**Abstract.** Histamine is a well-known mediator eliciting a broad range of responses in different cell types. Four different subtypes of G protein-coupled histamine receptors (H1-H4) have been cloned and pharmacologically characterized. However, involvement of the different histamine receptor subtypes in immunomodulatory functions of bronchial epithelium has only been investigated marginally. The expression and function of histamine receptor subtypes on the human bronchial epithelial cell line BEAS-2B was analyzed by PCR, intracellular Ca++-measurements and ELISA. We show mRNA expression of the histamine receptor subtypes H1, H2, and H3, but not H4 in the human bronchial epithelial cell line BEAS-2B. Using intracellular Ca++-measurements, we demonstrated functional expression of the H1 and H3 receptors. To characterize the biological properties of histamine in airway epithelial biology, we also investigated its effects on cytokine secretion by BEAS-2B cells. Thereby, we were able to show up-regulation of the proinflammatory mediators IL-6 and CXCL8/IL-8 via activation of the H1, H2, and H3 receptor subtypes. The Th1 cytokines CXCL9/MIG and CXCL10/IP-10 and the chemokine CCL5/RANTES were regulated in a distinct manner: Whereas histamine inhibited the IFN-γ/TNF-α-induced secretion of MIG via the histamine receptor subtypes H1, H2, and H3, the histamine-induced suppression of RANTES was due to activation of the H2 and H3 receptors, while reduction of cytokine-triggered IP-10 secretion was mediated only by triggering the H2 receptor. In summary our data provide evidence that histamine released during allergic lung diseases exerts regulatory influence on airway epithelial cells.

**Introduction**

Histamine [2-(4-imidazole)-ethylamine], one of the most intensely studied molecules in medicine, is a preformed mediator stored primarily in the cytoplasmic granules of basophils and mast cells (1). It is released upon activation of IgE receptors or stimulation with degranulating agents such as complement components, neuropeptides and cytokines. Histamine plays an essential role in cell-mediated immunity, but it is also involved in inflammatory lung diseases such as bronchial asthma (2-4).

The pleiotropic effects of histamine are mediated by different histamine membrane receptors. Four different subtypes of G protein-coupled histamine receptor, designated H1, H2, H3, and H4, have been described and pharmacologically characterized (5,6).

The H1 receptor which is coupled to pertussis toxin-insensitive Gq/11-proteins mediates most of the proinflammatory effects of histamine (7). Anti-inflammatory and immunosuppressive effects of histamine, such as inhibition of IL-12 secretion or induction of IL-10 production by dendritic cells, are mostly dependent on stimulation of the H2 receptor, which is coupled to the adenylate cyclase pathway (8,9). The H3 receptor elicits an increase in intracellular Ca++-concentration via activation of pertussis toxin-sensitive Gi/o-proteins. Furthermore, it is involved in the regulation of cytokine release from alveolar macrophages, mast cells, and dendritic cells (10). Previously, we demonstrated functional expression of the histamine receptors H1, H2, H3, and H4, have been described and pharmacologically characterized (5,6).

The H4 receptor which is coupled to pertussis toxin-insensitive Gq/11-proteins mediates most of the proinflammatory effects of histamine (7). Anti-inflammatory and immunosuppressive effects of histamine, such as inhibition of IL-12 secretion or induction of IL-10 production by dendritic cells, are mostly dependent on stimulation of the H2 receptor, which is coupled to the adenylate cyclase pathway (8,9). The H3 receptor elicits an increase in intracellular Ca++-concentration via activation of pertussis toxin-sensitive Gi/o-proteins. Furthermore, it is involved in the regulation of cytokine release from alveolar macrophages, mast cells, and dendritic cells (10). Previously, we demonstrated functional expression of the histamine receptors H1, H2, H3, and H4 in human monocyte-derived dendritic cells (DCs). Recently, functional expression of the H4 receptor on dendritic cells was shown, but its exact signal transduction pathways remain unclear (11).

Asthma is a lung disease characterized by acute bronchoconstriction, bronchial hyperreactivity and airway inflammation, leading to remodeling of the airways (12). The respiratory epithelium is believed to be important in the pathogenesis of allergic airway diseases since it is the first tissue to meet inhaled allergens (13). It has been shown previously that airway epithelial cells are capable of secreting a variety of mediators, including proinflammatory cytokines and chemokines. Therefore, they participate in the recruitment of different leukocytes into inflamed tissue (14,15).
this context, mediators such as interleukin (IL)-6, CXCL8/IL-8, CCL5/RANTES (regulated upon activation normal T-cell expressed and secreted) (16,17) or the Th1-chemokines CXCL10/IP-10 (interferon-inducible protein-10) and CXCL9/ MIG (monokine induced by γ-interferon) (14,18,19) play a prominent role. Airway epithelial cells from asthmatic patients have been shown to express a different pattern of proinflammatory cytokines compared to healthy individuals (20). Release of proinflammatory cytokines from airway epithelium can be provoked by a variety of agents, including proteases secreted by neutrophils, eosinophil granule proteins, or mast cell products such as histamine (18,21). However, little is known about the expression of the different histamine receptor subtypes on bronchial epithelial cells and their involvement in the activation of these cells.

To investigate the expression and function of histamine receptors on bronchial airway epithelial cells BEAS-2B cells were used. This adenovirus-12 SV40 hybrid virus transformed human bronchial epithelial cell line is widely used to explore the functional properties of bronchial epithelial cells (18,22). Furthermore, the aim of the present study was to characterize involvement of different histamine receptors in cellular responses and cytokine production.

Materials and methods

Materials. BEAS-2B cells were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). Human recombinant IFN-γ was obtained from ImmunoTools (Friesoythe, Germany) and recombinant TNF-α was from Strathmann Biotec (Hannover, Germany). Histamine and betahistine were obtained from Sigma (Deisenhofen, Germany), Dimaprit and (R)-α-methylhistamine (R-α-MeH) were purchased from ICN Biomedicals (Costa Mesa, CA, USA). ELISA kits for human CXCL8/IL-8, CXCL10/IP-10, CXCL9/MIG and CCL5/RANTES were purchased from R&D Systems (Minneapolis, USA). Matched pairs for human IL-6 were obtained from ImmunoTools.

Cell culture and stimulation. BEAS-2B cells were grown in MEM-medium (Gibco, Paisley, UK) containing 5% fetal calf serum gold (PAA Laboratories, Pasching, Austria) and 1% penicillin/streptomycin (Biochrom, Berlin, Germany) in 175-cm² culture flasks (BD Falcon, Bedford, USA) at 37°C, 5% CO₂, and 100% humidity, as described previously (23). To favor cell adhesion, flasks were coated with a solution containing 30 μg/ml rat collagen S, 1 mg/ml human fibronectin and 100 μg/ml BSA in MEM-medium. For subculture trypsin/EDTA-solution (Biochrom) was used to remove adherent cells. For subsequent experiments cells were seeded into 24-well tissue culture plates (Corning Inc., Corning, USA), at a density of 0.10x10⁶ cells/well. After 4 h of adherence, cells were serum-starved overnight. Then medium was changed and cells were stimulated. After an additional 24 h, cell supernatants were collected and stored at -70°C until analysis by ELISA.

Intracellular Ca²⁺-measurements. Ca²⁺ transients were measured in BEAS-2B cells loaded with the Ca²⁺ indicator fura-2/AM (Calbiochem, La Jolla, California, USA) by using the digital fluorescence microscope unit Attofluor (Zeiss, Oberkochen, Germany). Briefly, BEAS-2B cells were incubated with 2 μM Fura-2/AM for 30 min at 37°C in a Ca²⁺- and Mg²⁺-free Hanks' BSA solution. Cells were then washed twice and finally resuspended in the same buffer containing 1.5 mM CaCl₂ and 1.5 mM MgCl₂. Traces were followed spectrophotometrically and Ca²⁺ transients were determined by multiple cell acquisitions with the 340/380 wavelength excitation ratio at an emission wavelength of 505 nm. Curves shown are representative of the whole cell population.

Cytokine assays. IL-6, IL-8, IP-10, MIG, and RANTES were measured by ELISA and performed according to the manufacturer’s recommendations. Samples were assayed in duplicate for each condition.

Analysis of mRNA expression. Total RNA was extracted from the cells using Trizol-reagent (Gibco, Paisley, UK) as instructed by the manufacturer. To obtain cDNA, 5 μg of total RNA were primed with oligo-dT primers (Hermann GbR, Freiburg, Germany) and reverse transcribed with Stratascript reverse transcriptase (Stratagene, La Jolla, USA). Primers for the different human histamine receptors were designed based on published sequence data (H₁: NM_000861, H₂: NM_022304, H₃: NM_007232, H₄: NM_007232) employing Primer3-software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) retrieving the following sequences: H₁ receptor (403-bp product), sense 5'-TCGAATGGGTACTGTGATTTTGGAAAG-3', antisense 5'-ACGGCCACCATCAGGATTTTGGAAAG-3'; H₂ receptor (497-bp product), sense 5'-CCCGGCTCCGCAACCTTTATCACTGCAAATG-3', antisense 5'-CTTGGGGTTTTGATGGTGACT-3'; H₃ receptor (221-bp product), sense 5'-CTTCCTGCCCTAGCCGACCTTGT-3', antisense 5'-CTGATCCGGTGCTTGGTGACT-3'; H₄ receptor (184-bp product), sense 5'-TCGAATGGGTACTGTGATTTTGGAAAG-3', antisense 5'-ACGGCCACCATCAGGATTTTGGAAAG-3'.

PCR was performed using 10 μl of iQ-Supermix (Bio-Rad, Hercules, USA), 7 μl H₂O, 1 μl of each primer (final concentration 0.2 μM), and 2 μl cDNA. The reactions were subjected to multiple cell acquisitions with the 340/380 wavelength excitation ratio at an emission wavelength of 505 nm. Curves shown are representative of the whole cell population.
concentration 0.5 μM each) and 1 μl of cDNA. Amplification conditions were 12-min initial denaturation at 95˚C followed by 40 cycles of denaturation (94˚C) for 30 sec, annealing (60˚C, H₁/H₂; 62˚C, H₃; 57˚C, H₄) for 30 sec and amplification (72˚C) for 45 sec. PCR-products were subjected to electrophoresis on a 2.5% agarose gel and visualized by ethidium bromide staining.

Results

BEAS-2B cells express different histamine receptor subtypes. Expression of mRNA for the different histamine receptor subtypes in BEAS-2B cells was analyzed using RT-PCR. As shown in Fig. 1, BEAS-2B cells express H₁, H₂, and H₃ but not H₄ receptors. To confirm that the negative result was not due to incorrect experimental conditions, PCR was also performed with cDNA from human monocyte derived dendritic cells leading to the expected products for the H₄ receptor (data not shown).

Histamine induces intracellular Ca²⁺ mobilization. Changes in intracellular Ca²⁺ are crucial for cellular responses such as the release of cytokines or chemokines. Therefore, intracellular Ca²⁺ transients after stimulation with histamine were analyzed in fura-2-labeled BEAS-2B cells, using digital fluorescence microscopy. According to previous publications (9), histamine induced a rapid time- and concentration-dependent increase in intracellular Ca²⁺ (Fig. 2A). Maximal and half-maximal responses were at 10⁻⁵ mol/l and 10⁻⁷ mol/l histamine concentrations, respectively. To identify the histamine receptor subtypes involved in intracellular Ca²⁺ changes, BEAS-2B cells were stimulated with selective histamine receptor agonists. Fig. 2B shows that the H₁ receptor agonist betahistine induced a spiking concentration-dependent Ca²⁺ rise followed by a slow time-dependent declining phase. In addition, the H₃ receptor agonist R-α-MeH was also able to increase intracellular Ca²⁺ levels in a dose-dependent manner which was also followed by a slow time-dependent declining phase (Fig. 2C). In contrast, the H₂ receptor agonist dimaprit was not able to trigger an intracellular Ca²⁺ increase (Fig. 2D). Moreover, preincubation with diphenhydramine or thioperamide, which are H₁ and H₃ receptor antagonists, respectively, significantly reduced the histamine-induced calcium transient (Fig. 2E). In contrast, the H₂ receptor antagonist cimetidine was ineffective.
Stimulation of Beas-2B with histamine, betahistine or R-\(\alpha\)-MeH in a Ca\(^{++}\)-free ethyleneglycol-bis-(aminoethylether)-N,N,N’,N’-tetraacetic acid (EGTA)-containing saline solution (4 mmol/l EGTA) did not abrogate the histamine-induced intracellular Ca\(^{++}\) transients, suggesting that the Ca\(^{++}\) rise was due to Ca\(^{++}\) mobilization from the intracellular stores (data not shown).

Modulation of IL-6 and IL-8 release by histamine. It was previously shown that histamine influences the release of cytokines/chemokines, e.g. in fibroblasts, monocytes, lymphocytes or dendritic cells. As shown in Fig. 3A and 4A, histamine induces the secretion of the proinflammatory mediators IL-6 and IL-8 in a dose-dependent manner. In both cases, this effect was mimicked by the H\(_1\) receptor agonist betahistine, the H\(_2\) receptor agonist dimaprit, and the H\(_3\) receptor agonist R-\(\alpha\)-MeH, respectively (Fig. 3B and 4B).

Modulation of chemokine production by histamine. Bronchial epithelial cells are known to produce the chemokines RANTES, IP-10, and MIG, attracting preferably Th1-lymphocytes. As previously described, expression of these chemokines by BEAS-2B cells could be induced by a combination of the proinflammatory cytokines TNF-\(\alpha\) and IFN-\(\gamma\) (100 IU/ml each) (14). Addition of histamine dose-dependently inhibited the production of IP-10 (Fig. 5C), MIG (Fig. 5D), and RANTES (Fig. 5E). Inhibition of IP-10 secretion was mimicked by the H\(_1\) receptor agonist betahistine, whereas betahistine (H\(_1\) receptor agonist) and (R)-\(\alpha\)-methyl-histamine (H\(_3\) receptor agonist) showed no influence on the IP-10 production of BEAS-2B cells (Fig. 5B). In contrast, betahistine, dimaprit, as well as R-\(\alpha\)-MeH inhibited the secretion of the chemokine MIG in a dose-dependent manner (Fig. 5D). RANTES release of BEAS-2B cells was inhibited by both dimaprit, and R-\(\alpha\)-MeH. However the inhibitory effect could only be seen in higher concentrations (Fig. 5F).

Discussion

Histamine is a well-established mediator in various pathologies such as bronchial asthma. Following allergen exposure it is released primarily from mast cells and basophils causing acute symptoms such as bronchoconstriction, vasodilatation, and increased capillary permeability (4). Additionally, histamine influences chronic inflammatory processes by regulating numerous functions of different immune cells including proliferation, induction of adhesion molecule expression and cytokine production (9,24,25). The pleiotropic effects of histamine are mediated by different subtypes of histamine membrane receptors (6). To elucidate the influence of histamine on airway epithelial cells, we investigated the expression and function of the four known histamine receptor subtypes on the bronchial epithelial cell line BEAS-2B.

The present study revealed that BEAS-2B cells express functional H\(_1\), H\(_2\), and H\(_3\) but not H\(_4\) receptors, which are differently coupled to mobilization of intracellular Ca\(^{++}\), chemokine and cytokine production. Activation of both H\(_1\) and H\(_3\) receptors triggered intracellular Ca\(^{++}\) transients, which were insensitive to chelation of extracellular Ca\(^{++}\) and therefore were due to Ca\(^{++}\) mobilization from intracellular stores. In contrast, the H\(_2\) receptor was not involved in histamine-induced intracellular Ca\(^{++}\) increase. Our results match those of a former
study performed with immature dendritic cells (DCs) where H1 and H3 but not H2 receptors triggered intracellular Ca++ transients (9).

There is strong evidence that histamine is associated with bronchial asthma as increased levels of histamine were measured in bronchoalveolar lavage (BAL) fluids of asthmatics following segmental allergen challenge. Moreover, BAL histamine levels correlate with the degree of bronchial hyperresponsiveness (26,27). Inflammatory cytokines and chemokines released by airway epithelial cells have been shown to contribute significantly to the regulation of immune responses, as well as pathological features in bronchial asthma such as airway remodeling and airway fibrosis (28). Therefore, we investigated the influence of histamine on cytokine/chemokine secretion by BEAS-2B cells. We showed that histamine as well as the H1 receptor agonist betahistine, the H2 receptor agonist dimaprit, and the R-α-MeH (H3 receptor agonist), respectively. Supernatants were collected after 24 h and (A and B) IP-10, (C and D) MIG and (E and F) RANTES content was determined by ELISA. One representative of 3 similar experiments is shown.

Figure 5. Modulation of IP-10, MIG, and RANTES by histamine. BEAS-2B cells (∼1x10^5) were stimulated with a combination of TNFα/INFγ (100 IU each) and the indicated concentrations of (A, C and E) histamine or (B, D and F) the histaminergic agonists betahistine (H1 receptor agonist), dimaprit (H2 receptor agonist), and R-α-MeH (H3 receptor agonist), respectively. Supernatants were collected after 24 h and (A and B) IP-10, (C and D) MIG and (E and F) RANTES content was determined by ELISA. One representative of 3 similar experiments is shown.

endothelial cells and DCs, respectively (24,29,30). Furthermore, histamine triggered IL-6 production by peripheral blood mononuclear cells and by monocyte/macrophages. Several studies have shown the importance of IL-6 and IL-8 in the pathophysiology of bronchial asthma. Allergen challenge in humans caused an IL-8-mediated increase of neutrophils in the lungs (31). In contrast, IL-6 plays a key role in airway remodeling, another classical feature of the asthmatic lung. It contributes to both subepithelial fibrosis and dyscrine, since the regulation of mucin gene expression is mediated through an IL-6-dependent autocrine/paracrine loop (32,33).

The chemokines IP-10, MIG and RANTES are known to attract preferably Th1-lymphocytes (34,35). In this study we showed that histamine inhibited the release of these chemokines in a concentration-dependent manner. Inhibition of IP-10 production was mediated solely by H2 receptors. This is consistent with a previous study showing the involvement of H2 receptors and cAMP in the regulation of IP-10 secretion (36). In contrast, modulation of MIG was also mediated by H1 and H2 histamine receptors whereas RANTES secretion was mediated by H2 and H3 histamine receptors, indicating
These findings are consistent with the H2 receptor mediating the airway inflammation by reducing the recruitment of eosinophils and RANTES production by histamine could also limit allergic inflammation. Therefore, it can be concluded that inhibition of histamine levels with the degree of bronchial hyperresponsiveness.

Furthermore, RANTES levels in BAL fluids of asthmatic children were significantly increased compared to healthy controls. In contrast, IP-10-deficient mice airway hyperreactivity, eosinophilia, and IL-4 levels compared to wild-type controls. In contrast, IP-10 was increased in the lung after allergen challenge. It was reported that the CXCL9/MIG is also known to bind to CXCR3 and therefore attracts preferentially Th1-lymphocytes. However, it was found to be up-regulated in the airways of asthmatics. A murine model of asthma revealed that the expression of IP-10 was increased in the lung after allergen challenge. Mice overexpressing IP-10 in the lung showed enhanced airway hyperreactivity, eosinophilia, and IL-4 levels compared to wild-type controls. In contrast, IP-10-deficient mice demonstrated opposite effects, with a significant reduction in these measures of Th2-type allergic airway inflammation. The inhibitory effect of histamine on IP-10 secretion of BEAS-2B cells was mediated by activation of H2 receptors. These findings are consistent with the H2 receptor mediating the anti-inflammatory and immunosuppressive effects of histamine.

The role of IP-10 in the pathogenesis of asthma and its regulation by histamine is ambiguous. On the one hand, IP-10 secretion was inhibited by histamine, suggesting limited asthmatic inflammation. On the other hand, impaired chemotaxis of Th1-lymphocytes could promote a Th2-driven immune response. A possible explanation could be that inhibition of IP-10 production by histamine was mediated solely by H2 receptors, which are known to be involved in antiinflammatory and immunosuppressive processes.

Our study revealed that histamine inhibited the production of CCL5/RANTES which is like MIG and IP-10 known to attract preferably Th1-lymphocytes. Therefore, inhibition of RANTES release by histamine could amplify Th2 dominance, leading to augmented allergic airway inflammation. In contrast, RANTES was also found to play an important role in bronchial hyperresponsiveness and allergic inflammation of the lung, especially by strongly attracting eosinophils. Furthermore, RANTES levels in BAL fluids of asthmatic children were significantly increased compared to healthy children. Therefore, it can be concluded that inhibition of RANTES production by histamine could also limit allergic airway inflammation by reducing the recruitment of eosinophils. In fact, the effects of this chemokine are very versatile and sometimes controversial. The consequences of impaired RANTES production by histamine are complex and not fully understood at present.

The influence of histamine on secretion of chemotactic factors and the involvement of different histamine receptor subtypes underline the complex effects of histamine in human lung biology. It can be hypothesized that histamine released upon allergen challenge could activate epithelial cells, leading to enhanced release of IL-6 and IL-8, mediators known to be involved in inflammation and airway remodeling. Additionally, reduced secretion of IP-10, MIG, and RANTES, important chemotactic factors for Th1-lymphocytes could enhance a Th2-dominated surrounding.

In summary, our study provides evidence that stimulation of bronchial epithelial cells by histamine induces typical changes seen in allergy. Reduction in Th1-promoting cytokines favors allergic inflammation whereas the increase in IL-6 and IL-8 promotes airway remodeling. These effects are mediated by different histamine receptor subtypes, suggesting complex interactions of histamine released during allergic lung diseases.

References