Histamine Induces Interleukin-8 Secretion by Endothelial Cells

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It has been shown that histamine induces early changes on endothelial cells (EC), such as a transient expression of P-selectin and secretion and/or surface expression of early mediators (e.g., prostacyclin [PGI2], platelet-activating factor [PAF], and leukotriene B4 [LTB4]). However, delayed effects of histamine on EC and particularly on cytokine production are undefined. In this study, the effect of histamine on interleukin (IL)-8 production by EC was evaluated using an enzyme-linked immunosorbent assay (ELISA) method and mRNA expression. The results showed that histamine increased the secretion and the mRNA expression of IL-8 by EC. Histamine-induced IL-8 production was (1) dose-dependent (at a dose \( \geq 10^{-4} \) mol/L), (2) potentiated by costimulation with tumor necrosis factor (TNF)-α, (3) inhibited by H1 or H2 histamine receptor antagonists, and (4) significantly increased 4 hours after the initial stimulation. These data suggest that histamine may be involved in the control of the late inflammatory reaction associated with allergic disorders through IL-8 secretion by EC.

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Mast cells initiate immediate hypersensitivity reactions; after an IgE-dependent stimulation, mast cells rapidly release preformed mediators (e.g., histamine and tumor necrosis factor [TNF]-α) and newly synthesized mediators (such as PGI2 and sulfidopeptide leukotrienes [LTs]), which play a major role in the early clinical manifestations of allergic disorders (such as smooth muscle contraction, mucus hypersecretion, and edema formation). Histamine, able to elicit almost all of the pathologic processes involved in immediate reaction, is one of the most important.

In contrast, the local inflammatory response (that occurs after repeated exposure to allergens or during the late-phase reaction) seems to be the result of a complex network of mediators and interactions between recruited leukocytes and tissue cells like fibroblasts, epithelial cells, and endothelial cells (EC). Among these structural cells, EC represent critical elements in the inflammatory process. They control leukocytes’ transendothelial migration through the secretion of chemotactic factors (LTs, prostacyclin [PGI2], interleukin-8 [IL-8], and platelet-activating factor [PAF]) and amplify leukocytes’ activation through proinflammatory cytokine production (IL-1, IL-6, and IL-8). Moreover, they express various cell-surface proteins (P-selectin, VCAM-1, E-selectin, ICAM-1, etc), known as cell-adhesion molecules (CAM), that allow leukocyte adhesion to endothelium, a prerequisite to their emigration.2

 Mast cells reside in close proximity to potential target cells of mediator action such as fibroblasts, nerves, epithelial cells, and vascular EC.3 Various in vitro effects of mast cell-derived mediators, such as histamine and TNF-α, have been described on EC. TNF-α enhances the expression of numerous CAM and the secretion of various cytokines; in contrast, histamine induces early changes on EC, such as an increase in endothelial permeability, a transient expression of P-selectin, and the secretion and/or surface expression of early mediators (e.g., PGI2, PAF, and LTB4).5,6

Later effects of histamine onEC, in particular on cytokine production, have not been evaluated. The aim of this study was to assess the action of histamine on IL-8 production by EC to evaluate its potential involvement in the allergic inflammatory reaction.

MATERIALS AND METHODS

EC culture. Human umbilical vein endothelial cells (HUVEC) were obtained as previously described.7,8 Briefly, HUVEC were collected after treatment of umbilical vein by 0.2% collagenase in 37°C prewarmed Hank’s Balanced Salt Solution (M.A. Bioproducts, Walkerville, MD) for 15 minutes and centrifuged for 10 minutes at 1,000g. The cell pellet was resuspended at concentration of 1.2 × 106 cells/mL in RPMI 1640 supplemented with 100 U/mL penicillin G, 10 μg/mL streptomycin, 20% heat-inactivated fetal calf serum (vol:vol; GIBCO, Sergy Pontoise, France), 100 μg/mL heparin, and 25 μg/mL EC growth supplement (Sigma Chemical, St Louis, MO). Then, HUVEC, cultured in 35-mm diameter tissue culture wells at 37°C in 5% CO2, were collected after trypsinization and cultured in 96-well plates precoated with 1% gelatin. All the experiments were performed with cells at confluence.

Histamine and TNF-α Activation of EC. EC were incubated at 37°C in 5% CO2 in the presence or in the absence of various concentrations of histamine from 10-7 to 10-5 mol/L in a final volume of 100 μL. Supernatants were harvested at different time points (from 1 to 24 hours) and stored at -80°C until IL-8 quantification. In some experiments, EC were stimulated by incubation with both histamine (used at concentrations from 10-7 to 10-5 mol/L) and human recombinant TNF-α (used at 50 U/mL; Genzyme, Boston, MA). To investigate the type of histamine receptors involved, HUVEC were also preincubated with H1, H2, and H3 receptor antagonists (dexamethasone, dexamethasone and thiopropylamide [a generous gift of Dr J.Y. Bonnefoy, respectively]) at different concentrations before activation with 10-4 mol/L histamine.

IL-8 immunological assay. IL-8 was quantified using a commercial kit (R & D systems, Minneapolis, MN) and results were expressed in nanograms per milliliter or as percent of increase as follows: ([H-H]/T) × 100, where H = histamine-induced IL-8 production, and T = IL-8 production by resting cells. The inhibitory effect of histamine antagonists was expressed as the percentage inhibition by reference to histamine-induced IL-8 production, according to the following formula: ([H-A]/H) × 100, where A = IL-8 pro-

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duced after stimulation with histamine and histamine receptor antagonist, and \( H = \) histamine-induced IL-8 production.

**Northern blot analysis of IL-8 mRNA.** After 4 hours of EC activation with \( 10^{-4} \) mol/L histamine or with recombinant human TNF-\( \alpha \) (200 U/mL), total cellular RNA was isolated by a guanidium isothiocyanate method with cesium chloride modification. Equal amounts of RNA were denatured at 50°C for 1 hour in glyoxal buffer and then fractionated by electrophoresis through 1.0% agarose gel. RNA transfer to nylon membrane was accomplished by capillary blotting for 18 hours. After RNA transfer, membranes were dried and baked at 80°C in vacuum. Prehybridization was performed at 43°C in buffer containing 50% formamide, 50 mmol/L phosphate buffer, 5 \( \times \) SSC (1 \( \times \) SSC = 0.15 mol/L NaCl + 0.015 mol/L sodium citrate), 2 mmol/L EDTA, 0.1% (vol:vol) sodium dodecyl sulfate, and 2.5X Denhardt’s solution (Sigma). \(^{32}P\)-labeled DNA probe of IL-8 was obtained by Klenow fragment transcription of a Sma I-Xba I fragment of IL-8 cDNA (subcloned in pGEM-plasmid). Hybridization was performed for 18 hours at 43°C in prehybridization buffer with \( 2 \times 10^6 \) cpm/mL of labeled probe. After washings at 43°C, the blot was dried and exposed to Kodak X Omat x-ray film (Kodak, Rochester, NY). The Northern blots were then hybridized with \( \beta \)-actin probe. In each condition, the intensity of the signal was assessed using a densitometer. The results were normalized to represent equivalent RNA loading in each lane based on the intensity of the actin bands, and the intensity of the IL-8 bands were then expressed as a percentage of the maximal intensity for each blot.

**Statistical analysis.** Results were expressed as the mean \( \pm \) SEM and statistical analysis was performed using nonparametric tests.

## RESULTS

**Histamine induces IL-8 secretion by human EC.** HUVEC were incubated with increasing amounts of histamine (from \( 10^{-7} \) to \( 10^{-3} \) mol/L) and IL-8 was measured in the supernatants. Figure 1 shows the amounts of IL-8 produced by HUVEC following 6 hours (Fig 1A) and 24 hours (Fig 1B) of incubation with histamine. It appeared that IL-8 secretion by histamine-stimulated HUVEC was dose-dependent and that the highest amount of IL-8 was induced by histamine used at \( 10^{-3} \) mol/L. After a 6-hour incubation, histamine concentrations \( \geq 10^{-3} \) mol/L induced a significant increase in IL-8 production, as shown in Fig 1A. Nevertheless, after a 24-hour incubation, \( 10^{-6} \) mol/L histamine also induced a significant increase in IL-8 production.

**Kinetics of IL-8 production.** Kinetic studies presented in Fig 2 were performed with a single dose of histamine (\( 10^{-4} \) mol/L). When the incubation time was increased from 4 to 24 hours, increasing amounts of IL-8 were quantified in the supernatants of the resting EC. Higher levels of IL-8 were detected with HUVEC stimulated with histamine; the difference became significant after a 4-hour stimulation. The maximal percentage of IL-8 increase was observed 24 hours after stimulation (\( >200\% \)).

**Northern blot analysis.** IL-8 neosynthesis was assessed by Northern blot analysis (Fig 3). When cells were cultured for 4 hours with histamine (\( 10^{-4} \) mol/L) (lane c) or with the positive control, TNF-\( \alpha \) 200 U/mL (lane b), there was an increase of 1.8 kb IL-8 mRNA expression compared with basal expression in resting EC (lane a). The IL-8 results were adjusted based on the intensity of the actin bands to represent equivalent mRNA loading in each lane. Whereas the intensity of the IL-8 band in the unstimulated cells was 1% of that observed in TNF-stimulated cells (maximal intensity, 100%), IL-8 mRNA expression in cells activated with histamine was 36% of the maximal intensity.

**H1 and H2 histamine receptor antagonists inhibit histamine-induced IL-8 secretion by HUVEC.** The effects of the pretreatment with increasing amounts of H1 or H2 receptor antagonists on histamine (\( 10^{-4} \) mol/L)-induced IL-8 production by HUVEC are shown in Fig 4. Fifty percent inhibition of histamine-induced IL-8 production was observed with H1 and H2 receptor antagonists concentrations of \( 3.10^{-5} \) mol/L and \( 5.10^{-7} \) mol/L, respectively. Thus, histamine-induced IL-8 secretion by HUVEC appeared to be mediated through both H1 and H2 receptors. Only 30% inhibition was observed with an H3 receptor antagonist at \( 10^{-4} \) mol/L concentration (data not shown).

**Histamine and TNF-\( \alpha \) synergize in the induction of IL-8 production by HUVEC.** Previous reports have shown that TNF-\( \alpha \) is released concomitantly with histamine by mast
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Fig 2. Time course of IL-8 secretion by EC. At the indicated time points, IL-8 produced by EC either unstimulated or activated with histamine at $10^{-4}$ mol/L was quantified. Results are expressed in ng/mL as the mean ± SEM of six experiments.

Histamine acts on target cells through distinct H1, H2, and H3 receptors, which are detected on the surface of EC. H1 and H2 receptors (vasodilatation-related symptoms) induce IL-8 production by EC, as soon as 4 hours after stimulation. This production was dose-dependent and potentialized by TNF-α.

Histamine is a major contributor to the development of allergic diseases; its effects in immediate reaction include smooth muscle contraction, increased vasopermeability, and vasodilatation. Only early effects of histamine have been evidenced on EC. In this study, histamine induced significant IL-8 production by EC, as soon as 4 hours after stimulation. This production was dose-dependent and potentialized by TNF-α.

Fig 3. Northern blot analysis of IL-8 mRNA expression. Expression of IL-8 mRNA by EC in response to $10^{-4}$ mol/L histamine (lane c) or 200 U/mL human recombinant TNF-α (lane b) by comparison to unstimulated endothelial cells (lane a). Total mRNA were collected after 4 hours of culture. β-Actin probe was used to control that equivalent amount of mRNA were transferred onto the membrane.

DISCUSSION

Histamine is a major contributor to the development of allergic diseases; its effects in immediate reaction include smooth muscle contraction, increased vasopermeability, and vasodilatation. Only early effects of histamine have been evidenced on EC. In this study, histamine induced significant IL-8 production by EC, as soon as 4 hours after stimulation. This production was dose-dependent and potentialized by TNF-α.

Histamine acts on target cells through distinct H1, H2, and H3 receptors, which are detected on the surface of EC. Most of the effects of histamine in allergic disorders are mediated through H1 (smooth muscle contraction and increased vasopermeability) or H1 and H2 receptors (vasodilatation-related symptoms). Histamine-induced IL-8 production by EC appeared to be mediated through an interaction of histamine with H1 and H2 receptors, as it was reduced by both H1 and H2 receptor antagonists, whereas H3 receptor antagonist was ineffective.

Fig 4. Inhibition of histamine-induced IL-8 production by H1 and H2 receptor antagonists. HUVEC were pretreated with increasing concentrations of H1 (from $10^{-7}$ to $10^{-2}$ mol/L) or H2 (from $10^{-5}$ to $10^{-6}$ mol/L) receptor antagonists 1 hour before the addition of histamine ($10^{-4}$ mol/L). Six hours after the incubation with histamine, IL-8 was measured in the supernatants. Results are expressed as the percentage of decrease in IL-8 production induced by costimulation with histamine and histamine receptor antagonist versus histamine.
although it was difficult to define precisely the histamine concentration in the target organ, concentrations of histamine from $10^{-6}$ to $10^{-4} \text{ mol/L}$ have been reported to be comparable to those measured in tissues after mast cell degranulation.\textsuperscript{16-18} For example, concentrations such as 100 mmol/L were detected in nasal lavages collected in patients with allergic rhinitis after allergen challenge\textsuperscript{18}; however, the histamine concentration was diluted 10- to 100-fold by the lavage procedure. On the other hand, we observed that histamine and TNF-\alpha, which are in vivo concomitantly released by mast cells, synergized in vitro in the induction of IL-8 production by EC. Moreover, this costimulation increases the endothelial sensitivity to histamine (from $10^{-5}$ to $10^{-3} \text{ mol/L}$). Thus, according to these different points, these in vitro effects of histamine probably reflect its in vivo activity at inflammatory sites.

The production of IL-8 can have several consequences on the tissue environment. IL-8 has in vitro an attractant activity for neutrophils, lymphocytes, basophils, and eosinophils.\textsuperscript{39-42} Moreover, IL-8 induces activation and transendothelial migration of neutrophils\textsuperscript{31,32,33}; opposite effects have been reported that might be explained by neutrophil desensitization to IL-8 under some experimental conditions.\textsuperscript{25,27} In experimental studies, IL-1\beta, TNF-\alpha, or lipopolysaccharide-stimulated EC have been demonstrated to produce IL-8, which is responsible for leukocyte transmigration.\textsuperscript{11,29} In addition, only eosinophils primed, for example, with granulocyte-macrophage colony-stimulating factor or IL-3 respond in vitro to IL-8\textsuperscript{30} and this priming is suggested in allergic diseases.

Furthermore, as histamine-induced IL-8 production by EC was observed from 4 to 24 hours after stimulation, it may participate in vivo in the late inflammatory reaction that is sometimes observed in allergic patients several hours after allergen challenge. In fact, the involvement of histamine in the modulation of the leukocyte infiltrate has been confirmed by data showing a significant increase in neutrophil, mastocyte, and lymphocyte numbers in bronchoalveolar lavage from healthy subjects obtained after histamine challenge.\textsuperscript{30}

In addition to its chemotactic activity for basophils, IL-8 has been demonstrated to modulate basophil histamine release. IL-8 at very low concentration ($10^{-9} \text{ mol/L}$) could inhibit histamine release induced by cytokine- and histamine-releasing factor.\textsuperscript{19} In contrast, IL-8 could induce histamine release, either at high concentrations ($10^{-6} \text{ mol/L}$)\textsuperscript{2} or at lower levels on IL-3–primed basophils ($10^{-7}$ and $10^{-4} \text{ mol/L}$).\textsuperscript{33} In allergic patients, the IL-3 priming of basophils can be hypothesized. The IgE-dependent release of mast cell mediators observed immediately after allergen challenge is sometimes followed, in allergic patients developing a late-phase reaction several hours after challenge, by a second pick of histamine; different observations have suggested that basophils were the effector cell type in this late reaction.\textsuperscript{19,34}

Thus, if the local concentration of IL-8 that occurs several hours after histamine stimulation reaches $10^{-4} \text{ mol/L}$, it may also trigger the activation of basophils involved in the pathogenesis of the late inflammatory reaction. However, other studies are needed to confirm the involvement of histamine-induced IL-8 production in the development of the chronic inflammatory reaction associated with allergic asthama.

In conclusion, these data add arguments to recent studies suggesting that histamine should be considered as an integral component of immune and inflammatory responses.\textsuperscript{39} Indeed, this early mediator is not only involved in the early changes associated with allergic diseases, but also in the regulation of the production of proinflammatory cytokines involved in the late-phase reaction. Parallelly, these data underline the central and complex role of EC that respond with various changes (in CAM expression and secretion products) at different time points (concomitant with the early- or late-inflammatory-phase reaction) to histamine stimulation.

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