Histamine H₄ and H₂ Receptors Control Histamine-Induced Interleukin-16 Release from Human CD8⁺ T Cells

FLORIAN GANTNER, KATSUYA SAKAI, MICHAEL W. TUSCHE, WILLIAM W. CRUIKSHANK,¹ DAVID M. CENTER,¹ and KEVIN B. BACON

Bayer Yakuhin, Ltd., Research Center Kyoto, Therapeutic Research Area Asthma, Japan

Received April 12, 2002; accepted June 14, 2002

ABSTRACT

Histamine is known to trigger the release of interleukin (IL)-16 from human CD8⁺ cells. However, the individual roles of the presently known histamine receptor subtypes (H₁-H₄) in this inflammatory response have not been fully characterized. Histamine stimulation of human CD8⁺ T lymphocytes purified from peripheral blood led to a 5- to 8-fold increase in the basal release of IL-16 within 24 h, and this increase was significantly blocked by the H₂-selective antagonist, cimetidine, or by thiorperamide, an antagonist of H₃ and H₄ receptors, respectively. The H₁ antagonist pyrilamine showed limited effects. Agonists selective for H₂ (dimaprit), H₃/₄ (R(-)-α-methylhistamine), and H₄ (cloprenzepin) were capable of inducing the release of bioactive IL-16 because CD8⁺ cell supernatants induced CD4⁺ cell migration, which was abrogated by an anti-IL-16 antibody. Furthermore, preincubation of lymphocytes with pertussis toxin abolished IL-16 release triggered by activation of the G protein-coupled H₄ receptor but not by the H₂ receptor. Messenger RNA expression studies confirmed H₁, H₂, and H₄ expression in human CD8⁺ lymphocytes, whereas H₂ mRNA was completely absent. All leukocyte populations investigated expressed mRNA for H₄, with highest levels found in eosinophils, dendritic cells, and tonsil B cells. H₄ expression was also detected in human lung, trachea, and various cells of human lung origin, such as fibroblasts, bronchial smooth muscle cells, epithelial, and endothelial cells. Since many of those are known sources of IL-16, immune cell- and lung cell-expressed H₄ receptors may have a general role in the control of this mediator of inflammatory disorders such as asthma.

Histamine is an important endogenous amine that exerts numerous functions in central and peripheral tissues. These physiological processes are mediated through at least four receptors, H₁ to H₄, which are all members of the seven membrane-spanning G protein-coupled receptor (GPCR) family (Hough, 2001). H₁ receptors are widely expressed throughout the body, with high expression levels being found in the brain, smooth muscle cells, endothelial cells, adrenal medulla, and heart. By controlling smooth muscle and endothelial cell contraction (thereby increasing vascular permeability) and by stimulating nitric oxide formation, H₁ receptors modulate inflammatory and allergic responses; antihistamines have been in clinical use for allergy treatment for decades (reviewed in Walsh et al., 2001). H₂ receptor activation causes cAMP accumulation through activation of a stimulatory G protein in gastric cells stimulating gastric acid secretion (Black et al., 1972). H₂ receptors are involved in the regulation of cytokine and chemokine production and differentiation and maturation of a variety of cells in cardiac tissue, smooth muscle, and cells of the immune system (Elnekov et al., 1998; Poluektova and Khan, 1998; Kohka et al., 2000; Caron et al., 2001; Jutel et al., 2001). H₃ receptors are predominantly found in the brain, where they function as presynaptic autoreceptors on histamine-containing neurons and are believed to control the release of many brain mediators including histamine itself (Hough, 1999). Thus, several target indications for H₃ receptor-interacting compounds have been suggested: Alzheimer’s disease, sleep disorders, cognition and memory disorders, obesity, attention deficits, and others (Leurs et al., 1998). Phyllogenetic and homology analyses have revealed that H₃ receptors are surprisingly different, not only from H₁ and H₂ (Lovenberg et al., 1999; Leurs et al., 2000) but also from most of the known GPCRs. In the search of additional receptors more closely related to H₃, various groups recently cloned and pharmacologically characterized a novel histamine receptor, H₄ (Nakamura et al., 2000; Oda et al., 2000; Liu et al., 2001; Morse et al., 2001; Nguyen et al., 2001; Zhu et al., 2001). H₄ has about 40% homology to H₃ (58% in the transmembrane region), has a...
similar genomic structure, and like H₃, seems to be functionally coupled to G protein Gᵢₒ, thereby inhibiting forskolin-stimulated cAMP formation (Lovenberg et al., 1999). In contrast to these structural similarities, the expression pattern of H₄ dramatically differs from the expression profile of H₃.Hardly any evidence for H₄ expression in the brain and nervous tissues has been described. H₄ shows highest expression in the bone marrow and in leukocytes, moderate expression in spleen, thymus, lung, small intestine, colon, and heart (Nakamura et al., 2000; Oda et al., 2000; Liu et al., 2001; Morse et al., 2001; Nguyen et al., 2001; Zhu et al., 2001). This expression pattern and the presence of several putative regulatory elements mediating tumor necrosis factor-α and IL-6-stimulated transcription detected in the human H₄ gene promoter (Cuge et al., 2001) suggest significant roles for H₄ in the immune system, but the present biological knowledge about H₄ is very limited.

Histamine is known to trigger IL-16 production in CD8⁺ cells (Laberge et al., 1995) and in epithelial cells (Arima et al., 1999); however, the receptor(s) mediating this response have not been fully characterized in the human system. IL-16 is further produced by many other cell types, including mast cells, eosinophils, B cells, dendritic cells, and epithelial cells (reviewed in Cruikshank et al., 2000). IL-16 has been found in the bronchoalveolar fluid of allergen- or histamine-challenged asthmatics (Cruikshank et al., 1995; Mashikian et al., 1998; Krug et al., 2000) and is increasingly expressed in the bronchial mucosa of atopic asthmatics (Laberge et al., 1997) by eosinophils and mast cells (Laberge et al., 1999). Through binding to its receptor (CD4), IL-16 is believed to play an important role in the recruitment of CD4⁺ T cells into the lungs of asthmatic patients. A crucial role of IL-16 in asthma is further supported by the protection of animals from allergic asthma after pretreatment with an IL-16-blocking peptide (de Bie et al., 1999) or a neutralizing anti-IL-16 antibody (Hessel et al., 1998).

By using the pharmacological tools currently available for H₃/H₄ receptors, we describe a first immunologically relevant function of H₄. Together with H₂, H₄ is involved in the control of IL-16 release from human lymphocytes.

Materials and Methods

Chemicals. Histamine, dimaprit, clobenpropit, α-methyl-histamine, cimetidine, thioperamide, and pyrilamine were purchased from Sigma-Aldrich (St. Louis, MO). Pertussis toxin was purchased from Calbiochem (La Jolla, CA). Fetal calf serum was obtained from JRH Bioscience (Lenexa, KS). RPMI 1640 was purchased from Invitrogen (Carlsbad, CA). Sodium azide was purchased from Nacalai Tesque (Kyoto, Japan). All bulk chemicals not further specified were purchased from Wako Pure Chemicals (Osaka, Japan).

Blood Donors. Blood of both females and males were used for the studies. None of the donors was on any medication for at least 3 weeks before blood donation. All experiments were approved by the local ethical committee and performed in accordance with the Declaration of Helsinki.

Cell Purification and Cell Cultures. Peripheral blood mononuclear cells (PBMC) were prepared from heparinized human blood using Ficoll-HyPaque (Amersham Biosciences UK, Ltd., Buckinghamshire, UK) according to the manufacturer’s recommended protocol. Enriched monocytes were obtained from whole PBMC by incubation with RPMI 1640 + 10% fetal calf serum at 4°C for 30 min at constant rotation. Nonaggregated cells were removed, and the monocyte enriched pellet was resuspended in RPMI 1640 and plated at a density of 2 × 10⁶ cells/well in six-well plates with 10 ng/ml rhIL-4 (kindly provided by Drs. H-D. Hoerlein and J. Peters, Bayer AG, Wuppertal, Germany) and 25 ng/ml granulocyte/macrophage-colony-stimulating factor (Peprotech, Rocky Hill, NJ). After 7 days of culture, immature dendritic cells were collected and counted. Mature dendritic cells were obtained by incubation of immature dendritic cells for 2 more days in the presence of lipopolysaccharide (10 ng/ml; Sigma-Aldrich). FACS analysis revealed a purity of 90%, as assessed by the percentage of CD11c⁺ cells.

CD8⁺ cells were obtained from whole PBMCs by negative selection using magnetic antibody cell selection (MACS) beads, according to standard protocols. Briefly, cells were incubated with an antibody cocktail containing microbeads against CD4, CD11b, CD14, CD16, CD19, CD36, CD56, and IgE (Miltenyi Biotec, Bergisch-Gladbach, Germany) for 30 min at 4°C. The negative fraction was >85 to 90% CD8⁺, as determined by flow cytometric analysis. For some experiments, highly purified CD8⁺ cells (purity >98%, as determined by flow cytometry) were obtained from PBMC using anti-CD8 microbeads.

CD4⁺ T cells were positively selected from whole PBMC using anti-CD4 microbeads. The purity of CD4⁺ populations was found to be >95 to 98%. B cells were purified from surgically removed human tonsils (kindly provided by Dr. Okukubo, Kasai Municipal Hospital, Kasai, Japan). Tonsils were minced through a stainless mesh into phosphate-buffered saline. B cells were isolated according to a positive selection procedure based on CD19⁻/conjugated MACS antibodies according to standard protocols (Miltenyi Biotec). Purity of B cells, as determined by flow cytometry, was always >95%. Eosinophils were prepared from heparinized blood using Mono-polyresolving medium (Dainippon Pharmaceuticals, Osaka, Japan) and then by negative selection using CD16⁻/conjugated antibody (MACS). Eosinophils were assessed to be >90% pure by Diff-quick staining (International Reagents, Kobe, Japan).

All human lung cell samples were purchased from CLONTECH (Palo Alto, CA) and cultured according to the instructions given by the manufacturer. Generally, cells were used for experiments at passage 2 to 3. HMC-1 human leukemia mast cells (American Type Tissue Culture Collection, Manassas, VA) were maintained in Iscove’s modified Dulbecco’s medium (Invitrogen no. 12200-036) supplemented with 10% heat-inactivated fetal calf serum, 1.2 μM α-thioglycolate (M-6145; Sigma-Aldrich), and 100 μg/ml penicillin and streptomycin.

Flow Cytometry. Cells [2 × 10⁵ cells/tube in phosphate-buffered saline supplemented with 0.5% fetal calf serum and 0.1% sodium azide (FACS buffer)] were incubated with FACS buffer, isotype control antibody, or specific antibodies as specified for 30 min at 4°C in the dark. Samples were then washed once by centrifugation and resuspended in FACS buffer. Flow cytometric analysis was conducted using FACScan (Becton, Dickinson and Company, Mountain View, CA), and data were analyzed using CellQuest software.

Primers and PCR Conditions for mRNA Determination. All primers for quantitative RT-PCR were purchased from Nihon Iden Kenkyujo (Sendai, Japan). DNA was prepared using the TRizol reagent (Invitrogen no. 15596) strictly following the manufacturer’s instructions and treated with DNA-free (no. 1906; Ambion, Austin, TX) to remove any genomic DNA contamination. First-strand cDNA synthesis was performed by SUPERSCRIPT first-strand synthesis system for RT-PCR (Invitrogen no. 11904-018) using random primers. Copy DNA from human monocytes and from brain was purchased from CLONTECH (Palo Alto, CA).

Primers specificity and optimized PCR conditions were determined by using subcloned human H₄ (GenBank accession no. NM 000861), H₃ (GenBank accession no. NM 64799), H₂ (GenBank accession no. NM 007232), and H₁ (GenBank accession no. NM 021624) receptors as the corresponding templates. The primer sequences and the cycle numbers for gene amplification are summarized in Table 1. PCR analysis was performed in a 20-μl volume containing 20 ng of each cDNA sample and 5 μM each primer using the Hot Star Taq Master
mix kit (no. 203445; QIAGEN, Hilden, Germany). PCR conditions were as follows. All samples were preheated for 15 min at 95°C and then subjected to denaturing conditions at 95°C for 5 s. After annealing, genes were amplified for 15 s/cycle. Gene copy numbers were calculated after quantification of PCR fragments amplified by specific control primers using the real-time PCR Lightcycler method.

**IL-16 Determination.** CD8+ cells were seeded in RPMI 1640 supplemented with 5% fetal calf serum on 96-well plates at a density of 2 × 10^5 cells/well in 200 μl with or without histamine (Sigma-Aldrich). At the time points indicated, supernatants were removed and frozen at −30°C until further use. Total IL-16 concentrations were determined using a commercially available ELISA kit (Endogen, Woburn, MA) according to the manufacturer’s instructions.

**Migration Assay.** IL-16-containing cell culture supernatants were collected and stored at −30°C until further use. Migration was assessed by seeding 2 × 10^5 highly purified CD4+ cells over a removable 3-μm membrane of a Transwell migration chamber (Corning Costar, Corning, NY). Five hundred microliters of test supernatant at various dilutions was added to the lower well. The cell-containing membrane was placed over the test supernatants, and cells were allowed to migrate at 37°C for 2 h. For inhibition studies, a control mouse anti-human IgG antibody or anti-IL-16 antibody (clone 4.1; Cruikshank et al., 1995) was added to the lower well and incubated for 15 min at 37°C before the addition of cells. The amount of anti-IL-16 antibody added was able to block migration of CD4+ cells induced by 70 ng/ml rhu-IL-16, as determined in preliminary experiments. After a migration period of 2 h, cells were incubated on ice for 20 min to remove cells adhering to the membrane. The cells in the lower wells were collected and counted using FACS Cellquest software (Becton, Dickinson and Company). The migration index was calculated by dividing the absolute number of migrated cells by the number of cells spontaneously migrated in control wells.

**Statistics.** Unless otherwise stated, data are expressed as means ± S.D. of at least three independent experiments. Statistical significance was determined using the unpaired Student’s t test if applicable or with the Welch test if variances were nonhomogeneous using commercially available statistic software (GraphPad Software, Inc., San Diego, CA).

### Results

**Histamine Induced IL-16 Release from CD8+ Cells.** As previously reported (Laberge et al., 1995), histamine led to a concentration- and time-dependent release of IL-16 into the supernatant of freshly prepared CD8+ T cells from peripheral blood. IL-16 release reached a maximum at 1 μM histamine after 24 h. The basal release observed at this time point (51 ± 11 pg/2.5 × 10^5 cells) was increased approximately 6-fold in the presence of 1 μM histamine.

We next addressed the question of which H receptor(s) may trigger the release of IL-16 upon activation and performed a series of experiments in the presence or absence of selective histamine receptor antagonists or agonists. As shown in Fig. 2, cimetidine (H2 antagonist) and thioperamide (H3 antagonist) both concentration dependently suppressed histamine-induced IL-16 release. This inhibition was not complete but reached a plateau at approximately 70% inhibition, with half-maximal effects (IC50 values) of 30 nM for cimetidine and 590 nM for thioperamide. In contrast, the H1-selective

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers</th>
<th>Amplification Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>F 5′-AAGTCACCATCCTCAACCCCTGAC-3′</td>
<td>40 cycles, 70°C</td>
</tr>
<tr>
<td>H2</td>
<td>F 5′-TCAGCCCTGCTCATCATGCTGCTG-3′</td>
<td>40 cycles, 70°C</td>
</tr>
<tr>
<td>H3</td>
<td>F 5′-GCCTGCTGCTGCTGCTGCTGCTG-3′</td>
<td>35 cycles, 68°C</td>
</tr>
<tr>
<td>H4</td>
<td>F 5′-AGTCAGTCAGTCAGTCAGTCAGTC-3′</td>
<td>42 cycles, 70°C</td>
</tr>
<tr>
<td>GAPDH</td>
<td>F 5′-TGACGGGAGGTGATGATGATGATG-3′</td>
<td>25 cycles, 63°C</td>
</tr>
</tbody>
</table>

F, forward primer; R, reverse primer.
antagonist, pyrilamine, showed only 10 to 15% inhibition at the highest concentration used (10 μM). This suggested a contribution of H2 and H3/4 receptors in the process of IL-16 release. Of note, neither cimetidine nor thioperamide were able to block basal release, nor did they lead to a 100% block of IL-16 release when used in combination (data not shown).

To confirm our findings, we tested the capability of selective histamine receptor agonists to induce IL-16 release. In line with our antagonist studies, dimaprit (H2 agonist), α-methyl-histamine (H3/4 selective), and clobenpropit, the only selective H4 agonist described to date (Oda et al., 2000), all led to a concentration-dependent release of IL-16, the amounts of which were comparable to those maximally induced by histamine itself (Fig. 3A). Preincubation of the cells overnight with pertussis toxin abrogated IL-16 release induced by α-methyl-histamine and clobenpropit but not IL-16 released in response to dimaprit. This observation proves that H4 effects are mediated by coupling to pertussis toxin-sensitive G12/13 proteins, whereas H2 effects, which are known to signal via stimulatory G proteins, were not affected (Fig. 3B).

To check whether the liberated IL-16 was bioactive, we incubated CD4+ cells with supernatants from histamine- or clobenpropit-stimulated CD8+ cells. Indeed, a strong and concentration-dependent migration was noted when CD4+ cells were exposed to different dilutions of CD8+ supernatants collected 24 h after stimulation. Absolute migration activities differed considerably between individual CD4+ donors, even when exposed to the identical CD8+ supernatant. However, each CD8+ supernatant derived from cells activated by histamine or clobenpropit that was tested induced significant CD4+ migration in any single experiment, as exemplified in Fig. 4A.

Migration of CD4+ cells under these conditions was significantly inhibited in the presence of a neutralizing anti-IL-16

---

**Fig. 3.** Stimulation of IL-16 release by histamine receptor agonists. CD8+ cells were seeded at 2 x 10⁵ cells/well in 200 μl of medium and either immediately stimulated with various concentrations of histamine receptor agonists (A) or incubated with pertussis toxin (100 ng/ml) overnight before histamine receptor agonists were added at a final concentration of 10 μM (B). After 24 h, supernatants were removed and frozen until determination of IL-16 concentrations by ELISA. The effect of pertussis toxin was calculated based on the amount of agonist-induced IL-16 in the absence of pertussis toxin (set as 100%). Data represent mean ± S.D. of four to six (A) or three (B) independent experiments, respectively. *, p < 0.05; **, p < 0.01 versus control values without pertussis toxin; α-methyl-H2, α-methyl histamine.

**Fig. 4.** Lymphocyte migration activity of histamine and clobenpropit-induced IL-16. CD4+ migration was assessed in cell-free supernatants derived from CD8+ cells previously stimulated by histamine (1 μM; A and B) or clobenpropit (10 μM; B) for 24 h, as described under Materials and Methods. Supernatants were either diluted with cell culture medium (A) or pretreated with a neutralizing anti-IL-16 antibody, a control mouse anti-human IgG antibody (control antibody), or directly incubated with CD4+ cells (B). Antibody effects were calculated in percent inhibition versus controls (set as 100%). Data in A represent mean values of duplicate incubations and are shown for one representative experiment of five (see Results for details). Migratory activity is given as the migration index (see Materials and Methods for details). Data in B are given as mean ± S.D. from three to four individual experiments. *, P < 0.05 versus control values.
antibody. Equal amounts of a control murine anti-human IgG antibody was without effect (Fig. 4B). This clearly shows that H₄ activation on CD8⁺ cells leads to the release of bioactive IL-16 protein.

**Histamine H₄ Receptor Expression Studies.** Messenger RNA studies using primer pairs selective for human H₁ to H₄ receptor genes supported our pharmacological studies in CD8⁺-enriched cells; a high expression signal was noted for H₄ in all donors tested (n = 3), followed by moderate levels of H₂ and H₁. No evidence at all was noted for H₃ expression, an observation strongly supporting the suggestion that thioperamide exerted its inhibitory actions by antagonizing H₄ (Fig. 5).

The expression of H₄ was further examined in lung tissue and a variety of immune and lung cells. Human lung and tracheal tissue and various human lung cells, such as fibroblasts (NHLF), bronchial epithelial cells (NHBE), bronchial smooth muscle cells (BSMC), and microvascular endothelial cells (HMVEC-L), also expressed mRNA for H₄. In addition, all lung samples clearly showed H₁ expression, whereas H₂ expression was limited to smooth muscle, lung, and trachea (Fig. 6A). As expected, H₄ mRNA expression signals were observed in all immune cell populations investigated. Notably, positively selected CD8⁺ cells showed donor variation (samples from three individuals shown in Fig. 6B), and the mRNA signals found were generally lower compared with CD8⁺ samples obtained by negative selection. Highest expression levels were found in dendritic cells, eosinophils, and tonsil B cells. Lymphocytes showed moderate expression and monocytes only weak expression of H₄ mRNA (Fig. 6, B and C). The data obtained in the human mast cell-like cell line, HMC-1, suggest that mast cells do express H₄ but lack H₃. However, this needs further verification in freshly isolated or in vitro-differentiated mast cells of human origin. Importantly, H₂ mRNA was undetectable in all samples despite a faint signal in lung endothelial cells and immature dendritic cells (Fig. 6, A and C).

**Discussion**

The unique expression profile reported in the first publications on the novel histamine receptor H₄ (Nakamura et al.,

![Fig. 5. Histamine receptor subtype distribution in human CD8⁺ lymphocytes. After enrichment by negative selection, CD8⁺ cells were spun down by centrifugation, and histamine receptor expression was assessed by quantitative RT-PCR using the Lightcycler method, as described under Materials and Methods. Three samples representing cells from different individuals are shown based on normalization of the signals to β2 microglobulin.](image)

![Fig. 6. Histamine receptor mRNA expression in human lung tissue, various lung cell populations, and immune cells. Gene-specific primers were used for amplification (see Materials and Methods for details). As a representative summary of measurements performed in two to seven analogous samples of the respective cell type/tissue investigated, the distribution of H₁ to H₄ mRNA is shown for human total lung, trachea, fibroblasts (NHLF), epithelial cells (NHBE), bronchial smooth muscle cells (BSMC), and microvascular endothelial cells (HMVEC-L). Human umbilical vein endothelial cells (HUVEC) are shown in comparison (A). H₂ and H₄ receptor expression is shown for highly purified CD8⁺ lymphocytes (individual samples from three different donors), CD4⁺, monocytes (CD14), tonsil B cells and the human mast cell line HMC-1 (B), and for immature dendritic cells (iDC), mature dendritic cells (DC), and eosinophils (Eos; C). GAPDH served as a standard, numbers given correspond to defined copy numbers of the corresponding genes investigated. A human brain sample served as a positive control for H₃ and as a negative control for H₁.](image)
TABLE 2
Reported histamine receptor affinity values of histamine ligands used (ranges given in nanomolar)

<table>
<thead>
<tr>
<th>Compound</th>
<th>H4</th>
<th>H2</th>
<th>H3</th>
<th>H1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrilamine</td>
<td>0.4</td>
<td>5200</td>
<td>&gt;10000</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>&gt;10000</td>
<td>800</td>
<td>&gt;10000</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>Thiopropamide</td>
<td>&gt;10000</td>
<td>&gt;10000</td>
<td>25–93</td>
<td>27–519</td>
</tr>
<tr>
<td>Dimaprit</td>
<td>&gt;10000</td>
<td>1100</td>
<td>5–825</td>
<td>377–677</td>
</tr>
<tr>
<td>α-Methylhistamine</td>
<td>&gt;10000</td>
<td>&gt;10000</td>
<td>0.7</td>
<td>146–348</td>
</tr>
<tr>
<td>Clobenpropit</td>
<td>&gt;10000</td>
<td>10000</td>
<td>0.6</td>
<td>7–12</td>
</tr>
</tbody>
</table>

2000; Oda et al., 2000; Liu et al., 2001; Morse et al., 2001; Nguyen et al., 2001; Zhu et al., 2001) immediately suggested that a variety of immunological functions can be expected as a consequence of activation of this receptor subtype. One such function (i.e., the regulation of histamine-triggered IL-16 release from human CD8+ cells) is described in the present article. H2 expression analyses (PCR measurements), and pharmacological studies using selective antagonists, agonists, and inhibitors of signal transduction (pertussis toxin) leave no doubt on the involvement of H4 to the control of IL-16 release in human lymphocytes. A significant role of H2 is also clearly shown, which is in line with similar data reported earlier (Berman et al., 1984). The agonist and antagonist concentrations used are selective for the respective receptor subtype, i.e., cimetidine does not bind to H4 even at concentrations >10 μM (Liu et al., 2001; Morse et al., 2001; Nguyen et al., 2001; Zhu et al., 2001) and thiopropamide does not bind to H1 or H2 in the concentration ranges tested (Arrang et al., 1987). Also, the concentration range in which thiopropamide was efficacious is in accordance with those described previously by others in cellular systems (Oda et al., 2000). For further interpretation, literature-reported histamine receptor-binding data of the tool compounds used in the present study have been summarized in Table 2.

Clearly, H4 and H2 seem to be involved in the control of IL-16 release from human CD8+ T cells to a similar extent. A block of either receptor resulted in a 70 to 75% inhibition of cytokine levels (Fig. 2) and selective inhibition of the H4 signaling by pertussis toxin pretreatment abrogated histamine-induced IL-16 release by approximately 50% (Fig. 3B). Combined inhibition of both H2 and H4, however, did not completely abrogate IL-16 release (data not shown), pointing toward a minor contribution of H1 (10–15%) or to the involvement of an additional histamine receptor not yet known. Taking their opposite adenylyl cyclase coupling into consideration, an additive effect or synergism between H2 (leading to cAMP elevation) and H4 (leading to decrease of cAMP) was not expected. Rather, histamine seems to regulate IL-16 liberation from CD8+ cells by acting in parallel on various of its receptors, an observation made for this biogenic amine in several of its immune functions (Kimata et al., 1996; Sirios et al., 2000; Caron et al., 2001).

The following experimental evidence strongly argues for a direct action of histamine on H4 on CD8+ lymphocytes. First and most importantly, highly purified CD8+ cells (positive MACS selection, purity >98%) released similar amounts of bioactive IL-16 upon stimulation by histamine or the H4 agonist clobenpropit compared with the 85 to 90% pure CD8+-enriched population (Fig. 3; data not shown).

Secondly, mRNA message for H4 was also detectable, although donor variations were more pronounced and expression levels were lower as compared with samples derived from the enriched CD8+ cultures (Figs. 5 and 6B; some data not shown). The reasons for the discrepancy in mRNA levels are unclear. Possible explanations are differences in the purification protocol and the fact that H4 receptor mRNA expression seems to be quickly regulated by cell stimulation (Coge et al., 2001). In addition, significant donor-to-donor variations have also been reported for H4 mRNA expression levels in blood neutrophils (Oda et al., 2000).

Finally, such highly purified CD8+ cells indeed seemed to express H4 receptors, as suggested by flow cytometry analyses. The signal obtained in CD8+ cells after incubation with fluorescently labeled histamine was significantly competed by excess amounts of unlabeled histamine, cimetidine, thiopropamide, and clobenpropit. However, a proper quantitative analyses could not be done, possibly due to fast receptor down-regulation after histamine binding (see supplemental data set). Similarly, Western blot analyses of CD8+ cell lysates showed a reactive band at the expected molecular mass of 43 kDa, but data interpretation was hampered by the lack of specificity of the only commercially available anti-H4 antibody (data not shown).

The presence (Coge et al., 2001; Morse et al., 2001; Zhu et al., 2001) or absence (Oda et al., 2000; Liu et al., 2001) of H4 mRNA in lung tissue, and lung cells were under debate. Our data clearly show that a variety of human primary lung cells express H4. Whether H4 also contributes to the regulation of IL-16 production in lung epithelial cells is currently under investigation. In accordance with a putative role of H4 in inflammatory processes in the lung, several cytokine-modulated regulatory elements have been identified on the H4 gene, and modulation of H4 expression by IL-5, -10, and -13 in immune cells has recently been reported (Coge et al., 2001; Liu et al., 2001).

An important finding of this study is the lack of H4 expression in most of the cell types investigated. This puts many observations made in the past with thiopropamide, previously only known as an H3 antagonist, into a different perspective, since nearly all relevant findings using thiopropamide in the immune system may be attributed to H4 rather that to H3. Among those putatively H4-mediated immune functions of histamine are the inhibition of tumor necrosis factor release from mast cells (Bissonnette, 1996), stimulation of IL-10 in monocytes (Sirios et al., 2000), Ca2+ mobilization in human eosinophils (Raible et al., 1994), and IgE production from IL-4 + anti-CD58/LFA3-antibody stimulated human B cells (Kimata et al., 1996). However, alveolar macrophages, a population not studied here, were reported to express H4 mRNA (Sirios et al., 2000), and therefore a role of H4 in the immune system of the lung cannot be excluded.

The IL-16 protein released following H4 activation obviously was bioactive since migration of CD4+ cells induced by clobenpropit- or histamine-stimulated CD8+ cell supernatants was significantly blocked by preincubation with a neutralizing anti-IL-16 antibody. Furthermore, IL-16 seemed to be the major migration-inducing factor present in CD8+ supernatants following histamine receptor activation. IL-16 neutralization abrogated CD4+ cell migration by more than 60% (histamine) or more than 70% (clobenpropit), respec-
tively (Fig. 4B), an observation that is in accordance with previously published data (Lieberge et al., 1995). Moreover, the addition of a neutralizing anti-MCP-1 antibody was without effect (data not shown).

With regard to inflammation, the suppression of IL-16 release by histamine receptor antagonists may be of therapeutic relevance. Histamine has been discussed as a mediator of asthma for a long time (reviewed in Barnes et al., 1998) and one of the mediators induced by this biogenic amine is IL-16. Indeed, this CD4+ cell chemoattractant was found in lungs of asthma patients following challenge (Cruikshank et al., 1995; Laberge et al., 1995; Krug et al., 2000), and intervention strategies against IL-16 have been successful in experimental animal models mimicking asthma symptoms (Hessel et al., 1998; de Bie et al., 1999). However, cimetidine treatment of mice failed to significantly reduce IL-16 levels in the bronchoalveolar lavage fluid of Ag-challenged mice (de Bie et al., 1998). Since tiotropium has not been investigated in that study and detailed analyses on the regulation of IL-16 release by histamine in the mouse are not available, those data do not necessarily contradict our findings. In addition, the bronchoalveolar fluid of Ag-challenged mice contains few CD8+ cells (F. Gantner and K. B. Bacon, unpublished observation), and IL-16 levels in the plasma have not been investigated.

Based on present knowledge, it is difficult to speculate which anti-histamine approach would be the therapeutically most promising. Selective H4 antagonists became a standard therapy for allergic rhinitis, but their value in asthma-treatment is more than questionable (Van Ganse et al., 1997), although inhibition of bronchoconstriction and smooth muscle proliferation theoretically could be expected. H2-selective antagonists, tremendously successful as anti-ulcerative drugs, were initially considered as being contraindicated in asthma due to possible inhibition of bronchorelaxation. However, no complications have been observed over more than two decades of clinical use of H2 blockers. Rather, a significant therapeutic benefit was seen in a study analyzing asthmatics who received H2 antagonist medication to treat their gastritis (Field and Sutherland, 1998). Those beneficial effects are possibly due to H2 involvement in the regulation of many immunological processes relevant for asthma, such as stimulation of maturation, polarization, and chemokine induction in dendritic cells (Caron et al., 2001), IgE production in B cells (Kimata et al., 1996), IL-5 production in TH2 cells (Poluekova and Khan, 1998), and, finally, IL-16 release (Cruikshank et al., 1995; this study).

Neither direct bronchodilatory nor anti-inflammatory effects may be expected from compounds targeting H3 selectively since this receptor neither seems to be present in human lung cells nor in most leukocytes (this study). Nevertheless, the development of H3 agonists is considered for asthma treatment, primarily based on the observation that H3 seems to be involved in the regulation of cholinergic nerves in the human airways and in the release of neuropeptides from airway sensory nerves (Ichinose and Barnes, 1989). Based on its restricted expression profile and its crucial role in the regulation of IL-16 release, the development of H3-selective antagonist may be another promising anti-asthmatic approach. However, due to the limited knowledge of H3 biology a final conclusion cannot yet be drawn.

Acknowledgments

We thank Dr. S. Watanabe for help with the RNA isolation and cDNA preparation. Further thanks go to Drs. L. Sanchez-Pescador, P. Hermann, and N. Liu and to K. Nakashima and K. Takeshita for experimental support and helpful discussions.

References


**Address correspondence to:** Dr. F. Gantner, Bayer Yakuhin, Ltd., Research Center Kyoto, TRA Asthma, 6-5-1-3 Kunimidai, Kizu-cho, Soraku-gun, 619-0216 Kyoto, Japan. E-mail: florian.gantner.fg@bayer.co.jp