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*J Immunol* 2000; 164:2964-2970; doi: 10.4049/jimmunol.164.6.2964

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Importance of Histamine in the Cytokine Network in the Lung Through H2 and H3 Receptors: Stimulation of IL-10 Production

Jocelyne Sirois,* Geneviève Ménard,* Audric S. Moses,† and Elyse Y. Bissonnette2*

Histamine, a well-known inflammatory mediator, has been implicated in various immunoregulatory effects that are poorly understood. Thus, we tested the hypothesis that histamine inhibits the release of a proinflammatory cytokine, namely TNF, by stimulating the release of an anti-inflammatory cytokine, IL-10. Alveolar macrophages (AMs) from humans, Sprague Dawley rats, and the AM cell line, NR8383, were treated with different concentrations of histamine (10^{-5} - 10^{-7} M) for 2 h prior to their stimulation with suboptimal concentration of LPS (1 ng/ml) for 4 h. Histamine inhibited TNF release in a dose-dependent manner. This inhibition was mimicked by H2 and H3 receptor agonists, but not by H1 receptor agonist. Furthermore, we demonstrated the expression of H2 receptor mRNA in human AMs. Interestingly, treatment of AMs with anti-IL-10, anti-PGE2, or a NO synthase inhibitor (N^ω-nitro-arginine methyl ester) before the addition of histamine abrogated the inhibitory effect of the latter on TNF release. Histamine treatment (10^{-5} M) increased the release of IL-10 from unstimulated (2.2-fold) and LPS-stimulated (1.7-fold) AMs. Unstimulated AMs, NR8383, express few copies of IL-10 mRNA, as tested by quantitative PCR, but expression of IL-10 was increased by L5-fold with histamine treatment. Moreover, the stimulation of IL-10 release by histamine was abrogated by pretreatment with anti-PGE2 or the NO synthase inhibitor, Nω-nitro-arginine methyl ester. Thus, histamine increases the synthesis and release of IL-10 from AMs through PGE2 and NO production. These results suggest that histamine may play an important role in the modulation of the cytokine network. The Journal of Immunology, 2000, 164: 2964–2970.

Histamine was one of the first proinflammatory mediators to be described. Its main sources are basophils and mast cells, which are distributed throughout the body and are well known for their roles in allergic disorders and inflammation (1). Histamine has been involved in allergic reactions, vasodilatation and vasoconstriction, gastric acid secretion, and neurotransmission (2–4). In addition to its proinflammatory actions (5, 6), histamine possesses anti-inflammatory and immunosuppressive effects (7). In general, its inflammatory effects are mediated by histamine H1 receptors, whereas various immunoregulatory effects of histamine, such as induction of suppressor cells (8), inhibition of lymphocyte proliferation (9), and neutrophil chemotaxis (10), are mediated by H2 receptors. Furthermore, there is increasing evidence suggesting that histamine may be involved in the regulation of cytokine networks. Histamine can inhibit the release of IL-1, IL-2, IFN-γ, and TNF (11–15) and increase the release of IL-5 (13), IL-6 (16), and IL-8 (17).

A panoply of cytokines and cell types are involved in the pathogenesis of asthma. Alveolar macrophages (AMs), which are found throughout the respiratory tract and represent the most abundant cells in the airway lumen, play a crucial role in determining the development of immune responses, Th1/Th2, in the lung. AMs produce both Th1 (IL-12) and Th2 (IL-10 and IL-13) cytokines (18, 19) and secrete a panoply of mediators, including TNF, IL-1, IL-6, IL-8, and NO (19–22). TNF plays a pivotal role in inflammation by stimulating inflammatory cells and increasing the production of cytokines such as IL-1, IL-6, IL-8, and IL-12 (22, 23). Furthermore, there is some evidence suggesting an important role of TNF in the development of the Th1 response (24). Whereas TNF is often an inflammatory cytokine, IL-10 is usually considered an immunosuppressive and anti-inflammatory cytokine (25). IL-10 inhibits the production of IL-1, IL-6, IL-8, IL-12, and TNF by activated macrophages (26) as well as that of IFN-γ by Th1 cells (27). Thus, AMs produce both pro- and anti-inflammatory cytokines, which implies that a good balance in the production of these cytokines is crucial to maintain the homeostasis of the lung.

Histamine is released during allergic reactions and is found in bronchoalveolar lavages of asthmatic patients after allergic reactions (28). The presence of histamine in the airways may modulate cytokine production by AMs, thus affecting the inflammatory responses in the lung. Thus, we tested the hypothesis that histamine released during allergic reactions modifies cytokine production by AMs modulating the cytokine network. We investigated the effect of histamine pretreatment on the release of TNF and IL-10 by AMs stimulated with suboptimal concentrations of LPS. Here, we demonstrate that histamine inhibits the release of TNF from AMs in a dose-dependent manner by stimulating the synthesis and release of IL-10. The inhibitory effect of histamine on TNF release was mediated through H2 and H3 receptors and was modulated by the production of NO and PGE2. Furthermore, stimulation of IL-10 production by histamine was abrogated by the NO synthase inhibitor and anti-PGE2 Ab.

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Received for publication May 14, 1999. Accepted for publication January 5, 2000.

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This work was supported by the Medical Research Council of Canada and the Alberta Lung Association. E.Y.B. is a Medical Research Council/CLA Scholar.

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*Abbreviations used in this paper: AM, alveolar macrophage; i-NAME, N^ω-nitro-arginine methyl ester; MIP-1α, macrophage inflammatory protein-1α; NOS, NO synthase; cNOS, constitutive form of NOS; iNOS, inducible form of NOS.

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Materials and Methods

Animals

Outbred male Sprague Dawley rats were obtained from Charles River Canada (St. Constant, Canada) and were maintained in an isolation room with filter-topped cages to minimize unwanted infections. The animals were given food and water ad libitum and were maintained on a 12-h light, 12-h dark cycle. The experimental protocol was approved by the University of Alberta animal care committee in accordance with the guidelines of the Canadian Council on Animal Care.

Reagents

LPS from Salmonella enteritidis, histamine, H₂ receptor agonist (betahistine), Nω-nitro-L-arginine methyl ester (LNAME), and goat anti-mouse IL-10 Ab (IgG; bioactivity was assessed by IL-10 neutralization test in cell culture) were purchased from Sigma (St. Louis, MO). The H₂ receptor agonist, dimaprit, was obtained from ICN (Aurora, OH), and the H₂ receptor agonist, R-α-methyl-histamine, was purchased from RBI (Natick, MA). Rabbit anti-TGF-β neutralizing Ab (dilution of 1/50) inhibited the effect of 10⁻¹⁰ M TGF-β (29 pg/ml) on mast cells was obtained from R&D Systems (Minneapolis, MN), and Ab to PGE₂ was purchased from Biotechnology (Lake Placid, NY). The pH of all solutions was adjusted to 7.2 before use. RPMI 1640 medium (Life Technologies, Burlington, Canada) and PBS containing <0.05 endotoxin unit when tested using an E-Toxate kit (Sigma).

Cells

AMs were isolated as previously described (30). Briefly, animals were anaesthetized and exsanguinated by cutting abdominal aorta. The trachea was catheterized with a polyethylene tube, and airways were washed with 50 ml of cold PBS by repeated instillation of 8–10 ml. Cells from rat bronchoalveolar lavage contained 96.6 ± 0.5% AMs according to May-Grünwald Giemsa and nonspecific esterase staining. The viability always exceeded 95%.

The Sprague Dawley rat AM cell line (NR8383) was grown and maintained in Ham’s F-12 medium (Life Technologies, Grand Island, NY) supplemented with 1 mM glutamine, 15% FBS, and antibiotics as previously described (31).

Alveolar cells from normal nonsmoker subjects were obtained by bronchoalveolar lavages. AMs were purified by adherence on plastic for 2 h (95.5 ± 0.5% AMs) and were treated with histamine as indicated in the text.

Stimulation of cytokine release

AMs were incubated in RPMI 1640 medium for 2 h to allow adherence, washed gently, and resuspended in fresh medium. Cells were treated with histamine or histamine agonists for 2 h (as specified in the test) before addition of LPS (1–5 ng/ml) for 4 h. At the end of the treatment, cell-free supernatants were collected, and TNF and IL-10 contents were measured using an immunoassay kit for rat TNF and rat IL-10 (BioSource). The number of copies of IL-10 in the PCR reaction was quantified using a quantitative PCR detection kit for rat IL-10 (CytoXpress) from BioSource according to the manufacturer’s protocols. This assay can detect as few as 10 copies of IL-10/sample.

RNA from normal human AMs was isolated, and RT-PCR was performed as described above. The primers used were 1) human β-actin: sense primer, 5'-GTC CTT AAT GTC ACG CAC GAT TTC C-3' and 3' primer, 5'-TTC ATG GCC TGG TAG ACA CA TGC-3'; and 2) rat IL-10: 5' primer, 5'-CAC TGC TAT GTT GCC TGC TC-3' and 3' primer, 5'-TTC ATG GCC TGG TAG ACA CA TGC-3'. The PCR products for β-actin and IL-10 were 607 and 286 bp, respectively. The temperature and time were 95°C for 45 s, 62°C for 45 s, and 72°C for 2 min for both β-actin and IL-10. After a preliminary test of PCR cycle numbers, 35 cycles were used. Products were run on a 2% agarose gel and stained with ethidium bromide. The number of copies of IL-10 in the PCR reaction was quantified using a quantitative PCR detection kit for rat IL-10 (CytoXpress) from BioSource according to the manufacturer’s protocols. This assay can detect as few as 10 copies of IL-10/sample.

Results

Inhibition of TNF release by histamine

We have demonstrated that at least a 2-h pretreatment was needed to modulate TNF production by mast cells (15). Thus, the modulation of TNF release by histamine was investigated with and without a pretreatment period and using different concentrations of LPS. AMs from rat bronchoalveolar lavage were treated with histamine (10⁻⁷ M) for 2 h before adding LPS (1 and 5 ng/ml) for 4 h, or histamine was added at the same time as LPS. Histamine significantly (p < 0.005) inhibited the release of TNF by LPS-stimulated AMs only when the cells were pretreated with histamine for 2 h (Fig. 1a). No significant inhibition was observed when histamine was added at the same time as LPS. The spontaneous release of TNF was not modulated by histamine treatment alone (27 ± 2 pg/10⁶ AMs without histamine and 26 ± 4 pg/10⁶ with histamine). Furthermore, histamine pretreatment (2 h) significantly inhibited (68 ± 6%; n = 4) TNF release from LPS-stimulated AMs for 18 h (data not shown). These data were also confirmed using human AMs from normal volunteers. Histamine pretreatment (10⁻⁵ M for 2 h) inhibited (32 ± 3%; n = 4) TNF release by LPS-stimulated human AMs for 4 h.

To minimize the use of animals, similar experiments were performed using the AM cell line, NR8383 (Fig. 1b). These AMs produce significantly more TNF in the presence of 1 and 5 ng/ml LPS (6.5 ± 1.3 and 22 ± 5 ng, respectively) compared with freshly isolated AMs (1.8 ± 0.1 and 5.3 ± 0.7 ng, respectively). Histamine pretreatment (2 h) inhibited TNF release from NR8383 when they were stimulated with a low concentration of LPS (1 ng/ml) but not with 5 ng/ml, which stimulated NR8383 to release 4.2 times more TNF than freshly isolated AMs. Thus, NR8383, treated with 1 ng/ml LPS, were used to further investigate the immunomodulatory effects of histamine.

Specificity of histamine receptors on AMs

To investigate which histamine receptors were involved in the inhibition of TNF release from AMs, cells were pretreated for 2 h with different concentrations of histamine or H₁ (betahistine), H₂ (dimaprit), or H₃ (R-α-methyl-histamine)-specific histamine receptor agonists before being stimulated with LPS (1 ng/ml) for 4 h (Fig. 2A). Dose-dependent inhibition of TNF release was observed with histamine and H₁ and H₃ receptor agonists. A significant inhibition was observed at 10⁻⁷ M histamine, but at 10⁻⁶ M for...
The modulation of TNF through the H3 receptor on AMs has not been previously reported. Thus, to further investigate the presence of H3 receptors on AMs, RNA from normal human AMs was isolated, and RT-PCR for H3 receptor was performed. The PCR product for H3 receptor was detectable in AMs from four human volunteers, suggesting the presence of this receptor on AMs (Fig. 2B). However, additional PCR product (390 bp) was consistently detected in different PCR reactions with mRNA from subjects 1, 3, and 4 (Fig. 2B). Thus, this product was sequenced and had 100% identity with a portion of the human histamine H3 receptor. Thus, both PCR products (390 and 588 bp) correspond to human H3 receptors.

Mechanism of histamine inhibition of TNF release from AMs
To investigate the mechanism of the inhibitory effect of histamine on NR8383, Abs to TGF-β (dilution 1/25), IL-10 (20 μg/ml), and PGE2 (dilution 1/25) as well as an inhibitor of NOS, l-NAME (1 mM), were added 5 min before histamine (10^{-5} M). AMs were treated with histamine for 2 h, followed by 4-h treatment with LPS (1 ng/ml). The stimulation of TNF release by LPS was not modified by the addition of Abs or l-NAME. Furthermore, Ab to TGF-β did not modulate the inhibitory effect of histamine (Fig. 3). However, anti-IL-10, anti-PGE2, and l-NAME abrogated the inhibition of TNF release by histamine, suggesting that the inhibitory effect of histamine may be mediated by multiple mechanisms.

Stimulation of IL-10 production by histamine
To verify whether histamine can stimulate the release of IL-10, AMs were treated with histamine for 2 h before the addition of LPS (1 or 5 ng/ml) for 4 h, or histamine and LPS were added concurrently. Cell-free supernatants were tested for IL-10 content.
Pretreatment of AMs with histamine significantly increased the release of IL-10 stimulated by LPS, whereas concurrent treatment with histamine and LPS did not significantly modify the release of IL-10 (Fig. 4). The modulation of IL-10 release by histamine was observed only when AMs were treated with a low concentration of LPS (1 ng/ml). Interestingly, the significant augmentation of IL-10 observed only when AMs were treated with a low concentration of histamine and LPS did not significantly modify the release of IL-10 by AMs pretreated with histamine and LPS (data not shown). After 20 h of treatment, histamine significantly increased the release of IL-10 at a level similar to LPS pretreatment (10^{-5} M) before LPS stimulation (1 ng/ml for 4 h). Histamine significantly (*, p < 0.05; **, p < 0.01) inhibited the release of TNF with and without anti-TGF-β. However, anti-IL-10, anti-PGE_2, and L-NAME abrogated the inhibitory effect of histamine. The results are the mean ± SEM of eight experiments performed in duplicate.

To determine whether IL-10 release observed in culture supernatants reflected an increase in steady state levels of mRNA for IL-10, RNA was isolated from sham-treated cells or from cells treated with and without histamine (2 h) and LPS (1 ng/ml) for 3 h.

To verify whether NO and PGE_2 were involved in the stimulation of IL-10 release by histamine, AMs were treated with L-NAME and anti-PGE_2 before the addition of histamine. Histamine alone significantly increased the release of IL-10 at a level similar to LPS and further increased LPS-stimulated IL-10 release (Fig. 6). However, in the presence of anti-PGE_2 or an inhibitor of NO, histamine did not modify IL-10 release from AMs, suggesting that these two mediators are involved in the immunomodulatory effects of histamine.

To determine whether histamine can stimulate the release of NO from AMs, cells were treated with histamine (10^{-5} M) for 48 h, and NO_2^- was measured in supernatants. The production of NO by AMs (0.9 ± 0.1 μM) was significantly increased by histamine treatment (1.4 ± 0.2 μM; p < 0.01; n = 7). However, histamine did not modify the release of NO when stimulated with LPS (41 ± 10 μM without histamine compared with 40 ± 9 μM with histamine).

Modulation of chemokine release by histamine

To investigate the modulatory effect of histamine on chemokine release, AMs were treated for 2 h with histamine (10^{-5} M) followed by LPS (1 ng/ml) for 4–20 h. Cell-free supernatants were tested for the presence of MIP-1α and RANTES. LPS stimulated the release of both chemokines (3-fold), but histamine treatment (20 h) did not modify their release (4.9 ± 0.1 ng of MIP-1α and 151.4 ± 23.5 pg of RANTES/10^6 AMs without histamine and RT-PCR analysis was performed. Unstimulated AMs expressed low amounts of IL-10 mRNA, but histamine treatment alone stimulated the expression of IL-10 mRNA (Fig. 5A). LPS treatment increased the expression of IL-10 mRNA, which was further increased by pretreatment with histamine (10^{-5} M). Quantification of mRNA for rat IL-10 using a quantitative PCR detection kit showed an increase of 1.5-fold with histamine treatment alone and of 1.2-fold in the presence of LPS (Fig. 5B).
HISTAMINE MODULATES CYTOKINE RELEASE VIA H₂ AND H₃ RECEPTORS

FIGURE 6. Mediators implicated in histamine stimulation of IL-10 release from AMs. Anti-PGE₂ (aPGE₂; dilution 1/25) or the NO inhibitor, L-NAME (1 mM), was added to the histamine (10⁻⁵ M) pretreatment (2 h) before LPS stimulation (1 ng/ml for 4 h). Histamine and LPS significantly (**, p < 0.01; †, p < 0.001) increased the release of IL-10. Furthermore, histamine significantly (**, p < 0.01) increased LPS-stimulated IL-10. However, anti-PGE₂ and L-NAME abrogated the stimulatory effect of histamine. The results are the mean ± SEM of five to seven experiments performed in duplicate.

5.0 ± 0.5 ng of MIP-1α and 157.4 ± 24.8 pg of RANTES/10⁶ AMs with 10⁻⁵ M histamine).

Discussion

Allergic inflammation is triggered by mast cell activation, which releases inflammatory mediators such as histamine (33). The role of histamine in the early- and late-phase reactions in asthma is well known (2). However, its immunomodulatory effects are not as well understood. We have previously demonstrated that histamine pretreatment inhibits mast cell TNF production (15). This inhibition was mediated through H₂ and H₃ receptors and was abrogated by anti-PGE₂. The present study shows that histamine pretreatment inhibits TNF release from AMs in a concentration-dependent manner (10⁻⁵-10⁻⁷ M). This inhibition required a minimum of 2-h pretreatment with histamine and was mediated through H₂ and H₃ receptors. Interestingly, histamine does not modulate cytokine production of human monocytes through H₄ receptors (34), demonstrating an additional difference between AM and monocytes.

This is the first evidence of the presence of H₃ receptors on AMs. The H₃ receptor agonist used in this study (R-α-methyl-histamine) has a relative activity related to histamine of 0.49, 1.02, and 1550 on H₂, H₃, and H₄ receptors, respectively (35). Thus, the high specificity of H₃ receptor agonist strongly indicates the presence of H₃ receptors on AMs. Furthermore, we demonstrated the presence of H₃ receptor mRNA in human AMs from four volunteers. Interestingly, AMs from one subject did not show any PCR product for H₃ receptor (data not shown), whereas three volunteers showed more than one PCR product (Fig. 2B). The sequencing of 588-bp (32) and 390-bp PCR products showed 100% homology with human histamine H₃ receptor. This may be explained in part by H₂ receptor heterogeneity (36). There is increasing evidence suggesting the presence of H₃ receptor subtypes (37, 38), which may correspond to the additional PCR product observed. Thus, our data suggest that histamine modulates AM functions through both H₂ and H₃ receptors. However, further investigations are needed to identify the subtype of H₃ receptor involved.

Inhibition of TNF release by histamine has been shown in different sources of macrophages, but not in human AMs (39). In that study there was no histamine pretreatment before LPS stimulation, and a higher concentration of LPS was used. Similar results were obtained in our laboratory in these conditions. When AM stimu-
cells to AMs, the concentration of histamine surrounding AMs can easily reach $10^{-7}$–$10^{-5}$ M, as studied here. Thus, histamine released by mast cells during allergic reactions can induce inflammation and stimulate AMs to express adhesion molecules (47) through $H_2$ receptors. At the same time, histamine may modulate the cytokine network toward a Th2 type response by stimulating IL-10 production. Although IL-10 shows anti-inflammatory properties, its role in the differentiation of Th2 cells (48) and in the inhibition of IFN-γ and IL-12 (49) may potentiate the Th2-type response seen in allergic asthma. Furthermore, histamine has been shown to inhibit IFN-γ production by Th0 and Th1 cells (12). Recently, Elenkov et al. (34) showed the suppression of IL-12 and the stimulation of IL-10 production by human Th1 cells (12). Recently, Elenkov et al. (34) showed the suppression of IL-12 and the stimulation of IL-10 production by human Th1 cells (12).

In their experiments, cells were pretreated for 10 min with histamine before adding LPS. They demonstrated that the effects of histamine were mediated through $H_2$ receptors, but not $H_1$ or $H_3$ receptors, and that monocytes were the source of IL-12 and IL-10. Our study shows that histamine stimulates IL-10 production from purified AMs at the protein and mRNA levels and that this effect was mediated through NO and PGE2 production. Furthermore, histamine modulates cytokine production by AMs through $H_2$ and $H_3$ receptors, in contrast to monocytes. The presence of $H_3$ receptors on AMs may explain in part the difference in histamine responsiveness between AMs and monocytes. Interestingly, an increase in IL-10 production by purified mast cells was also observed after histamine treatment (data not shown). Fig. 7 summarizes the immunomodulatory effects of histamine on the cytokine network.

Given the differences between monocytes and AMs in their responsiveness to various secretagogues (50, 51) and the local relevance of AMs during allergic reactions in asthma, it was essential to demonstrate the effects of histamine on these cells. Thus, our study provides additional information on the modulatory effects of histamine: induction of anti-inflammatory effects and potential contributions to the perpetuation of Th2-type response. These effects should be taken into consideration in other diseases using anti-histamine receptor treatment. Interestingly, a critical review demonstrated that patients with asthma treated for gastroesophageal reflux with $H_2$ receptor antagonist showed 69% improvement in their asthma symptoms and 62% reduction in their medication (52). These beneficial effects of $H_2$ antagonist may be partially explained by inhibiting histamine’s effects on the Th2 response. $H_2$ antagonists are also used in patients with colorectal cancer (53). This treatment may abrogate the inhibitory effects of histamine on TNF, IL-12, and IFN-γ production, which are particularly important for NK cell and macrophage functions. Thus, although several roles of $H_2$ and $H_3$ receptors have been characterized, further investigation is needed to understand the modulation of the immune system of the host treated with $H_2$ and $H_3$ receptor antagonists in different diseases.

References


