Oxidative Stress Regulates IL-4 Gene Expression in Mast Cells through the Reduction of Histone Deacetylase

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

Abstract

Objective. Many proinflammatory cytokines are regulated by the acetylation and deacetylation of the core histone. Since dysregulation of T helper 2 cytokine production is a key in the pathogenesis of allergic diseases, we examined the role of histone deacetylase (HDAC) on interleukin (IL)–4 gene expression in mast cells. We also examined whether oxidative stress has any impact on HDAC activity.


Setting. Academic research laboratory.

Methods. An IgE-sensitized mast cell line (RBL-2H3 cells) was treated with varying concentrations of the HDAC inhibitors trichostatin A (TSA) and H2O2 and stimulated with antigens. The amount of IL-4 gene expression was quantified by real-time polymerase chain reaction. Quantitative measurement of IL-4 in the cell supernatant was performed using enzyme-linked immunosorbent assay. Moreover, HDAC activity was measured with the use of a nonisotopic assay that utilized an 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 5-fold in a dose-dependent manner. Furthermore, 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 cells via a decrease in HDAC activity.

Keywords

histone deacetylase, interleukin (IL)–4, mast cell, oxidative stress

Received June 15, 2014; revised August 29, 2014; accepted October 21, 2014.
The objective of this study was to investigate whether oxidative stresses affect cytokine production in mast cells through reductions in HDAC activity.

Materials and Methods

Cell Cultures and Stimulation

The rat basophilic leukemia cell line RBL-2H3 was cultured in Dulbecco’s modified Eagle’s medium (Nissui, Tokyo, Japan) supplemented with 20% heat-inactivated fetal calf serum (JRH Biosciences, Lenexa, Kansas), 2 mM L-glutamine (Irvine Scientific, Santa Ana, California), 100 U/mL penicillin (Sigma-Aldrich Co, St Louis, Missouri), and 100 μg/mL neomycin (Sigma). RBL-2H3 cells were sensitized by IgE receptor cross-linking using 100 ng/mL of purified anti-dinitrophenyl (DNP) IgE (Sigma) to saturate 2.5 \( \times 10^6 \) cells/mL. The IgE-sensitized RBL-2H3 cells were treated with varying concentrations of the HDAC inhibitor trichostatin A (TSA; Sigma) for 15 minutes and stimulated with 100 ng/mL DNP-KLH (Sigma). For H2O2 stimulation, the IgE-sensitized RBL-2H3 cells were treated with 10 nM, 1 nM, or 0.1 nM of H2O2 for 16 hours and stimulated with 100 ng/mL DNP-KLH (Sigma). After a 2-hour incubation, the cells were washed twice with ice-cold phosphate buffered saline. Total RNA was obtained as described below.

Cytokine Detection

Quantitative measurement of interleukin (IL)-4 in the cell supernatant was performed using Biotrak rat IL-4 enzyme-linked immunosorbent assay (Amersham, Arlington Heights, Illinois). Briefly, 4 mL of the cell supernatant from stimulated or, as a control, unstimulated cells was concentrated to a final volume of 200 μL. Aliquots of 50 μL 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 (Pierce, Rockford, Illinois). The cytokine level in each sample was adjusted against the protein concentration.

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 minutes, the supernatant was collected. The leukotriene C4 concentration was measured using a rat leukotriene C4 EIA Kit (Cayman Chemical, Ann Arbor, Michigan) according to the manufacturer’s instructions.

Real-time Polymerase Chain Reaction

Total RNA was extracted from approximately \( \times 10^6 \) cells using an RNaseasy kit (Qiagen). Reverse transcription was performed with the use of TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, California). Gene transcript levels of rat IL-4 and housekeeping genes (ribosomal RNA) were quantified by real-time polymerase chain reaction (PCR) with the use of a TaqMan Gene Expression Assay on a 7500 Real Time PCR system (Applied Biosystems). Variations in the amount of transcript in different samples were corrected against housekeeping gene expression.

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 minutes at 1200 rpm, and nuclear extracts were obtained using a Nuclear Extract Kit (Active Motif, Carlsbad, California).

Moreover, HDAC activity was measured with the use of a nonisotopic assay that utilized a fluorescent derivative of N-acetyl lysine (HDAC Fluorescent Activity Assay Kit, Biomol, Plymouth Meeting, Pennsylvania). The results are expressed as micromolar values of the provided standard per microgram of protein.

Statistics

We performed all experiments at least 3 times to confirm reproducibility. Values represent the mean ± standard error of the mean of 3 experiments. Comparisons between experimental groups were performed using the Kruskal-Wallis statistical and Mann-Whitney U test.

Results

Effect of TSA on IL-4 mRNA Expression in RBL-2H3 Cells

To investigate the effect of HDAC inhibition on cytokine expression in mast cells, TSA was used to inhibit the HDAC activity of the mast cells. RBL-2H3 cells expressed IL-4 mRNA in an unstimulated state, and TSA administration did not up-regulate IL-4 mRNA expression without IgE cross-linking. IgE/DNP incubation increased IL-4 mRNA expression from the basal level. The expression of IL-4 mRNA was enhanced in the presence of TSA in a dose-dependent manner after IgE cross-linking (Figure 1A).

TSA Enhances the Secretion of IL-4 from RBL-2H3 Cells

To evaluate whether TSA stimulates IL-4 protein secretion from mast cells, we measured IL-4 concentrations in the culture supernatants of RBL-2H3 cells. RBL-2H3 cells secreted IL-4 proteins in an unstimulated state. Stimulation with TSA alone did not have any effects; however, stimulation with TSA and IgE/DNP up-regulated IL-4 secretion from mast cells in a dose-dependent manner (Figure 1B).

TSA Does Not Affect LTC4 Secretion from RBL-2H3 Cells

We measured LTC4 secretion from mast cells to evaluate whether HDAC inhibition has an effect on mast cell degranulation and leukotriene synthesis. RBL-2H3 cells secreted LTC4 proteins in an unstimulated state and secreted LTC4 with IgE cross-linking. However, TSA stimulation did not influence the secretion of LTC4 in RBL-2H3 cells, regardless of IgE/DNP stimulation (Figure 1C).

H2O2 Enhances Antigen-induced IL-4 Expression in RBL-2H3 Cells

To determine the effect of oxidative stress on cytokine expression in mast cells, RBL-2H3 cells were stimulated...
with different concentrations of H$_2$O$_2$ with or without IgE cross-linking. Total RNA was isolated and used in real-time PCR to evaluate the mRNA levels of IL-4. RBL-2H3 cells expressed basal levels of IL-4 mRNA in the unstimulated state. H$_2$O$_2$ stimulation did not result in a significant increase in IL-4 mRNA expression in RBL-2H3 cells without IgE cross-linking (Figure 2A). IgE/DNP incubation increased the expression of IL-4 mRNA from the basal level, whereas treatment with 0.1 to 10 nM of H$_2$O$_2$ for 16 hours with IgE cross-linking dose-dependently enhanced the levels of IL-4 mRNA (Figure 2B).

**H$_2$O$_2$ Decreased HDAC Activity in RBL-2H3 Cells**

To determine the effect of oxidative stress on HDAC activity in mast cells, we stimulated cells with H$_2$O$_2$ (100 μM) for 4 or 24 hours. The HDAC activity of mast cells was decreased in the presence of H$_2$O$_2$ in a time-dependent manner. Oxidative stress also decreased HDAC activity in RBL-2H3 cells (Figure 2C).

**Discussion**

In this study, we found that H$_2$O$_2$ treatment enhanced the IgE cross-linking–induced increase in IL-4 expression in RBL-2H3 cells. Further, HDAC inhibition enhanced antigen-induced IL-4 mRNA expression and IL-4 protein secretion in mast cells. Moreover, the HDAC activity in RBL-2H3 cells was reduced by H$_2$O$_2$ treatment.

Many allergic diseases, including allergic rhinitis and asthma, present with inflammation characterized by elevated T helper 2 (Th2) cytokines such as IL-4. This involves a complex cascade of inflammatory mediators whose expression is enhanced during the disease process. Under resting conditions, many of these inflammatory genes are not expressed in normal cells. The increased expression of these proteins must result from enhanced gene transcription occurring in a cell-specific manner.

Recently, molecular mechanisms that switch on inflammatory genes have been reported. Chromatin structural changes were shown to be essential to the regulation of gene expression. In resting cells, DNA is wound tightly around the core histones, inhibiting the binding of the RNA polymerase II enzyme, which activates the formation of mRNA. Acetylation of histone residues results in the unwinding of DNA wrapped around the histone core. This process opens up the chromatin structure, and these changes enable transcription factors and RNA polymerase II to bind more readily to DNA, thereby increasing gene transcription. The inhibition of gene transcription is possible by the reversal of this process by histone deacetylation. These processes are controlled by histone acetyltransferases (HATs) and HDACs. Increased HAT activation or a reduction in HDAC activity or expression leads to hyperacetylation and increased gene expression.

It was reported that HDAC activity in bronchial biopsies and in alveolar macrophages in patients with asthma was lower than that in healthy controls. A significantly greater reduction in HDAC activity was observed in bronchial biopsies in patients with asthma who smoke than in nonsmoking patients with asthma. Moreover, a good correlation between HDAC activity and asthmatic severity was observed in bronchial biopsies of patients with asthma.
These data suggest that, in patients with asthma, oxidative stress affects the disease state via a reduction in HDAC.

In T cells, IL-4 expression is associated with changes in the chromatin structure. For example, silencing of the IL4 gene in developing Th1 cells is associated with repositioning into heterochromatin. During the differentiation of naive T cells into effector Th2 cells, the IL4 gene becomes demethylated. Additionally, overexpression of HDAC inhibits IL-4 promoter–driven transcription, whereas co-transfection of the HAT CREB-binding protein potentiated IL-4 promoter activity. Apart from the above, there have been few studies on the relation between chromatin structures and IL-4 production in mast cells.

It has been shown that IL-4 is an essential factor for Th2 cell differentiation and IgE production. Overproduction of IL-4 can result in tissue-damaging autoimmune diseases, such as asthma and allergic diseases. The major source of IL-4 is non-T, non-B cells, which belong to the mast cells and are activated by the cross-linking of FcεRI. Mast cells are known to be exposed to an oxidative environment in the course of allergic and inflammatory reactions. Therefore, it is thought to be worthwhile to study whether oxidative stress alters IL-4 production.

Our present study demonstrates that H2O2 treatment and HDAC suppression enhance the IgE cross-linking–induced increase in IL-4 expression in mast cells. Oxidative stress, which is produced as a consequence of normal cell function or derived from external sources, induces various responses, ranging from proliferation to growth arrest and cell death. Oxidative stress can also induce an appropriate environment for allergic inflammation, not only via T cells but also via mast cells.

Although recent advances in the use of topical steroid drugs have improved the control of allergic diseases such as allergic rhinitis and asthma, control in some patients remains poor. These patients need a continuous intake of systemic steroids for asthma or require surgery for allergic rhinitis. This poor control of allergic diseases may arise from corticosteroid resistance. It has been reported that corticosteroid resistance is, at least in some part, caused by HDAC reduction, and the restoration of HDAC can restore steroid responsiveness. There are several potential drugs that can ameliorate the loss of HDAC expression due to oxidative stress by reversing steroid resistance through HDAC activation.

Investigating the details of HDAC status in cases of inflammation characterized by elevated Th2 cytokines such as IL-4 may help us to better understand disease mechanisms and yield new therapeutic targets for allergic diseases such as asthma and allergic rhinitis.

**Conclusion**

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

**Author Contributions**

Yuji Nakamaru, data analysis, drafting, final approval, accountability for all aspects of the work; Dai Takagi, data analysis,
revising, final approval, accountability for all aspects of the work; 
Akihiro Homma, interpretation of data, revising, final approval, accountability for all aspects of the work; 
Shigetsugu Hatakeyama, data analysis, revising, final approval, accountability for all aspects of the work; 
Satoshi Fukuda, interpretation of data, revising, final approval, accountability for all aspects of the work.

Disclosures
Competing interests: None.
Sponsorships: None.

References