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Functional Expression of H4 Histamine Receptor in Human Natural Killer Cells, Monocytes, and Dendritic Cells

Bassam B. Damaj, Cecilia Barrena Becerra, Henry J. Esber, Ying Wen, and Azzam A. Maghazachi

We describe here the protein expression of H4 histamine receptor in cells of the innate immune system, which include NK cells, monocytes, and dendritic cells (DCs). Anti-H4R specifically stained permeabilized NK cells, THP-1 clone 15 monocytes, and DCs. This binding was inhibited by incubating anti-H4R Ab with its corresponding peptide. Histamine induced NK cells, THP-1 clone 15 cells, and DCs chemotaxis with high affinity. The ED50 chemotactic effect was 5 nM, 6.8 nM, and 2.7 nM for NK cells, THP-1 clone 15 cells, and DCs, respectively. Thioperamide, an H3R/H4R antagonist, inhibited histamine-induced chemotaxis in all these cells. However, histamine failed to induce the mobilization of [Ca2+]i in NK cells and THP-1 clone 15 cells, but it induced calcium fluxes in DCs. Using a new method of detecting NK cell-mediated cytolysis, it was observed that NK cells efficiently lysed K562 target cells and that histamine did not affect this NK cell activity. In summary, this is the first demonstration of the protein expression of H4 receptor in NK cells. Also, the results of the chemotactic effects of histamine on NK cells and THP-1 cells are novel. These results may shed some light on the colocalization of cells of innate immune arm at sites of inflammation. They are also important for developing drugs that target H4R for the treatment of various disorders, such as autoimmune and immunodeficient diseases. The Journal of Immunology, 2007, 179: 7907–7915.

Histamine is a dibasic vasoactive amine that is located in most body tissues but is highly concentrated in the lungs, skin, and gastrointestinal tract. It is produced by the decarboxylation of histidine by histidine decarboxylase and is considered as one of the most important mediators of allergy and inflammation. It is released from mast cells after the interaction of allergens with surface IgE. It is also produced during acute asthmatic episodes and is associated with asthmatic attacks (1). Hence, histamine exerts multiple effects causing allergic symptoms, such as smooth muscle contraction, vasodilatation, peptic ulcers, and intestinal bleeding (2–4). In the CNS, histamine is involved in regulating drinking, body temperature, and in the control of blood pressure and the perception of pain (5, 6).

Histamine effects are mediated through four pharmacologically distinct subtypes of receptors. These are the H1R, H2R, H3R, and H4R, which are all members of the G-protein-coupled receptors (GPCRs) family. H1R is distributed in the brain, most smooth muscle cells, endothelial cells, adrenal medulla, and heart. It plays roles in smooth muscle contraction, stimulation of NO formation, endothelial cell contraction, and increasing vascular permeability. H1 receptor preferentially couples to the Gαq/11 family of G-proteins and causes mobilization of intracellular Ca2+, activates the phosphorylation of protein kinase C, and enhances the activation of MEK, ERK, and NF-κB (7).

The H2R causes cAMP accumulation and inhibition of IL-12 production (8) through coupling to Gαq (9). A third subtype of histamine receptor, human H3R, was identified as a presynaptic autoreceptor on histamine neurons in the brain. Subsequently, the H3 receptor has been cloned (10) and was shown to be a presynaptic heteroreceptor in nonhistamine-containing neurons in both the central and peripheral nervous systems (11). H3R is primarily expressed in the brain. Several studies using H3 selective agonists revealed that H3 receptor couples to pertussis toxin-sensitive Gq protein, and it induces the mobilization of intracellular calcium, activates the MAPKs, but inhibits cAMP production (12).

A fourth GPCR, named H4R, has been cloned and characterized (13, 14). It has ~37% homology to H3R, but unlike H3R it has a distinct tissue distribution and is localized in the peripheral blood leukocytes, spleen, thymus, and colon (13–15). Activation of H4 receptors by histamine primed eosinophils for increased chemoattractive responses to eotaxin, (16). The H4R is expressed in mononuclear cells, CD4+ and CD8+ cells, but its expression in CD14+ monocytes was hardly detectable (14). Recent work by Dijkstra et al. showed that H4R is expressed on the surface of monocytes and that such expression is up-regulated by IFN-γ (17). Morse et al. (13) observed expression of this receptor in human peripheral T cells as well as in monocytes particularly those incubated with anti-IL-10. Hence, culture conditions might affect the expression of this receptor on hemopoietic cells.

NK cells perform several important functions. Among them is the regulation of the adaptive immune response by secreting cytokines such as IFN-γ, shaping the innate immune system by interacting with dendritic cells (DCs), defending against viral infection, and lysing and destroying tumor cells (18, 19). Human NK cells comprise ~10–15% of total blood lymphoid cells. Under physiological conditions, NK cells are not normally found in lymphoid and nonlymphoid tissues, except for the spleen. IL-2 activated NK cells have been used for the treatment of cancer and...
other diseases (18). They express a variety of GPCRs, such as receptors for chemokines (20), and lysophosphatidyls (21). These receptors are important in recruitment of these cells toward the sites of inflammation and viral infection. They are also vital in guiding NK cells toward the sites of tumor growth where NK cells, particularly activated cells, are involved in recognizing and eradicating abnormally proliferating cells.

DCs subsets differing in phenotype, function, activation state, and location have been identified, but their relationship and developmental origins are still a matter of debate. The wide variety of cytokines/growth factors and conditions that have been shown to influence DC differentiation and activity further highlights the extreme heterogeneity of DC populations, reflecting the diverse phenotype, functions, and ontogeny displayed by these cells (22). DCs endowed with a high migratory and allostimulatory capacity can be rapidly generated by a single step procedure in the presence of GM-CSF and type I IFNs (23), or GM-CSF plus IL-4 (24).

In this report, we investigated in detail the functional expression of histamine receptors and, in particular, H4R in cells of the human innate immune system which include NK cells, monocytes and DCs.

Materials and Methods

Generation of THP-1 clone 15 cells

By limiting dilution, cells were plated in fifteen 96-well plates at these concentrations: 3 cells/well, 1 cell/well, and 0.3 cell/well. Five plates per cell concentration were prepared. The wells were monitored until they were confluent. Supernatants were collected from these wells after 24 h of stimulation with LPS, and were tested for TNF-α, IL-1β, IL-6, and IL-10 secretion. This was done until the number of clones was narrowed to five. THP-1 clone 15 cells secreted high levels of TNF-α and IL-1β. Other cell lines used in this study were the human promyelomonocytic cell line U937, the human B cell lymphoma DAUDI, the human T cell leukemia MOLT-4, and the human T cell leukemia cell line SR (American Type Cell Collection).

Isolation of monocytes derived DCs

The procedure of isolating monocytes derived human DCs has been previously described (24). In brief, PBMCs were isolated from the buffy coats of human volunteers (SD Blood Bank) by Ficoll-Paque gradient centrifugation (GE HealthCare/Amersham), incubated (1 × 10⁵ cells/ml) in 100 mm petri dishes at 37°C for 2 h, and the adherent cells were collected. These cells were cultured at 5 × 10⁵/ml with 6 ng/ml IL-4 (eBioscience) and 25 ng/ml rhGM-CSF (PeproTech) for 5 days at 37°C to generate the immature DCs. Cells were fed after 2.5 days with fresh medium. Mature DCs (mDCs) were generated by incubating immature DCs for an additional 2 days with 1 µg/ml LPS (Sigma-Aldrich).

Preparation of IL-2 activated NK cells

PBMCs were isolated from buffy coats by Ficoll-Paque gradient centrifugation. NK cells were separated from PBMCs by the use of nylon wool columns (Polysciences). Briefly, PBMCs were incubated over nylon wool column for 1 h at 37°C, and the nonadherent cells were rinsed from the nylon wool column by RPMI complete medium. These cells contained ~90–95% T cells and 5–10% NK cells. They were cultured with 500 U/ml rHL-2. After ~7–8 days culture, the cells were collected and T cells were depleted by incubation with anti-CD3 Ab coated Dynal beads (Invitrogen Life Technologies). The purity of NK cells after this isolation is typically between 95 and 99% (CD16+).

NK cell lysis assay

Here we describe a new method for detecting NK cell lysis. The NK-sensitive K562 cells were labeled with 5 µg/ml calcein AM (TelFlabs) for 45 min. The cells were pelleted by centrifugation and resuspended in RPMI. To obtain total lysis, these cells were incubated in 96-well plates with 0.05% Triton X, whereas they were incubated with medium alone to obtain total viability. In other cultures, calcein-AM-labeled K562 were incubated with activated NK cells at different NK target:cell ratios (designated as E:T cell ratio). After 4-h incubation, the plates were centrifuged, supernatants were removed and replaced with PBS. Fluorescence units (FU) were measured in Cytofluor plate reader. The percentage of cytotoxicity was calculated according to the following formula: % viability = FU of targets incubated with IL-2-activated NK cells (experimental) minus FU of targets incubated with Triton X, divided by FU of targets incubated in medium only (total viability) minus FU of targets incubated with Triton X (total lysis). Percent cytotoxicity was then calculated as 100 minus percent viability.

In vitro chemotaxis assay

NK, DCs, and THP-1 cell migration was quantitated by blind wells Boyden chamber technique. THP-1 cells were suspended at 0.5 × 10⁶ cells/ml in RPMI 1640 plus 0.5% BSA. The cell concentration for IL-2 activated NK and DCs were 1 × 10⁵ cells/ml and 0.5 × 10⁵ cells/ml, respectively. To determine the antagonists effects, the cells were incubated with various concentrations of antagonists (thioperaamide, cimetidine, or diphenhydramine; all from Sigma-Aldrich) at 37°C for 30 min, washed, and then placed in the top wells of Boyden chambers. The bottom wells of the chambers were loaded with different concentrations of histamine. Eight-micrometer pore polycarbonate filters were placed between the top wells and the bottom wells. The chambers were incubated for 2 h at 37°C in 5% CO₂. After incubation, the cells in the top wells were removed and the filters were fixed with methanol and stained with Giemsa stain. Cell movement was measured by counting the total number of cells in the filters.

For 96-well chemotactic plates, THP-1 cells were washed twice in RPMI with 0.1% BSA and starved for 2 h in RPMI containing 0.1% BSA at 37°C in 5% CO₂. After starving, the cells were resuspended at 1 × 10⁶ cells/ml in RPMI containing 0.1% BSA and stained with 1 µg/ml calcein-AM for 30 min at 37°C in 5% CO₂. Stained cells were washed twice with PBS and resuspended at 1 × 10⁵ cells/ml in RPMI 0.1% BSA. Approximately 25 µl of the cells were added into the upper chambers of the 96-well NeuroProbe plates with 8 µm pore size filter (NeuroProbe). Chemotactic factors were diluted to the indicated concentrations in RPMI 0.1% BSA, and 30 µl of the mixture was added into the lower wells of the 96-well Neuroprobe plates. After 2 h at 37°C in 5% CO₂, the cells remaining in the upper chambers were removed and rinsed with PBS once. Migrated cells in the lower surface of the filters and low chamber were determined by measuring the fluorescence at 450−530 by Cytofluor plate reader.

Immunoblot analysis

Cells were collected in tubes and washed twice with 1 ml of PBS. For cells collected in one T150 flask, 0.5 ml of lysis buffer containing 145 mM sodium chloride, 1% Triton X-100, 10 mM EDTA, 2 mM PMSF, 1% protease mixture inhibitor tablet (Sigma-Aldrich) was added, and then incubated for 30 min at 4°C with rocking. The cells were spun down at 13,000 rpm for 10 min at 4°C. The supernatants were removed and protein levels were quantitated using BCA assay kit from Novagen (EMD Chemicals). A 30 µg total lysate per sample was used. Reducing agent and LDS sample buffer (Invitrogen Life Technologies) were added and the samples were boiled at 80°C for 10 min. Using Invitrogen 4−12% Bis-Tris gel, the protein was loaded and run at 120 volts for 1 h. The gel was transferred to a polyvinylidene difluoride membrane presoaked in methanol for 1 h at 100 volts. The membranes were blocked with 5% milk in TRBS at room temperature for 1 h. The milk was removed and 10 ml of primary Ab solution prepared in milk/TBS with Tween was added. Abs used were rabbit anti-human H4R (Alpha Diagnostics International) used at 1:500 or 1:1000 dilutions and rabbit IgG (Sigma-Aldrich) used as a control. The gels were washed twice in TBS and then incubated for 30 min at 4°C in 5% milk; all from Sigma-Aldrich) at 37°C for 30 min, washed, and then placed in the top wells of the chambers. The bottom wells of the chambers were loaded with different concentrations of histamine. Eight-micrometer pore polycarbonate filters were placed between the top wells and the bottom wells. The chambers were incubated for 2 h at 37°C in 5% CO₂. After incubation, the cells in the top wells were removed and the filters were fixed with methanol and stained with Giemsa stain. Cell movement was measured by counting the total number of cells in the filters.

For 96-well chemotactic plates, THP-1 cells were washed twice in RPMI with 0.1% BSA and starved for 2 h in RPMI containing 0.1% BSA at 37°C in 5% CO₂. After starving, the cells were resuspended at 1 × 10⁶ cells/ml in RPMI containing 0.1% BSA and stained with 1 µg/ml calcein-AM for 30 min at 37°C in 5% CO₂. Stained cells were washed twice with PBS and resuspended at 1 × 10⁵ cells/ml in RPMI 0.1% BSA. Approximately 25 µl of the cells were added into the upper chambers of the 96-well NeuroProbe plates with 8 µm pore size filter (NeuroProbe). Chemotactic factors were diluted to the indicated concentrations in RPMI 0.1% BSA, and 30 µl of the mixture was added into the lower wells of the 96-well Neuroprobe plates. After 2 h at 37°C in 5% CO₂, the cells remaining in the upper chambers were removed and rinsed with PBS once. Migrated cells in the lower surface of the filters and low chamber were determined by measuring the fluorescence at 450−530 by Cytofluor plate reader.

Mobilization of intracellular calcium assay

Cells were resuspended at 1 × 10⁶/ml in Hanks’ buffer (10 mM HEPES, 1.6 mM calcium chloride, HBSS, and pH 7.4) and loaded with 5 µM Indo-1 AM (Telfluor) at 37°C for 45 min. The cells were washed twice at room temperature and resuspended at 5 × 10⁴/2 ml in the cuvettes. Several concentrations of histamine or 20 µM lonomycin (Iono; Sigma-Aldrich) were used. For THP-1 clone 15 cells, 10 nM of MCF-1 (PeproTech) was used as a control. In certain experiments, DCs were treated with 10 nM thimerosal for 30 min, washed, and then examined for the release of intracellular calcium. Continuous monitoring of the fluorescence ratio (350/405 nm) was performed using SLM.AMINCO.
Staining with anti-H1, H2, H3, or H4 Ab and flow cytometric analysis

Cells were washed twice with 1× sterile DPBS and normalized to 0.5 × 10^6 cells/condition, distributed in each Eppendorf tube, and fixed with 1% paraformaldehyde for 10 min at room temperature. After 10 min, the cells were washed once with PBS containing 1% BSA. They were resuspended into 100 μl of PBS/1% BSA/0.1% saponin solution plus first Ab H1R, H2R, H3R, or H4R (Alpha Diagnostics International) used at 1 μg/ml. Rabbit IgG was used at the same concentration. The cells were incubated for 30 min at room temperature. They were washed twice with 1 ml of PBS/1% BSA/0.1% saponin solution and centrifuged at 2500 rpm for 5 min. The cells were then resuspended in 100 μl of PBS/1% BSA/0.1% saponin solution plus secondary Ab F(ab’)2 anti-rabbit FITC-conjugated from Calbiochem. They were incubated for 30 min at room temperature in the dark. After this, they were washed twice with 1 ml of FACS buffer. After the last wash, the cells were resuspended in 200 μl FACS buffer and analyzed using Beckman Coulter XL-MCL II. To investigate the up-regulation of H4R after cytokine stimulation, THP-1 wild type (WT) cells (1 × 10^7/ml) were incubated with 100 ng/ml TNF-α, IL-1β, or their combination for 24 or 48 h. The cells were washed and then examined for the expression of H4R.

Significant values were generated using the Student’s t test. The ED_{50} and IC_{50} were calculated using the one-site competition of nonlinear regression (Prism).

Results
Expression of histamine receptors in human IL-2-activated NK cells

The expression of receptors for histamine in IL-2-activated NK cells was determined by flow cytometric analysis. Fig. 1A demonstrates that 93% of these cells were positive for H1 receptor, and ~63% were positive for H4 receptors. In contrast, these cells did not express H2 or H3 receptors (Fig. 1B). To demonstrate the specificity of the expression of H4 receptor, IL-2-activated NK cells were labeled with anti-H4 in the presence of two different concentrations of blocking peptide (BP) against this receptor. Results in Fig. 1C indicate that 100 ng/ml H4R blocking peptide completely abrogated the binding of anti-H4R Ab to NK cells, whereas 1 ng/ml BP inhibited ~60% of the binding. These results are in line with those described by Dijkstra et al. (17), who showed that specific staining of human monocyes by anti-H4R is inhibited after binding the Ab with the respective H4R peptide.

Effect of histamine on IL-2-activated NK cell chemotaxis, calcium mobilization, and cytosis

Next, we examined the ability of histamine to induce the chemotaxis of IL-2-activated NK cells. Results in Fig. 2A demonstrate that 10, 1, 0.1, or 0.01 μM of histamine significantly induced the chemotaxis of these cells in a typical bell-shape response (p values of 0.04, 0.007, 0.0001, and 0.04, respectively). The ED_{50} effect of histamine was calculated at 5.18 × 10^{-8} M (Fig. 2B). Thioperamide (H3/H4 antagonist) inhibited histamine induced IL-2-activated NK cell migration with an IC_{50} of 1.07 × 10^{-8} M (Fig. 2C).

In contrast to its effect on the chemotaxis of IL-2-activated NK cells, histamine used up to 10 μM failed to induce the mobilization of intracellular calcium in these cells, whereas Iono, which was used as a positive control, robustly induced the mobilization of intracellular calcium in these cells (Fig. 2D). Similarly, different concentrations of histamine failed to affect NK cell cytotoxicity against K562 target cells. IL-2-activated NK cells efficiently lysed K562 when used at 1:5, 1:10, and 1:20 E:T cell ratios. Addition of 1 nM, 10 nM, 100 nM, 1 μM, 10 μM, or 100 μM histamine to the cultures of NK plus K652 cells did not affect NK cell mediated cytotoxicity (Fig. 2E).

Expression of the H4 receptor in monocytes

We next examined the expression of histamine receptors in other cells of the innate immune system. We have chosen the human monocytic cell line THP-1 that is widely used to examine the biological activities of monocytes (25). Three different cell concentrations and two different concentrations of anti-H4R Ab were used to stain these cells. Results in Fig. 3 show that 2 μg/ml rabbit anti-H4R stained 74.9%, 87.5%, and 50.3% of 3 × 10^6, 6 × 10^5, and 7 × 10^6 THP-1 clone 15 cells, whereas 1 μg/ml Ab stained 49.9%, 64.6%, and 29.1% of these cells (Fig. 3A–C, respectively). The binding of anti-H4 receptor to THP-1 clone 15 cells was completely abrogated by incubating this Ab with 100 ng/ml or 1 ng/ml H4R blocking peptide (Fig. 3D). The expression of H4R was also examined by immunoblot analysis. The results indicate that THP-1 clone 15 cells and the monocytic cell line U937 cells expressed the 45 kDa band representing H4R. PBMCs expressed this band with a much lower intensity than the cell lines, perhaps due to the small number of cells in this preparation that express this receptor. In contrast, the B cell lymphoma DAUDI and two T cell leukemia cells lines, MOLT-4 and SR, failed to express this receptor (Fig. 3E).

Histamine failed to induce significant chemotaxis of THP-1 WT cells. Only a very high concentration, i.e., 1 μM of histamine, induced the in vitro chemotaxis of these cells and this activity did not fit with the typical bell-shaped chemotaxis response (not shown). However, THP-1 clone 15 cells responded to histamine with a bell-shape dose-response curve and an ED_{50} of ~6.8 nM (Fig. 4A). Two different chemotaxis assays were used to evaluate the effect of histamine on THP-1 clone 15-induced chemotaxis.
Both blind well Boyden chambers (Fig. 4A) and 96-well chemotaxis plates (Fig. 4B) supported histamine-induced chemotaxis of these cells.

We entertained the possibility that THP-1 clone 15 cells might express the H4R receptor, because they secrete high levels of TNF-α and IL-1β, whereas THP-1 WT cells do not. Hence, we incubated THP-1 WT cells with 100 ng/ml TNF-α, IL-1β, or their combination for 24 or 48 h and then examined the expression of H4R on the surface of these cells. Although there was some up-regulation of H4R after incubating THP-1 WT cells with TNF-α for 48 h, this was not impressive and resulted in only ~14% of the cells expressing the H4R. In contrast, IL-1β failed to induce the expression of this receptor in THP-1 WT cells. Also, there were no synergy or additive effects of these two cytokines on the expression of H4R (Table 1).

Blocking histamine receptors with specific antagonists was then used to examine the contribution of various histamine receptors in mediating histamine-induced chemotaxis of these cells. The H3/H4 antagonist Thioperamide inhibited the chemotaxis of THP-1 clone 15 cells induced by 100 nM histamine (similar results were obtained with 10 nM histamine). In two separate experiments, the IC₅₀ for the effect of this antagonist were very comparable, i.e., 78.31 nM and 114.2 nM (Fig. 5A). In contrast, neither the H1R antagonist Diphenhydramine nor the H2R antagonist Cimetidine affected histamine-induced THP-1 clone 15 cell chemotaxis even when these antagonists were used at 1 mM concentration (Fig. 5B), confirming that these cells do not express H1 and H2 receptors (data not shown).

Next, the effect of histamine on the mobilization of intracellular calcium in THP-1 clone 15 cells was examined. Results in Fig. 5C show that histamine used at 100 and 1000 nM concentrations failed to induce calcium fluxes in these cells. In contrast, 10 nM of MCP-1, which was used as a positive control, induced the mobilization of intracellular calcium in THP-1 clone 15 cells (Fig. 5D).

Effect of histamine on monocytes derived mDCs chemotaxis and intracellular calcium mobilization

Using flow cytometric analysis, it was observed that human monocytes derived mDCs express H4 receptors. In one donor, >80% of these cells expressed H4 receptor (Fig. 6A). Approximately 65% of monocytes derived mDCs, generated from another donor, expressed this receptor (Fig. 6B). The binding of anti-H4R to permeabilized...
mDCs was found to be specific, blocking peptide for anti-H4R used at 100 ng/ml and to a lesser extent 1 ng/ml inhibited the binding of anti-H4R to human monocytes derived mDCs (Fig. 6B). The ability of histamine to induce the chemotaxis of these cells was next examined. Histamine induced their chemotaxis with an ED50 of 2.7 × 10⁻⁹ M (Fig. 6C). Thioperamide inhibited the chemotaxis induced by histamine, with an IC50 of 4.129 × 10⁻¹⁰ M for donor one and 4.349 × 10⁻¹¹ M for donor 2 (Fig. 6D and E, respectively).

Histamine also induced the mobilization of intracellular Ca²⁺ concentration ([Ca²⁺]i) in human monocytes derived mDCs. In the experiment shown in Fig. 7A, a 100 nM concentration was the lowest dose that induced this mobilization. This concentration, however, did not desensitize the calcium flux response as addition of 1 μM, after the addition of 100 nM also resulted in a calcium flux response (Fig. 7A). Addition of another 1 μM afterward did not induce the mobilization of [Ca²⁺]i, indicating that the 1 μM concentration desensitized the mobilization of intracellular calcium induced by 1 μM histamine. Results in Fig. 7B indicate that addition of 1 μM did not desensitize the response to 10 μM of histamine, but 10 μM concentration did.

To correlate the effect of histamine with the expression of H4R in monocytes derived mDCs, we incubated these cells with 10 μM concentration of Thioperamide for 30 min before examining these cells in the calcium mobilization assay. Results in Fig. 7C demonstrate that treatment with Thioperamide inhibited ~50% of the calcium flux response induced by 10 μM histamine. Dijkstra et al. showed similar results in human monocytes where the H4 antagonist inhibited ~50% of the calcium flux response induced by the H4R agonists clobenpropit and 4-methylhistamine (17).

Discussion
Histamine is a pleotropic inflammatory mediator stored and secreted by inflammatory cells such as mast cells and basophils. It exerts its activities by binding to H1, H2, H3, and H4 receptors.
These receptors are differentially expressed in various organs, tissues, and cell types. Histamine receptors are important targets for treatment of various diseases, such as asthma, heart disease, peptic ulcer, and Alzheimer’s disease, among many others (15). All histamine receptors are seven transmembrane spanning domain receptors, also known as GPCRs. H4R is the latest histamine receptor that has been cloned and characterized. In this report, we describe the functional expression of H4 receptor in cells of the innate immune system NK cells, monocytes, and DCs.

Messenger RNA of H4R was shown to be expressed in NK cells (13), however, neither the protein expression nor the function of H4R in NK cells is known. Here we demonstrate that H4R protein is expressed in IL-2-activated NK cells and that this receptor mediates these cell chemotaxis induced by histamine. IL-2-activated NK cells expressed H1R and H4R but not H2R or H3R, and therefore the effect of Thioperamide, an antagonist of H3R and H4R, is specifically exerted on H4R, since NK cells lack the expression of H3 receptor. Histamine induced NK cell chemotaxis with an ED_{50} of 5.2 nM, indicating a high affinity binding to H4R in these cells. Consistent with this high affinity binding, we observed that Thioperamide inhibited histamine activity with an IC_{50} of ~10 nM. Based on this information, it can be concluded that in addition to chemokines and lysophospholipids, which are chemoattractant for NK cells (21), histamine is a potent chemotactic factor for these cells. Hence, it would not be surprising to observe that IL-2-activated NK cells may accumulate at sites that are devoid of chemokines and lysophospholipids but are rich in histamine secretion.

It was previously shown that histamine protects NK cells mediated cytotoxicity from the inhibitory effect of reactive oxygen species (26). The mechanism of this protection may be due to the ability of histamine to restore the expression of NKp46 and NKG2D, which are down-regulated upon the interaction of NK cells with target cells. The restoration of these molecules allows NK cells to recognize and lyse target cells more efficiently.

Table I. *Up-regulation of H4R on the surface of THP-1 after cytokine stimulation*

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>24 h</th>
<th>48 h</th>
</tr>
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<tbody>
<tr>
<td>TNF-α</td>
<td>1.3%</td>
<td>14.4%</td>
</tr>
<tr>
<td>IL-1β</td>
<td>1%</td>
<td>1%</td>
</tr>
<tr>
<td>TNF-α plus IL-1β</td>
<td>1%</td>
<td>13.4%</td>
</tr>
<tr>
<td>Cells alone</td>
<td>1.6%</td>
<td>1%</td>
</tr>
</tbody>
</table>

*Wild-type THP-1 cells were incubated with 100 ng/ml TNF-α, IL-1β, or their combination for 24 or 48 h. The expression of H4R was then examined by flow cytometry. Shown are the numbers of positive cells after subtracting the background. Cells alone indicate THP-1 cells that were incubated with media only. One of three experiments performed.*
regard, it is interesting that TNF-α might induce the expression of H4 receptor in these cells. In this regard, they are comparable, if not better, than those obtained using the radioisotopes. The results obtained are highly reproducible and incubating THP-1 WT cells with TNF-α induced the expression of H4 receptor on only a small number of these cells. It is plausible that other cytokines secreted by these cells may contribute to the expression of this receptor. Dijkstra et al. (17) showed that IFN-γ induced the H4R on the surface of monocytes. We did not add IFN-γ in our assay because THP-1 clone 15 cells do not secrete this cytokine.

Depending on the cell number and on the concentration of anti-H4R used, between 50 and 88% of THP-1 clone 15 cells expressed H4 receptor, and this binding was inhibited by incubating the Ab with its corresponding blocking peptide, indicating the specificity of the staining procedure. The expression of this receptor on THP-1 clone 15 cells was also confirmed by immunoblot analysis. Histamine induced the chemotaxis of these cells, an activity that was inhibited by Thioperamide but not by the H1 receptor antagonist Diphenhydramine or the H2 antagonist Cimetidine. Finally, histamine failed to induce the mobilization of [Ca²⁺]i in these cells, which is similar to what was observed in NK cells. This conflicts with the results observed in human monocytes in which histamine induces calcium mobilization through H4R (17).

In contrast to NK cells and monocytes, the expression of histamine receptors in DCs was examined by many investigators. Mazzi et al. reported that activated mast cells releasing histamine affected the T cell polarizing capability of DCs (30). Hence, DCs exposed to histamine secreted by activated mast cells reduced Ag-presenting activity and IL-1β production. It remains to be seen whether H4R mediates this activity. A recent study shows that blockade of H4 receptor on DCs resulted in decreased cytokine and chemokine production of Th1 type (1).

In addition, Jawdat et al. (31) reported that mast cells secreting histamine induce the recruitment of Langerhans cells into the peripheral lymph nodes, but the nature of the histamine receptor was not examined in this study. A more direct involvement of H4 receptor in DCs chemotaxis was provided by Gutzmer et al., who showed that DCs migrate in vitro toward histamine concentration gradients.
and that this activity is mimicked by H4R agonist (32). Furthermore, it was shown that CCL-16 induces eosinophil chemotaxis by binding to H4 receptor (33). These results provide new insights on the chemotactic activity of chemokines which can bind and activate receptors other than chemokine receptors.

Here, we described the specific expression of H4R protein in human DCs as determined by flow cytometric analysis. We also showed that histamine induced the chemotaxis of these cells with high potency (ED50 = 2.7 nM) and that Thioperamide inhibited histamine-induced DC chemotaxis with an IC50 of between 0.4 and 0.04 nM. In contrast to NK cells and monocytes, histamine induced the mobilization of [Ca2+]i in human DCs. This activity was desensitized by a second addition of histamine, indicating the selectivity of histamine-induced [Ca2+]i in these cells. The reasons why histamine induced the mobilization of intracellular calcium in DCs but not in NK cells or in monocytes are not clear at the present time. What is important, however, is the ability of histamine to recruit both DCs and NK cells. A number of studies highlighted the importance of the interaction between NK cells and DCs resulting in the regulation of DC maturation as well as NK activation (19). The interaction between NK and DCs is bidirectional and involves cell-to-cell contact. DCs activate NK cells by enhancing their proliferation, cytotoxic activity, and IFN-γ production. In turn, activated NK cells provide either maturation signals for DCs or induce their death by direct killing. Our results indicate that in addition to other factors, histamine may play an important role in bringing these cells into the sites of inflammation and, perhaps, induces their cognate interaction.

Our results demonstrate different efficacies for histamine and Thioperamide in human IL-2-activated NK, THP-1 clone 15, and monocytes derived DCs vs those reported in human eosinophils. For example, Buckland et al. (16) reported that between 0.004 and 2 μM concentrations of histamine induced shape change in human eosinophils, whereas Ling et al. (34) showed that 0.01–30 μM histamine exerted similar effect. Also between 0.01 and 10 μM concentrations of histamine induced the chemotaxis of human eosinophils (34). Furthermore, the activity of 1 μM histamine was inhibited by two different IC50 effects of Thioperamide depending on the assay examined. Thioperamide inhibited histamine-induced eosinophil shape change with an IC50 of 1.4 μM, whereas less than half, an IC50 of 519 nM, inhibited histamine-induced eosinophil chemotaxis (34). In another study, even a higher concentration, i.e., 10 μM of Thioperamide abolished histamine-induced eosinophil shape change (16). In our hands, histamine-induced IL-2-activated NK cell chemotaxis was inhibited by Thioperamide with an IC50 of 10 nM, whereas the same antagonist inhibited histamine-induced THP-1 clone 15 cell chemotaxis with IC50 of 78.3 and 114.2 nM. However, Thioperamide was most potent against...
histamine-induced monocytes derived DC chemotaxis where it inhibited this response with IC50s of 0.4 and 0.04 nM. In contrast, the 10 µM concentration only partially inhibited histamine-induced monocytes derived DC calcium mobilization. Collectively, these results indicate that histamine and/or ThIOperamide may have differential potencies on different cell types in different biological assays.

In summary, we described the protein expression of H4 receptor in cells of the innate immunity, which include human NK cells, monocytes and DCs. We also demonstrated that histamine is a robust chemotactic factor for these cells. Our results may explain how these cells might localize at sites of inflammation, particularly those enriched with histamine. These results may also be of significant importance in developing drugs that target H4 receptor for the treatment of autoimmunity and other diseases.

Disclosures
The authors have no financial conflict of interest.

References

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