

HISTONE MODIFICATIONS OF NOTCH1 PROMOTER AFFECT LUNG CD4⁺ T CELL DIFFERENTIATION IN ASTHMATIC RATS

Z-L. CUI, W. GU, T. DING, X-H. PENG, X. CHEN, C-Y. LUAN, R-C. HAN,
W-G. XU and X-J. GUO

*Department of Respiratory Medicine, XinHua Hospital Affiliated to Shanghai Jiao Tong University
School of Medicine, Shanghai, China*

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Asthma is an inflammatory disease of the airways, and the current treatment in managing asthma is the control of inflammation. Notch signaling pathway has been linked to T-cell imbalance. The present study aimed to explore the histone modifications of Notch1 promoter in normal and asthmatic lung CD4⁺ T cells. Chromatin immunoprecipitation analysis showed that the acetylation levels of total H3, H4, site-specific H3K9, H3K14, H3K27, H3K18, H4K16, and the trimethylation levels of H3K4, H3K79 of Notch1 gene promoter were increased significantly in asthmatic lung CD4⁺ T cells compared to the control group, which correlated with increased P300, PCAF activity and decreased HDAC1, HDAC2 activity. After intervention of garcinol, a potent inhibitor of histone acetyltransferases, in asthmatic lung CD4⁺ T cells, HAT activity decreased significantly and the increased Notch1 and hes-1 expression was reversed. The total H3ac, H4ac, site-specific H3K9ac, H3K14ac, H3K27ac, H3K18ac, H4K16ac and H3K79me3 levels of Notch1 gene promoter decreased significantly, and the H3K4me3, H3K9me3, H4K20me3 levels had no significant difference. We further investigated the suppressive effects of GAR on asthmatic parameters. Results showed that the levels of IL-4, IL-5 and IL-13 were significantly reduced and a small reverse trend was found in the level of IFN- γ after GAR treatment. Furthermore, the expression of NF- κ B and AP-1 reduced significantly. These results suggest that asthma is associated with changes in the epigenetic status of Notch1 promoter, including abnormal histone acetylation and methylation, and GAR may have applications in the treatment of asthma.

Asthma is a chronic airway inflammatory illness caused by both heritable and environmental factors. Imbalance in effector T-cell populations plays an important role in the development of airway inflammation (1). The most extensively studied of these T-helper subsets are called Th1 and Th2. Th1 cells are characterized by production of IFN- γ and are responsible for generating immunity against intracellular pathogens. On the other hand, Th2 cells produce IL-4, IL-5 and IL-13, are necessary

for protection against helminth worms, and mediate in allergic reactions. Allergic bronchial asthma is a prototypic disease of dominant Th2 phenotype activation, although other T-cell subtypes might be involved (2, 3).

The Notch pathway is an evolutionarily conserved signaling cascade that regulates different aspects of cellular differentiation, proliferation and apoptotic events in multicellular organisms and has been shown to play a pathogenetic role in a variety of diseases,

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Mailing address: Prof. Xue-Jun GUO and Wei-Guo XU,
Department of Respiratory Medicine, XinHua Hospital
Affiliated to Shanghai Jiao Tong University
School of Medicine, 1665 Kongjiang Road,
Shanghai, 200092, China
Tel.: +86 21 25077370
e-mail: guoxjz@126.com; xuweiguo@xinhua@126.com

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including inflammation and tumor (4). In mammals, there are four Notch receptors (Notch1, 2, 3 and 4) and five ligands (Jagged1 and 2, and Delta-like1, 3, and 4). Upon binding to its ligands, these receptors undergo a series of proteolytic steps initiated by presenilin-dependent gamma secretase-like protease that result in the release of the Notch intracellular domain (ICN). The ICN next translocates to the nucleus where it activates the expression of Notch target proteins such as Hes, Hey and Cux1 (5).

Although rather controversial, new evidence has assigned a role of the Notch signaling pathway to peripheral T-cell responses such as CD4⁺ T cell activation and polarization (6). For instance, it has been shown that the inhibition of Notch signaling by γ -secretase inhibitors (GSI) decreases IFN- γ production and the proliferation of activated T cells (7). On the other hand, the first evidence of Notch involvement in T-helper cell differentiation came from the observation that the Notch ligands, Jagged and Delta, are expressed on dendritic cells following Th1 and Th2 polarizing conditions (8). Studies showed that treatment with GSI blocks the generation of Th2 cells, suggesting that Notch signaling is involved in the regulation of Th2 responses (9, 10). However, the precise role of Notch signaling pathway in T-cell polarization remains to be further explored.

Epigenetics is defined as the study of mitotically and meiotically heritable changes in gene function that are not dependent on DNA sequence. The molecular mechanism of epigenetic processes is complex and involves histone modifications, DNA methylation, positioning of histone variants, and gene regulation by non-coding RNAs (11, 12). Changes in histone modifications and DNA methylation selectively activate or inactivate genes that control cell growth, proliferation and apoptosis and determine when and where genes are expressed in the process of development (13). Epigenetic mechanisms are thought to play a key role in different complex diseases of the lungs as recently reviewed. Chronic obstructive pulmonary disease (COPD) and asthma are strongly influenced by environmental factors, including pollution and cigarette smoking (14, 15). Epigenetic modifications are potentially reversible, therefore, a comprehensive understanding of these changes may identify novel therapeutic targets for many diseases (16).

In this study, we focused on the epigenetic changes of Notch1 promoter. The purpose of this study was to verify the hypothesis that asthma is associated with gene-activating epigenetic changes in Notch1 promoter. Histone modifications were investigated in normal and asthmatic lung CD4⁺ T cells via Chromatin Immunoprecipitation (CHIP). The effects of garcinol (GAR) on Notch signaling pathway and asthmatic parameters were also investigated.

MATERIALS AND METHODS

OVA-sensitized chronic airway inflammation

Six-to-eight-week-old male Wistar rats (mean weight 160 $\pm$ 10 g) were bred under specific pathogen-free conditions. Animals were sensitized with 2 mg of OVA (grade V; Sigma-Aldrich, St. Louis, MO, USA) and 200 mg of Al(OH)₃ in 1 ml 0.9% NaCl applied subcutaneously. As a second adjuvant, concentrated preparations of 56109 heat-killed *Bordetella pertussis* bacilli were given intraperitoneally at the same time. All sensitization procedures were repeated on day 7 after the first sensitization. The rats were then exposed to aerosolized OVA for 30 min per day from day 14 to day 28 in a whole body inhalation exposure system. The rats in the control group were administered normal saline with Al(OH)₃ intraperitoneally on 0 and 7th days of the experiment, and exposed to aerosolized saline for 30 min per day from day 14 to day 28. All animal research was approved by the Shanghai Jiaotong University School of Medicine Animal Care and Use Committee.

Bronchoalveolar Lavage (BAL)

Rats were sacrificed 48 h after the last challenge and tracheotomies were performed. After ice-cold PBS having been instilled into the lungs, bronchoalveolar lavage fluid (BALF) was obtained via tracheal cannulation. The BALF was centrifuged and supernatants were collected. Total inflammatory cell numbers were assessed by counting cells in at least five squares of a hemocytometer. One hundred microliters of BALF was loaded onto a slide and centrifuged to fix the cells onto the slide using a cytospin machine.

Measurement of airway hyperresponsiveness (AHR)

In vivo airway responsiveness to methacholine was measured twenty-four hours after the last OVA challenge. The rats were challenged with aerosolized saline or methacholine (12.5, 25, 50 mg/ml). The degree of bronchoconstriction was recorded for an additional 5 min after each dose of methacholine. The highest Penh

value during each methacholine challenge was expressed as a proportion of the basal Penh value in response to PBS challenge.

Lung histopathology

For histologic analysis, the right lung was fixed with 4% paraformaldehyde through the trachea, removed, and stored in 4% paraformaldehyde. Lung tissues were cut into 3 μ m sections and stained with hematoxylin and eosin. The degree of airway inflammatory cell infiltration, mucus occlusions of the airway diameter, and airway edema were determined by morphometry.

Isolation and culture of lung CD4⁺ T Cells

Lungs were removed following perfusion with 15 ml ice-cold PBS via the left ventricle of the heart, and single-cell suspensions were prepared by collagenase I (1 mg/ml; Invitrogen) and DNase I (0.2 mg/ml, Invitrogen) treatment. Digested lung tissue was isolated by Histopaque gradient centrifugation (Sigma-Aldrich) and further dispersed by passage through a 75-mm-pore nylon tissue strainer (BD Biosciences); the resultant single-cell suspension was treated with RBC lysis buffer (BD Biosciences) to remove residual RBCs and washed twice. The cells were then subjected to positive selection with anti-CD4 magnetic beads on MS-positive selection columns (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's protocol. FACS analysis revealed a purity of greater than 90%.

GAR treatment

Isolated lung CD4⁺ T cells were cultured in complete medium (RPMI 1640 containing 10% heat-inactivated FCS, 100 U/ml penicillin, 100 mg/ml streptomycin, 2 mM L-glutamine) with anti-CD3 (5 μ g/ml) and anti-CD28 (2 μ g/ml). Cells were treated with DMSO or 10 μ M GAR (Enzo Life Sciences, New York, USA) in DMSO, and then harvested for analysis after 48 h.

Real-time PCR

Total RNA was extracted from asthmatic lung CD4⁺ T cells with Trizol Reagent (Invitrogen) and transcribed to cDNA (Takara). SYBR Green Master Mix kit (Takara) was used for real-time PCR with ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). The real-time PCR protocol consisted of 95°C for 30 sec, followed by 40 cycles of 95°C for 5 sec and 60°C for 34 sec, with a final dissociation stage. The Ct of target genes was normalized to GAPDH gene transcript levels. Relative quantification and calculation were performed using the comparative threshold cycle method. All experiments were performed in triplicate. The real-time PCR primer sequences are shown in Table I.

Western blotting

Lung CD4⁺ T cells were lysed and denatured. Equal quantities of protein were loaded onto SDS-PAGE and transferred to PVDF membranes. The membranes were blocked in Tris-buffered saline, 0.1% Tween20, and 5% milk, and then incubated with primary antibodies overnight at 4°C. Specific HRP-conjugated secondary antibodies were incubated with the membranes for 2 h at room temperature. Finally, the membranes were incubated in ECL reagents (Pierce) and exposed to film. For the quantification of expression levels, the blots were scanned and the density of each band was determined using image analysis software and normalized against the density of the GAPDH band. All experiments were repeated in three samples.

HAT activity assay in vitro

Lung CD4⁺ T cells were collected and nuclear proteins were extracted. HAT activity of nuclear extracts was measured with a nonisotopic assay using fluorescent derivative of acetyl lysine (HAT Assay Kit; Upstate Biotechnology). The detection plates were pre-coated with reconstituted histone and incubated overnight at 4°C. Nuclear extraction was incubated with HAT reaction cocktail at room temperature for 15 min. Then the HAT assays were performed according to the manufacturer's instructions and emitted light was detected at 460 nm in a fluorometric plate reader. Each assay group was determined in triplicate samples.

Chromatin immunoprecipitation assays

Chromatin was prepared and precipitated using the ChIP kit (EZ-CHIP™; Upstate Biotechnology) as described by the manufacturer. Purified Lung CD4⁺ T cells (1×10^6 for a single precipitation) were re-suspended in growth media and crosslinked in 1% formaldehyde for 10 min at 37°C. Crosslinking was quenched by the addition of glycine, cells were lysed and chromatin was sonicated. Chromatin was incubated overnight with the antibody of interest. The next day, immunoprecipitation complexes were harvested by incubation with 60 μ l Protein G Agarose. Immunoprecipitation complexes were eluted from the protein G Agarose and reverse crosslinked to free DNA. DNA was extracted with phenol-chloroform, precipitated with ethanol, and dissolved in elution buffer. The abundance of immunoprecipitated DNA fragments was quantified by real-time PCR using SYBR Green Master Mix kit.

Measurements of cytokines

The cytokine levels in the BAL fluid and cell culture supernatants were measured by ELISA. IL-4, IL-5, IL-13 and IFN- γ (R&D Systems, Minneapolis, MN, USA)

ELISAs were performed according to the manufacturer's protocols.

Statistical analysis

Statistical significance between groups was examined by one-way ANOVA followed by Tukey's test using SPSS version 13.0 (SPSS Inc, Chicago, IL, USA). Data are representative of three independent experiments with six rats in each group. Significance was defined as $P < 0.05$.

RESULTS

OVA-sensitized asthmatic rat models were established successfully

Airway responsiveness to inhaled methacholine was measured. Results showed a significant increase of AHR in the OVA group compared with saline-treated rats (Fig. 1A). The total cell numbers in BALF were increased in the OVA group compared with the control group, especially eosinophilia (Fig. 1B). The

concentrations of IL-4 and IFN- γ in the serum and BAL were detected by ELISA as described above. In the asthmatic group, the IL-4 levels increased significantly, while IFN- γ decreased significantly in both the serum and BAL. (Fig. 1C). HE staining of asthmatic lung tissue showed apparent inflammation of the airways mainly caused by an influx of eosinophils and lymphocytes (Fig. 1D).

Expression of Notch1 and hes-1 in lung CD4⁺ T Cells

To investigate the involvement of Notch signaling pathway in asthma, the mRNA expression levels of Notch1 and hes-1 in normal and asthmatic lung CD4⁺ T cells were measured by real-time PCR. As shown in Fig. 2A, compared with the control group, Notch1 and hes-1 mRNA expression in asthmatic lung CD4⁺ T cells increased significantly. Western blotting was used to detect the protein levels of Notch1 and hes-1. Notch1 and hes-1 protein was expressed higher in

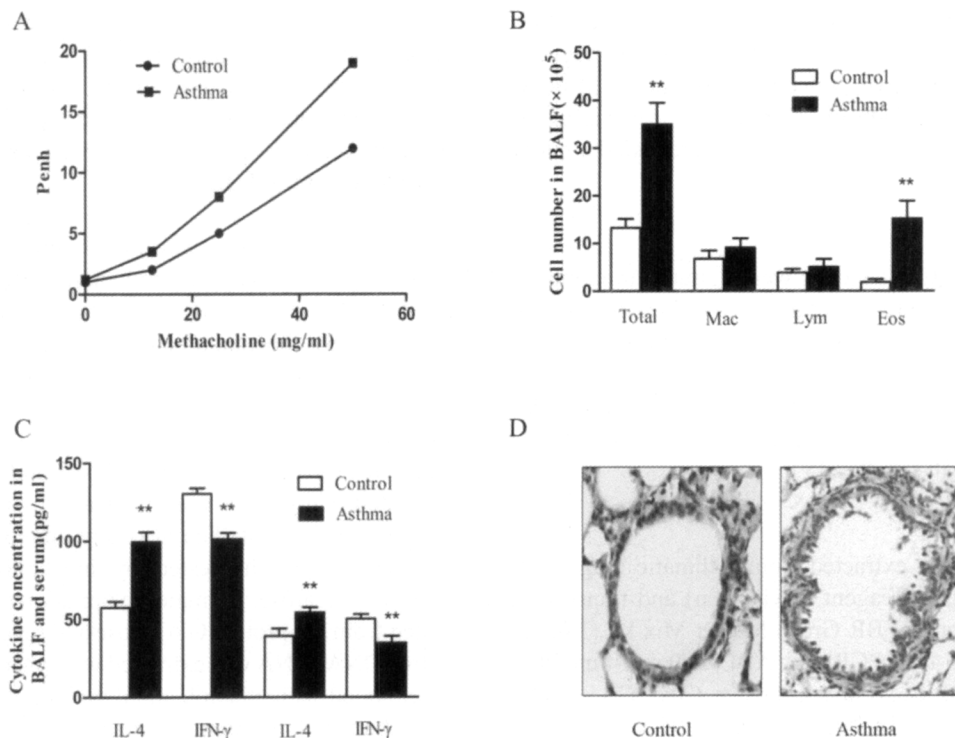


Fig. 1. OVA-sensitized asthmatic rat models were established successfully. **A)** The degree of bronchoconstriction in response to inhaled methacholine was determined in saline-treated group (SAL; $n=6$) and the OVA group (OVA; $n=6$). **B)** Cell numbers and composition of BALF in the OVA group and the control group. Data are presented as the mean $\pm$ SEM of three independent experiments. ** $p < 0.01$. **C)** The concentrations of IL-4 and IFN- γ in the serum and BALF. Data are presented as the mean $\pm$ SEM ($n=6$ in each group). ** $p < 0.01$. **D)** HE staining of lung tissue in the asthmatic group and the control group. The asthmatic group showed apparent inflammation cell infiltration in the airways.

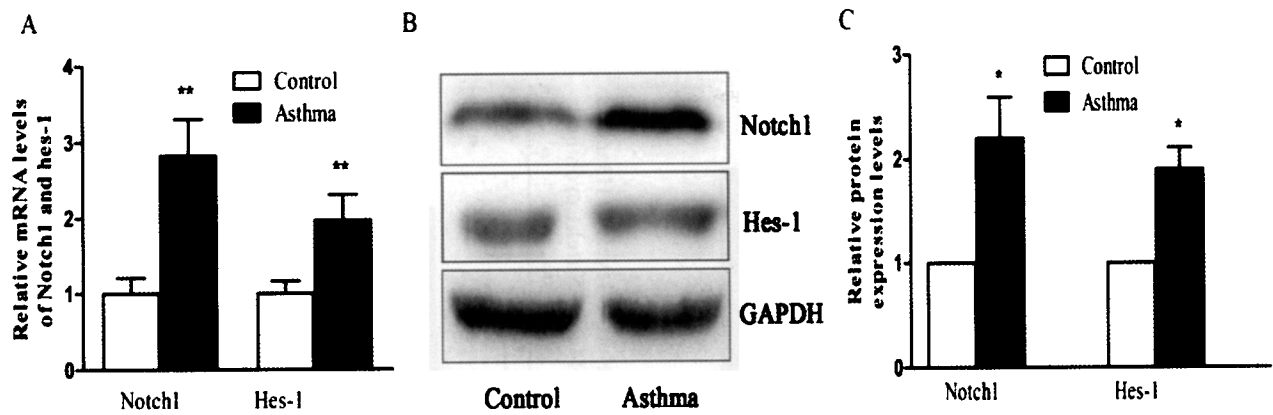


Fig. 2. Expression of Notch1 and hes-1 in lung CD4⁺ T cells. **A)** The mRNA expression levels of Notch1 and hes-1 in lung CD4⁺ T cells were detected by real-time PCR, normalized to GAPDH. Results are from three independent experiments and the results for each group are expressed as mean $\pm$ SEM. ** $P < 0.01$. **B, C)** Western blotting was performed to detect the protein levels of Notch1 and hes-1. GAPDH was used as a loading control. The results are representative of three independent experiments. * $P < 0.05$.

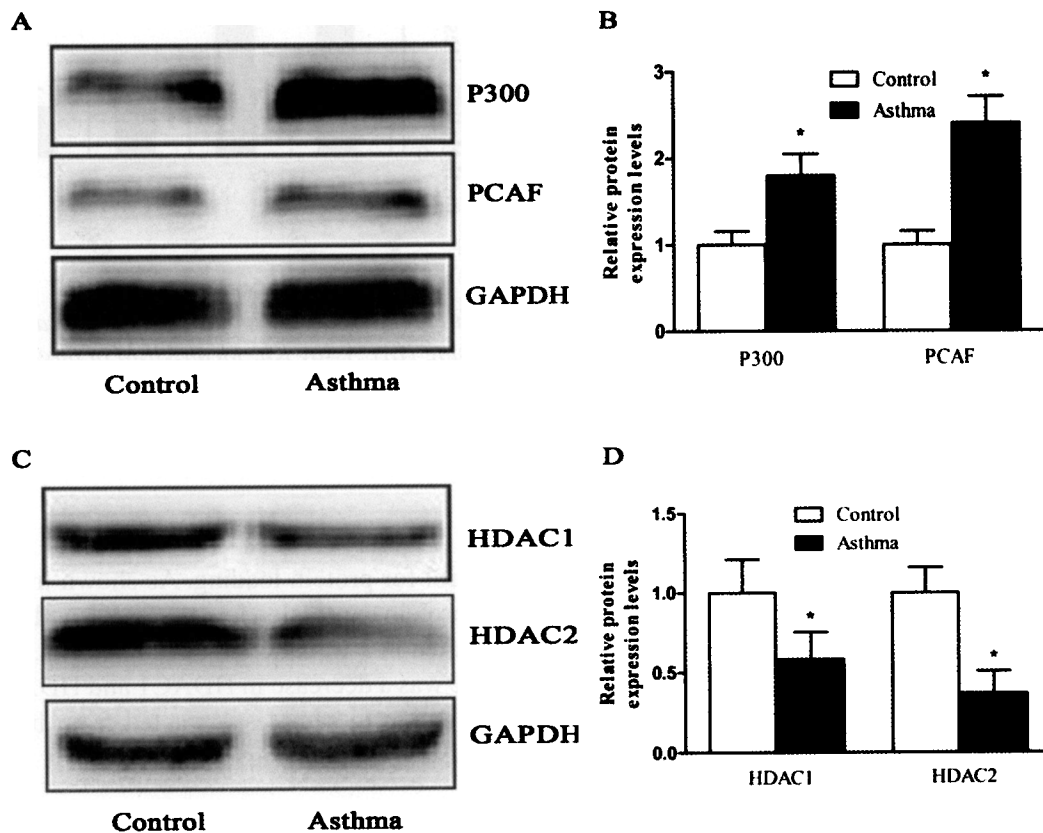


Fig. 3. Expression of P300, PCAF, HDAC1 and HDAC2 in lung CD4⁺ T cells. **A, B)** The relative expression of P300 and PCAF in lung CD4⁺ T cells was detected by Western blotting. GAPDH expression was used as a control for protein loading. Results are representative in each group. * $P < 0.05$. **C, D)** The expression of HDAC1 and HDAC2 was also detected by Western blotting. Experiments were repeated thrice. * $P < 0.05$.

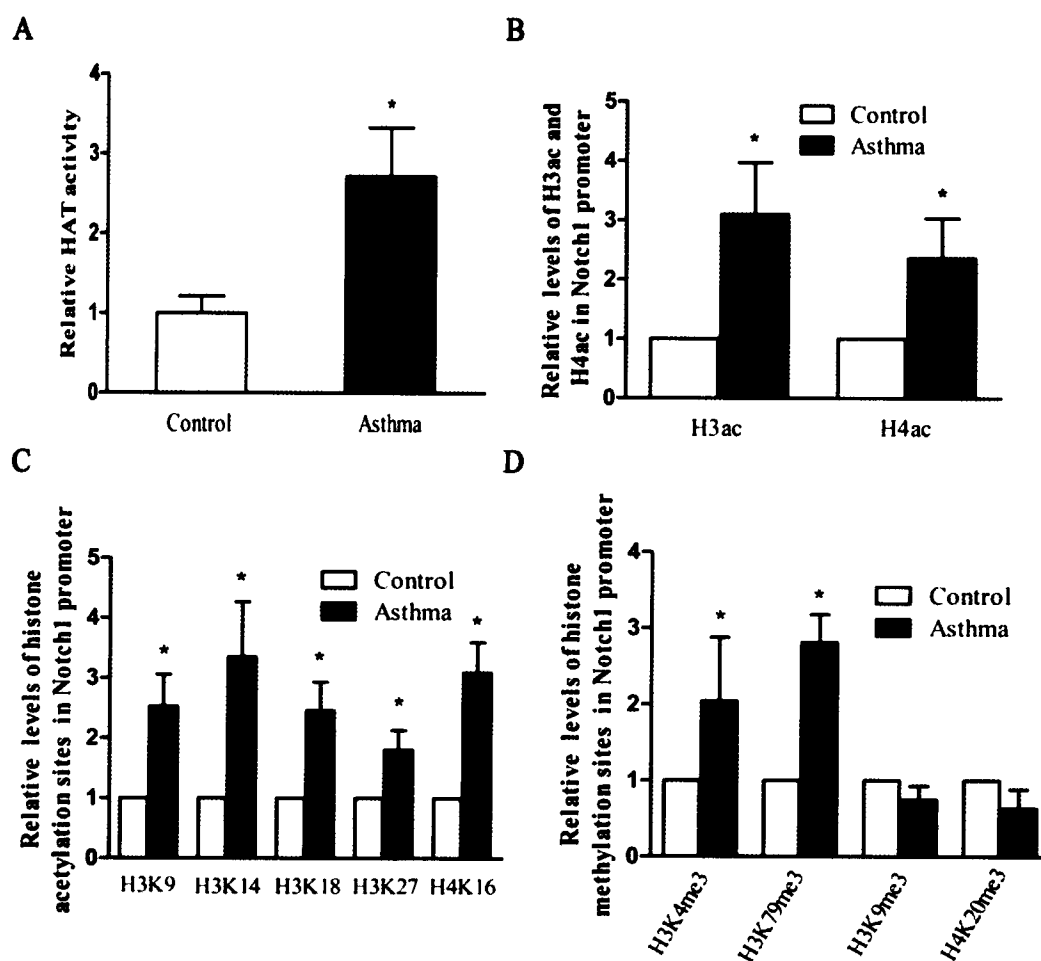


Fig. 4. HAT activity and histone modifications are associated with upregulation of Notch1 promoter in asthmatic lung CD4⁺ T cells. **A)** HAT activity of nuclear extracts was measured using HAT Assay Kit. Mean $\pm$ SEM of three independent experiment is shown. * P <0.05. **B, C)** The histone acetylation levels of total H3, H4, site-specific H3K9, H3K14, H3K27, H3K18 and H4K16 were assessed via CHIP. Experiments were repeated thrice. * P <0.05. **D)** The trimethylation levels of H3K9, H3K4, H3K79 and H4K20 at Notch1 promoter of lung CD4⁺ T cells were also investigated. Results are from three independent experiments. * P <0.05.

asthmatic lung CD4⁺ T cells, which was consistent with the mRNA levels (Fig. 2B-C).

Expression of P300, PCAF, HDAC1 and HDAC2 in lung CD4⁺ T cells

Modulation of chromatin structure by histone acetylation and deacetylation plays an important role in inflammatory gene transcription. Increased gene expression is associated with enhanced histone acetylation or diminished deacetylation. The expression of the P300 and PCAF in lung CD4⁺ T cells of normal and asthmatic rats was detected by Western blotting. GAPDH expression was used as a

control for protein loading. As shown in Fig. 3A-B, the protein expression levels of P300 and PCAF were elevated in asthmatic lung CD4⁺ T cells. In contrast, there was a decrease in HDAC1 and HDAC2 protein expression in samples extracted from lung CD4⁺ T cells with asthma (Fig. 3C-D).

HAT activity and Histone modifications are associated with upregulation of Notch1 promoter in asthmatic lung CD4⁺ T cells

Lung CD4⁺ T cells were collected and nuclear proteins were extracted, HAT activity of nuclear extracts was measured with a nonisotopic assay

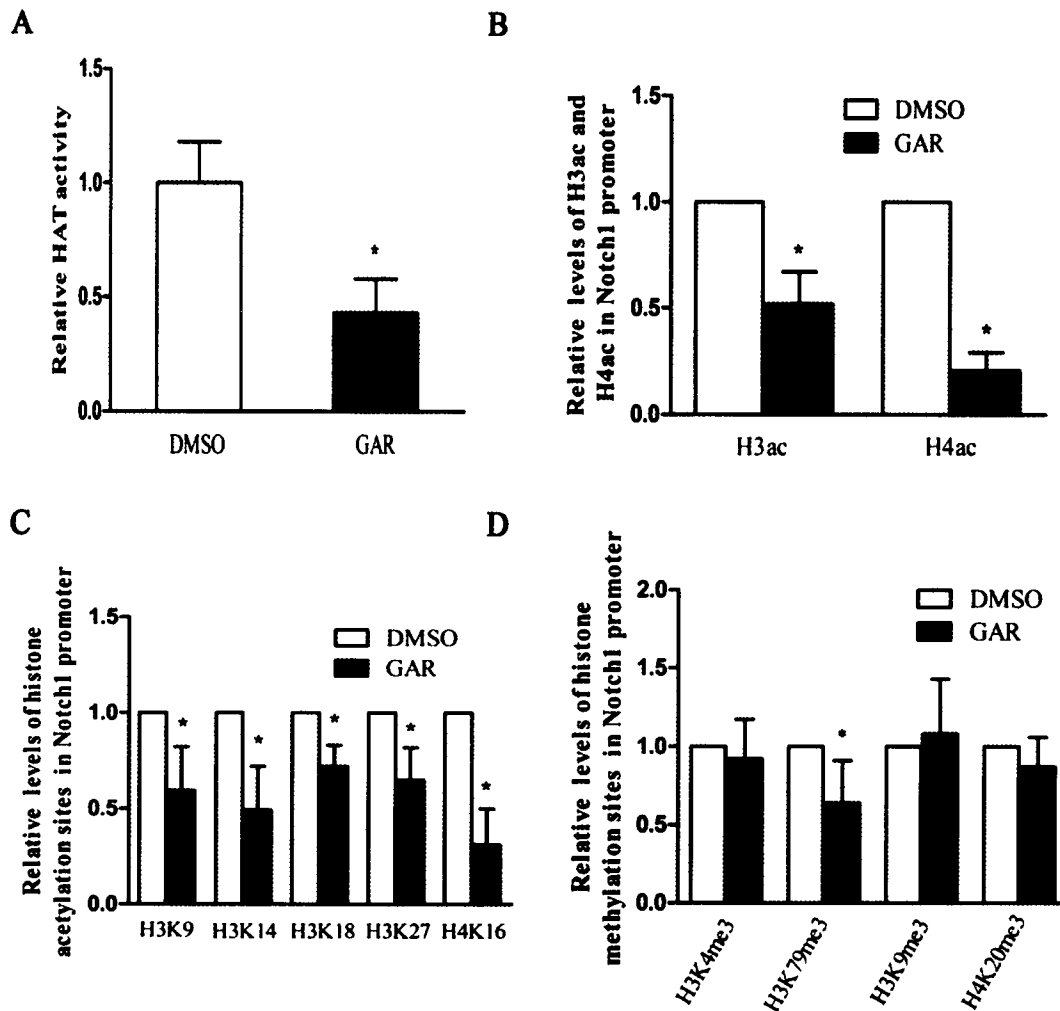


Fig. 5. GAR inhibits the HAT activity and alters histone modifications of Notch1 promoter in asthmatic lung CD4⁺ T cells. **A)** After treatment with GAR (10 mM), the HAT activity of asthmatic lung CD4⁺ T cells was determined using HAT Assay Kit. Mean $\pm$ SEM of three independent experiments is shown. * P <0.05. **B, C)** After treatment with GAR (10 mM), the histone acetylation levels of total H3, H4, site-specific H3K9, H3K14, H3K27, H3K18 and H4K16 were determined via CHIP. Experiments were repeated thrice. * P <0.05. **D)** The trimethylation levels of H3K9, H3K4, H3K79 and H4K20 at Notch1 promoter were also investigated. Experiments were repeated thrice. * P <0.05.

using fluorescent derivative of acetyl lysine. As shown in Fig. 4A, total HAT activity was significantly increased in subjects with asthma compared with control subjects.

The histone acetylation levels of total H3, H4, site-specific H3K9, H3K14, H3K27, H3K18 and H4K16 at Notch1 promoter of lung CD4⁺ T cells were investigated. Chromatin was prepared and precipitated using the ChIP kit. The abundance of immunoprecipitated DNA fragments was quantified

by real-time PCR using SYBR Green Master Mix kit. There were significant increases in the acetylation levels of histone modifications above, compared to the control group (Fig. 4B-C). The levels of H3K9me3, H3K4me3, H3K79me3 and H4K20me3 at Notch1 promoter were also assessed. As shown in Fig. 4D, the levels of H3K4me3 and H3K79me3 which are known to activate gene transcription, were significantly increased. The levels of H3K9me3 and H4K20me3, which inactivate gene transcription, had

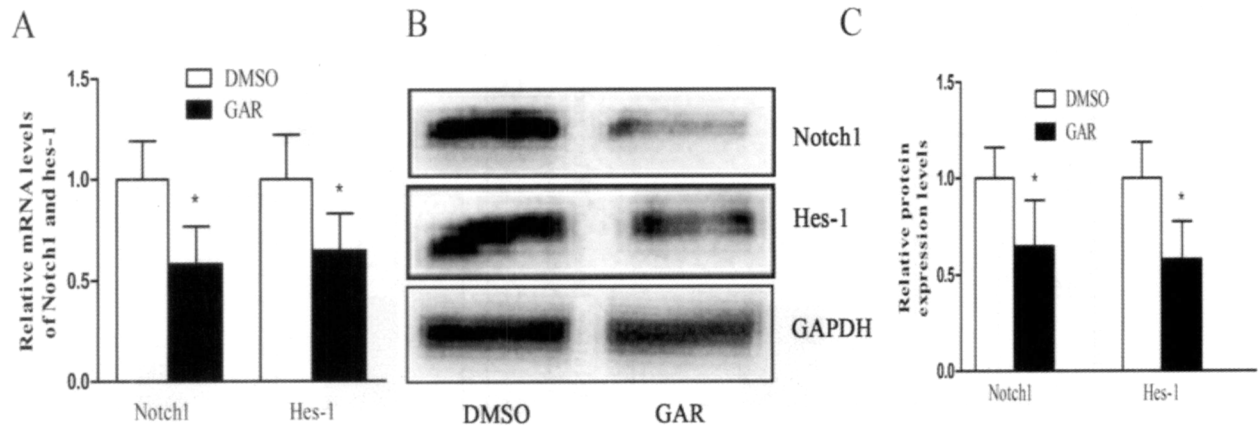


Fig. 6. Effect of GAR on the Notch1 signaling pathway in asthmatic lung CD4⁺ T cells. **A)** After treatment with GAR (10 mM), the mRNA expression levels of Notch1 and hes-1 in asthmatic lung CD4⁺ T cells were detected by real-time PCR, normalized to GAPDH. Results are from three independent experiments and the results for each group are expressed as mean $\pm$ SEM. **P* < 0.05. **B, C)** Western blotting was also performed to detect the protein levels of Notch1 and hes-1. GAPDH was used as a loading control. The results shown are representative of three independent experiments. **P* < 0.05.

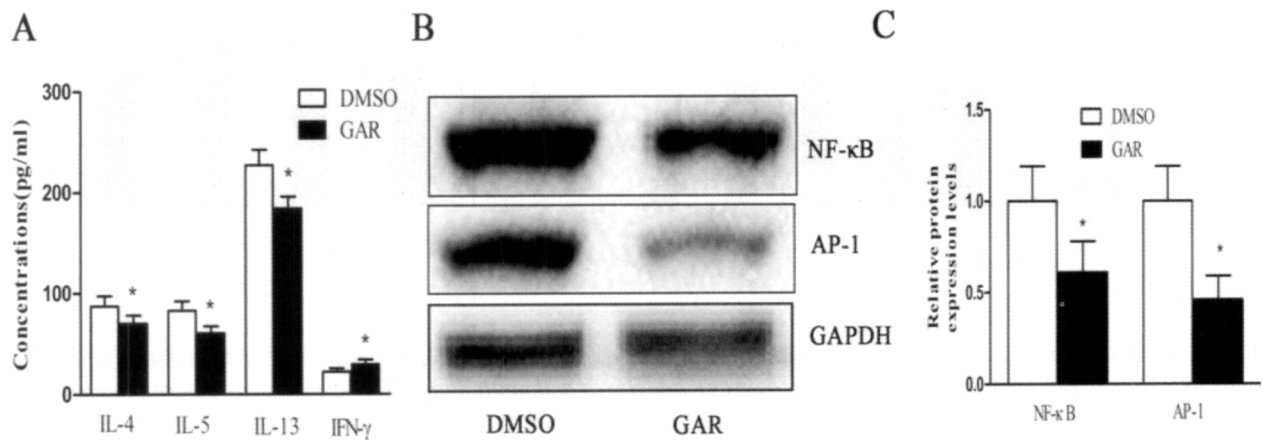


Fig. 7. GAR inhibits inflammatory cytokines and pro-inflammatory transcription factors. **A)** The levels of IL-4, IL-5, IL-13 and IFN- γ were determined using ELISA 48 h after GAR (10 mM) treatment. Results are from three independent experiments and values shown are mean $\pm$ SEM, *P* < 0.05. **B, C)** The expression levels of NF- κ B and AP-1 were detected by Western blotting. The results shown are representative of three independent experiments. **P* < 0.05.

no significant difference.

GAR inhibits the HAT activity and alters histone modifications of Notch1 promoter in asthmatic lung CD4⁺ T cells

GAR has been characterized as a HAT inhibitor. To confirm the effect of GAR on intracellular HAT

activity, lung CD4⁺ T cells isolated from asthmatic rats were treated with GAR (10 μ M) or DMSO as a vehicle control for 48 h. After treatment with GAR, the cultured cells were collected and nuclear protein was extracted. The HAT activity was determined using HAT Assay Kit. The lung CD4⁺ T cells treated with GAR showed reduced HAT activity (Fig. 5A).

Table I. Summary of primer used for real-time PCR.

Primer		sequence(5'-3')
Notch1	sense	TTTAGAAACAGGCCAAGACCTTGAA
	antisense	AGCCCATGCGGTGGTTACA
Hes-1	sense	TGGGTCCTAACGCAGTGTCG
	antisense	GGGAGTTTATGATTAGCAGTGGC
GAPDH	sense	GGCACAGTCAAGGCTGAGAATG
	antisense	ATGGTGGTGAAGACGCCAGTA

We also examined whether the histone modifications of Notch1 promoter could be altered by GAR. As shown in Fig. 5B-D, the total H3ac, H4ac, site-specific H3K9ac H3K14ac, H3K27ac, H3K18ac, H4K16ac and H3K79me3 levels of Notch1 gene promoter decreased significantly, the H3K4me3, H3K9me3 and H4K20me3 levels had no significant difference.

Effect of GAR on the Notch1 signaling pathway in asthmatic lung CD4⁺ T cells

It has been shown that GAR inhibits HAT activity and predominantly down-regulates gene expression in cells. Purified asthmatic lung CD4⁺ T cells were treated with GAR (10 μ M) or DMSO as a vehicle control for 48 h. Cells were collected and analysed for Notch and hes-1. The mRNA expression levels of Notch1 and hes-1 were measured by real-time PCR. As shown in Fig. 6A, compared with the DMSO group, Notch1 and hes-1 mRNA expression in the GAR group reduced significantly. Western blotting was used to detect the protein levels of Notch1 and hes-1. Notch1 and hes-1 protein were expressed lower in the GAR group, which was consistent with the mRNA levels (Fig. 6B-C).

GAR inhibits inflammatory cytokines and inhibits pro-inflammatory transcription factors

Because Th2 cytokines promote airway inflammation in asthma, we investigated the

expression of inflammatory cytokines. All cytokines were determined by ELISA at 48 h after GAR treatment. As shown in Fig. 7A, the levels of IL-4, IL-5 and IL-13 were significantly reduced in the GAR group compared with the DMSO group, and a small reverse trend was found in the level of IFN- γ . Results showed that GAR effectively blocked Th2 differentiation and promoted Th1 response.

Pro-inflammatory transcription factors including NF- κ B and AP-1 regulate the expression of genes that code for many inflammatory proteins, such as cytokines, inflammatory enzymes, adhesion molecules and inflammatory receptors. We further examined whether GAR reduces the expression of NF- κ B and AP-1 using Western blot analysis. As shown in Fig. 7B-C, GAR treatment significantly inhibited the protein expression levels of NF- κ B and AP-1, compared to the DMSO group.

DISCUSSION

Th1/Th2 imbalance plays an important role in the development of airway inflammation. Although the role of Notch signaling pathway in the regulation of T-cell differentiation into effector T-cell lineages remains inconsistent, there is increasing evidence to sustain the function of Notch signaling pathway in the induction of Th2 differentiation (17, 18). It is therefore possible that restriction of Th2 and inflammatory responses through the intervention of

Notch signaling pathway may provide new treatment strategies of asthma. Our previous study suggested that GSI inhibits the Notch signaling pathway, and has dual effects: inhibition of Th2 cytokine production and amplification of Th1 cytokine production. It seems likely that the skewing of the cytokine production from Th2 to Th1 responses provides therapeutic value through the inhibition of Notch signaling pathway (19, 20).

The mutation of Notch1 gene was not observed in asthmatic model in our previous study (20). Therefore, the analysis of epigenetics, such as histone modifications and DNA methylations, might help to provide an explanation of abnormal gene expression (21). Histone acetylation often results in an open structure of chromatin, which facilitates transcription factors and activates related gene expression. HATs such as CREB-binding protein CBP and its close homolog p300 execute these modifications. HDACs reverse HAT activity by making chromatin more condensed and promote gene repression (22). Histone methylation could keep chromatin in either an activated or a repressed state. H3K4me3 and H3K79me3 facilitate open chromatin for active transcription. In contrast, H3K9me3 and H4K20me3 are generally associated with a condensed chromatin and gene silencing. While the changes in histone modifications have been reported regarding developmental process or allergic diseases, there is a paucity of data in asthma (23, 24).

Environmental exposures and dietary factors influence epigenetic marks, which in turn regulate gene expression. Earlier studies suggested that acetylation of histone may play an important role in asthma. Increased acetylation of H4 has been demonstrated in asthmatic patients and is associated with the up-regulation of proinflammatory genes (25, 26). It has also been shown that increased acetylation of histone results in decreased HDAC activity, which might be responsible for enhanced expression of proinflammatory genes. Modulation of HAT and HDAC activity may prove to be important new targets for treatment of asthma (27, 28).

In this study, we observed that lung CD4⁺ T cells of asthmatic rats displayed increased HAT activity and decreased HDAC expression level. Treatment of GAR appears to alter histone modifications that regulate the Notch signaling pathway and alleviates

allergic inflammatory response via the inhibition of HAT activity, suggesting that targeting histone acetylation might lead to novel anti-inflammatory therapies.

Altered patterns of acetylation and methylation have been reported in inflammatory airway diseases and cancer and may provide biomarkers of early states. As the evidence for cross-talk among different types of epigenetics marks is increasing, it becomes difficult to ascribe observed results to histone modifications alone (29). However, the genome-wide distribution of histone modifications has provided new insights into the flexibilities of gene expression programs (30). More detailed analysis of the T-cell epigenome is required to understand how the cell fate decisions are made and to elucidate the mechanisms of specificity and plasticity of polarized cells.

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