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

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References

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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 H_{2} and H_{3} receptor agonists, but not by H_{1} receptor agonist. Furthermore, we demonstrated the expression of H_{2} receptor mRNA in human AMs. Interestingly, treatment of AMs with anti-IL-10, anti-PGE_{2}, or a NO synthase inhibitor (N^{ω-}nitro- ω-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 1.5-fold with histamine treatment. Moreover, the stimulation of IL-10 release by histamine was abrogated by pretreatment with anti-PGE_{2} or the NO synthase inhibitor, N^{ω-}nitro-ω-arginine methyl ester. Thus, histamine increases the synthesis and release of IL-10 from AMs through PGE_{2} and NO production. These results suggest that histamine may play an important role in the modulation of the cytokine network.

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Histamine is released during allergic reactions and is 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 H_{2} and H_{3} receptors and was modulated by the production of NO and PGE_{2}. Furthermore, stimulation of IL-10 production by histamine was abrogated by the NO synthase inhibitor and anti-PGE_{2} Ab.
Materials and Methods

Animals
Oblivred male Sprague Dawley rats were obtained from Charles River Canada (St. Constant, Canada) and were maintained in an isolation room with filter-tipped 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, H<sub>2</sub> receptor agonist (betahistine), AT<sub>a</sub>-nitro-L-arginine methyl ester (l-NAME), and goat anti-mouse IL-10 Ab (IgG; bioactivity was assessed by IL-10 neutralization test in supernatants) were collected, and TNF and IL-10 contents were measured using an ELISA developed in our laboratory as previously described (30). Neutralizing dose (50%), 7.8 pg/ml. MIP-1α, RANTES were also measured using a kit (Beckman, Mississauga, Canada) 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 TCT AAT GTC ACC CAG TCT GCC TGG C-3′; and antisense primer, 5′-TGG GGC CCC ACC AGC CA-3′ (526 bp); and 2) human H<sub>2</sub> receptor: sense primer, 5′-CAG GTA CCA CGG CCT CCT CTC GC-3′; and antisense primer, 5′-GGG GCT TTT TTC GAG TGA GC-3′ (588 bp). The PCR product (390 bp) for H<sub>2</sub> receptor was sequenced at Laval University, whereas the PCR product (588 bp) has been previously cloned (32).

Statistical analysis
ANOVA combined with Fisher’s protected least significant difference test or Student’s t test for paired data were used to compare treatments. Differences were considered significant at p < 0.05.

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<sup>−7</sup> 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/10<sup>6</sup> AMs without histamine and 26 ± 4 pg/10<sup>6</sup> 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<sup>−5</sup> 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 H<sub>1</sub> (betahistine), H<sub>2</sub> (dimaprit), or H<sub>3</sub> (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 H<sub>2</sub> and H<sub>3</sub> receptor agonists. A significant inhibition was observed at 10<sup>−7</sup> M histamine, but at 10<sup>−6</sup> M for...
dimaprit and R-α-methyl-histamine. Betahistine did not significantly modulate the release of TNF from AMs. TNF release from human AMs was also inhibited by treatment with H₂ (22.63%) and H₃ (32.68%), but not with H₁ (5.61%), receptor agonists (n = 4). These data suggest that histamine inhibits TNF release from AMs through both H₂ and H₃ receptors.

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, Abs 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 release corresponded to the significant inhibition of TNF release with histamine and LPS (data not shown). After 20 h of treatment, histamine significantly (p < 0.05) increased the release of both chemokines (3-fold), but histamine treatment did not modify IL-10 release from AMs, suggesting that these two mediators are involved in the immunomodulatory effects of histamine.

The time-course analysis demonstrated that at least 4 h of treatment with LPS were necessary to increase the release of IL-10 by histamine and LPS (data not shown). After 20 h of treatment, histamine significantly (p < 0.05) increased the release of IL-10 (69 pg/10^6 AMs without histamine and 127 ± 26 pg/10^6 AMs with histamine; n = 6). However, histamine did not further increase the release of IL-10 stimulated with LPS (414 ± 80 pg/10^6 AMs without histamine and 418 ± 56 pg/10^6 AMs with histamine; n = 6).

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, 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).

**Mechanism of action of histamine**

To verify whether NO and PGE2 were involved in the stimulation of IL-10 release by histamine, AMs were treated with l-NAME and anti-PGE2 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-PGE2 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
Histamine) has a relative activity related to histamine of 0.49, 1.02, and 1.05 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. 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 H3 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 H3 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 stimulated by TNF (0.17 ± 0.08 ng/10⁶ cells) from human AMs, which was not inhibited by histamine, can modulate its production by stimulating the release of IL-10.

IL-10 and TGF-β are two anti-inflammatory cytokines that inhibit TNF release (25, 29). Our data showed that TGF-β was not implicated in the inhibitory effect of histamine on AMs. However, anti-IL-10 abrogated the inhibition caused by histamine. Furthermore, we showed that histamine stimulated the release of IL-10 from AMs. However, after 18 h of treatment with LPS, histamine did not significantly increase the release of IL-10, but still inhibited TNF production. It is possible that the presence of more IL-10 in the beginning of the incubation was sufficient to further inhibit TNF production and that, with time, IL-10 production by LPS-stimulated AMs overcomes the increase by histamine. Furthermore, we cannot exclude the possibility that other mechanisms may be involved in the inhibition of TNF production by histamine after 18 h. Interestingly, the release of IL-10 seemed to be mediated through PGE2 and NO production. Although histamine is known to stimulate the release of PGE2 and NO (40, 41), the roles of these mediators in IL-10 production are not well understood. Some evidence shows that PGE2 and NO stimulate IL-10 release (42–44). Our data suggest that a small amount of NO production stimulated by histamine may increase IL-10 release. NO synthesis from L-arginine can be catalyzed by different NO synthases, a constitutive form (cNOS) and an inducible form (iNOS). These two forms of NO can be differentiated using specific inhibitors, such as L-NAME for cNOS. Thus, given our results with L-NAME, it seems that histamine stimulates NO release through a cNOS pathway. Although AMs are well known to produce NO through iNOS, unstimulated AMs can also produce NO via cNOS (45). This may explain why histamine alone stimulated the release of NO, but did not potentiate its release when histamine-pretreated AMs were stimulated with LPS, which increases iNOS. Thus, histamine stimulates the release of PGE2 and NO, which, in turn, may stimulate the release of IL-10 that can inhibit TNF release from AMs.

In the airways, mast cells and AMs are in close proximity, and cooperation between these two cell types may be important in diseases such as asthma. An increased amount of histamine is found in bronchoalveolar lavage, reaching a concentration of 2.8 ng/ml after allergic reactions in asthmatic patients (46). Given the dilution of bronchoalveolar lavage fluid and the proximity of mast
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. 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 of the effects of histamine in asthma symptoms and 62% reduction in their medication usage. Histamine suppresses tumor necrosis factor (TNF) release by Th1 and Th2. This treatment may abrogate the inhibitory effects of histamine on the Th2-type response seen in allergic asthma. Furthermore, histamine treatment (data not shown). Fig. 7 summarizes the immunomodulatory effects of histamine on the cytokine network.

References