Histamine Induces Exocytosis and IL-6 Production from Human Lung Macrophages Through Interaction with H₁ Receptors

Massimo Triggiani, Marco Gentile, Agnese Secondo, Francescopaolo Granata, Alfonso Oriente, Maurizio Taglialetela, Lucio Annunziato and Gianni Marone

J Immunol 2001; 166:4083-4091; doi: 10.4049/jimmunol.166.6.4083
http://www.jimmunol.org/content/166/6/4083

References
This article cites 54 articles, 14 of which you can access for free at:
http://www.jimmunol.org/content/166/6/4083.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Histamine Induces Exocytosis and IL-6 Production from Human Lung Macrophages Through Interaction with H\textsubscript{1} Receptors\textsuperscript{1}

Massimo Triggiani,\textsuperscript{2,}* Marco Gentile,\textsuperscript{*} Agnese Secondo,\textsuperscript{†} Francescopaolo Granata,\textsuperscript{*} Alfonso Oriente,\textsuperscript{*} Maurizio Taglialatela,\textsuperscript{†} Lucio Annunziato,\textsuperscript{†} and Gianni Marone\textsuperscript{*}

Increasing evidence suggests that a continuous release of histamine from mast cells occurs in the airways of asthmatic patients and that histamine may modulate functions of other inflammatory cells such as macrophages. In the present study histamine (10\textsuperscript{−9}–10\textsuperscript{−6} M) increased in a concentration-dependent fashion the basal release of \(\beta\)-glucuronidase (EC\textsubscript{50} = 8.2 ± 3.5 × 10\textsuperscript{−9} M) and IL-6 (EC\textsubscript{50} = 9.3 ± 2.9 × 10\textsuperscript{−8} M) from human lung macrophages. Enhancement of \(\beta\)-glucuronidase release induced by histamine was evident after 30 min and peaked at 90 min, whereas that of IL-6 required 2–6 h of incubation. These effects were reproduced by the H\textsubscript{1} agonist (6-[2-(4-imidazolyl)ethylamino]-N-(4-trifluoromethylphenyl)heptane carboxamide) and by the H\textsubscript{1} antagonist dimaprit. Furthermore, histamine induced a concentration-dependent increase of intracellular Ca\textsuperscript{2+} concentrations ([Ca\textsuperscript{2+}]) that followed three types of response, one characterized by a rapid increase, a second in which [Ca\textsuperscript{2+}] displays a slow but progressive increase, and a third characterized by an oscillatory pattern. Histamine-induced \(\beta\)-glucuronidase and IL-6 release and [Ca\textsuperscript{2+}] elevation were inhibited by the selective H\textsubscript{1} antagonist fexofenadine (10\textsuperscript{−5}–10\textsuperscript{−3} M), but not by the H\textsubscript{2} antagonist ranitidine. Inhibition of histamine-induced \(\beta\)-glucuronidase and IL-6 release by fexofenadine was concentration dependent and displayed the characteristics of a competitive antagonism (\(K_A = 89 \text{ nM}\)). These data demonstrate that histamine induces exocytosis and IL-6 production from human macrophages by activating H\textsubscript{1} receptor and by increasing [Ca\textsuperscript{2+}], and they suggest that histamine may play a relevant role in the long-term sustainment of allergic inflammation in the airways. The Journal of Immunology, 2001, 166: 4083–4091.

Histamine is a chemical mediator synthesized and stored within cytoplasmic granules of human basophils and mast cells (1, 2). Immunologic and nonimmunologic activation of these cells induces the release of this proinflammatory mediator (3). The central role played by histamine in the pathophysiology of inflammatory and allergic disorders has been clearly established. Histamine is released in vivo during allergic reactions (4), and administration of exogenous histamine reproduces signs and symptoms typical of allergic diseases (5). Histamine exerts a variety of proinflammatory and immunomodulating effects through the interaction with three receptors, H\textsubscript{1}, H\textsubscript{2}, and H\textsubscript{3} (6, 7). The pivotal role of histamine in allergic inflammation is supported also by the observation that antagonists of the H\textsubscript{1} receptor are effective in alleviating some of the acute symptoms of allergic disorders (8). The immediate actions of histamine on vascular endothelium and on bronchial and vascular smooth muscle cells have been clearly elucidated. These effects are mostly mediated by the activation of the H\textsubscript{1} receptor, and they are responsible for the majority of the acute symptoms in bronchial asthma, allergic rhinitis, and urticaria (8). Histamine also exerts a variety of other regulatory functions by modulating the activity of T cells (9, 10), monocytes (11), neutrophils (12), and eosinophils (13). Increasing evidence suggests that histamine not only is massively released during acute allergic reactions, but can be secreted in low concentrations in the airways of patients with bronchial asthma. In fact, detectable levels of histamine have been found in the bronchoalveolar lavage (BAL)\textsuperscript{3} of patients with bronchial asthma during asymptomatic periods (14–16), and these levels have been found to correlate with asthma severity and bronchial hyperreactivity (17). In addition, basophils and BAL mast cells from patients with asthma have an increased capacity to release histamine in vitro as compared with those from healthy donors (14). Finally, an increased number of degranulated mast cells and basophils also has been documented in the airways of asthmatics when biopsies were performed at a time distant from the acute attack (18, 19). These observations are compatible with the hypothesis that histamine is chronically released in the airways of asthmatic patients and may have a role in persistent airway inflammation and tissue remodeling typical of bronchial asthma (20).

The macrophage is the predominant cell in the lung parenchyma and in the BAL of both healthy individuals and asthmatic patients (21). This cell plays a major role in the defense against infections and in the local modulation of immune and inflammatory responses (22). Lung macrophages produce a wide spectrum of mediators including lytic enzymes, lipid mediators, reactive oxygen

\textsuperscript{1}Division of Clinical Immunology and Allergy and \textsuperscript{2}Section of Pharmacology, Department of Neurosciences, University of Naples Federico II, Naples, Italy

Received for publication August 7, 2000. Accepted for publication January 3, 2001.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

\textsuperscript{*}This work was supported in part by grants from the Consiglio Nazionale delle Ricerche (Target Project Biotechnology Grants 99.00216.PF31 and 99.00401.PF49), from the Istituto Superiore di Sanità (AIDS Project 9403, Rome, Italy), and from Aventis (Milan, Italy).

\textsuperscript{†}Address correspondence and reprint request to Dr. Massimo Triggiani, Division of Clinical Immunology and Allergy, University of Naples Federico II, Via Pansini 5, 80131 Naples, Italy. E-mail address: triggian@unina.it

\textsuperscript{3}Abbreviations used in this paper: BAL, bronchoalveolar lavage; fura-2 AM, 1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxyl]-2-[2-(4-imidazolyl)ethylamino]-N-(4-trifluoromethylphenyl)heptane carboxamide; [Ca\textsuperscript{2+}], intracellular Ca\textsuperscript{2+} concentration; PLA\textsubscript{2}, phospholipase A\textsubscript{2}.
Materials and Methods

Reagents and buffers

Histamine dihydrochloride, ranitidine hydrochloride, Percoll, l-glutamine, antibiotic-antimycotic solution (10,000 IU/ml penicillin, 10 mg/ml streptomycin, and 25 μg/ml amphotericin B), group IA secretory phospholipase A2, (PLA2) (from Neisseria meningitidis). LPS (from Escherichia coli type 026:B6), fatty acid-free human serum albumin, Triton X-100, 1-[2-(5-carboxyoxazol-2-yl)-6-amino-2-benzoxuron-5-oxyl]-2-(2'-amino-5'-methylphenoxy)ethane-N,N,N',N'-tetraacetic acid pentaacetate, bovine serum albumin, rabbit IgG, efector IgM, SDS-PAGE, and 5 mM KCl, pH 7.4. Glycine buffer was composed of 400 mM glycine and purified by the Johns Hopkins DNA Core Facility. All other reagents

Materials and Methods

Isolation and purification of human lung macrophages

Macrophages were obtained from the lung parenchyma of patients undergoing thoracic surgery as reported previously (23). Macrophages were purified by a variety of stimuli acting on specific membrane receptors including the three histamine receptors (H1, H2, and H3; Refs. 24 and 25). Activation of histamine receptors modulates several macromolecule release (26) and exocytosis (24). Macrophages are often found in close proximity to mast cells in the airways of asthmatic patients (18, 19). The anatomical association between these two cells suggests that lung macrophages may be exposed to histamine released locally from immunologically activated mast cells.

In this study, we have examined the effect of low concentrations of histamine on human lung macrophages in vitro. Our results indicate that histamine induces the release of β-glucuronidase, a marker of exocytosis, and the production of IL-6 by activating the H3 receptor and by increasing the intracellular Ca2+ concentrations ([Ca2+]i). These effects are reproduced by selective H3 receptor activation and are inhibited in a competitive fashion by fexofenadine, a second generation H1 receptor antagonist.

IL-6 ELISA

IL-6 in the culture supernatant of macrophages was measured in duplicate determinations by a commercially available ELISA (Euro Clone, Torquay, U.K.) according to the manufacturer’s instructions. The linearity range of the assay was between 5 and 150 pg/ml. Because the number of adherent macrophages can vary in in vitro and in different experimental conditions, the results were normalized for the total protein content in each well, determined in the cell lysates (0.1% Triton X-100) by the method of Lowry et al. (29).

IL-6 gene expression

Macrophages (5 × 104/ml) were incubated (37°C, 1 h) in RPMI 1640 containing 5% FCS in six-well plates. The cells then were washed and incubated (37°C, 1–9 h) in FCS-free medium alone or with histamine (10–6 M). At the end of the incubation, RNA was isolated with the TRIzol reagent (Life Technologies, Grand Island, NY) according to the manufacturer’s instructions. Diethylpyrocarbonate-treated water without SDS was used for the final resuspension step; RNA was stored at –80°C. Reverse transcription was performed with 5 mM MgCl2, dideoxynucleoside triphosphates, and murine leukemia virus reverse transcriptase according to the manufacturer’s instructions (Perkin-Elmer, Norwalk, CT) on a thermocycler (GeneAmp PCR System 2400; Perkin-Elmer). PCR was performed by using Taq polymerase (1–2.5 U/reaction) at the annealing temperature of 60°C with target-specific primers for IL-6 (5'–AGGATCTCTTCTCCAAAGGCG3' and 3'–GGAGGACCCTCAAGGGCTGAGTCG–5' or 0.2–1 μM primer) at sub saturating cycle number (30 cycles). Normalization of RNA was achieved by RT-PCR for the constitutive marker gene β-actin by sub saturating cycle number. Specific RNase-free conditions were maintained throughout the procedure (30). All PCR products were visualized by ethidium bromide-stained gel electrophoresis and photographed. Analysis of relative band intensity was performed by using a digital scanner (Celbio, Milan, Italy).

$[Ca^{2+}]_{i}$ measurements and analysis of $[Ca^{2+}]_{i}$ oscillations

$[Ca^{2+}]_{i}$, was measured by a microfluorometric technique, as reported previously (31). Briefly, the cells grown on glass coverslips were loaded with 5 μM fura-2 AM in Krebs-Ringer saline solution for 1 h at 22°C. At the end of fura-2 AM loading, the coverslip was introduced into a microscope chamber (Medical System, Greenevale, NY) on an inverted Nikon Diaphot fluorescence microscope (Nikon, Melville, NY). The cells were kept in Krebs-Ringer saline solution throughout the experiment. All the drugs tested were introduced into the microscope chamber by fast injection. A fast-scan xenon lamp (Osram, Berlin, Germany) with a computer-operated filter wheel bearing two different interference filters (340 and 380 nm) illuminated the microscopic field with UV light, alternating the wavelength at an interval of 500 ms. The interval between each pair of illuminations was 2 s, and the interval between filter movements was 1 s. Consequently, $[Ca^{2+}]_{i}$ was measured every 3 s. Emitted light was passed through a 400-nm dichroic mirror, filtered at 510 nm, and collected by a charge-coupled device camera (Photonic Science, Robertsbridge, East Sussex, U.K.) connected to a light amplifier (Applied Imaging, Dukesway Gateshead, U.K.) and digitized and analyzed by a computerized image processor (Applied Imaging). By using a calibration curve, the Tardis software (Applied Imaging) calculated the $[Ca^{2+}]_{i}$, corresponding to each pair of images from the ratio between the intensity of the light emitted when the cells were illuminated at both 340 and 380 nm.

$[Ca^{2+}]_{i}$ oscillations were defined as an increase of $[Ca^{2+}]_{i}$, above the mean of the basal value ± 2 SD. The frequency of $[Ca^{2+}]_{i}$ oscillations was calculated as the number of peaks occurring per min, and the amplitude was determined as the difference between the maximal $[Ca^{2+}]_{i}$ value of the peak and the minimal $[Ca^{2+}]_{i}$ value just before the occurrence of the peak.
Statistical analysis

The data are expressed as the mean ± SE of the indicated number of experiments, and p values were determined with t test for unpaired samples with Bonferroni’s correction (32). The threshold for statistical significance was set at p < 0.05. The data subjected to linear regression analysis were calculated by the least squares method (y = a + bx) in which a was the y-axis intercept and b the slope of the line. For each pharmacological treatment in the experiments on [Ca\(^{2+}\)], at least five cells in at least three experimental sessions were evaluated.

Results

Effect of histamine on β-glucuronidase release from human lung macrophages

We first examined whether histamine induced the release of β-glucuronidase, a marker of exocytosis (33), from human lung macrophages. Fig. 1 shows the results of six experiments indicating that histamine induces a concentration-dependent release of β-glucuronidase from macrophages. After 2 h of incubation, a significant effect of histamine was evident at a concentration of 10\(^{-9}\) M, whereas the maximum release of β-glucuronidase occurred at 10\(^{-7}\) M, with an EC\(_{50}\) of 8.2 ± 3.5 × 10\(^{-9}\) M. The highest concentration of histamine (10\(^{-7}\) M) induced a release that was 2.4-fold higher than that from unstimulated cells (7.2 ± 0.5 vs 3.0 ± 0.3% of total cellular content; p < 0.01). Concentrations of histamine higher than 10\(^{-7}\) M did not further enhance β-glucuronidase release. Viability of macrophages was routinely assessed at the end of each experiment by trypan blue exclusion and was found to be always >95%.

Fig. 2 shows the kinetics of histamine (10\(^{-7}\) M)-induced β-glucuronidase release from macrophages. The release was relatively rapid, starting after 30 min and reaching a plateau 2 h after the addition of the stimulus. These data indicate that submicromolar concentrations of histamine rapidly enhance β-glucuronidase release from macrophages.

Effect of histamine on IL-6 production from human lung macrophages

Macrophages are an important source of cytokines regulating inflammatory and immune responses in the lung. Although histamine has been reported either to induce (25, 34) or inhibit (35, 36) cytokine synthesis in human monocytes and macrophages, there are no data on the effect of this mediator on IL-6, a major cytokine synthesis in human monocytes and macrophages. Fig. 3 shows the kinetics of IL-6 release from three preparations of human lung macrophages challenged with 10\(^{-6}\) M of histamine. Maximum enhancement of IL-6 release was evident between 2 and 6 h after addition of histamine (Fig. 3) with a maximum enhancement of 2-fold the basal release (1.6 ± 0.3 vs 0.8 ± 0.1 ng of IL-6/mg of protein) at 10\(^{-6}\) M. Histamine increased the basal secretion of IL-6 from macrophages in a concentration-dependent fashion (Fig. 3) with a maximum enhancement of 2-fold the basal release (1.6 ± 0.3 vs 0.8 ± 0.1 ng of IL-6/mg of protein) at 10\(^{-6}\) M. Histamine also appeared to be less effective in inducing IL-6 release than it was on β-glucuronidase because a significant effect on IL-6 release was achieved only at a concentration of 10\(^{-7}\) M and the EC\(_{50}\) was 9.3 ± 2.9 × 10\(^{-8}\) M.

Fig. 4 shows the kinetics of IL-6 release from three preparations of human lung macrophages challenged with 10\(^{-6}\) M of histamine. It can be seen that the effect of histamine on IL-6 release was concentration-dependent and dose-dependent in all three preparations. The data are expressed as the mean ± SE of six experiments. *p < 0.01 vs control.
data are the mean ± SE of five experiments.

To establish the relative contribution of histamine to IL-6 production from human lung macrophages, we compared the release induced by histamine with that induced by three known agonists of these cells, i.e., secretory PLA2, platelet-activating factor and LPS (Fig. 5). The results indicate that histamine is the least potent stimulus; it induced a production of IL-6 that was approximately one-half of that induced by other stimuli.

**Effect of H1 and H2 agonists and antagonists on histamine-induced β-glucuronidase and IL-6 release from human lung macrophages**

To determine the type of receptor activated by histamine on macrophages, the cells were incubated (37°C, 2 h for β-glucuronidase and 6 h for IL-6) with either HTMT, a selective H1 agonist (38), or dimaprit, a selective H2 agonist (39). Table I shows that HTMT but not dimaprit induces the release of both β-glucuronidase and IL-6 from macrophages. HTMT had an effect comparable to that of histamine as it increased ~2-fold the basal release of both β-glucuronidase and IL-6. These data indicated that activation of the H1 receptor was involved in histamine-induced exocytosis and IL-6 release.

To confirm these data, macrophages were preincubated with fexofenadine, a second generation H1 receptor antagonist (40), or with ranitidine, a selective H2 antagonist (39), before the addition of histamine and the release of β-glucuronidase was determined. Fig. 6A shows that increasing concentration of fexofenadine (10⁻⁷–10⁻⁴ M) induced a parallel rightward shift of the histamine concentration-response curve. In addition, the inhibitory effect of fexofenadine was progressively reduced by increasing the concentrations of the agonist (histamine). This observation suggested that fexofenadine acted as a competitive antagonist at the receptor level. In contrast, ranitidine (10⁻³ M) had no effect on histamine-induced β-glucuronidase release.

The results of the experiments shown in Fig. 6A were used for Schild plot analysis. In case of a competitive antagonism, Schild plot analysis provides a straight line with an intercept on the abscissa corresponding to log Kᵣ. Fig. 6B shows the least squares fit to the experimental points from four separate experiments. The mean ± SE value for Kᵣ of fexofenadine was 89.2 ± 22.6 nM, a value that is close to the Kᵣ reported for fexofenadine in binding the H1 receptor in rat brain tissue (160 nM; Ref. 41).

Additional experiments were performed to determine whether the H2 antagonist fexofenadine also inhibited histamine-induced IL-6 release. Fig. 7 shows that preincubation of macrophages with fexofenadine for 15 min inhibits in a concentration-dependent fashion the release of IL-6 induced by 10⁻⁷ M histamine. The inhibitory effect of fexofenadine was significant at 10⁻⁶ M, and it reached a maximum inhibition of 85 ± 15% of histamine response at a concentration of 10⁻⁴ M. Together these data indicate that both β-glucuronidase and IL-6 release are mediated by the activation of the H1 receptor on macrophages.

**Effect of histamine on cytosolic [Ca²⁺], in human lung macrophages**

Activation of the H1 receptor is associated with phospholipase C activation and inositol 1,4,5-triphosphate generation leading to a raise in [Ca²⁺], (42). The aforementioned results indicated that human macrophages express a functionally active H1 receptor.
Therefore, in the next group of experiments, we monitored with the help of a microfluorometric technique at a single-cell level the histamine-induced changes in $[\text{Ca}^{2+}]$. When human lung macrophages were exposed to $10^{-7}$ M histamine, an elevation of $[\text{Ca}^{2+}]$, occurred in $\sim 45\%$ of the cells examined. Moreover, responsive macrophages displayed three different patterns of $[\text{Ca}^{2+}]$ elevation (Fig. 8A). Sixty-five percent of the cells exhibited a rapid and long-lasting elevation of baseline $[\text{Ca}^{2+}]$ (Fig. 7A, upper tracing). In a second subgroup of cells (23%), histamine response was characterized by a slower but progressive increase of $[\text{Ca}^{2+}]$ (Fig. 8A, middle tracing). Interestingly, in the remaining 11% of lung macrophages an oscillatory pattern was elicited by histamine (Fig. 8A, lower tracing). In these cells, histamine-induced $[\text{Ca}^{2+}]$ oscillations were characterized by a frequency of 1–3/min and an amplitude ranging from 40 to 60 nM. Histamine-induced $[\text{Ca}^{2+}]$ elevation in responsive macrophages was concentration dependent and reached a maximum increase of 80% as compared with baseline at $10^{-6}$ M (Fig. 8B).

Preincubation (37°C, 1 min) of macrophages with the H1 antagonist fexofenadine ($10^{-7}$ M) inhibited all kind of $[\text{Ca}^{2+}]$ elevations induced by the subsequent addition of $10^{-7}$ M histamine (Fig. 9A). The concentration-response curve of the inhibitory effect of fexofenadine on histamine-induced $[\text{Ca}^{2+}]$ changes is shown in Fig. 9B.

**Influence of Ca$^{2+}$ on histamine-induced IL-6 release from human lung macrophages**

The data reported above indicated that histamine induced IL-6 release and increased $[\text{Ca}^{2+}]$ in human lung macrophages. To understand whether the increase in $[\text{Ca}^{2+}]$, was required for IL-6 release from macrophages, the cells were stimulated with histamine either in the absence or in the presence of the Ca$^{2+}$ chelating agent EDTA (10 mM). Table II shows that incubation of macrophages with EDTA completely inhibits IL-6 release in response to histamine but not the spontaneous release. In separate experiments we explored whether the Ca$^{2+}$ ionophore A23187 induces IL-6 release from macrophages. In three experiments, A23187 ($10^{-6}$ M) significantly increased IL-6 release from human lung macrophages ($2.51 \pm 0.26$ vs $0.19 \pm 0.19$ ng/mg of protein; $p < 0.05$). These data are compatible with the hypothesis that the increase in $[\text{Ca}^{2+}]$, is necessary for histamine-induced IL-6 release.

**Effect of histamine on IL-6 expression in human lung macrophages**

It has been reported that Ca$^{2+}$ signals differentially activate nuclear transcription factors depending on their amplitude, duration, and oscillatory pattern (43, 44). To test the hypothesis that histamine may activate gene expression for IL-6, we evaluated the expression of IL-6 mRNA in macrophages incubated with medium alone (control) or with histamine ($10^{-6}$ M) for 1–9 h. Fig. 10A depicts specific RT-PCR amplification products from one representative experiment of three. Adequate normalization of RNA for
each sample was confirmed by the equality of RT-PCR amplification products for the constitutive marker gene of β-actin (first row). Histamine increased IL-6 mRNA expression after 3 and 6 h of incubation. The enhancing effect of histamine was no longer evident after 9 h of incubation. Fig. 10B shows the densitometric analysis of the IL-6 bands, expressed as the ratio of the signal in histamine-treated and -untreated cells. These data indicate that histamine enhances IL-6 production in macrophages by increasing its specific mRNA.

Discussion
In this study, we have examined the effects of low concentrations of histamine on human lung macrophages in vitro. Our results indicate that incubation of macrophages with submicromolar concentrations of histamine activates exocytosis and IL-6 production and increases [Ca\(^{2+}\)]\(_i\) with three different patterns of response. These effects of histamine are all mediated by the activation of H\(_1\) receptors.

Both experimental and clinical evidence suggests that low levels of histamine are chronically released in the airways of asthmatic patients even in the absence of clinical symptoms (14–17). The level of histamine detected in the BAL fluid of asthmatics is ~1–2 ng/ml (15, 16). Considering the dilution of the BAL and the intimate contact between mast cells and macrophages in the airways, the concentrations of histamine surrounding lung macrophages may reach 10\(^{-7}\)–10\(^{-6}\) M. Our results demonstrate that these concentrations of histamine are effective to increase exocytosis and IL-6 release from macrophages.

Our data provide the first evidence that physiologically relevant concentrations of histamine induce the release of IL-6 from human macrophages isolated from the lung parenchyma. Histamine increases IL-6 production presumably by increasing its mRNA expression. However, our data do not exclude the possibility that histamine increases the stability of IL-6 mRNA. IL-6 is a multifunctional cytokine involved in inflammatory and immune responses (37). Previous studies have shown that the levels of IL-6 are increased in plasma and BAL (45–47) and that this cytokine is overexpressed in the bronchial mucosa of patients with bronchial asthma (48). Although its role has not been completely elucidated, IL-6 may participate to allergic inflammation in a number of ways. IL-6 is a stem cell factor and a B cell proliferating and activating factor (37). For example, IL-6 is involved in mast cell proliferation.
and differentiation (49, 50), it may favor Th2 responses and IgE production (51, 52) and stimulates airway epithelial cells to release IL-8 (53). These observations support the hypothesis that IL-6 may have an important role in modulating allergic inflammation in asthma. Our results are in agreement with previous data showing that histamine induces IL-6 production in bronchial epithelial cells (54), endothelial cells (55), and B cells (56). Therefore, histamine appears to activate a common pathway leading to IL-6 expression in all the major sources of this cytokine in the human lung.

The conclusion that histamine induces exocytosis and IL-6 production through the activation of H1 receptors on macrophages is supported by two lines of evidence: 1) these events are induced by HTMT, a selective H1 agonist, but not by the H2 agonist dimaprit, and 2) they are inhibited by the H1 antagonist fexofenadine but not by the H2 antagonist ranitidine. Human macrophages express all types of histamine receptors, H1, H2, and H3 (24, 25). Several studies have started to highlight the complexity of histamine’s effects on cytokine network in human cells depending on the type of receptor activated. For example, activation of IL-6 production generally occurs via H1 receptors (54–56), whereas inhibition of IL-1, TNF-α, and IL-12 production by LPS-stimulated human monocytes is mediated by H2 receptors (35, 36, 57). Furthermore, activation of H4 and H3 receptors stimulates IL-10 release from human monocytes and macrophages (11, 25). These findings suggest that the local release of histamine in inflamed tissues may play a role in the modulation of the cytokine network more complex than originally thought. Even more interesting is the hypothesis that histamine may differentially modulate cytokine synthesis depending on the type of receptor predominantly expressed on a given cell. Studies are currently ongoing to define whether macrophages isolated from patients with bronchial asthma express a pattern of histamine receptors different from those of nonasthmatic individuals.

Stimulation of macrophages with histamine results in the increase in [Ca2+]i, and this effect is inhibited by the H1 competitive antagonist fexofenadine. These results are in line with the general observation that H2 receptor activation is associated with intracellular Ca2+ influx (39) and they indicate that this signaling pathway is also active in human macrophages. Moreover, our results suggest that the increase in [Ca2+]i, induced by histamine is required for the activation of IL-6 production in these cells.

A number of studies support the hypothesis that subsets of macrophages with different morphological, biochemical, and functional properties exist in the human lung (58). Whether these differences are related to a different state of maturation or activation of macrophages is presently unclear. We add a novel observation to support the hypothesis of lung macrophage heterogeneity by showing that the same concentration of histamine may induce three distinct profiles of Ca2+ response in these cells: a slow increase, a rapid increase, and a series of phasic oscillations. These different profiles of Ca2+ response may reflect either the activation of diverse macrophage populations or qualitative differences in the expression of histamine receptors on macrophages. In any event, lung macrophages display a heterogenous Ca2+ response to histamine. This finding acquires further relevance in light of the recent demonstration that different profiles of Ca2+ signaling may selectively activate nuclear transcription factors such as NF-kB, c-Jun N-terminal kinase, and NF-AT (43, 44). Therefore, the induction of different Ca2+ responses may be a mechanism by which histamine modulates the expression of various cytokines in the human macrophages.

The second generation H1 antagonist fexofenadine inhibits histamine-induced exocytosis, IL-6 production, and Ca2+ signaling. The observation that fexofenadine is a competitive antagonist at the histamine H1 receptor level on human lung macrophage with a
HISTAMINE-MEDIATED ACTIVATION OF LUNG MACROPHAGES

\( K_a \) comparable to that obtained in other tissues is of interest for a number of reasons. First, it indicates that the H\(_1\) receptor on macrophages displays pharmacological characteristics similar to the H\(_1\) receptor expressed in other tissues. Second, the observation that the \( K_a \) of fexofenadine is close to the \( K_a \) indicates that the inhibitory effect of this drug is truly a pharmacological event at the receptor level and it does not represent a nonspecific interaction of fexofenadine with the cell membrane. Finally, the observation that fexofenadine not only antagonizes the exocytotic effect of histamine on macrophages, but also the synthesis of IL-6 is of potential clinical importance. In fact, it suggests that administration of this drug in patients with allergic disorders may prevent not only the acute symptoms, but that it might interfere with some of the mechanisms involved in chronic inflammation and in tissue damage associated with the activation of macrophages.

Taken together, our data demonstrate that histamine activates human lung macrophages via H\(_1\) receptor. In these cells, histamine enhances the release of a preformed mediator (\( \beta \)-glucuronidase) as well as the expression and release of IL-6. These effect of histamine are associated with a rise in cytosolic Ca\(^{2+} \) concentrations. These novel actions of histamine on a cell that plays a central role in inflammatory lung diseases provide an additional mechanism by which histamine contributes to maintain chronic inflammation in bronchiol asthma. The ability of fexofenadine, a selective H\(_1\) blocker, to inhibit histamine-induced activation of human macrophages opens new perspectives on the long-term use of H\(_1\) receptor antagonists in the treatment of allergic and inflammatory lung diseases.

References


