



Short Communication

Histamine Receptor Expression on Peripheral Blood Lymphocytes Is Influenced by Specific and Nonspecific Activation

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Abstract. Histamine is a physiological mediator which exerts both effector and regulatory functions through its receptors on various cells. The aim of the study was to investigate changes in histamine receptor expression on peripheral blood lymphocytes affected by stimulation with both specific and nonspecific stimuli. Lymphocytes were obtained from both healthy and allergic subjects. Cells were incubated with various allergens (mixed grass pollen, *Lolium perenne*, *Dermatophagoides pteronyssinus* 1, bee venom, phospholipase A₂) and nonspecific (fMLP, PMA/ionomycin, LPS) stimuli. The percentage of histamine-binding cells was determined with a fluorescence microscope after incubation with histamine-fluorescein. In control subjects histamine binding after stimulation with allergens was not significantly changed. In contrast, in allergic subjects stimulation with specific allergens resulted in significantly increased histamine binding. Nonspecific stimulation caused increased histamine binding to lymphocytes in both allergic subjects and healthy controls. We conclude that specific and nonspecific activation of lymphocytes is associated with increased expression of histamine receptors.

Key words: histamine receptors; lymphocytes; specific activation.

Introduction

Histamine is a ubiquitous mediator of various biologic reactions. In humans and other mammals, histamine is continuously produced and stored in granules of mast cells and basophils. Histamine binds to 4 types of histamine receptors: H₁, H₂, H₃ membrane proteins, and H₄ which is an intracellular receptor^{5, 9, 16}. Binding of histamine to the H₁ receptor mediates allergic type 1 response, which manifests clinically as bronchial and intestinal smooth muscle contraction, periph-

eral vasodilatation, increased vascular permeability and pruritus^{8, 22}. Histamine is also shown to suppress inflammatory responses, acting through the H₂ receptor^{3, 4, 16}. Histamine differentially affects the immune response. At concentrations higher than 10⁻⁵ mol/l it seems to suppress both humoral and cellular responses, whereas at lower concentrations it acts as an enhancer of both types of responses^{3, 18}.

Histamine receptors are heterogeneous proteins, which bind different regions of histamine molecule. This different spatial relationship between histamine

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and its receptors allows developing antagonists that bind specifically to various types of histamine receptors. Therefore, the final result of histamine action depends on both the number and type of receptors as well as their affinity to histamine present at the site of mediator release.

We investigated whether expression of histamine receptors on lymphocytes, involved in the regulation of allergic responses, is influenced by changes in the cellular milieu and whether specific and nonspecific activation of these cells induces changes in the expression of histamine receptors.

Materials and Methods

Subjects. The study was performed on 44 subjects. The control group comprised 10 healthy subjects aged 21–48 years with negative histories of atopy and without any symptoms of allergic diseases, 34 patients with documented histories of allergy: 6 house dust mite allergic, 8 bee venom allergic and 20 pollen allergic patients, aged 17–41 years. All allergic patients had positive skin prick tests with the specific allergen.

Reagents. Reagents used were: mixed grass pollen allergen (Bencard-Beecham, UK), *Lolium perenne* antigen extract (Biomed, Poland), bee venom (*Apis mellifera*) (ALK, Denmark), phospholipase A₂ (PLA₂), a major allergen of bee venom (Boehringer Ingelheim, Germany), Der. p. 1, a major allergen of *Dermatophagoides pteronyssinus* (ALK, Denmark), fMLP – N-formylmethionyl-leucyl-phenylalanine methylester (Sigma, USA), PMA – phorbol-12-myristate 13-acetate (Sigma, USA), ionomycin (Sigma, USA) LPS – lipopolysaccharide (Serva, Germany), Gradisol G (Polfa, Poland), histamine fluorescein (Molecular Probes, USA). An important feature of this histamine preparation is the direct binding to fluorescein. This conjugate is superior to those used in previous reports, where histamine and fluorescein were bound to a protein carrier capable of inducing nonspecific binding¹⁷. In addition, we demonstrated in skin prick testing that the biological activity of the labeled histamine was preserved and was as comparable to histamine dihydrochloride.

Separation of cells. Peripheral blood lymphocytes were isolated by density gradient centrifugation on Gradisol G according to ZEMAN et al.²³ using blood drawn into a heparinized (10 UI/ml) tube. Monocytes were removed from the mononuclear cell fraction using the plate method¹⁰. Viability was tested by trypan blue exclusion and exceeded 96% in each isolation.

Stimulation of cells. 10⁶ cells/ml were incubated

(30 min, 37°C, 5% CO₂) with either fMLP (10⁻⁸ mol/l), or LPS (1 µg/ml) or PMA/ionomycin (1 ng, 200 ng/ml) or with various allergens: mixed grass pollen (1 ng/ml), *Lolium perenne* (100 pg/ml), bee venom (100 pg/ml), PLA₂ (5 pg/ml), Der. p. 1 (10 ng/ml). Stimulation with allergens was always specific. Control cells were incubated in PBS only. The concentrations used were those which appeared to be optimal in the previous experiments (not shown). The cells were then washed and resuspended in PBS. No significant cytotoxicity of the substances tested was observed at the concentrations used.

Staining with labeled histamine. The specificity of histamine fluorescein binding to lymphocytes was investigated by competitive inhibition with either histamine dihydrochloride or histamine receptors' antagonists or agonist. A hundred-fold excess of both the H₂ receptor agonist (Dimaprit) and the antagonist (Ranitidine) as well as the unlabeled histamine strongly limited binding of histamine fluorescein. However, high concentrations (>10⁻⁵ mol/l) of a number of commonly used H₁ receptors' antagonist and agonists were shown to be toxic to the cells, so they could not be used for testing. We observed that histamine fluorescein did not bind to erythrocytes, which are known not to express histamine receptors on their surface¹⁵.

The cells incubated with either specific or nonspecific stimuli or left in medium alone (negative control) were stained with labeled histamine. 10⁶ alive cells were suspended in 90 µl PBS and fluorescein-labeled histamine was added at the final concentration of 10⁻⁵ mol/l. The cell suspension was incubated for 15 min at room temperature in a dark chamber. The percentage of fluorescein-labeled cells was then counted using a fluorescence microscope (Olympus BX-50, magnification ×400).

Statistical analysis. Mean values and standard deviations (SD) were calculated. Differences were considered statistically significant with $p < 0.05$ in Student's *t*-test.

Results

As shown in Fig. 1, nonspecific stimulation (fMLP, LPS, PMA/ionomycin) of lymphocytes from both normal and allergic subjects resulted in a significant increase of histamine fluorescein binding ($p < 0.01$). No significant difference in histamine receptor expression between normal and allergic subjects was observed. Figure 2 shows the effect of incubation of lymphocytes from allergic and normal subjects with various aller-

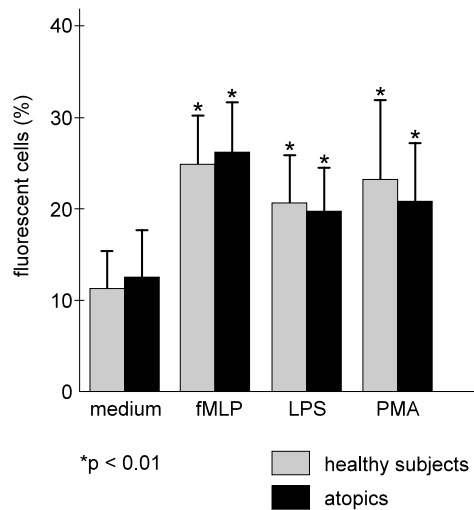


Fig. 1. Effect of nonspecific stimulation on histamine receptor expression on lymphocytes from healthy and allergic subjects. Peripheral blood lymphocytes after incubation with either fMLP, LPS, PMA/ionomycin were stained with fluorescein-labeled histamine. The percentage of histamine binding cells was determined

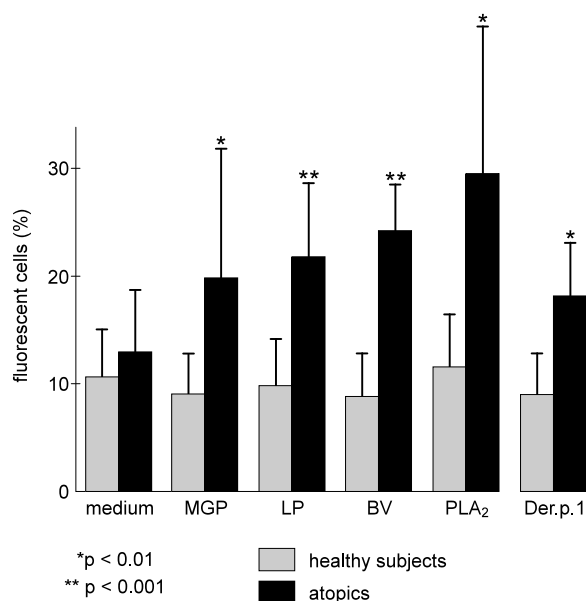


Fig. 2. Effect of specific stimulation on histamine receptor expression on lymphocytes from healthy and allergic subjects. Peripheral blood lymphocytes after incubation with mixed grass pollen (MGP), *Lolium perenne* extract (LP), bee venom (BV), Phospholipase A₂ (PLA₂), *Dermatophagoides pteronyssinus* allergen (Der.p.1) were stained with fluorescein-labeled histamine. The percentage of histamine binding cell was determined

gens (mixed grass pollen, *Lolium perenne*, bee venom, PLA₂, *Dermatophagoides pteronyssinus*). Stimulation with specific allergens resulted in a significant increase in lymphocyte histamine binding in allergic subjects. Although this effect was observed with all allergens

used, it was most pronounced when the major allergen of bee venom (PLA₂) was used.

Stimulation of cells from normal subjects with the same allergens did not result in changes of histamine receptor expression.

Discussion

The data in this study show that nonspecific activation of lymphocytes leads to an increase in histamine binding in both allergic and healthy subjects. In contrast, antigen-specific stimulation by allergen extract results in an increase of the percentage of histamine-binding cells only in allergic patients. It can be therefore assumed that activation of cells, independent of the specific or nonspecific nature of the activation, leads to an increase of histamine receptor expression on lymphocytes. Our data are in line with other studies showing the modulation of the expression of histamine receptors by a variety of factors. SHIBATA et al.²⁰ demonstrated that activation by concanavalin A (Con A) and IL-2 induced a significant increase of H₂ histamine receptor expression on suppressor T lymphocytes. IRIYOSHI et al.¹¹ observed significantly increased expression of mRNA for the H₁ histamine receptor in nasal mucosa of patients with dust mite allergy as compared to healthy controls. An increase in the number of histamine receptors on lymphocytes might have important clinical implications. Numerous studies indicate multiple immunoregulatory functions of histamine^{2, 6, 8, 12, 13, 19, 21, 24}. Macrophages and lymphocytes have also been shown to synthesize histamine *de novo* through induced histidine decarboxylase¹. Histamine is capable of modulating several lymphocyte functions, including proliferation and secretion of active agents such as LCF (lymphocyte chemoattractant factor), IL-2, IFN- γ or IL-5^{1, 2, 7, 12, 13, 19, 21}. Histamine is believed to limit the extent of inflammatory reactions by suppressing local cytokine synthesis by T cells, mast cells, synovial cells, chondrocytes or alveolar macrophages^{3, 8}. Histamine receptors are targets of anti-allergic therapy. Histamine type1 receptor antagonists are widely used to control allergic symptoms¹⁴. The effects of histamine are determined by the receptor type (H₁, H₂, H₃) expressed on the target cell. In most systems, signaling through the H₁ receptor has an enhancing effect, whereas triggering of the H₂ receptor is suppressive^{2, 8}.

Our results show that an increase of histamine receptor expression is one of the events accompanying both specific and nonspecific activation of lymphocytes, which in turn might enable the histamine to exert

its regulatory functions and modulate immune response.

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