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Histamine induces IL-6 production by human endothelial cells

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SUMMARY

Histamine is one of the major mediators implicated in the physiopathology of allergy. On vascular endothelium, histamine mainly induces early effects: an increase in vasopermeability leading to oedema, a release of lipid mediators and a transient expression of P-selectin. The aim of this study was to evaluate the effects of histamine on adhesion molecule expression and IL-6 production by human endothelial cells. Histamine did not modulate the expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin, but induced a transient expression of P-selectin as previously reported. In addition, histamine increased in a dose- (from 10^{-5} to 10^{-3} M) and time- (from 4 h to 24 h) dependent fashion the IL-6 synthesis by endothelial cells. Tumour necrosis factor-alpha (TNF-α)-induced IL-6 production was also potentiated in a dose-dependent manner by histamine, without modification of the time course of IL-6 secretion. Moreover, this increase of IL-6 production induced by histamine was inhibited in a dose-dependent manner by H1 and H2 histamine receptor antagonists (50% inhibition of IL-6 production at 5×10^{-4} M and 4×10^{-5} M, respectively). So, histamine induces, besides already well known effects, a late stimulation of endothelial cells, i.e. the production of IL-6.

Keywords histamine IL-6 endothelial cells adhesion molecule

INTRODUCTION

Mast cells are the main effector cells of the early allergic response. The reticulation of the high-affinity IgE receptor (by cross-linking of IgE by allergens) induces the degranulation of mast cells and the release of several mediators, including histamine [1]. Histamine is a potent vasoactive and bronchoconstrictor mediator [2,3]. Indeed, pretreatment with histamine receptor antagonists prevents the development of the early response [4]. After allergen challenge, a peak of histamine release in serum is detected early at 15 min, and in some cases later on, between 3 and 8 h. Histamine plays an important role in the pathological features accompanying allergic rhinitis or asthma (particularly hyperreactivity, vasodilatation and smooth muscle contraction) [5–7]. Although histamine is unable to modulate directly the inflammatory reaction accompanying the allergic reaction, it induces the synthesis of other mediators potentially implicated in this process (such as neutrophil and eosinophil chemotactic factors, leucotrienes, prostaglandins or platelet-activating factor (PAF)) [8]. Moreover, mast cells produce several other mediators after activation, which may potentiate their respective effects, such as tumour necrosis factor-alpha (TNF-α) [9,10] (a potent pro-inflammatory cytokine, produced by mast cells and alveolar macrophages [11]).

The endothelial cell is one of the first targets triggered by histamine, since an increase of vasopermeability is detected during the immediate reaction following allergen challenge or after histamine bronchial instillation [7]. Endothelial cells are now recognized as important cells implicated in the allergic response [12]. They participate in early and late events of the allergic reaction: (i) during the early response through an increase in vasopermeability (oedema formation) and the production of vasoactive mediators [13,14]; and (ii) during the late phase reaction through adhesion molecule expression [12] (leading to the adhesion and further transvascular migration of leucocytes) and the production of several proinflammatory mediators (such as IL-1, IL-6, IL-8 or granulocyte-macrophage colony-stimulating factor (GM-CSF)) [15–17].

Previous reports showed that histamine activates endothelial cells, inducing (i) the production of PGI2 and PAF [13,18], (ii) the transient expression of P-selectin [18], and (iii) an increase in vasopermeability [7]. Because histamine is rapidly released after the activation of mast cells by allergens, the known effects of this mediator on endothelial cells have been studied during early events. The aim of this study was to analyse the histamine effects on endothelial cells which could participate in the development of the late phase reaction,

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namely, the modulation of adhesion molecule (intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin) expression and the production of IL-6.

MATERIALS AND METHODS

Endothelial cell culture

Endothelial cells were derived from human umbilical vein (HUVEC), according to the method previously described [19]. Briefly, HUVEC were collected after treatment of umbilical vein by 0·2% collagenase in 37°C prewarmed Hanks' balanced salt solution (HBSS) for 15 min (M.A. Bioproducts, Walkersville, MD) and pelleted by centrifugation (1000 *g*, 10 min). They were resuspended at $1{\cdot}2 \times 10^5$ cells/ml in culture medium consisting of RPMI 1640 supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 10 mg/ml streptomycin, 20% heat-inactivated fetal calf serum (FCS; GIBCO BRL, Courbevoie, France), 100 μg/ml heparin, and 25 mg/ml endothelial cell growth supplement (Sigma Chemical Co, St Louis, MO). HUVEC were cultured in 35 mm diameter tissue culture wells at 37°C in 5% CO_2. They were collected after trypsinization and then cultured in gelatin-coated 96-well flat-bottomed culture plates until confluency. The purity of endothelial cell culture was assessed by the detection of the factor VIII-related antigen by a fluorescein-conjugated antibody against this antigen: more than 98% cells were positive. Only cells of the third and the fourth passage were used. Experiments were performed using endothelial cells derived from eight different umbilical veins.

Histamine and TNF-α activation of endothelial cells

Histamine (Sigma) was diluted in RPMI 1640 medium as a concentrated solution, stored at −80°C and used within 1 month.

At confluency, culture medium was replaced before performing all experiments. Endothelial cells were incubated in culture medium at 37°C and 5% CO_2 with different concentrations of histamine (from 10^{-7} to 10^{-3} M).

Endothelial cell supernatants were collected at different time points (from 2 h to 24 h), centrifuged, aliquoted and stored at −80°C until IL-6 measurement.

Endothelial cells were also concomitantly stimulated with different concentrations of histamine (from 10^{-7} to 10^{-3} M) and a single suboptimal dose of human recombinant TNF-α (50 U/ml) (Genzyme, Boston, MA). For these experiments we calculated the ratio of potentiation as the net value of IL-6 produced in the presence of TNF-α plus histamine divided by the sum of the net levels of IL-6 produced with TNF-α and histamine separately (after subtraction of the baseline level).

In order to investigate mechanisms by which endothelial cells were activated under histamine stimulation, cells were preincubated with H1 receptor antagonist (dexchlorpheniramine maleate) (Polaramine; Schering-Plough, Levallois-Perret, France) (from 10^{-2} to 10^{-10} M) or H2 receptor antagonist (dichlorisoproterenol) (Atarax; UCB Pharma, Nanterre, France) (from 10^{-1} to 10^{-10} M), before activation with 10^{-4} M histamine. The inhibition of histamine-induced IL-6 production by histamine receptor antagonists was expressed as a percentage of inhibition of IL-6 production as follows: $(Hi - Ho)/(Ha - Ho) \times 100$, where Ho was the IL-6 production by resting endothelial cells, Hi was IL-6 production by histamine-stimulated endothelial cells pretreated with histamine antagonist receptor, and Ha was the IL-6 level produced by 10^{-4} M histamine-stimulated endothelial cells.

IL-6 biological assay

IL-6 production was quantified in the HUVEC culture supernatant according to the technique described by Van Snick *et al.* [20] with some modifications. Briefly, the hybridoma cells 7TD1 (a generous gift of Dr J. Van Snick, Institut Ludwig, Brussels, Belgium) were incubated with serial dilutions of endothelial cell supernatants in microtitre plates. After 4 days of incubation, the number of cells was evaluated by a colourimetric assay. A 4 mg/ml solution of 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) (Sigma) was added at 10%. After 4 h of incubation, the supernatants were discarded and 0·2 ml of 0·04 N HCl-isopropanol solution was added to each well to solubilize the reduced MTT precipitate. After homogenization, the optical density was read at 570 nm with a reference at 650 nm. All analysis was performed in duplicate, and concentrations were calculated by a probit analysis in comparison with a standard of rhIL-6 (Boehringer Mannheim, Mannheim, Germany). The specificity of the test was controlled by the inhibition of cell proliferation after addition of a neutralizing anti-IL-6 rabbit antibody (Genzyme). In all cases, specific antibody addition to endothelial cell supernatants induced an inhibition greater than 90%. Results were expressed in units of IL-6 activity per ml.

Modulation of cellular adhesion molecule expression by histamine

The modulation of cellular adhesion molecule expression by histamine was analysed using an endothelial cell ELISA.

Endothelial cells were cultured in gelatin-coated 96-well flat-bottomed culture plates until confluency. Cells were incubated with histamine over 15 min for the analysis of P-selectin expression or over 8 h for ICAM-1, E-selectin and VCAM-1 expression. After incubation at 37°C and 5% CO_2, cells were washed with 37°C prewarmed PBS and then fixed in 0·05% glutaraldehyde in PBS for 10 min at 4°C. After four washings in 0·1 M PBS pH 7·4 containing 5 mM EDTA and 5% (w/v) bovine serum albumin (BSA) (washing-assay buffer), plates were incubated for 1 h in this same buffer. Positive controls for ICAM-1, E-selectin and VCAM-1 expression included optimal concentrations of hrTNF-α (200 U/ml).

Adhesion molecule expression was measured as follows. Glutaraldehyde-fixed endothelial cells were incubated for 1 h with 100 μl/well of anti-VCAM-1 MoAb (clone BBIG-V1), E-selectin (clone BBIG-E6) (British Biotechnology Ltd, Oxford, UK), ICAM-1 (clone 84H10) (Immunotech, Marseille-Luminy, France), P-selectin (clone AC1.2*) (Becton Dickinson, San Jose, CA) or control IgG1 MoAb (Immunotech) at the experimentally defined dose of 2 μg/ml. Cells were washed four times in washing-assay buffer and then incubated for 1 h with 100 μl per well of 1:5000 (v/v) diluted peroxidase-conjugated anti-mouse IgG rabbit antiserum (Pasteur Production, Courbevoie, France) in washing-assay buffer. After four washings, bound IgG antibodies were detected by addition of 100 μl of detection buffer (0·1 M citrate buffer pH 5·0 containing *o*-phenylenediamine and H_2O_2). Colour development reaction was stopped by addition of 100 μl/well of 4 N HCl. Optical density (OD) was

read at 492 nm, and results were expressed as OD values. All analyses were performed in triplicate.

Northern blot analysis of IL-6 mRNA

After 4 h of EC activation with 10^{-4} M histamine or with hrTNF-α (200 U/ml), total cellular RNA was isolated by a guanidium isothiocyanate method and purified by caesium chloride modification. Equal amounts of RNA were denatured at 50°C for 1 h 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 h.

After RNA transfer, membranes were dried and baked at 80°C under vacuum. Prehybridization was performed at 50°C in buffer containing 50% formamide, 50 mM phosphate buffer, 5 × SSC, 2 mM EDTA, 0·1% (v/v) SDS and 2·5× Denhardt's solution (Sigma). ^{32}P-labelled IL-6 DNA probe was obtained by Klenow fragment transcription of an EcoRI-XbaI fragment of IL-6 cDNA (cloned in pBluescript plasmid). Hybridization was performed for 18 h at 50°C in prehybridization buffer with 2×10^6 ct/min per ml of labelled probe. After washings at 50°C, the blot was then dried and exposed to Kodak X Omat x-ray film (Kodak, Rochester, NY).

Statistical analysis

Statistical analysis was performed using Wilcoxon's or the Mann–Witney *U*-test.

RESULTS

Histamine did not modulate ICAM-1, VCAM-1 and E-selectin expression

The expression of P-selectin was enhanced after stimulation of HUVEC for 15 min with histamine as previously reported (Table 1). In contrast, ICAM-1, VCAM-1 and E-selectin expression was not modulated after 8 h incubation of HUVEC with histamine (Table 1); positive controls included optimal concentration of hrTNF-α (200 U/ml). Moreover, the enhanced expression of these adhesion molecules induced by hrTNF-α was not modulated by histamine (data not shown). The OD value obtained with the control MoAb was 0·08 ± 0·02 OD.

Table 1. Modulation of cellular adhesion molecule expression onto endothelial cells after stimulation with histamine or tumour necrosis factor-alpha (TNF-α)

	Medium	Histamine (10^{-4} M)	hrTNF-α (200 U/ml)
ICAM-1	0·225 ± 0·050	0·250 ± 0·070	0·450 ± 0·050*
VCAM-1	0·120 ± 0·012	0·060 ± 0·050	0·252 ± 0·060*
E-selectin	0·08 ± 0·015	0·084 ± 0·060	0·460 ± 0·080*
P-selectin	0·106 ± 0·010	0·325 ± 0·015*	0·125 ± 0·040

The expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin was evaluated after 8 h, and P-selectin after 15 min of stimulation. Results are expressed in optical density (OD) values (mean ± s.e.m.) ($n = 6$). The OD value with the control MoAb was 0·08 ± 0·01.

* $P < 0·05$ compared with the result obtained in medium alone.

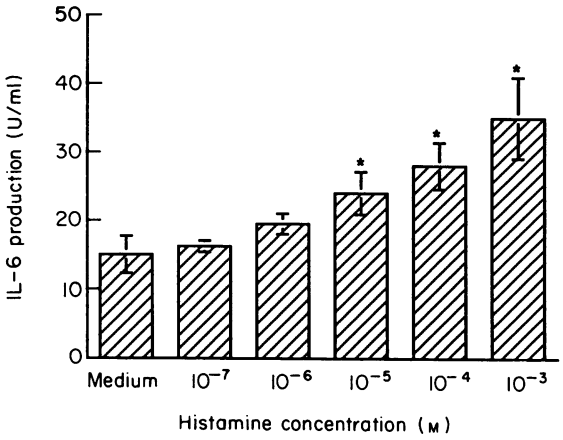

Fig. 1. Dose-dependent increase of IL-6 production by human umbilical vein endothelial cells (HUVEC) cultured for 6 h in response to histamine stimulation. Results are expressed in U/ml, mean ± s.e.m. ($n = 8$). * $P < 0·05$ compared with the resting cells.

Histamine-induced IL-6 production by human endothelial cells

IL-6 production by endothelial cells stimulated with histamine was quantified at 6 h ($n = 8$). This production was increased by histamine in a dose-dependent manner from 10^{-5} to 10^{-3} M ($P < 0.05$), and was maximal at 10^{-3} M histamine (35 ± 5·9 U/ml compared with 15 ± 2·7 U/ml produced by resting endothelial cells; mean ± s.e.m.) (Fig. 1).

The time course of IL-6 production was studied between 2 and 24 h of incubation using a single dose of histamine: 10^{-4} M (Fig. 2). An increase of IL-6 production was not detectable before 4 h (14·5 ± 1 compared with 10·5 ± 0·25 U/ml by resting HUVEC, mean ± s.e.m., $P < 0·05$). This production significantly increased from 6 h (28 ± 3·4 U/ml) to 24 h (69 ± 6 U/ml) when compared with IL-6 production by unstimulated cells at

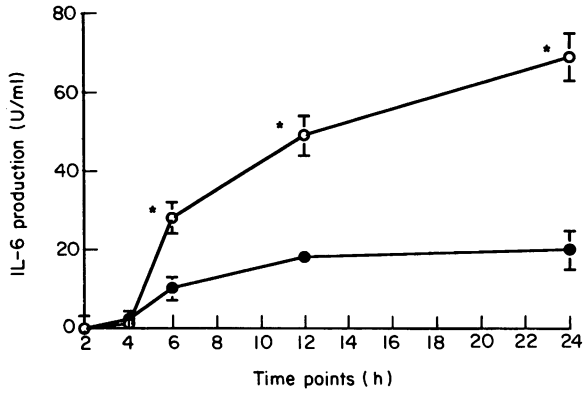

Fig. 2. Kinetics of IL-6 production by human umbilical vein endothelial cells (HUVEC) in response to 10^{-4} M histamine (○) compared with IL-6 production by resting cells (●). Results are expressed in U/ml, mean ± s.e.m. ($n = 8$). * $P < 0·05$ compared with the resting cells.

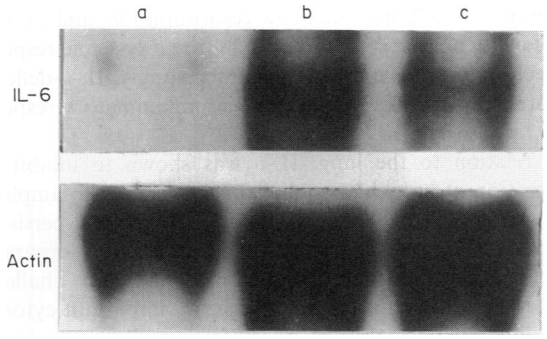

Fig. 3. Northern blot analysis of IL-6 mRNA expression by human umbilical vein endothelial cells (HUVEC) in response to 10^{-4} M histamine (lane b) or 200 U/ml human recombinant tumour necrosis factor-alpha (hrTNF-α) (lane c) in comparison with unstimulated endothelial cells (lane a). Total RNA was collected after 4 h of culture. This is a representative experiment out of three.

the same time points (15 ± 2·7 and 20 ± 4·5 U/ml, respectively; $P < 0.05$).

IL-6 mRNA expression was assessed after histamine stimulation by Northern blot analysis (Fig. 3). An increase in IL-6 mRNA expression was observed 4 h after histamine stimulation (lane c) compared with the basal level of IL-6 mRNA expression in resting endothelial cells (lane a). The positive control consisted of 200 U/ml hrTNF-α-activated endothelial cells (lane b). This experiment was repeated three times with similar results.

H1 and H2 receptor antagonists inhibited histamine-induced IL-6 production

Incubation of endothelial cells with H1 and H2 histamine receptor antagonists before stimulation with a single dose of 10^{-4} M histamine induced a dose-dependent inhibition of IL-6 production evaluated at 6 h (Fig. 4). Maximal inhibition of IL-6 production when compared with that obtained following histamine stimulation was 86 ± 2% ($P < 0.05$) (Fig. 4a) with 10^{-2} M H1 receptor antagonist, and 58 ± 14% ($P < 0.05$) with

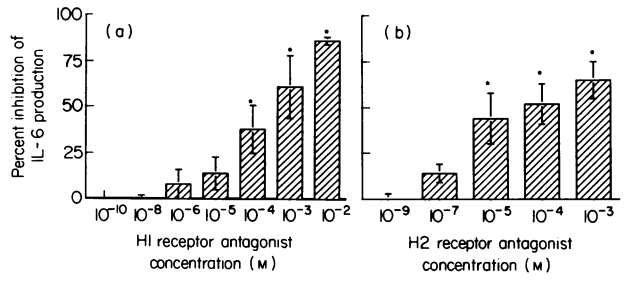

Fig. 4. (a) The augmentation of IL-6 production was inhibited by H1 receptor antagonists after histamine activation. Results are expressed as the inhibition percentage of IL-6 production at 6 h. The positive control was the IL-6 level obtained with 10^{-4} M histamine; mean ± s.e.m. ($n = 6$). * $P < 0.05$ compared with the positive control. (b) The augmentation of IL-6 production was inhibited by H2 receptor antagonists after histamine activation. Results are expressed as the inhibition percentage of IL-6 production at 6 h. The positive control was the IL-6 level obtained with 10^{-4} M histamine; mean ± s.e.m. ($n = 6$). * $P < 0.05$ compared with the positive control.

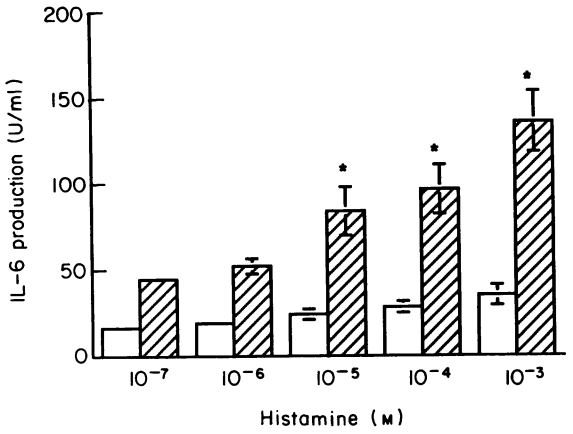

Fig. 5. Potentiation by histamine of tumour necrosis factor-alpha (TNF-α)-induced IL-6 production (▨) compared with the augmentation of IL-6 production induced by histamine alone (□). IL-6 was quantified in supernatants from 6 h culture. Results are expressed in U/ml, mean ± s.e.m. ($n = 8$). * $P < 0.05$ compared with the sum of the results obtained with both activators alone.

10^{-3} M H2 receptor antagonist (Fig. 4b). Higher levels of H2 receptor antagonist were cytotoxic for endothelial cells.

The concentrations of histamine receptor antagonists inducing an inhibition of IL-6 production of 50% (IC_{50}) were 5×10^{-4} M and 4×10^{-5} M with H1 and H2 receptor antagonists, respectively.

Histamine and TNF-α synergized to induce IL-6 production

Human recombinant TNF-α at a suboptimal dose of 50 U/ml induced a significant increase in IL-6 production (48·5 ± 4 U/ml) compared with IL-6 production by resting endothelial cells (15·5 ± 2·6 U/ml) (mean ± s.e.m.; $P < 0.05$).

The 10^{-5} M histamine dose was the lowest concentration which significantly increased hrTNF-α-induced IL-6 production by endothelial cells (84 ± 14·2 compared with 24 ± 3·1 U/ml for histamine alone) (ratio of potentiation = 1·61; $P < 0.05$ compared with the sum of the net value of IL-6 obtained with TNF-α and histamine separately). Synergistic effects were also observed at 10^{-4} M (96·6 ± 16·4 compared with 28 ± 3·4 U/ml for histamine alone) and were maximal at 10^{-3} M (136 ± 7·7 compared with 35 ± 5·9 U/ml for histamine alone), inducing an increase in IL-6 production that was 1·78- and 2·27-fold greater than the additive effects of each mediator alone, respectively ($P < 0.05$ compared with the sum of the net value of IL-6 obtained with TNF-α and histamine separately) (Fig. 5).

DISCUSSION

In this study, histamine was shown to induce a significant increase of IL-6 synthesis by human endothelial cells in a dose- (from 10^{-5} to 10^{-3} M) and time- (from 4 h to 24 h) dependent manner which was associated with an increase in specific mRNA expression. This effect was inhibited by specific H1 (IC_{50} 5×10^{-4} M) and H2 (IC_{50} 4×10^{-5} M) receptor antagonists. TNF-α-induced IL-6 production was also potentiated by histamine.

The expression of the endothelial cell adhesion molecules ICAM-1, VCAM-1 and E-selectin was not modulated by

histamine, except for P-selectin expression which showed a transient increase, as previously reported [18]. The cellular adhesion molecule P-selectin is essentially implicated in the adhesion of neutrophils to endothelial cells [21]. Infiltrative neutrophils are more abundant in nasal epithelium after nasal challenge with histamine or allergen [22–24], which may be related in part to an increase of P-selectin expression. In contrast, neutrophils did not infiltrate bronchial tissue in patients with allergic asthma [25–27], suggesting the complexity and diversity of the mechanisms controlling the infiltrate. The absence of modulation of ICAM-1, VCAM-1 and E-selectin expression onto endothelial cells after stimulation with histamine corroborates the complexity of pathways implicated in the activation of endothelial cells during the development of the inflammatory late phase reaction.

Histamine-induced increase of IL-6 production by endothelial cells was dependent on the concentration and duration of incubation. Previous reports have shown that histamine is also able to induce production of cytokines by monocytes [28] at concentrations similar to those used in the present study. The concentrations of histamine measured in bronchoalveolar lavage (BAL) obtained from allergic asthmatics at baseline are around 10^{-8} M to 10^{-7} M [29], whereas in allergic rhinitis concentrations such as 100 nM were detected in nasal lavages collected after an allergen challenge [23]. However, the nasal secretions were diluted 10–100-fold by instillation of physiological saline. Since histamine after its release by mast cells rapidly diffuses into the surrounding tissues, the amounts of histamine able to stimulate IL-6 production by endothelial cells are consistent with those found locally in inflammatory sites. Moreover, IL-6 production induced by TNF-α was potentiated by histamine. Indeed, both histamine and TNF-α are preformed in mast cell granules and are rapidly produced after IgE-dependent stimulation [1,9]. TNF-α is a potent endothelial cell activator, as shown by the induction of cytokine production and the enhancement of cellular adhesion molecule expression [30]. Both TNF-α and histamine may act independently or in combination to activate endothelial cells, and this potentiation could be a potent stimulatory pathway in the development of the inflammatory reaction.

The augmentation of IL-6 production is effective at 4 h, and this effect increases until 24 h, consecutively to cytokine neosynthesis (as demonstrated by the neotranscription of mRNA encoding for IL-6). During the allergic reaction, the late phase reaction, characterized by the transvascular migration of inflammatory cells, appears around 6 h and persists for at least 24 h [31]. Similar kinetics of IL-6 production by endothelial cells has been also reported after activation with other mediators (IL-1, TNF-α, interferon-gamma (IFNγ), PAF or IL-4) [32,33]. Thus, the augmentation of IL-6 production by endothelial cells is concomitant with the initiation of the late phase reaction and with the inflammatory reaction persisting during the following days.

The effects of histamine on its target cells are mediated by its binding to specific receptors (H1, H2 and H3) which are present on endothelial cells [34]. Our finding that IL-6 production was inhibited by both H1 and H2 receptor antagonists suggests that endothelial cells may be activated by two histamine pathways.

Several recent reports have proposed that IL-6 be considered as a major mediator of inflammatory reactions and immune response [35,36]. IL-6 induces B cell proliferation and differentiation [37], bone marrow resorption [38] and production of acute phase proteins, which induce a systemic response to infection and tissue injury [39]. Interestingly, IL-6-deficient mice are unable to develop an efficient inflammatory response [36].

In relation to the lung, IL-6 was shown to inhibit the proliferation of bronchial epithelial cells, leading to amplified desquamation during bronchial asthma, and to persistent bronchial hyperreactivity [40]. IL-6 production occurs in different allergic diseases following an allergen challenge [22,40,41], and therefore appears to be an important cytokine in the inflammatory process acompanying allergic reactions. Moreover, by inducing IL-6 production by endothelial cells, histamine could serve as an agonist of the acute and of the late phase of the allergic response.

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REFERENCES

1 Ishizaka T. Mechanisms of IgE-mediated hypersensitivity. In: Middleton E Jr, Reed CE, Ellis EF, Adkinson NF Jr, Yninger JW, eds. St Louis: Mosby, 1988.
2 Hill SJ. Multiple histamine receptors: properties and functional characteristics. Biochem Soc Trans 1992; **20**:122–5.
3 Barnes PJ. Histamine receptors in the lung. Agents Actions 1991; **33**:103–22.
4 Rafferty P, Holgate ST. Histamine and its antagonists in asthma. J Allergy Clin Immunol 1989; **84**:144–51.
5 Falus A, Meterey K. Histamine: an early messenger in inflammatory and immune reactions. Immunol Today 1990; **13**:154–6.
6 Naclerio RM. The role of histamine in allergic rhinitis. J Allergy Clin Immunol 1990; **86**:628–32.
7 White MV. The role of histamine in allergic disease. J Allergy Clin Immunol 1990; **86**:599–605.
8 Noah TL, Paradiso AM, Madden MC, McKinnon KP, Devlin RB. The response of a human bronchial epithelial cell line to histamine: intracellular calcium changes and extracellular release of inflammatory mediators. Am J Respir Cell Mol Biol 1991; **5**:484–92.
9 Plaut M, Pierce JH, Watson CJ, Hanley-Hyde J, Nordan RP, Paul WE. Mast cell lines produce lymphokines in response to cross-linkage of FcεRI or to calcium ionophores. Nature 1989; **339**:64–67.
10 Galli SJ, Gordon JR, Wershil BK. Cytokine production by mast cells and basophils. Curr Opin Immunol 1992; **31**:865–73.
11 Gosset P, Tsicopoulos A, Wallaert B, Joseph M, Capron A, Tonnel AB. Tumor necrosis factor alpha and interleukin-6 production by human mononuclear phagocytes from allergic asthmatics after IgE-dependent stimulation. Am Rev Respir Dis 1992; **146**:768–74.
12 Hogg JC. The pathology of asthma. Clin Chest Med 1984; **5**:567–78.
13 Majno G, Palade GE. Studies on inflammation 1. Effect of histamine and serotonin on vascular permeability; an electron, microscopic study. J Biophys Biochem Cytol 1961; **11**:571–605.
14 Baezinger NL, Force LE, Bechner PR. Histamine stimulates prostacyclin synthesis in cultured human umbilical vein endothelial cells. Biochem Biophys Res Commun 1980; **92**:1435–40.
15 Locksley RM, Heinzel FP, Shepard HM, Agosti J, Eessalu TE, Aggarwal BB, Harlan JM. Tumor necrosis factors differ in their capacities to generate interleukin-1 release from human endothelial cells. J Immunol 1987; **139**:1891–5.
16 Jirik FR, Podor TJ, Hirano T, Kishimoto T, Lostukoff DJ, Carson DA, Lotz M. Bacterial lipopolysaccharide and inflammatory

mediators augment IL-6 secretion by human endothelial cells. J Immunol 1989; **142**:144–7.

17 Strieter RM, Kunkel SL, Showell HJ, Remick DG, Phan SH, Ward PA, Marks RM. Endothelial cell gene expression of a neutrophil chemotactic factor by TNF-α, LPS, and IL-1β. Science 1989; **243**:1467–9.

18 Lorant DE, Patel KD, McIntyre TM, McEver RP, Prescott SM, Zimmerman GA. Coexpression of GMP-140 and PAF by endothelium stimulated by histamine or thrombin: a juxtacrine system for adhesion and activation of neutrophils. J Cell Biol 1991; **115**:223–34.

19 Jaffe EA, Nachman R, Becher CG, Minick CR. Culture of human endothelial cells derived from umbilical veins: identification by morphologic and immunologic criteria. J Clin Invest 1973; **52**:2745–56.

20 Van Snick J, Cayphas S, Vinck A, Uyttenhove C, Coulie PG, Rubira M, Simpson RJ. Purification and NH_2-terminal amino acid sequence of a T cell derived lymphokine with growth activity for B cell hybridomas. Proc Natl Acad Sci USA 1986; **83**:9679–84.

21 Geng JG, Bevilacqua MP, Moore KL *et al.* Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. Nature 1990; **343**:757–60.

22 Gosset P, Malaquin F, Delneste Y, Wallaert B, Capron A, Joseph M, Tonnel AB. IL-6 and IL-1α production is associated with antigen-induced late nasal response. J Allergy Clin Immunol 1993; **92**:878–90.

23 Naclerio RM, Proud D, Togias AG *et al.* Inflammatory mediators in late antigen-induced rhinitis. N Engl J Med 1985; **313**:65–70.

24 Varney VA, Jacobson MR, Sudderick RM *et al.* Immunohistology of the nasal mucosa following allergen-induced rhinitis. Identification of activated T lymphocytes, eosinophils, and neutrophils. Am Rev Respir Dis 1992; **146**:170–6.

25 Robinson DR, Hamid Q, Ying S *et al.* Predominant Th2-like bronchoalveolar T-lymphocyte population in atopic asthma. N Eng J Med 1992; **326**:298–304.

26 Soderberg M, Lundgren R, Bjermer L, Stjernberg N, Rosenhall L. Inflammatory response in bronchoalveolar lavage fluid after inhaling histamine. Allergy 1989; **44**:98–102.

27 Azzawi M, Bradley B, Jeffery PK *et al.* Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. Am Rev Respir Dis 1990; **142**:1407–13.

28 Vannier E, Dinarello CA. Histamine enhances interleukin (IL)-1-induced IL-1 gene expression and protein synthesis via H2 receptors in peripheral blood mononuclear cells. Comparison with IL-1 receptor antagonist. J Clin Invest 1993; **92**:281–7.

29 Casale TB, Wood D, Richerson HB, Trapp S, Metzger WJ, Zavala D, Hunninghake G. Elevated bronchoalveolar lavage fluid histamine levels in allergic asthmatics are associated with metacholine bronchial hyperresponsiveness. J Clin Invest 1987; **79**:1197–203.

30 Mantovani A, Bussolino F, Dejana E. Cytokine regulation of endothelial cell function. FASEB J 1992; **6**:2591–9.

31 Swerlick RA, Lawley TJ. Role of microvascular endothelial cells in inflammation. J Invest Dermatol 1993; **100**:111S-115S.

32 Leeuwenberg JFM, Von Asmuth EJU, Jeunhomme TMAA, Buurman WA. IFN-γ regulates the expression of the adhesion molecule ELAM-1 and IL-6 production by human endothelial cells *in vitro*. J Immunol 1990; **145**:2110–4.

33 Moutabarrik A, Ishibashi M, Namiki M *et al.* Disparate regulation of interleukin-6 secretion from blood monocytes and vascular endothelial cells by interleukin-4. Transpl Proc 1992; **24**:2898–9

34 Ottosson A, Jansen I, Edvinsson L. Pharmacological characterization of histamine receptors in the human temporal artery. Br J Clin Pharmacol 1988; **27**:139–45.

35 Choi I, Kang H-S, Yang Y, Pyun K-H. IL-6 induces hepatic inflammation and collagen synthesis *in vivo*. Clin Exp Immunol 1994; **95**:530–5.

36 Kopf M, Baumann H, Freer G *et al.* Impaired immune and acute-phase responses in interleukin-6-deficient mice. Nature 1994; **368**:339–42.

37 Muraguchi A, Hirano T, Tang B, Matsuda T, Horii Y, Nakajima K, Kishimoto T. The essential role of B-cell stimulatory factor (BSF-2/IL-6) for the terminal differentiation of B cells. J Exp Med 1988; **167**:332–44.

38 Kishimoto T. The biology of interleukin-6. Blood 1989; **74**:1–10.

39 Gauldi J, Richards C, Harnish D, Lansdorp P, Baumann H. Interferon β2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. Proc Natl Acad Sci USA 1987; **84**:7251–6.

40 Takizawa H, Ohtoshi T, Ohta K *et al.* Interleukin-6 can function as an inhibitory autocrine growth factor for human bronchial epithelial cells. Am Rev Respir Dis 1992; **145**:123A.

41 Lee CE, Neuland ME, Teaford HG *et al.* Interleukin-6 is released in the cutaneous response to allergen challenge in atopic individuals. J Allergy Clin Immunol 1992; **89:** 1010–20.

42 Gosset P, Tsicopoulos A, Wallaert B *et al.* Increased secretion of TNF-α and Interleukin-6 by alveolar macrophages consecutive to the development of the late phase reaction. J Allergy Clin Immunol 1991; **88**:561–71.