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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 H3 and H4 receptor agonists, but not by H2 receptor agonist. Furthermore, we demonstrated the expression of H3 receptor mRNA in human AMs. Interestingly, treatment of AMs with anti-IL-10, anti-PGE2, or a NO synthase inhibitor (N⁶-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 L-5-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, N⁶-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 H3 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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Abbreviations used in this paper: AM, alveolar macrophage; i-NAME, N⁶-nitro-L-arginine methyl ester; MIP-1α, macrophage inflammatory protein-1α; NOS, NO synthase; cNOS, constitutive form of NOS; iNOS, inducible form of NOS.
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, H₄ receptor agonist (betahistine), 6-Nitro-L-arginine methyl ester (L-NAME), and goat anti-mouse IL-10 Ab (IgG; bioactivity was assessed by IL-10 neutralization test in an immunoassay kit for rat TNF and rat IL-10 (BioSource, Cambridge, MA). Supernatants were collected, and TNF and IL-10 contents were measured. Histamine or histamine agonists for 2 h (as specified in the text).

AMs were isolated as previously described (30). Briefly, animals were anesthetized and exsanguinated by cutting abdominal aorta. The trachea was cannulated 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, Burlington, Canada) and PBS contained <0.05 endotoxin unit when tested using an E-Toxate kit (Sigma).

Cells

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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 text) 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, Cambridge, MA), with a sensitivity of <5 pg/ml. MIP-1α and RANTES were also measured using an ELISA developed in our laboratory as previously described (30).

Measurement of NO₂⁻ production.

AMs were treated with histamine for 48 h, and cell-free supernatants were assayed for NO₂⁻ using the Griess reaction. Briefly, 500 µl of supernatant was incubated with 250 µl of 1% sulfanilamide (Sigma) and 250 µl of 0.1% N-(1-naphthyl)enediamine dihydrochloride (Sigma) in 5% H₃PO₄ at room temperature for 5 min. The NO₂⁻ concentration, proportional to OD₅₄₀, was determined using a spectrophotometer (Beckman, Mississauga, Canada) with reference to a standard curve (NaNO₂) with a sensitivity of 0.05 µM.

RNA isolation and RT-PCR

AMs, NR8383, were treated with or without histamine for 2 h, followed by LPS (1 ng/ml) for 3 h, and total RNA was extracted using an RNaseasy minikit (Qiagen, Mississauga, Canada) according to the manufacturer’s protocols. Total RNA was quantified using RiboGreen RNA quantitation reagent (Molecular Probes, Eugene, OR), and results were read on a Fluoroskan Ascent FL (Labsystems, Franklin, MA). For cDNA synthesis, 1 µg of total RNA was reverse transcribed by Moloney murine leukemia virus RT (Life Technologies) using a Peltier Thermal Cycler 200 (M2 Research, Watertown, MA). PCR was performed using the Qiagen Taq DNA polymerase kit. The primers used were: 1) rat β-actin: sense primer, 5’-ATG CCA TCC TGC GTC TGG ACC TGG C-3’; and antisense primer, 5’-AGC ATT TGC GGT GCA CGA TGG C-3’; and 2) rat IL-10: 5’- primer, 5’-CAC TGC TAT GTT GCC TGC TC-3’; and 3’ primer, 5’-TTC ATG GCC TTG TAG ACA CC-3’. The PCR products were 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.

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 CCT CCA TTG CAT TGC CTA-3’ and antisense primer, 5’-GTG GGC GCC CCC AGG CAC CA-3’ (526 bp); and 2) human H₂ receptor: sense primer, 5’-CAG CTA CGA CCG CCT CCT TGT GTC-3’; and antisense primer, 5’-GGG GCC CTT CTT GAG TGA GC-3’ (588 bp). The PCR product (390 bp) for H₂ 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 (10⁻⁵ 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/10⁵ AMs without histamine and 26 ± 4 pg/10⁵ 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 H₂ and H₃ receptor agonists. A significant inhibition was observed at 10⁻⁷ M histamine, but at 10⁻⁶ 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 (22.6%±3%) and H₃ (32.6%±8%), but not with H₁ (5%±1%), 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. 2A). 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.

FIGURE 1. A, Inhibition of TNF release by histamine. AMs from bronchoalveolar lavage (BAL) of Sprague Dawley rats were pretreated for 2 h with histamine (10⁻⁵ M) before adding LPS (H 2h), were treated with histamine and LPS concurrently (H), or were sham treated for 2 h before adding LPS (C). Pretreatment with histamine significantly (‡, p < 0.005) inhibited the release of TNF from AMs. The results are the mean ± SEM of four to six experiments performed in duplicate. B, Inhibition of TNF release from the AM cell line NR8383. Cells were treated as described in A. Histamine significantly (*, p < 0.05) inhibited the release of TNF when NR8383 cells were pretreated for 2 h before being stimulated with LPS (1 ng/ml). The results are the mean ± SEM of six experiments performed in duplicate.

FIGURE 2. A, Concentration-dependent inhibition of TNF release by histamine and H₂ and H₃ histamine receptor agonists. NR8383 were treated for 2 h with 10⁻⁷-10⁻⁵ M histamine (H), betahistine (H₁ receptor agonist), dimaprit (H₂ receptor agonist), or R-α-methyl-histamine (H₃ receptor agonist) before being stimulated with LPS (1 ng/ml) for 4 h. Histamine and H₂ and H₃ receptor agonists significantly (*, p < 0.05; **, p < 0.01) inhibited the release of TNF, whereas H₁ receptor agonist did not significantly modify its release. The results are the mean ± SEM of six experiments performed in duplicate. B, Expression of H₃ receptor mRNA in human AMs. AMs were purified by adherence, and RT-PCR was performed for H₃ receptor and β-actin. Lanes 1–4, Volunteers; lane 5, negative control; lane 6, DNA ladder.
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 and without anti-TGF-β. However, anti-IL-10, anti-PGE₂, and L-NAME abrogated the inhibitory effect of histamine. The results are the mean ± SEM of eight experiments performed in duplicate.

FIGURE 3. Mediators implicated in histamine inhibition of TNF release from AMs. Anti-TGF-β (αTGFβ, dilution 1/25), anti-IL-10 (αIL-10; 20 μg/ml), anti-PGE₂ (αPGE₂; dilution 1/25), and NO inhibitor, (l-NAME; 1 mM) were added to the histamine (10⁻⁵ M) pretreatment (2 h) 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-PGE₂, and l-NAME abrogated the inhibitory effect of histamine. The results are the mean ± SEM of eight experiments performed in duplicate.

FIGURE 4. Stimulation of IL-10 release by histamine. AMs were pretreated for 2 h with histamine (10⁻⁵ M) before adding LPS (H 2h), were treated with histamine and LPS at the same time (H), or were sham treated for 2 h before adding LPS (C). Pretreatment with histamine (2 h) significantly (*, p < 0.01) increased the release of IL-10 from AMs when stimulated with LPS (1 ng/ml). The results are the mean ± SEM of six or seven experiments performed in duplicate.

FIGURE 5. A. Stimulation of IL-10 mRNA by histamine. AMs were pretreated, or not, with histamine (10⁻⁵ M, 2 h) followed by LPS stimulation (1 ng/ml), and RT-PCR was performed for IL-10 and β-actin (A). Lane 1, Sham-treated AMs; lane 2, AMs treated with histamine alone (5 h); lane 3, AMs treated with LPS alone; lane 4, AMs pretreated with histamine for 2 h and stimulated with LPS. A representative experiment of four different experiments is shown. The number of IL-10 copies measured using quantitative PCR is presented in B (mean of two experiments).

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.

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/10⁶ AMs without histamine and...
and 1550 on H1, H2, and H3 receptors, respectively (35). Thus, histamine has a relative activity related to histamine of 0.49, 1.02, and 157.4 ± 24.8 pg of RANTES/10⁶ AMs with 10⁻⁵ M histamine). 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.

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 To 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 H2 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 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 stimulation is too potent, a substantial amount of TNF is released that histamine does not significantly inhibit (Fig. 1b). Interestingly, human AMs release 17 times more TNF than monocytes (34 ng/10⁶ AMs and 2 ng/10⁶ monocytes) when stimulated with LPS (500 ng/ml), which may explain in part the unresponsiveness of human AMs to histamine inhibition under these conditions (39). In our study LPS (1 ng/ml) stimulated the release of a moderate amount of TNF (0.17 ± 0.08 ng/10⁶ cells) from human AMs, which was inhibited by histamine treatment. Thus, when TNF release is not maximal, 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 NOS 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 through H_{2} receptors. At the same time, histamine may modulate inflammation by reducing TNF release through H_{2} and H_{3} receptors and increasing IL-10 release. However, histamine may also modulate the cytokine network toward a Th2 type response by stimulating IL-12 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). Recently, Elenkov et al. (34) showed the suppression of IL-12 and the stimulation of IL-10 production by human Th1 cells (12).

Given the differences between monocytes and AMs in their responses 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. Our study provides additional information on the modulatory effects of histamine on the cytokine network.

References


