as nonhistone chromosomal proteins) are thought to inhibit the accessibility of transcription factors and components of the basal machinery to their target promoters. A variety of epigenetic biochemical processes can alter the intrinsic repressive structure of chromatin, including DNA demethylation¹ and nucleosome remodeling. Depending on the promoter context, nucleosome remodeling can involve both adenosine triphosphate-dependent events, as well as posttranslational modifications of histone proteins, such as acetylation.²⁻⁶ Histone deacetylation, on the other hand, is now strongly linked with transcriptional repression.⁷⁻⁹

Histone deacetylases (HDACs) have been grouped into 2 families on the basis of sequence similarities with yeast homologues: the class I family (Rpd3-like) includes HDACs 1, 2, 3, and 8, and the class II family (Hda1-like) includes HDACs 4, 5, 6, 7, and 9.^{10,11} HDACs are expressed in a tissue-specific manner, possess different intrinsic deacetylation abilities, and associate in distinct multiprotein complexes.^{7,10,12} HDACs do not contain canonical DNA-binding domains and are thought to be recruited to chromatin by means of protein-protein interactions with other factors.^{7,9,13} Because changes in histone acetylation states can be inherited in an epigenetic fashion, they can have long-lasting effects on gene expression.¹⁴ Relatively little is known about how changes in histone structure affect cytokine gene expression in human T cells.

Dysregulated expression of IL-4, a pleiotropic cytokine,¹⁵ has been strongly linked with allergic, inflammatory, and autoimmune diseases.¹⁶⁻¹⁸ The precise molecular basis for enhanced IL-4 production in allergic diseases is unknown. In T cells *IL4* gene expression is controlled at the level of transcription by

From ^athe Department of Medicine, Division of Pulmonary and Critical Care Medicine, and ^bthe Division of Allergy and Clinical Immunology, The Johns Hopkins Asthma and Allergy Center, Baltimore.

*These authors contributed equally to this work.

Supported by research grants from the National Institutes of Health, the American Lung Association (056N), the American Heart Association, and the Johns Hopkins University School of Medicine Clinician Scientist Program.

Received for publication November 27, 2000; revised October 8, 2001; accepted for publication October 15, 2001.

Reprint requests: Steve N. Georas, MD, Rm 4B.41, The Johns Hopkins Asthma and Allergy Center, 5501 Hopkins Bayview Circle, Baltimore, MD 21224.

Copyright © 2002 by Mosby, Inc.

0091-6749/2002 \$35.00 + 0 1/10/121145

doi:10.1067/mai.2002.121145

the assembly of a complex set of factors on the proximal promoter,¹⁹ as well as by distal enhancer elements.²⁰⁻²² Genetic polymorphisms in some of these regions have been linked with allergic phenotypes^{23,24} and presumably act by either enhancing or inhibiting the affinity for transcriptional activators or repressors, respectively. In addition, enhanced activation of IL-4 promoter-binding transcription factors may also be a cardinal feature of the allergic phenotype.^{16,25}

IL-4 expression in T cells is also associated with changes in chromatin structure. For example, silencing of the *IL4* gene in developing T_H1 cells is associated with repositioning into heterochromatin.²⁶ Additionally, during the differentiation of naive T cells into effector T_H2 cells, the *IL4* gene becomes demethylated,²⁷ and multiple DNase-hypersensitive sites appear in the IL-4 cytokine gene cluster, at least one of which is located near the IL-4 promoter.²⁸⁻³⁰ Most of the studies linking chromatin remodeling with *IL4* gene expression have been performed by using mouse lymphocytes studied under T_H-polarizing conditions. Relatively little is known about how chromatin remodeling contributes to gene expression in activated human T cells. We speculated that *IL4* gene expression would involve changes in the acetylation states of histones near the IL-4 promoter. In this article we show that the HDAC inhibitor trichostatin A (TSA) significantly enhances IL-4 protein production from activated human peripheral blood T (PBT) cells. Using reporter constructs in a T-cell line, we show that coexpression of the histone acetyltransferase CREB-binding protein (CBP) potentiates, and overexpression of the type 1 HDACs downregulates, human IL-4 promoter activity. Finally, we used chromatin immunoprecipitation (ChIP) assays to investigate the acetylation status of nucleosomes in or near the IL-4 promoter in resting and activated T cells.

METHODS

Plasmid construction

Luciferase-based human IL-4 promoter constructs containing 270, 145, and 65 bp upstream from the IL-4 transcription start site (referred to as 270 luc, 145 luc, and 65 luc, respectively) were synthesized as previously described.³¹ CMV promoter-driven expression vectors encoding HDAC 2 and HDAC 3 were kindly donated by Dr Edward Seto (University of South Florida).^{9,12} Expression vectors encoding wild-type and catalytically inactive HDAC 1 mutants H141A and H199A were provided by Dr Stuart Schreiber (Harvard University).⁸ The CBP expression vector was a kind gift of Dr Michael Rosenfeld (University of California at San Diego).³²

PBT cells, basophils, and ELISA

PBT cells were purified from leukapheresis packs of anonymous donors with countercurrent elutriation. This procedure routinely yields approximately 80% pure CD3⁺ cells, as determined by means of immunofluorescence and flow cytometry (not shown). Cells (2 × 10⁶/condition) were stimulated as indicated in the presence of TSA (Wako Chemicals) or solvent (dimethyl sulfoxide) control, followed by analysis of cytokine concentrations in supernatants with sensitive ELISA kits. The following kits were purchased and used according to manufacturer's instructions: IL-4 (Biosource Interna-

tional), IL-13, and IL-2 (both from Immunotech). Basophils were enriched from peripheral blood of additional donors and stimulated with anti-IgE antibodies (10 ng/mL for 4 hours), as previously described.³³

Cell lines and transfections

Jurkat T cells (courtesy of Dr Jack Strominger, Harvard University) were maintained and transfected, as previously reported,³¹ with 1 μg of reporter, the indicated amounts of expression vector, and empty vector to keep the total amount of DNA constant with 3 μL of Superfect (Qiagen) per microgram of plasmid DNA. Cell lysates were analyzed by means of luminometry with a Monolight 3010C luminometer and luciferase assay kit (Analytical Bioluminescence). Cell extracts were normalized for protein content before assays for reporter gene expression. Similar results were obtained when IL-4 reporter luciferase activity was normalized to control luciferase values with a cotransfected Tk-Renilla vector and the Dual-Luciferase assay method (Promega).

Western blot analysis

Whole-cell lysates from Jurkat, HeLa, and Cos-7 cells (10 μg of each) were separated by means of 7.5% SDS-PAGE and then transferred to Trans-Blot Transfer Medium PVDF membrane (BioRad). After blocking in PBS-5% BSA/0.1% Tween-20 for 1 hour, membranes were probed with an anti-HDAC 2 antibody (1:2000, courtesy of Dr Edward Seto) for 18 hours, followed by incubation for 1 hour with a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1:5000, Amersham Pharmacia Biotech). Immunoreactive bands were visualized by means of enhanced chemiluminescence and autoradiography with the ECL Western Blotting Detection kit (Amersham Pharmacia Biotech).

ChIP assays

Solubilized chromatin was prepared from Jurkat cells or PBMCs (5 × 10⁶ per antibody condition) isolated from 4 subjects. PBMCs were cultured in the presence of PHA (5 μg/mL) for 18 hours, stimulated with A23187 (0.5 μmol/L) and phorbol myristate acetate (20 ng/mL) for 6 hours, and then fixed in 1% formalin for 15 minutes at 37°C. Cell pellets were sonicated with 3 pulses of 10 seconds each with a Heat Systems Ultrasonics sonicator (approximately 40% of maximal output). These settings were determined in pilot experiments to yield DNA fragments of approximately 400 to 600 bp in length (data not shown). The resulting sheared and cross-linked DNA was then immunoprecipitated with antibodies specific for acetylated forms of histone H4 (αAcH4, 5 μL, Upstate Biotechnologies) by using the Acetyl-Histone ChIP Assay Kit (Upstate Biotechnologies), according to the manufacturer's instructions. After reversal of cross-links at 65°C for 4 hours, the immunoprecipitated DNA was extracted and recovered by means of ethanol precipitation. DNA was divided into equal aliquots and analyzed by means of PCR with a series of primers (matched for Tm) surrounding the IL-4 transcription start site (primer sequences available on request). Each PCR reaction contained 2 mmol/L MgCl₂, 2 μg/mL primers, and 2.5 units of HotStarTaq (Qiagen) and was subject to 35 one-minute cycles at 94°C, 66°C, and 72°C, followed by a 10-minute extension cycle at 72°C. One tenth of the final reaction was then analyzed by means of 1% agarose gel electrophoresis with ethidium bromide staining.

RESULTS

TSA enhances IL-4 production in PBT cells

We first studied the effects of stimulating PBT cells in the presence or absence of the HDAC inhibitor TSA.³⁴ As shown in Fig 1, A, cocubation with TSA signifi-

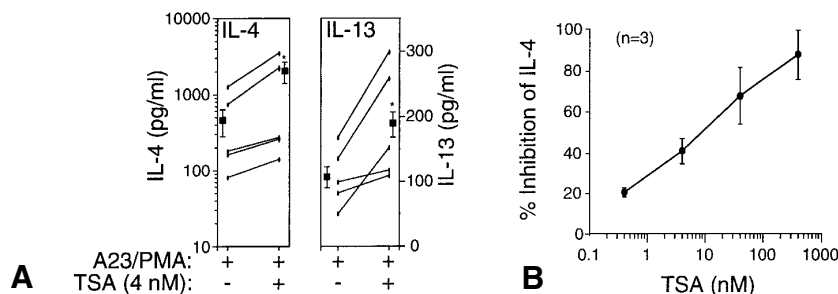


FIG 1. TSA increases IL-4 and IL-13 production from activated PBT cells. **A**, T cells (2×10^6 /condition) were enriched from peripheral blood by using countercurrent elutriation and stimulated with calcium ionophore plus phorbol myristate acetate in the presence or absence of TSA, as indicated (see "Methods" section). Data points from individual donors are indicated by ovals, and means $\pm$ SEM are indicated by squares. $*P < .05$. **B**, In contrast, TSA inhibited anti-IgE-mediated IL-4 production in enriched human basophils (9.0% $\pm$ 0.6% pure) in a concentration-dependent manner (median effective concentration, approximately 10 nmol/L). Data shown are the average of 3 experiments in which IL-4 production in the absence of TSA was 607 ± 154 pg per 10^6 basophils.

cantly increased IL-4 production in T cells stimulated with calcium- and protein kinase C-mediated signals. TSA increased IL-4 and IL-13 production with PBT cells from multiple donors (Fig 1, A). Unstimulated cells secreted no detectable IL-4 or IL-13, and even prolonged incubation with TSA alone (up to 72 hours) had no effect on cytokine production (data not shown). Dose-response experiments revealed that higher concentrations of TSA (40–400 nmol/L) did not further enhance (and occasionally inhibited) IL-4 secretion (data not shown). Two observations suggest that basophils, which can produce abundant IL-4 in cultures of PBMCs,³³ did not contribute to the TSA response. First, the PBT cell preparations used for these experiments contained less than 1% basophils, as determined by means of alcian blue staining (data not shown). Second, we analyzed the effects of TSA on anti-IgE-mediated IL-4 production with basophils enriched from the peripheral blood of additional donors. In these experiments TSA inhibited IL-4 secretion in a concentration-dependent manner (Fig 1, B). Histamine release was unaffected by TSA (<5% inhibition) at all tested concentrations (data not shown). To determine the specificity of TSA for enhanced cytokine secretion, we analyzed supernatants of stimulated PBT cells obtained from 4 additional donors for IL-2 secretion. Interestingly, we found that TSA did not significantly affect IL-2 secretion in A23187/phorbol myristate acetate-stimulated cells (data not shown).

Regulation of IL-4 transcriptional activation by coexpressed HDAC or CBP

Although TSA is currently thought to be a specific, reversible inhibitor of HDAC,³⁴ enhancement of IL-4 expression by this agent could clearly be an indirect effect caused by regulated expression of other gene products. To determine whether histone deacetylation played a direct repressive effect on *IL4* gene transcription, we studied the effect of overexpressing 3 different HDACs on IL-4 promoter activity with a transfectable T-cell line. Fig 2 shows that HDAC 3 potently inhibited both constitutive and calcium-induced transcription driven by a full-

length IL-4 promoter construct in cotransfected Jurkat T cells. In contrast, overexpression of CBP, which possesses intrinsic histone acetyltransferase activity, enhanced both constitutive and calcium-driven IL-4 promoter activation (Fig 2, B). In parallel experiments each type 1 HDAC (HDACs 1, 2, and 3) inhibited IL-4 promoter activity to a comparable degree (Fig 3, A). Thus, although HDACs may associate with different cofactors and possess distinct deacetylation activities, inhibition of IL-4 transcription is shared by 3 different HDAC molecular species. A recent structure-function analysis concluded that a series of conserved histidine residues was required for the repressive effects of HDAC 1 on gene transcription, in part because of their interaction with the corepressor mSin3A.^{8,35} We therefore analyzed the ability of 2 HDAC 1 histidine mutants (H141A and H199A) to repress IL-4 promoter activity in cotransfection assays and found that both were less efficient repressors than wild-type HDAC 1, although at high concentrations of cotransfected plasmid, we did observe some inhibition of the IL-4 promoter (Fig 3, B).

To map the HDAC-responsive elements in the IL-4 promoter, we next analyzed the repressive effects of HDAC 2 on transcription driven by a series of IL-4 promoter deletional fragments. In these experiments we chose HDAC 2 because Jurkat cells are relatively deficient in this factor (Fig 4, A, lane 1).⁸ We verified that cotransfection of the HDAC 2 expression vector significantly enhanced HDAC 2 protein expression in Jurkat cells (Fig 4, A, lane 4). As shown in Fig 4, B, the repressive effect of HDAC 2 maps to the proximal 145 bp of the IL-4 promoter. None of the factors that bind within this region of the promoter (Fig 4, C) is currently known to interact with an HDAC.

Nucleosomes near the IL-4 promoter are acetylated after cell activation

We next used ChIP assays to specifically study the structure of histones associated with the native *IL4* gene. These assays rely on the ability of specific DNA sequences to be immunoprecipitated with antibodies against associ-

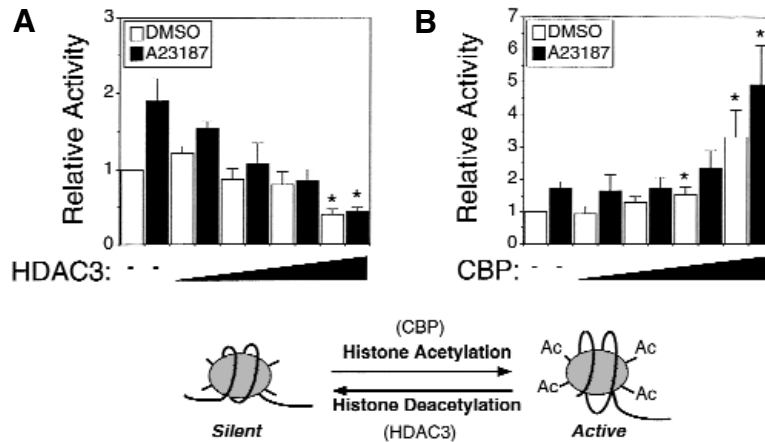


FIG 2. Regulation of IL-4 promoter activity by an HDAC or a histone acetyltransferase. Jurkat T cells were cotransfected with *IL4* 270 luc (1 µg, see "Methods" section) and with expression vectors encoding HDAC 3 (0.5, 1, 2, and 4 µg; **A**) or the histone acetyltransferase CBP (0.5, 1, 2, and 4 µg; **B**). Cells were incubated for 18 hours in the absence (open bars) or presence (filled bars) of a calcium ionophore (0.5 µmol/L A23187), followed by cell lysis and analysis of reporter gene activity by luminometry. Results are means ± SEM of 3 experiments expressed relative to the activity of *IL4* 270 luc in unstimulated cells (which averaged 14,785 ± 6821 RLU). **P* < .05 for effect of cotransfection. DMSO, Dimethyl sulfoxide.

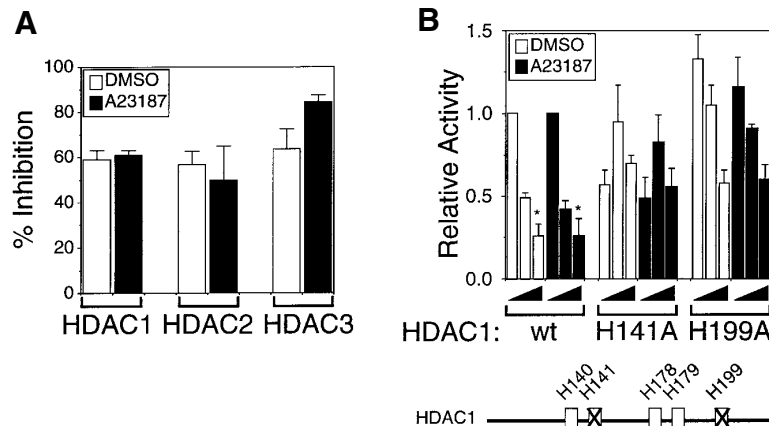


FIG 3. Inhibition of IL-4 promoter activity by type 1 HDAC. Jurkat T cells were cotransfected with *IL4* 270 luc (1 µg), together with expression vectors for the type 1 HDACs (HDACs 1, 2, and 3; 4 µg each; **A**) or wild-type and mutant HDAC1 constructs (0.5, 1, and 2 µg; **B**; see "Methods" section). Cells were stimulated and analyzed as in the legend for Fig 2. Results are means ± SEM of 3 to 5 experiments expressed as percentage of inhibition (**A**) or luciferase activity (**B**) relative to the cotransfection of empty vector alone. Averages in unstimulated and stimulated cells were 17,437 RLU and 30,874 RLU, respectively. **P* < .05 for effect of cotransfection. The 5 histidine residues conserved among type 1 HDACs, 2 of which are mutated in the expression vectors, are indicated (open boxes) below **B**. DMSO, Dimethyl sulfoxide.

ated proteins after protein-DNA cross-linking by formaldehyde.³⁶ Solubilized chromatin was prepared from resting and activated cells by means of sonication after formalin cross-linking and then immunoprecipitated with antibodies specific for acetylated forms of histone H4. In preliminary experiments with Jurkat cells, we detected a promoter-specific band with an anti-acetyl H4 antibody in calcium-ionophore activated cells (Fig 5). Equal amounts of DNA were analyzed in resting and activated cells with the ChIP assay, as determined by analyzing a fraction of the chromatin preparation before immunoprecipitation

(input control; Fig 5, *B*). This result indicates that nucleosomes in or near the IL-4 promoter are acetylated on calcium-mediated cell activation and are in keeping with the transient transfection data.

We next studied PBMCs isolated from 4 subjects. Using a panel of specific primers (each of which produced a single band of the expected molecular weight with genomic DNA as a control, data not shown), we found that the induction of H4 acetylation extended both upstream and downstream of the IL-4 transcription start site (Fig 6). Because the resolution of the ChIP technique is relatively

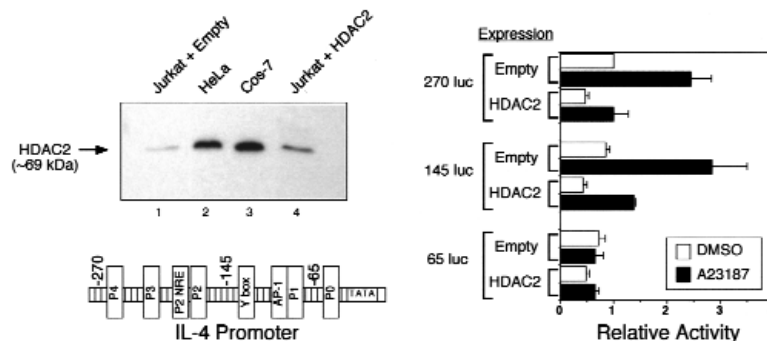


FIG 4. Deletion analysis of the repressive effects of HDACs on IL-4 promoter activity. **A**, Whole-cell lysates from the indicated cell lines (10 μ g each) were analyzed by means of immunoblotting with an anti-HDAC2 antibody (see "Methods" section). Transfection with the HDAC 2 expression vector (lane 4) significantly increased HDAC 2 protein levels, which were barely detectable in cells transfected with empty vector (lane 1). Equal amounts of protein were loaded in each lane, and the results shown are representative of 2 additional experiments. **B**, Jurkat T cells were cotransfected with the indicated reporter constructs (1 μ g) and HDAC 2 expression vector (4 μ g), as indicated. Results are means $\pm$ SEM of 3 experiments expressed relative to the activity of IL4 270 luc in unstimulated cells (which averaged 96,702 RLU). **C**, Schematic diagram of the IL-4 promoter (not drawn to scale), indicating 5 NFAT-binding P elements (P0-P4), as well as a binding site for NF-Y (Y box),⁴¹ a recently characterized repressor element (P2NRE),⁶¹ and an AP-1 site adjacent to P1.⁶² DMSO, Dimethyl sulfoxide.

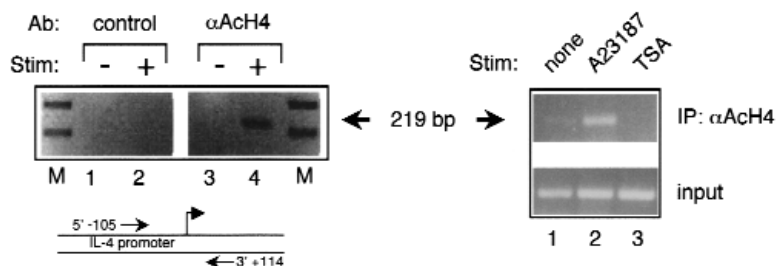


FIG 5. Nucleosomes near the IL-4 promoter are acetylated in activated Jurkat T cells. Solubilized chromatin was prepared from formalin-fixed Jurkat T cells that had been stimulated for 18 hours in the absence or presence of a calcium signal (0.5 μ M/L A23187) or TSA (40 nmol/L), as indicated, and immunoprecipitated with antibodies directed against acetylated forms of histone H4 (α ACh4), or mouse IgG (mIgG). Coprecipitated DNA fragments were eluted and analyzed by means of PCR with primers specific for the IL4 gene (indicated on the lower part of the figure), followed by agarose gel electrophoresis of the amplified products. In **B**, a fraction (approximately 10%) of the sheared and cross-linked chromatin was analyzed before administration of immunoprecipitation as a control (input). The gels were visualized with ethidium bromide staining, and the inverse image is shown in **A** for better resolution.

large, it is not possible to precisely correlate these data with single binding sites for individual factors. Nonetheless, H4 acetylation was detected in promoter regions known to bind nuclear factor of activated T cells (NFAT)³⁷ (primer pair C) and other factors (primer pair D).¹⁹ The negative control antibody (rabbit IgG) gave no signal when analyzed with primer pair D (data not shown).

DISCUSSION

Knowledge of how chromatin structure affects gene expression in T cells is accumulating at a rapid pace. Research in this area has been greatly accelerated by the availability of specific pharmacologic and molecular probes to study chromatin remodeling. Our study was prompted by the observation that TSA enhanced IL4 and IL13 gene expression in pharmacologically activated

human PBT cells (Fig 1). Because even prolonged incubation with TSA alone did not induce IL-4 expression, other factors in addition to HDAC-dependent repression are involved in silencing the IL4 gene in unstimulated T cells. TSA has been proven to be a remarkably specific antagonist that specifically contacts an active site conserved across the HDAC family.³⁸ That TSA selectively enhanced IL-4 production in T cells and not basophils underscores the differences between these 2 cell types in the molecular regulation of cytokine production.³⁹ Future studies will be needed to determine whether TSA-mediated inhibition of basophil IL-4 secretion involves indirect effects on other gene products (eg, enhanced expression of a basophil-specific inhibitor) or other novel effects of this compound. The fact that histamine release was not affected argues against, for example, a proapoptotic effect of TSA in basophils.⁴⁰

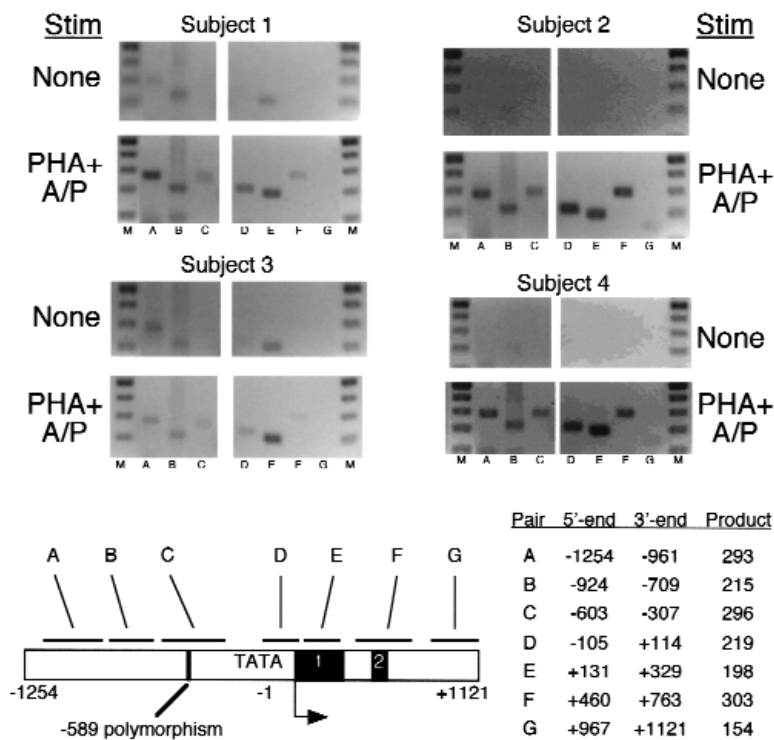


FIG 6. Nucleosomes surrounding the IL-4 transcription start site are acetylated on activation of PBMCs. PBMCs isolated from 4 subjects were primed with PHA (5 μ g/mL) for 20 hours and then stimulated for 6 hours with phorbol myristate acetate (20 ng/mL) plus A23187 (0.5 μ mol/L). DNA sequences surrounding the *IL4* gene were analyzed by means of ChIP assay with an anti-acetyl H4 antibody and PCR primers specific for the *IL4* gene (indicated on the lower part of the figure). Equal amounts of DNA per condition were subject to immunoprecipitation. The gel was visualized with ethidium bromide staining, and the inverse image is shown for better resolution. Primer pairs are indicated below the respective lanes, and a 100-bp ladder (with intense 500-bp band) is indicated as M. Dark boxes in the cartoon denote exons, and the asthma-associated polymorphism is indicated.²³

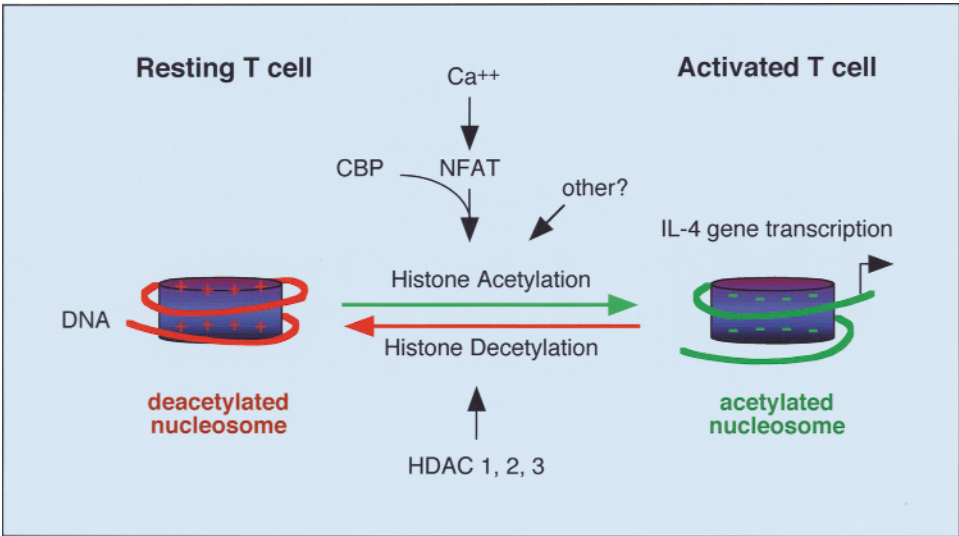


FIG 7. Schematic diagram of histone acetylation near the *IL4* gene in resting and activated T cells. Cellular activation enhances NFAT nuclear translocation, which recruits CBP (and possibly other factors) to DNA, which then acetylates lysine residues in the histone octamer (blue cylinder). Acetylated histones do not associate as strongly with DNA, allowing transcription of the *IL4* gene by means of RNA polymerase and associated factors.

IL4 gene expression is regulated at the level of transcription by a proximal promoter and other regulatory elements.^{15,37,41-44} Although essential for tissue-restricted gene expression,^{45,46} the *IL-4* promoter alone is a relatively weak activator of transcription. We have previously shown that maximal *IL-4* promoter activity is induced in Jurkat T cells by a calcium signal alone, reflecting the need for NFAT activation.^{31,43} The noninducibility of the minimal promoter construct 65 luc (Fig 4) is not surprising because it lacks the P1 NFAT element, which is known to be required for promoter activation.⁴⁷ Our observation that cotransfected CBP potentiated *IL-4* promoter activity likely reflects its ability to interact with NFAT⁴⁸ and is reminiscent of results recently obtained with a multimerized NFAT response element.⁴⁹ CBP is a versatile coactivator, and different domains appear to activate transcription in a cell type- and promoter-specific manner.⁴⁹ Studies aimed at identifying the precise basis by which CBP transactivates the *IL-4* promoter are under way.

We found that overexpression of 3 type 1 HDACs inhibited *IL-4* promoter activity in transiently transfected Jurkat T cells. In future studies it will be important to determine whether HDAC family members globally repress T-cell cytokine gene transcription or if they act in a subset-specific fashion. The ability of histone-modifying enzymes to regulate transcription driven by transiently transfected reporter constructs has been observed in other model systems^{9,32,50} and likely reflects the assembly of nucleosomal structures on nonintegrated plasmid templates.⁵¹ The precise mechanism by which chromatin is organized in transfected plasmids is not known, although it has been observed that acetylated histones can faithfully associate with transfected DNA.⁵⁰ To ensure that there was at least some association of histones with reporter plasmids in our experiments, we performed ChIP assays by using transiently transfected Jurkat cells and analyzed DNA precipitated with the anti-acetyl H4 antibody by means of PCR with a 5' *IL-4* promoter primer and a 3' primer specific for the luciferase gene. Interestingly, in these experiments we did detect a plasmid-specific transcript in transfected but not nontransfected cells (data not shown). This finding supports the use of transfection assays to study histone-modifying enzymes, although we can not exclude the possibility that these enzymes regulate the expression of integrated and chromatinized DNA templates in a distinct fashion.

The molecular basis of T_H2 differentiation has been studied extensively with transgenic and knock-out mouse models.⁵² By using these approaches, several critical transcription factors have been identified, including NFATc, cMaf, and Stat6.⁵³⁻⁵⁵ Recent studies have also uncovered chromatin-based regulatory mechanisms involved in T_H2 differentiation that are dependent on GATA-3 and other factors.^{28,30,56-59} In different model systems chromatin remodeling at the T_H2 locus in differentiating T cells has been shown to involve histone acetylation, DNA demethylation, and positioning away from heterochromatin.^{26,27,58} A picture is emerging in which

multiple regulatory elements interact to favorably alter chromatin structure and allow transcription of the *IL4* gene in activated T_H2 cells.^{59,60} It is difficult to extrapolate results from mouse T cells studied in vitro under highly polarizing conditions to peripheral blood human lymphocytes. Our results provide the first demonstration to our knowledge that epigenetic modifications can alter cytokine gene expression in freshly isolated human T cells (Fig 7). Specifically, the data presented in this article show that *IL4* gene expression is inhibited in T cells by means of histone deacetylation and that this is mediated by regulatory elements contained within the proximal *IL-4* promoter. Future studies aimed at uncovering the specific cell types and signal-transduction pathways involved in this response should prove to be worthwhile. It will also be interesting to determine whether alterations in histone structure contribute to dysregulated *IL4* gene expression in allergic diseases.

We thank our collaborators, Drs Donald MacGlashan, Edward Seto, Michael Rosenfeld, and Stuart Schreiber, for their generous gifts of cells and reagents and Dr Robert Eisenman for helpful suggestions.

REFERENCES

1. Bird AP, Wolffe AP. Methylation-induced repression—belts, braces, and chromatin. *Cell* 1999;99:451-4.
2. Workman JL, Kingston RE. Alteration of nucleosome structure as a mechanism of transcriptional regulation. *Annu Rev Biochem* 1998;67:545-79.
3. Struhl K. Histone acetylation and transcriptional regulatory mechanisms. *Genes Dev* 1998;12:599-606.
4. Zhao K, Wang W, Rando OJ, Xue Y, Swiderek K, Kuo A, et al. Rapid and phosphoinositide-dependent binding of the SWI/SNF-like BAF complex to chromatin after T lymphocyte receptor signaling. *Cell* 1998;95:625-36.
5. Kim J, Sif S, Jones B, Jackson A, Koipally J, Heller E, et al. Ikaros DNA-binding proteins direct formation of chromatin remodeling complexes in lymphocytes. *Immunity* 1999;10:345-55.
6. Jenuwein T, Allis CD. Translating the histone code. *Science* 2001;293:1074-80.
7. Knoepfler PS, Eisenman RN. Sin meets NuRD and other tails of repression. *Cell* 1999;99:447-50.
8. Hassig CA, Tong JK, Fleischer TC, Owa T, Grable PG, Ayer DE, et al. A role for histone deacetylase activity in HDAC1-mediated transcriptional repression. *Proc Natl Acad Sci U S A* 1998;95:3519-24.
9. Yang W-M, Inouye C, Zeng Y, Bearss D, Seto E. Transcriptional repression by YY1 is mediated by interaction with a mammalian homolog of the yeast global regulator RPD3. *Proc Natl Acad Sci U S A* 1996;93:12845-50.
10. Grozinger CM, Hassig CA, Schreiber SL. Three proteins define a class of human histone deacetylases related to yeast Hda1p. *Proc Natl Acad Sci U S A* 1999;96:4868-73.
11. Bertos NR, Wang AH, Yang XJ. Class II histone deacetylases: structure, function, and regulation. *Biochem Cell Biol* 2001;79:243-52.
12. Yang WM, Yao YL, Sun JM, Davie JR, Seto E. Isolation and characterization of cDNAs corresponding to an additional member of the human histone deacetylase gene family. *J Biol Chem* 1997;272:28001-7.
13. Laherty CD, Yang WM, Sun JM, Davie JR, Seto E, Eisenman RN. Histone deacetylases associated with the mSin3 corepressor mediate mad transcriptional repression. *Cell* 1997;89:349-56.
14. Wolffe AP, Matzke MA. Epigenetics: regulation through repression. *Science* 1999;286:481-6.
15. Brown MA, Hural J. Functions of *IL-4* and control of its expression. *Crit Rev Immunol* 1997;17:1-32.
16. Chan SC, Brown MA, Willcox TM, Li SH, Stevens SR, Tara D, et al. Abnormal *IL-4* gene expression by atopic dermatitis T lymphocytes is reflected in altered nuclear protein interactions with *IL-4* transcriptional regulatory element. *J Invest Dermatol* 1996;106:1131-6.
17. Rivas D, Mozo L, Zamorano J, Gayo A, Torre-Alonso JC, Rodriguez A,

- et al. Upregulated expression of IL-4 receptors and increased levels of IL-4 in rheumatoid arthritis patients. *J Autoimmun* 1995;8:587-600.
18. Wilson SB, Kent SC, Patton KT, Orban T, Jackson RA, Exley M, et al. Extreme Th1 bias of invariant α 24 β T cells in type 1 diabetes. *Nature* 1998;391:177-81.
19. Szabo SJ, Glimcher LH, Ho IC. Genes that regulate interleukin-4 expression in T cells. *Curr Opin Immunol* 1997;9:776-81.
20. Ranganath S, Ouyang W, Bhattacharya D, Sha W, Grupe A, Peltz G, et al. GATA-3-dependent enhancer activity in IL-4 gene regulation. *J Immunol* 1998;161:3822-6.
21. Loots GG, Locksley RM, Blankespoor CM, Wang ZE, Miller W, Rubin EM, et al. Identification of a coordinate regulator of interleukins 4, 13, and 5 by cross-species sequence comparisons. *Science* 2000;288:136-40.
22. Agarwal S, Avni O, Rao A. Cell-type-restricted binding of the transcription factor NFAT to a distal IL-4 enhancer in vivo. *Immunity* 2000;12:643-52.
23. Burchard EG, Silverman EK, Rosenwasser LJ, Borish L, Yandava C, Pillari A, et al. Association between a sequence variant in the IL-4 gene promoter and FEV(1) in asthma. *Am J Respir Crit Care Med* 1999;160:919-22.
24. Graves PE, Kabesch M, Halonen M, Holberg CJ, Baldini M, Fritsch C, et al. A cluster of seven tightly linked polymorphisms in the IL-13 gene is associated with total serum IgE levels in three populations of white children. *J Allergy Clin Immunol* 2000;105:506-13.
25. Keen JC, Sholl L, Wills-Karp M, Gaurus SN. Preferential activation of nuclear factor of activated T cells c correlates with mouse strain susceptibility to allergic responses and interleukin-4 gene expression. *Am J Respir Cell Mol Biol* 2001;24:58-65.
26. Grogan JL, Mohrs M, Harmon B, Lacy DA, Sedat JW, Locksley RM. Early transcription and silencing of cytokine genes underlie polarization of T helper cell subsets. *Immunity* 2001;14:205-15.
27. Bird J, Brown D, Mullen A, Moskowitz N, Mahowald M, Sider J, et al. Helper T cell differentiation is controlled by the cell cycle. *Immunity* 1998;9:229-37.
28. Agarwal S, Rao A. Modulation of chromatin structure regulates cytokine gene expression during T cell differentiation. *Immunity* 1998;9:765-75.
29. Takemoto N, Koyano-Nakagawa N, Yokota T, Arai N, Miyatake S, Arai K. Th2-specific DNase I-hypersensitive sites in the murine IL-13 and IL-4 intergenic region. *Int Immunol* 1998;10:1981-5.
30. Agarwal S, Viola JP, Rao A. Chromatin-based regulatory mechanisms governing cytokine gene transcription. *J Allergy Clin Immunol* 1999;103:990-9.
31. Chen R, Burke T, Cumberland J, Brummet M, Beck L, Casolaro V, et al. Glucocorticoids inhibit calcium- and calcineurin-dependent activation of the human IL-4 promoter. *J Immunol* 2000;164:825-32.
32. Korzus E, Torchia J, Rose DW, Xu L, Kurokawa R, McInerney EM, et al. Transcription factor-specific requirements for coactivators and their acetyltransferase functions. *Science* 1998;279:703-7.
33. Schroeder JT, MacGlashan DW Jr, Kagey-Sobotka A, White JM, Lichtenstein LM. IgE-dependent IL-4 secretion by human basophils. The relationship between cytokine production and histamine release in mixed leukocyte cultures. *J Immunol* 1994;153:1808-17.
34. Yoshida M, Horinouchi S, Beppu T. Trichostatin A and trapoxin: novel chemical probes for the role of histone acetylation in chromatin structure and function. *Bioessays* 1995;17:423-30.
35. Hassig CA, Fleischer TC, Billin AN, Schreiber SL, Ayer DE. Histone deacetylase activity is required for full transcriptional repression by mSin3A. *Cell* 1997;89:341-7.
36. Orlando V, Strutt H, Paro R. Analysis of chromatin structure by in vivo formaldehyde cross-linking. *Methods* 1997;11:205-14.
37. Burke TF, Casolaro V, Gaurus SN. Characterization of P5, a novel NFAT/AP-1 site in the human IL-4 promoter. *Biochem Biophys Res Commun* 2000;270:1016-23.
38. Finnin MS, Donigian JR, Cohen A, Richon VM, Rifkind RA, Marks PA, et al. Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. *Nature* 1999;401:188-93.
39. Schroeder JT, MacGlashan DW, Jr. New concepts: the basophil. *J Allergy Clin Immunol* 1997;99:429-33.
40. Dangond F, Gullans SR. Differential expression of human histone deacetylase mRNAs in response to immune cell apoptosis induction by trichostatin A and butyrate. *Biochem Biophys Res Commun* 1998;247:833-7.
41. Szabo S, Gold J, Murphy T, Murphy K. Identification of cis-acting regulatory elements controlling interleukin-4 gene expression in T cells: roles for NF-Y and NF-AT. *Mol Cell Biol* 1993;13:4793-805.
42. Chuvpilo S, Schomburg C, Gerwig R, Heinfli A, Reeves R, Grummt F, et al. Multiple closely-linked NFAT/octamer and HMG I(Y) binding sites are part of the interleukin-4 promoter. *Nucl Acids Res* 1993;21:5694-704.
43. Casolaro V, Gaurus S, Song Z, Zubkoff I, Abdulkadir S, Thanos D, et al. Inhibition of NF-AT-dependent transcription by NF-kB: implications for differential cytokine gene expression. *Proc Natl Acad Sci U S A* 1995;92:11623-7.
44. Casolaro V, Keane-Myers AM, Swendeman SL, Steindler C, Zhong F, Sheffery M, et al. Identification and characterization of a critical CP2-binding element in the human interleukin-4 promoter. *J Biol Chem* 2000;275:36605-11.
45. Todd M, Grusby M, Lederer J, Lacy E, Lichtman A, Glimcher L. Transcription of the interleukin 4 gene is regulated by multiple promoter elements. *J Exp Med* 1993;177:1663-74.
46. Wenner CA, Szabo SJ, Murphy KM. Identification of IL-4 promoter elements conferring Th2-restricted expression during T helper cell subset development. *J Immunol* 1997;158:765-73.
47. Abe E, De Waal Malefyt R, Matsuda I, Arai K, Arai N. An 11 base-pair DNA sequence motif apparently unique to human interleukin 4 gene confers responsiveness to T-cell activation signals. *Proc Natl Acad Sci U S A* 1992;89:2864-70.
48. Garcia-Rodriguez C, Rao A. Nuclear factor of activated T cells (NFAT)-dependent transactivation regulated by the coactivators p300/CREB-binding protein (CBP). *J Exp Med* 1998;187:2031-6.
49. Avots A, Buttmann M, Chuvpilo S, Escher C, Smola U, Bannister AJ, et al. CBP/p300 integrates Raf/Rac-signaling pathways in the transcriptional induction of NF-ATc during T cell activation. *Immunity* 1999;10:515-24.
50. Luo RX, Postigo AA, Dean DC. Rb interacts with histone deacetylase to repress transcription. *Cell* 1998;92:463-73.
51. Camerini-Otero RD, Zasloff MA. Nucleosomal packaging of the thymidine kinase gene of herpes simplex virus transferred into mouse cells: an actively expressed single-copy gene. *Proc Natl Acad Sci U S A* 1980;77:5079-83.
52. Murphy KM, Ouyang W, Farrar JD, Yang J, Ranganath S, Asnagli H, et al. Signaling and transcription in T helper development. *Annu Rev Immunol* 2000;18:451-94.
53. Kaplan MH, Schindler U, Smiley ST, Grusby MJ. Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. *Immunity* 1996;4:313-9.
54. Ranger A, Hodge M, Gravalles E, Oukka M, Davidson L, Alt F, et al. Delayed lymphoid repopulation with defects in IL-4-driven responses produced by inactivation of NF-ATc. *Immunity* 1998;8:125-34.
55. Ho IC, Lo D, Glimcher LH. c-maf promotes T helper cell type 2 (Th2) and attenuates Th1 differentiation by both interleukin 4-dependent and -independent mechanisms. *J Exp Med* 1998;188:1859-66.
56. Ouyang W, Lohning M, Gao Z, Assenmacher M, Ranganath S, Radbruch A, et al. Stat6-independent GATA-3 autoactivation directs IL-4-independent Th2 development and commitment. *Immunity* 2000;12:27-37.
57. Lee HJ, Takemoto N, Kurata H, Kamogawa Y, Miyatake S, O'Garra A, et al. GATA-3 induces T helper cell type 2 (Th2) cytokine expression and chromatin remodeling in committed Th1 cells. *J Exp Med* 2000;192:105-15.
58. Kimura M, Koseki Y, Yamashita M, Watanabe N, Shimizu C, Katsumoto T, et al. Regulation of Th2 Cell Differentiation by mel-18, a Mammalian Polycomb Group Gene. *Immunity* 2001;15:275-87.
59. Lee RL, Fields PE, Flavell RA. Regulation of IL-4 gene expression by distal regulatory elements and GATA-3 at the chromatin level. *Immunity* 2001;14:447-59.
60. Mohrs M, Blankespoor CM, Wang ZE, Loots GG, Afzal V, Hadeiba H, et al. Deletion of a coordinate regulator of type 2 cytokine expression in mice. *Nat Immunol* 2001;2:842-7.
61. Gaurus S, Cumberland J, Burke T, Chen R, Park E, Ono S, et al. Characterization of a novel repressor element in the interleukin 4 promoter. *Leukemia* 2000;14:629-35.
62. Rooney J, Hoey T, Glimcher L. Coordinate and cooperative roles for NF-AT and AP-1 in the regulation of the murine IL-4 gene. *Immunity* 1995;2:473-83.