

Letter to the Editor

Childhood allergic asthma is associated with increased IL-13 and FOXP3 histone acetylation

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Efficient maturation of the immune system in early childhood is essential for protection against allergy and asthma later in life. In addition to genetic predisposition, epigenetic modifications can influence the risk for development of chronic inflammatory diseases such as asthma. Epigenetic mechanisms including DNA methylation and histone acetylation play a major role in controlling the polarization of naive T cells toward different effector T cells such as T_H1 , T_H2 , and regulatory T (Treg) cells and may thus modify the risk for disease development by modulating the developmental function of these T-cell populations.¹ Recently, the link between epigenetic modifications and the risk for asthma development was investigated in several cohort studies, which mainly focused on DNA methylation,² whereas little is known about the role of histone modifications including histone acetylation during asthma manifestation. Here, we present data on histone acetylation of specific T_H1 , T_H2 , and Treg-cell-related gene loci in isolated $CD4^+$ T cells of children with allergic asthma (AA) compared with healthy control (HC) children.

Histone acetylation as an epigenetic marker for transcriptional activation was measured in a subgroup of 4- to 15-year-old steroid-naïve children with AA and HC children from the Munich Clinical Asthma Research Association (CLARA) study population ($n(AA) = 14$, $n(HCs) = 18$).³ Children with AA were characterized by increased eosinophils, IgE, and fractional exhaled nitric oxide compared with HC children and showed a significant bronchodilator response⁴ according to American Thoracic Society/European Respiratory Society guidelines. Clinical and population characteristics of this subpopulation were comparable to those of the whole population (data not shown).

PBMCs were isolated within 24 hours after blood drawing by density-gradient centrifugation with Ficoll-Hypaque (Amersham Bioscience, Uppsala, Sweden). $CD4^+$ cells were further isolated by negative selection using AutoMACS separation (Miltenyi Biotec, Bergisch Gladbach, Germany), with a purity of 93.8% on average, determined by flow cytometry. Following isolation, $CD4^+$ cells were directly frozen in FCS with dimethyl sulfoxide at a final dimethyl sulfoxide concentration of 7.5%. The cells were carefully thawed on ice not interfering with chromatin stability (unpublished data) and subsequently fixed with formaldehyde, sonicated with a Bioruptor (Diagenode, Seraing, Belgium) for 30 cycles (30 seconds on, 30 seconds off, high power), and Chromatin Immuno-precipitated with antibodies against acetylated histones H3 and H4 (Millipore, Darmstadt, Germany). The promoter regions of selected T_H1 , T_H2 , and Treg-cell signature gene loci were analyzed for enrichment of acetylated H3 and H4 histones. Acetylation at *RPL32* was measured as positive control, with mean enrichment of 1.27% for histone H3 and 1.31% for histone H4 of the input control. All following measurements were normalized for acetylation enrichment at the *RPL32* locus. MS4A2 served as a negative control and was not enriched in any sample. Statistical analysis was performed using Kruskal-Wallis test or Tobit regression analysis, depending on the distribution of data. As representative T_H1 genes, histone acetylation at the *TBX21* and *IFNG*-loci was measured. No differences in H3 and H4 acetylation levels were observed between children with AA and HC children (Fig 1, A). Consistent with this, Raedler et al³ did not observe differences in IFN- γ protein levels between children with AA and HC children of the whole CLARA study population.

Within the T_H2 gene loci, we observed no significant differences in histone acetylation for *IL4* and *IL5* between children with AA and HC children, but a significant increase in histone H3 acetylation ($P = .04$) and in parallel a trend for increased H4 acetylation at the *IL13* locus in children with AA compared with HC children (Fig 1, B; $P = .09$).

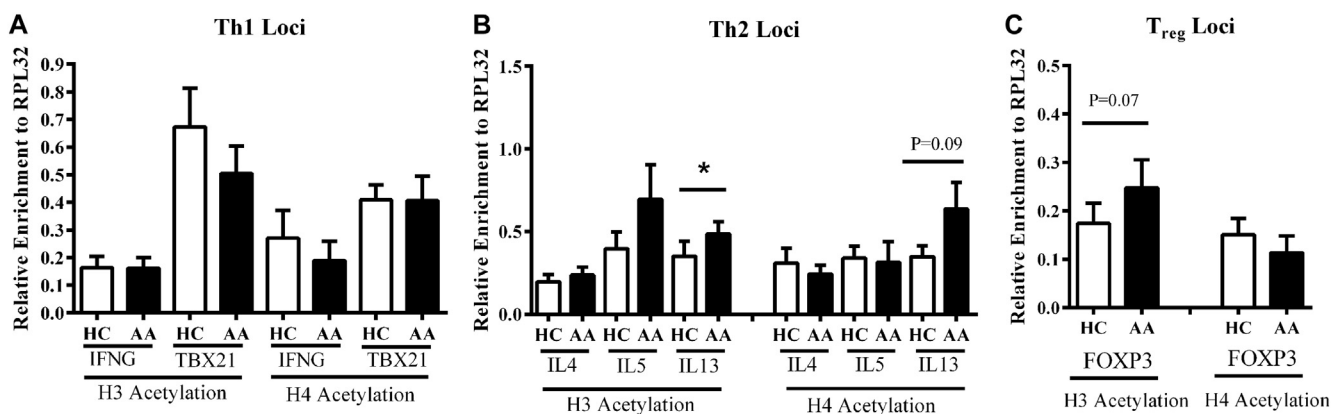


FIG 1. Histone acetylation at both H3 and H4 histones. **A**, Histone acetylation for T_H1 genes *IFNG* and *TBX21* showing no difference between HC children and children with AA. **B**, Histone acetylation for T_H2 genes *IL4*, *IL5*, and *IL13* showing a significant increase in histone acetylation at histone H3 at the *IL13* locus in parallel with a trend for increased H4 acetylation in children with AA compared with HC children. **C**, Histone acetylation for *FOXP3* showing a trend for increased H3 acetylation in children with AA compared with HC children; data presented as mean $\pm$ SE; $n(HCs) = 18$, $n(AA) = 14$; P value from Kruskal-Wallis test (*IL13* H3/H4) or Tobit regression analysis (*FOXP3* H3). * $P < .05$.

This difference in H3 acetylation correlated with higher IL-13 protein levels in supernatants of anti-CD3/CD28-stimulated PBMCs of children with AA compared with HC children (not shown; $r = .66$; $P = .02$). This may indicate a functional role of *IL13* acetylation for subsequent protein secretion. IL-13 is considered a critical T_H2 lineage cytokine being associated with asthma exacerbations.⁴ In addition, several single nucleotide polymorphisms (SNPs) at this locus have been associated with a higher risk for asthma development.⁵ Interestingly, the amplicon used in our study with focus on histone acetylation at the *IL13* promoter is spanning the *IL13* SNP rs1881457. This SNP was previously associated with atopy and asthmatic symptoms.⁶ For further studies, it would be interesting to investigate whether this genotype influences histone acetylation at the *IL13* promoter; however, this was beyond the scope of this study.

Analyzing the Treg-cell locus *FOXP3*, we identified that histone H3 acetylation at this locus was trendwise higher in children with AA than in HC children (Fig 1, C; $P = .07$). *FOXP3* encodes the master transcription factor for Treg-cell differentiation and is also important for Treg-cell function.⁷ Differentiation of Foxp3⁺ Treg cells is associated with a strong increase in *FOXP3* H3 acetylation in parallel with only marginal changes in histone H4 acetylation,⁸ indicating a crucial link between *FOXP3* H3 acetylation and the development of Treg cells. In murine models, histone deacetylase treatment and increased Foxp3 acetylation was associated with enhanced Treg-cell numbers and increased suppressive function of Treg cells.⁹ If increased *FOXP3* H3 acetylation promotes Treg-cell differentiation, a higher frequency of Treg cells would be expected in the asthmatic group. In accordance, Treg-cell numbers were increased in children with AA compared with HC children from the CLARA study population as recently shown.³ To date, the role of *FOXP3*-expressing Treg cells in asthma is not completely understood. Although Treg cells are mostly suggested to play an anti-inflammatory role in allergic disease, increased numbers of Treg cells have been described for patients with asthma before,¹⁰ potentially indicating a counterregulatory mechanism yet not sufficient to control allergic inflammation.³ Within the CLARA population, we did not observe differences in the suppressive capacity of Treg cells from children with AA compared with HC children, indirectly indicating no association between *FOXP3* H3 acetylation and Treg-cell function.³ Of note, acetylation data and suppressive function were assessed in different children with AA and HC children of the CLARA study and can consequently not provide a direct link between acetylation and functional data within this study. Also, additional posttranslational and epigenetic mechanisms beyond protein acetylation and DNA methylation are relevant to the modulation of Foxp3⁺ Treg-cell function, and should be included in future studies.

This study provides important insight into the complexity of epigenetic regulation, specifically histone acetylation, of immunoregulatory genes in isolated CD4⁺ peripheral blood T cells of asthmatic versus healthy children. Although our study is an exploratory study with a limited number of cases, we had the power to detect differences in the acetylation of the *IL13* and *FOXP3* locus in a subgroup of children with AA and HC

children from the CLARA study population, which is comparable to the whole population. In parallel, higher IL-13 protein levels and increased Treg-cell numbers were observed in children with AA versus HC children from the whole CLARA population,³ indicating that differences in acetylation may be associated with differences in T-cell differentiation and function between children with AA and healthy children. Nevertheless, presently we cannot distinguish whether the observed differences in histone acetylation at the analyzed loci are the cause or the consequence of disease development. For further investigation it would also be interesting to analyze CD4⁺ T cells from healthy children during immune maturation until manifestation of disease to determine a potential contribution of histone acetylation to the development of asthma symptoms later in life.

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