ANTI-INFLAMMATORY ACTIVITY OF ANTIHISTAMINES*

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ABSTRACT

The central role of histamine in the pathogenesis of the early-phase allergic response is well established; however, increasing evidence shows that histamine can modulate the activity of several cell types, including eosinophils, T-cells, monocytes, and macrophages. This evidence has resulted in the hypothesis that the role of histamine may extend to the regulation of the late-phase responses and airway remodeling. This article describes a series of studies that examine the effects of histamine on activation of human lung macrophages and the molecular mechanisms responsible for this interaction. Understanding this wider role of histamine may provide new therapeutic opportunities for the treatment of allergic diseases.

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Studies of allergic disease have shown that histamine, a biogenic amine synthesized and stored in mast cells and basophils, plays a central role in the pathogenesis of the early-phase allergic response and inflammatory symptoms.[1] Several immunologic and nonimmunologic stimuli induce the release of histamine, which diffuses rapidly into the surrounding tissues and exerts its effects via interaction with histamine receptors. Four histamine receptors—H_1, H_2, H_3, and H_4—have been characterized as belonging to the superfamily of G-protein–coupled receptors.[2] In addition to histamine, mast cells and basophils release other proinflammatory mediators, including the cysteinyl leukotrienes and, in particular, leukotrienes C_4 (LTC_4) and D_4 (LTD_4). Interaction of histamine with H_1-receptors mediates a variety of classic immediate effects, including bronchoconstriction, vasodilation, increased vascular permeability, and stimulation of sensory receptors.[1,3] These effects alone or in combination can lead to bronchial obstruction in asthma; nasal congestion, sneezing, itching, and rhinorrhea in rhinitis; and itchy skin wheal and flares in urticaria. The efficacy of antihistamines in relieving the symptoms of allergic inflammation has further confirmed the role of histamine.[4,5]

Increasing evidence shows that histamine can modulate the activity of several cell types, including eosinophils, T-cells, monocytes, and macrophages.[6] Consequently, it has been hypothesized that the role of histamine is not limited to the early inflammatory reaction, but may be wider than presently considered.[7,8] Consistent with such a role, a continuous release of histamine and other mediators from activated mast cells and basophils has been documented in patients with bronchial asthma.[9-11] Similarly, increased numbers of degranulated mast cells and basophils have been detected in the airways of asthmatics.[10] This increase was reported even when biopsies were performed in patients with asthma during apparent clinical remission. In addition, histamine has been detected in the bronchoalveolar lavage fluid (BALF) of asthmatics, even during asymptomatic periods and clinical remission.[9,11]

One of the key cellular targets of histamine is the macrophage. Macrophages are the predominant

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inflammatory cells in the airways, both in the BALF and lung parenchyma of healthy and asthmatic patients, and play a major role in defense against infections and in the local modulation of immune and inflammatory responses.[12] Furthermore, they synthesize and release numerous proinflammatory mediators, enzymes, cytokines, and chemokines, which modulate the function of T-cells, epithelial cells, fibroblasts, and eosinophils.[13] Evidence from immunohistochemical studies has revealed a close anatomical association between macrophages and mast cells at sites of allergic inflammation,[10] suggesting that lung macrophages may be exposed to local histamine released from immunologically activated mast cells. Moreover, these cells have been shown to express at least 3 types of histamine receptor—H_1, H_2, and H_3—with increasing evidence for selective expression of H_1- and H_2-receptors.[14,15] These observations are compatible with the hypothesis that histamine is chronically released in the airways of patients with bronchial asthma and allergic rhinitis and may have a role in persistent airway inflammation and tissue remodeling. Understanding this wider role of histamine may provide new therapeutic opportunities for the treatment of allergic disease.

Interactions of Histamine and Macrophages

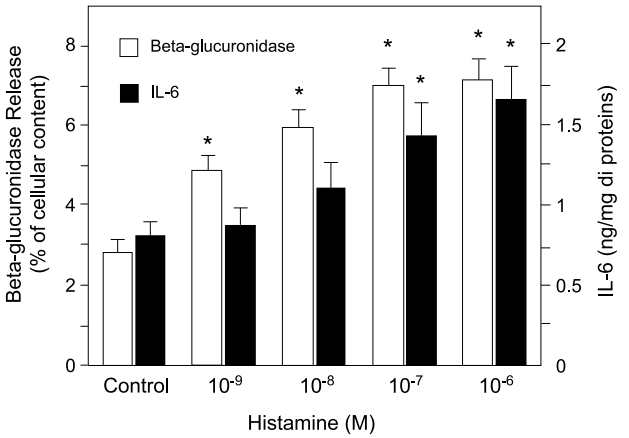
In order to explore the relationship between histamine and macrophages, we conducted a series of experiments using purified human lung macrophages.[16,17] Using a novel technique for isolating and purifying macrophages, we have been able to generate highly purified (>95%) preparations of human lung macrophages.[17] These cells have been used to investigate the effect of low concentrations of histamine on the release of mediators from macrophages.

Effect of Histamine on Beta-glucuronidase Release

We first examined the effects of histamine on the release of the lysosomal enzyme beta-glucuronidase. Beta-glucuronidase is stored preformed in macrophages and is released following activation, thereby acting as a useful marker of macrophage activation and exocytosis.[16] The release of lysosomal enzymes, such as beta-glucuronidase, is thought to be an important factor in inducing epithelial damage and basal membrane disruption, ultimately leading to airway remodeling in asthma.[18] Although effects of histamine on inflammatory cells have been previously reported, these have been obtained using high concentrations of histamine.[14,19,20] In contrast, the low concentrations of histamine (10^{-9} M to 10^{-6} M) used in the studies reviewed here are equivalent to concentrations that occur at the sites of allergic inflammation.

Incubation of macrophages with low concentrations of histamine resulted in a rapid and dose-dependent induction of beta-glucuronidase release (Figure 1).[16,17] This effect was statistically significant at a concentration of 10^{-9} M and maximal at a concentration of 10^{-7} M. Following incubation with 10^{-7} M histamine, levels of beta-glucuronidase were more than twofold higher compared with unstimulated cells. Characterization of the kinetic parameters of this reaction showed that maximum release occurred between 30 minutes and 2 hours and continued for up to 18 hours. Interestingly, incubation with LTD_4 alone had no effect on the levels of beta-glucuronidase release from macrophages; however, preincubation of macrophages with LTD_4 before stimulation with histamine resulted in a synergistic interaction of these mediators on the activation of macrophages.[21] These

Figure 1. Concentration-Response Curve of Histamine on the Release of Beta-glucuronidase and Interleukin-6 from Human Lung Macrophages



Cells were incubated (37°C, 2-6 h) with the indicated concentrations of histamine. Beta-glucuronidase release was determined by a colorimetric technique. The values are expressed as the percentage of the total cellular content determined in cell aliquots lysed with 0.1% Triton X-100. IL-6 release was determined by ELISA. The values are expressed as ng of IL-6/mg of total cellular protein. The data are the mean ± SE of 6 experiments. *$P < .05$ vs control.
IL = interleukin; ELISA = enzyme-linked immunosorbent assay.
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findings suggest that the release of LTD_4 from mast cells may potentiate the activating effects of histamine on lung macrophages.

Effect of Histamine on Cytokine Production

Interleukin (IL)-6 is a major cytokine produced by human macrophages and is thought to play a central role in allergic inflammation.[22] It is a hematopoietic stem cell proliferation and differentiation factor and is involved in mast cell development and maturation.[23] In addition, this cytokine is thought to be involved in immunoglobulin E (IgE) synthesis[24] and has been shown to stimulate IL-8 release from epithelial cells.[25] The increased levels of IL-6 in the blood and BALF of patients with asthma following bronchial challenge further support an integral role for IL-6 in modulating allergic inflammation.[26,27]

Previous studies have reported that histamine results in both induction[14,20] and inhibition[28,29] of cytokine synthesis in human monocytes and macrophages. Our experiments showed that physiologic concentrations of histamine (10^{-7} M to 10^{-6} M) increased the basal secretion of IL-6 from macrophages in a dose-dependent manner, with a twofold maximum enhancement above basal levels (Figure 1).[16,17] Analysis of the release kinetics revealed that this response was observed after only 4 to 6 hours and continued for up to 18 hours of incubation.[16,17]

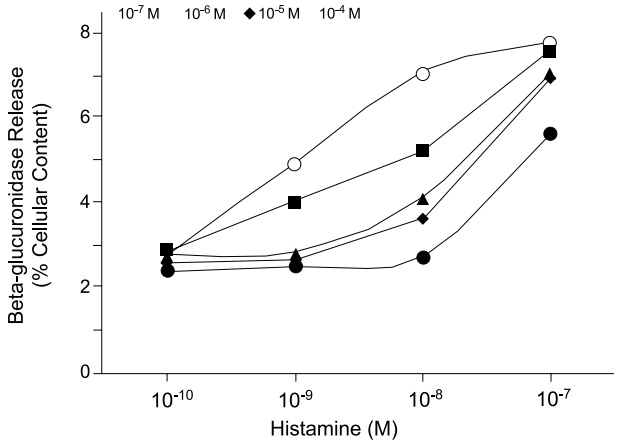
The difference in release kinetics between beta-glucuronidase and IL-6 from macrophages can be explained by the requirement for de novo synthesis of IL-6 from macrophages. Using reverse transcriptase–polymerase chain reaction the effect of histamine on IL-6 mRNA expression levels was investigated. It was observed that IL-6 mRNA expression levels in macrophages increased following 3 to 6 hours of incubation with histamine,[17] indicating that histamine enhances IL-6 production by increasing its specific mRNA. More recent data indicate that histamine induces the release of tumor necrosis factor (TNF)-alpha, another proinflammatory cytokine produced by macrophages. In addition, histamine inhibits the production of lipopolysaccharide-induced IL-12 (Triggiani M et al, unpublished observations). These results indicate that histamine exerts complex effects on the production of inflammatory and immunoregulatory cytokines from macrophages.

Molecular Mechanisms of Histamine-Induced Release of Beta-glucuronidase and IL-6

Pharmacologic analyses have been performed to elucidate further the molecular mechanisms of histamine-induced exocytosis and IL-6 production from macrophages. By incubating the cells with HTMT (6-[2-{4-imidazolyl}ethylamino]-N-[4-trifluoromethylphenyl] heptanecarboxamide), a selective H_1-receptor agonist, or dimaprit, a selective H_2-receptor agonist, the type of receptor activated by histamine on macrophages was investigated. These experiments showed that HTMT induced the release of both beta-glucuronidase and IL-6 from macrophages, with an effect comparable to that of histamine.[17] In contrast, dimaprit had no effect on the release of these mediators compared with nonstimulated cells.

To further confirm these findings, macrophages were preincubated with the highly selective H_1-receptor antagonist fexofenadine and the H_2-receptor antagonist ranitidine. Preincubation with increasing concentrations of fexofenadine (10^{-7} M to 10^{-4} M) induced parallel rightward shifts of the histamine dose-response curve for

Figure 2. Effect of the H_1-Receptor Antagonist Fexofenadine on Histamine-Induced Release of Beta-glucuronidase from Human Lung Macrophages



Macrophages were preincubated (37°C, 15 min) with RPMI (O) or with increasing concentrations of fexofenadine followed by incubation (2 h, 37°C) with histamine (10^{-7} M). Beta-glucuronidase release was determined by a colorimetric technique. The values are expressed as the percentage of the total cellular content determined in cell aliquots lysed with 0.1% Triton X-100. The data are the mean ± SE of 4 experiments.
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both beta-glucuronidase and IL-6 release from macrophages (Figure 2).[17] In contrast, the H_2-receptor antagonist ranitidine at concentrations of up to 10^{-4} M had no effect on histamine-induced beta-glucuronidase or IL-6 release from macrophages and, furthermore, had no additive effect with fexofenadine. Schild plot analysis of the inhibition of histamine-induced beta-glucuronidase release revealed a K_d of approximately 90 nM, which is consistent with an interaction between fexofenadine and H_1 receptors. Together, these findings indicated that both IL-6 and beta-glucuronidase release from lung macrophages is mediated via the activation of H_1-receptors on macrophages.

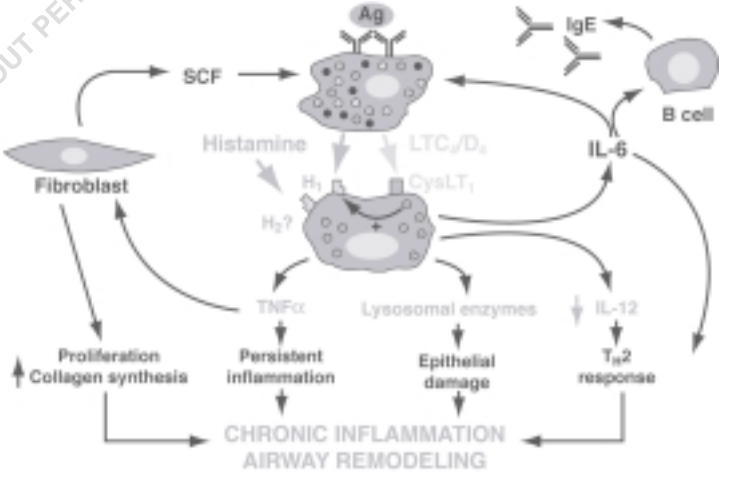
Effect of Histamine on Cytosolic Calcium

It is well established that activation of H_1-receptors is associated with phospholipase C activation and inositol-1,4,5-triphosphate generation, leading to an increase in cytosolic calcium $[Ca^{2+}]_i$.[30] The data presented thus far indicate the presence of functionally active H_1-receptors on lung macrophages. We therefore sought to examine the histamine-induced changes in $[Ca^{2+}]_i$ on a single-cell level using a microfluorometric technique.[17] Our findings showed that stimulation of macrophages with histamine results in an increase in $[Ca^{2+}]_i$ and that this effect is inhibited by fexofenadine. These results are consistent with a molecular mechanism whereby activation of H_1-receptors by histamine increases $[Ca^{2+}]_i$ and this, in turn, is necessary for histamine-induced IL-6 release.

Further investigation of the Ca^{2+} response in these cells showed that the effects of histamine encompassed 1 of 3 distinct profiles: a slow increase, a rapid increase, and a series of phasic oscillations.[17] These differences may be the result of either activation of diverse macrophage populations or qualitative differences in the expression of histamine receptors on macrophages, and they represent the first data to suggest the possibility of a functional heterogeneity of human lung macrophages. A number of different techniques were used to further explore this potential heterogeneity. Percoll gradient experiments identified 2 distinct bands of macrophages: low-density macrophages, representing approximately 35% of cells, and high-density macrophages, representing approximately 65% of cells. Density characteristics of these cells were stable over a period of 12 to 24 hours at 37°C. Additionally, continuous density gradients showed a bimodal population of lung macrophages from a single preparation of cells. Similarly, optical microscopy analysis distinguished 2 morphologic patterns of macrophages with different surface areas and densities, corresponding to low- and high-density macrophages (Triggiani M et al, unpublished data).

It could be speculated that these populations of cells produce distinct patterns of cytokines and express different distributions of cell-surface receptors. Indeed, in a preliminary study, exogenous histamine was shown to selectively stimulate the release of IL-6 from high-density macrophages (Marone G et al, unpublished data). In addition, recent studies have demonstrated that different calcium responses might selectively activate nuclear transcription factors.[31,32] Further investigation of these findings is now required in order to elucidate the role of these different cell types in lung diseases.

Figure 3. Histamine-Mediated Mast Cell–Macrophage Interaction in Airway Remodeling



Histamine immunologically released by activated FcERI+ cells induces the release of lysosomal enzymes and the production of IL-6 and TNF-alpha from human lung macrophages by interacting with H_1-receptors. Histamine also inhibits the release of IL-12, presumably by binding the H_2-receptors. The effect of histamine on macrophages is potentiated by cysteinyl LTC_4 and LTD_4. IL-6 is a T_H2-polarizing cytokine enhancing IgE synthesis in B lymphocytes. TNF-alpha is a proinflammatory cytokine also inducing fibroblast activation and production of SCF. Histamine-induced cytokine profile (stimulation of IL-6 and inhibition of IL-12) promotes the T_H2 response. Together these effects of histamine on macrophages contribute to development of chronic inflammation and airway remodeling associated with bronchial asthma.
FcERI+ = high-affinity receptor for IgE; IgE = immunoglobulin E; IL = interleukin; LTC_4 = leukotriene C_4; LTD_4 = leukotriene D_4; SCF = stem cell factor; TNF = tumor necrosis factor.

Histamine in Inflammation and Airway Remodeling

Traditionally, histamine has been considered a mediator of acute symptoms of allergic inflammation. The results outlined in this paper show that concentrations of histamine, such as those found in the airways of asthmatic patients, are an effective stimulus for exocytosis and cytokine production from human lung macrophages. These actions of histamine on macrophages outline a model for the role of histamine in the pathogenesis of persistent inflammation and airway remodeling that occurs in diseases such as chronic asthma and in symptoms of allergic rhinitis (Figure 3).[33]

According to the model of mast cell–macrophage interaction in Figure 3, the release of low concentrations of histamine and cysteinyl leukotrienes from activated basophils and mast cells results in the release of numerous mediators from macrophages. These include IL-6, which acts on B-lymphocytes and potentiates IgE synthesis,[24] lysosomal enzymes, which are important in tissue damage and remodeling,[18] and TNF-alpha, which is a potent activator of fibroblasts inducing the accumulation of collagen and the synthesis of stem cell factor (a major growth factor for mast cells).[34] This model emphasizes the importance of a dynamic interaction between proinflammatory mediators and cytokines. Indeed, our findings show that the release of proinflammatory mediators from mast cells and basophils can influence the de novo synthesis of cytokines that are central in allergic reactions and tissue remodeling. Administration of the highly selective H_1-receptor antagonist fexofenadine antagonized not only the exocytotic effect of histamine on macrophages, but also the synthesis of IL-6 and the synergistic interaction of histamine and LTD_4.

Therapeutic Implications

In addition to the above data, several studies have shown that fexofenadine can inhibit the expression and release of proinflammatory mediators in vitro and ex vivo at clinically relevant therapeutic doses.[17,35,36] For example, fexofenadine (10^{-9} mol/L to 10^{-3} mol/L) significantly inhibited the release of RANTES (regulated upon activation, normal T-cell expressed and secreted), IL-8, granulocyte-macrophage colony-stimulating factor, and soluble intercellular adhesion molecule-1 from human nasal epithelial cell cultures derived from patients with allergic rhinitis.[35] Fexofenadine also significantly attenuated eosinophil chemotaxis and adherence to human endothelial cells in the same study. Therefore, administration of fexofenadine in patients with allergic disorders may not only prevent acute symptoms, but may also interfere with some of the mechanisms involved in the late-phase immune responses and in airway remodeling. It is important to note that several other antihistamines, particularly those of the newer generation, have shown similar anti-inflammatory effects in vitro and in vivo.

Overall, our observations raise the possibility that allergen-induced basophil/mast cell activation in the lower airways triggers early stimulation of the lung macrophages, the predominant cell in BALF and lung parenchyma. Lung macrophages are a major source of lysosomal enzymes and several cytokines that play a central role in tissue damage and remodeling. Taking into account the mast cell–macrophage amplification loop described above, it can be postulated that there is a parallel dynamic interaction between allergic inflammation and tissue remodeling. Accordingly, tissue remodeling may initiate early in the sequence of events underlying allergic disorders, indicating that treatment to prevent tissue remodeling in allergic disease should begin at an early stage. This hypothesis has yet to be tested in the clinical setting.

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