Oxidative stress regulates IL-4 gene expression in mast cells through the reduction of histone deacetylase

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Abstract

Objective: Many pro-inflammatory cytokines are regulated by the acetylation and deacetylation of the core histone. Since dysregulation of Th2 cytokine production is a key in the pathogenesis of allergic diseases, we examined the role of histone deacetylase (HDAC) on IL-4 gene expression in mast cells. We also examined whether oxidative stress has any impact on HDAC activity.

Methods: An IgE-sensitized mast cell line (RBL-2H3 cells) was treated with varying concentrations of the HDAC inhibitors Tricostatin A (TSA) and H2O2, and stimulated with antigens. The amount of IL-4 gene expression was quantitated by real-time PCR. Quantitative measurement of IL-4 in cell supernatant was performed using ELISA. HDAC activity was measured with the use of a nonisotopic assay that utilized a HDAC Fluorescent Activity Assay Kit.

Results: IL-4 mRNA expression was induced by antigens in IgE-sensitized RBL-2H3 cells. Pretreatment with TSA and H2O2 enhanced IL-4 mRNA expression up to five-fold in a dose-dependent manner. HDAC activity in RBL-2H3 cells was reduced after treatment with H2O2.

Conclusion: Our results suggest that oxidative stress may up-regulate IL-4 gene expression in mast cell via a decrease in HDAC activity.

Introduction

The prevalence of allergic diseases has increased over the last two decades in developed countries1,2. As genetic changes cannot occur so rapidly, one possible reason for this phenomenon is change in our lifestyle and environment. A number of environmental factors, including air pollution, cigarette smoking3 and diet4 have been proposed to explain the increased prevalence of allergic diseases, with most of these environmental factors known to lead to oxidative stress on our bodies. One ROS, hydrogen peroxide (H2O2), significantly enhanced cytokine production in bronchial epithelial (BEAS-2B) cells through decreased histone deacetylase (HDAC) 2 activity5. There has been a good deal of evidence suggesting that H2O2 affects antigen-induced responses in mast cells6-8. However, the mechanism underlying this effect is not understood yet. The objective of this study was to investigate whether oxidative stresses affect cytokine production in mast cells through reductions in HDAC activity.

Methods and Materials

1. Cell cultures and stimulation

The rat basophilic leukemia cell line RBL-2H3 was cultured in Dulbecco’s modified Eagle’s medium. RBL-2H3 cells were sensitized by IgE receptor cross-linking using 100 ng/ml of purified anti-dinitrophenyl (DNP) IgE to saturate 2.5×106 cells/ml. The IgE-sensitized RBL-2H3 cells were treated with varying concentrations of the HDAC inhibitor Tricostatin A (TSA) for 15 min and stimulated with 100 ng/ml of DNP-KLH. For H2O2 stimulation, the IgE-sensitized RBL-2H3 cells were treated with 10nM, 1nM or 0.1nM of H2O2 for 16 hours and stimulated with 100 ng/ml of DNP-KLH. After 2 hour incubation, the cells were washed twice with ice-cold PBS. Total RNA was obtained.

2. Cytokine detection

Quantitative measurement of IL-4 in cell supernatant was performed using BiotrakTM rat IL-4 ELISA. Briefly, 4 ml of cell supernatant from stimulated or, as a control, unstimulated cells was concentrated to a final volume of 200 µl. Aliquots of 50µl were used for the assay according to the manufacturer’s instructions. The protein concentration in the supernatants was determined using a BCA Protein Assay Kit. The cytokine level in each sample was adjusted against the protein concentration.

3. Measurement of Leukotriene C4

RBL-2H3 cells were sensitized with anti-DNP IgE, treated with TSA and stimulated with DNP-KLH as mentioned above. After incubation for 15 min, the supernatant was collected. The leukotriene C4 concentration was measured using a rat leukotriene C4 EIA Kit according to the manufacturer’s instructions.

4. Real-time PCR

Total RNA was extracted from approximately 1x10⁶ cells using an RNeasy kit. Reverse transcription was performed with the use of TaqMan Reverse Transcription Reagents. Gene transcript levels of rat IL-4 and housekeeping genes (ribosomal RNA) were quantitated by real-time polymerase chain reaction (PCR) with the use of a TaqMan Gene Expression Assay on a 7500 Real Time PCR system. Variations in the amount of transcript in different samples were corrected against housekeeping gene expression.

5. HDAC Activity

RBL-2H3 cells were also stimulated with H2O2 for 4 and 24 hours. H2O2-treated and untreated control cells were collected by centrifugation for 5 min at 1,200 rpm and nuclear extracts were obtained using a Nuclear Extract Kit. HDAC activity was measured with the use of a nonisotopic assay that used a fluorescent derivative of epsilon-lysine histone deacetylase activity. The results are expressed as micromolar values of the provided standard per microgram of protein.

6. Statistics

We performed all experiments at least three times to confirm reproducibility. Values represent the means ± SEM of three experiments. Comparisons between experimental groups were performed using the Kruskal-Wallis and Mann-Whitney U test.

Conclusions

We demonstrated that oxidative stress up-regulated IL-4 production in mast cell via a reduction in HDAC activity. This result suggests that the exacerbation of allergic diseases by oxidative stress may occur via mast cells.

References


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