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Human basophil chemotaxis and activation are regulated via the histamine H4 receptor

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Abstract

Background:

IgE-mediated cross-linking of FcεRI results in the release of mediators stored in basophil granules, such as histamine and proteases, and in the *de novo* synthesis of sulfidoleukotrienes.

Objective:

In this study we investigated the role of the histamine receptors, in particular that of the histamine H4 receptor (H4R), in modulating human basophil function.

Methods:

The mRNA expression of the histamine receptors was measured by real-time PCR. Migration of basophils was assessed by using the modified Boyden chamber technique. The expression levels of CD63 and CD203c on the cell surface and the sulfidoleukotriene release were determined by flow cytometry and ELISA, respectively.

Results:

We could show that highly purified basophils express the H1R, H2R and H4R but not the H3R mRNA. Human basophils expressed higher H4R mRNA levels as compared to the expression levels of the H1R ($p < 0.01$). Histamine and the H4R-agonist ST-1006 initiated active migration of basophils ($p < 0.001$). A significant reduction of FcεRI cross-linking-mediated surface expression of CD63 and CD203c was observed on basophils after pre-incubation with histamine or the specific H4R-agonist ST-1006 ($p < 0.01$).

The synthesis and release of sulfidoleukotrienes from basophils after activation with different stimuli, by FcεRI cross-linking or by stimulation with hymenoptera venom allergens were significantly reduced by histamine or the H4R-agonist ST-1006 ($p < 0.05-0.01$).

Conclusion:

These data imply that the H4R regulates IgE-dependent processes in human basophils and provides a novel function of the H4R preventing an overwhelming immune reaction by engagement of a negative feedback loop.

Key words:

Human basophils, histamine H4 receptor, immuno-silencing, sulfidoleukotrienes

Abbreviations used:

FcεRI: High-affinity IgE-receptor

FMLP: Formyl-Methionyl-Leucyl-Phenylalanine,

HXR: Histamine X receptor

PAF: Platelet-activating-factor.

Introduction

Basophils, first described by Paul Ehrlich in 1879, circulate in the peripheral blood at relatively low levels and migrate to sites of inflammation. Despite the fact that basophils derive from a distinct cell lineage, they show phenotypical and functional analogies to tissue resident mast cells. Both cell types abundantly express the high-affinity IgE-receptor (FcεRI) on their cell surfaces and were outlined as important effector cells in allergic diseases (1-3).

It has been shown that human basophils, compared to mast cells, represent a more accessible source to examine allergic reactions. The *in vitro* allergy diagnostics are based on two different basophil activation signals: First, the expression of activation markers on the cell surface of basophils, in particular of the markers CD63 and CD203c which are up-regulated during anaphylactic degranulation (4); second, the release of a broad array of mediators of allergic inflammation, either preformed and stored in granules such as histamine and proteases or newly synthesized and immediately released such as the phospholipid metabolite sulfidoleukotriene or newly formed but slowly secreted mediators such as cytokines or chemokines (1). Histamine is the most prominent mediator mainly released from basophils and mast cells during activation. Histamine orchestrates the inflammatory and allergic response by triggering four pharmacologically distinct histamine receptors which are differentially expressed on immune cells that are involved in the course of

allergic reactions. An increasing body of literature describes the role of the histamine receptors, in particular that of the newest member the H4R, which is predominantly expressed on cells of the hematopoietic lineage (5-7). Studies from different research groups provided evidence for the functional expression of the H4R on mast cells (8), monocytes (9), eosinophils (10), T-cells (11, 12) or keratinocytes (13). In consequence, it has been shown that the H4R triggers calcium mobilisation, actin polymerisation and cell shape change, thereby inducing chemotaxis and modulating cytokine and chemokine release (8, 14-16)

IgE-mediated secretion of histamine from basophils and mast cells is a hallmark of allergic reaction. Therefore research in allergic diseases is mainly focused on the process of the release of this multifunctional messenger molecule from basophils or mast cells, but only few studies highlight autocrine effects of histamine on respective cells during the anaphylactic degranulation (17-19).

In our study we could show that isolated human basophils express the H1R, H2R and H4R mRNA but not the H3R mRNA. Of note, the mRNA expression of the H4R was more pronounced when compared to the H1R- or H2R mRNA expression. Therefore we hypothesized a role for the H4R in basophil function notably during the process of degranulation when high amounts of histamine are present. Functional activity of the H4R was confirmed by the induction of chemotaxis which was inhibited using the H4R-antagonist JNJ7777120. In the current work we used a multi-colour flow cytometry test, which is well evaluated in *in vitro* allergy diagnostics, to show that histamine and the H4R-agonist ST-1006 influence the FcεRI cross-linking-induced cell surface expression of CD63 and CD203c on human basophils. Furthermore we examined whether histamine or the H4R-agonist ST-1006 regulates the release of sulfidoleukotrienes from human basophils following stimulation with activating stimuli, anti-FcεRI antibodies or allergen challenge with hymenoptera venom.

Our data present the novel finding that anaphylactic as well as anaphylactoid sulfidoleukotriene release from human basophils is attenuated by histamine which is mainly mediated via the H4R.

Materials and Methods

Histamine receptor ligands

The following histamine receptor ligands were used in this study: Histamine agonist for all histamine receptors; Alk-Scherax, Wedel, Germany, 2-pyridylethylamine selective H1R-agonist; Tocris Bioscience, Bristol, UK, amthamine selective H2R-agonist; Tocris Bioscience, Bristol, UK, ST-1006 H4R-agonist; (Institute of Pharmaceutical and Medicinal Chemistry, Heinrich Heine University, Germany) (20, 21), JNJ7777120 selective H4R-antagonist; Sigma Aldrich, Deisenhofen, Germany. All histamine receptor ligands were used at a concentration of 10 μ M. In extensive previous studies we could show that the concentration of 10 μ M is optimal to demonstrate and reproduce robust H4R-agonist mediated effects (20).

Isolation of human basophil granulocytes

Residual blood samples from platelet (PLT) apheresis disposables used for routine PLT collection and of regular anonymous healthy donors served as source material for the isolation of human basophil granulocytes. Basophils were isolated with a magnetic cell sorting kit (Stem Cell Technologies, Grenoble, France) by using established protocols (22). The isolated basophils had a purity of at least 96%.

Peripheral blood samples were taken from healthy donors, patients with a history of allergic diseases and hymenoptera venom sensitized patients. All participants gave their written informed consent.

Real time quantitative LightCycler RT-PCR

Total RNA was isolated from 96-100% pure human basophils derived from healthy donors using the RNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The cDNA was synthesized by reverse transcription (QuantiTect reverse transcription kit, Qiagen, Germany). Real-time quantitative PCR was performed with Quantitect® primer assays for H1R (QT00199857), H2R (QT00210378), H3R (QT00210861), H4R (QT00032326) and GAPDH (glyceraldehyde-3-phosphate-dehydrogenase) (QT00079247) using SYBR® Green according to the manufacturer's instructions (Qiagen, Hilden, Germany).

The following settings in the LightCycler PCR were used: An initial activation step of 15 min at 95 °C and a ramp of 20 °C/s was followed by 3-step cycling (42 cycles): denaturation; 15 s, 94 °C, annealing; 20 s, 55 °C, extension; 20 s, 72 °C (all 3 with a ramp of 2 °C/s). The melting curve analysis, 60-90 °C, ramp: 20 °C/s. The amount of the target mRNA relative to the amount of the reference GAPDH mRNA in the same sample was calculated using the comparative Ct method also known as the [delta] [delta] Ct method provided by the Relative Quantification Software (Roche Molecular Biochemicals). The Ct values of both the calibrator and the samples of interest are normalized to the appropriate endogenous housekeeping gene GAPDH.

Boyden chamber chemotaxis assays

Chemotactic activity of basophils was determined using a modified Boyden chamber technique. In brief, Boyden chambers (Nucleopore) were filled with stimuli (FMLP, histamine or H4R-agonist) dissolved in phosphate-buffered saline (PBS) and covered with polycarbonate filters (pore size 8 nm; Nucleopore). One hundred microliters of a basophil suspension in PBS (1×10^6 cells/ml) were added on top of each filter. After incubation for 120 min at 37 °C, fluids on top of these filters were discharged, filters carefully removed and migrated cells in the lower part of the Boyden chambers

were lysed by adding 0.1% Triton X-100, and β -glucuronidase activity in the lysates was determined photometrically using *p*-nitrophenyl β -D-glucuronide as a substrate (all from Sigma-Aldrich). For calculation of the number of migrated cells, equivalent to β -glucuronidase activity determined in the lower parts of the Boyden chambers, values were calculated from a standard curve using known numbers of basophils. The results are shown as a chemotactic index which is defined as the ratio of FMLP, histamine or H4R-agonist attracted basophils divided by the number of basophils which were migrated spontaneously in response to the negative control (PBS without stimuli).

Basophil activation test

Surface expression of the basophil activation markers CD63 and CD203c was measured by using multi-colour flow cytometry. Briefly, 100 μ l of whole blood from healthy subjects was diluted in 100 μ l stimulation buffer consisting of PBS supplemented with IL-3 (2 ng/ml, ImmunoTools, Friesoythe, Germany). Cells were pre-incubated for 15 min at 37 °C with stimulation buffer only, histamine (10 μ M) or the H4R-agonist ST-1006 (10 μ M). For dose kinetic experiments, the cells were stimulated with different concentrations of histamine or the H4R-agonist (0,1 μ M-100 μ M). For the time kinetic experiments the cells were pre-incubated for different periods of time including 15, 10, 5 and 0 min with histamine or the H4R-agonist before the anti-IgE antibody was added for 20 min. To prove that the effects were specific for the H4R, a pre-incubation of the cells with the H4R-antagonist JNJ7777120 for 30 min preceded histamine or H4R stimulation. Thereafter the cells were activated for 20 min by adding the monoclonal anti-human IgE antibody (Sigma Aldrich) which binds to the ϵ -chain of human IgE. Degranulation was stopped by chilling the cells on ice for 5 min. The cells were stained with FITC-conjugated anti-CD63 (BD Pharmingen), phycoerythrin-conjugated anti-CD203c (eBioscience) and PerCP-conjugated anti-Fc ϵ RI (BioLegend). Erythrocytes were removed by a lysing reaction and the cells were analyzed by flow cytometry (FACS Calibur, Becton Dickinson, Heidelberg, Germany).

Sulfidoleukotriene ELISA

A commercially available cellular antigen stimulation test ELISA (CAST ELISA) from Bühlmann, Schönenbuch, Switzerland was used to quantify the release of sulfidoleukotrienes including leukotriene C4 (LTC4) and its derivatives LTD4 and LTE4, produced by isolated leukocytes upon contact with allergens or stimuli which fosters the degranulation. Isolation of leukocytes, cell stimulation and leukotriene determination were performed according to the protocol of the supplier. Whole blood was collected from patients with a history of allergic diseases or from hymenoptera venom sensitized patients. In brief, leukocytes were separated from whole blood by density gradient centrifugation on dextran. The isolated leukocytes were resuspended in stimulation buffer containing IL-3. Each sample was tested for the basal release and for the release after stimulation with different stimuli. To activate the basophils, the cells were stimulated for 40 min at 37 °C (i) with an antibody directed against the high affinity IgE-receptor or (ii) with a mixture of C5a (0,1 µM), Formyl-Methionyl-Leucyl-Phenylalanine (FMLP) (1 µM) and platelet-activating-factor (PAF) (10 µM) (all from Sigma Aldrich). To test the allergen dependent degranulation of basophils, the cells were incubated for 40 min at 37 °C with bee and wasp venom (200 µg/ml, Alk, Scherax). Histamine (10 µM), the H4R-agonist ST-1006 (10 µM), the H1R-agonist 2-pyridylethylamine (10 µM) and the H2R-agonist amthamine (10 µM) were added simultaneously with the activating stimuli to all approaches as indicated. To show the concentration dependency the cells were stimulated with different concentrations (0,1 µM-100 µM) of the H2R-agonist amthamine or the H4R-agonist ST-1006. For the time kinetic experiments the cells were pre-incubated for different periods of time including 10, 5 and 0 min with the H2R-agonist or the H4R-agonist each 10 µM before the wasp venom allergen was added for 40 min. Finally, the cells were centrifuged and the supernatants were collected. Sulfidoleukotriene release was estimated using the CAST ELISA according to manufacturer's instructions.

Statistics

For statistical analyses, the software GraphPad Prism Version 5.0 was used. Wilcoxon matched pairs test was performed and the median or mean $\pm$ SEM is shown in the graphs. A p-value < 0.05 was regarded as statistically significant (p < 0.05 was labelled with *, p < 0.01 was labelled with **, p < 0.001 was labelled with ***).

Ethics

The investigation of the role of the histamine receptors in inflammatory diseases was approved by the local ethics committee of the Hannover Medical School (Vote 4253) and was conducted according to the declaration of Helsinki Principles.

Results

The H4R is highly expressed on human basophils

The expression of the H1R, H2R and the H4R was detected at the mRNA level on highly purified human basophils from healthy donors (Fig. 1A). The H3R was not expressed on basophils (data not shown). The histamine receptor subtypes were differentially expressed on these cells. Interestingly, basophils expressed higher H4R mRNA levels compared to the expression levels of the H1R and the H2R (Fig. 1B). Due to the fact that currently no specific antibodies against the human and murine H4R are commercially available we were not able to show the H4R expression on protein level on human basophils (23, 24).

Stimulation of the H4R induces chemotaxis of basophils

The effect of the H4R on the migration of basophils was assessed using a modified Boyden chamber technique. We demonstrated that FMLP, as positive control, histamine and the H4R-agonist ST-1006 significantly induced migration of basophils (Fig. 2A). Reflecting the high expression level of the H4R on human basophils, the H4R-agonist was a more potent inducer of basophil migration as compared to histamine. In additional experiments with basophils from different patients we could show that the histamine effect (Fig. 2B) as well as the H4R effect (Fig. 2C) were convincingly blocked by pre-incubation of the basophils with the well characterized H4R-antagonist JNJ7777120.

Histamine and the H4R-agonist ST-1006 suppress FcεRI-mediated basophil activation

Basophil activation test was performed with blood obtained from healthy donors to show whether histamine or the H4R-agonist ST-1006 influences the FcεRI-mediated basophil activation (Fig. 3). Therefore we investigated the surface expression of CD63 and CD203c, which are known to consistently parallel basophil degranulation, following stimulation. Our data show that pre-incubation with histamine and the H4R-agonist ST-1006 significantly reduced the CD63 and CD203c expression levels on FcεRI-activated basophils (Fig. 3A, 3B, 3G, 3H). Pre-incubation with the specific H4R-antagonist JNJ7777120 for 30 min before histamine and ST-1006 stimulation restored the histamine as well as the ST-1006-induced suppression of FcεRI-mediated basophil activation. Histamine and the H4R-agonist ST-1006 had no effect on the basal expression levels of CD63 and CD203c (Fig. 3C, D and 3I, J).

By performing dose kinetic experiments with different concentrations of histamine or the H4R-agonist we observed that the suppressive effect of histamine and the H4R-agonist on basophil activation was mostly pronounced at a concentration of 10 μM (Fig. 3E, K). We found that the expression of CD63 and CD203c on FcεRI-activated basophils was gradually reduced over time. The lowest expression of CD63 and CD203c was observed when the cells were incubated with histamine

and the H4R-agonist for 15 min before the cells were stimulated with an anti-IgE antibody (Fig. 3F, L).

The release of sulfidoleukotrienes upon activation from IL-3 primed leukocytes, obtained from allergic patients, is suppressed by histamine or the H4R-agonist ST-1006.

Basophil degranulation is a key event in the pathogenesis of allergic diseases. Cross-linking of the FcεRI by an antibody leads to degranulation and the release of mediators, comparable to the events following the cross-linking of surface-bound specific IgE by allergens on sensitized basophils. In *in vitro* diagnostic tests, especially basophil degranulation tests, such as the sulfidoleukotriene or histamine release assays are very important tools in the daily routine diagnosis of patients with IgE-mediated allergies. Since it was found that the sulfidoleukotriene release test is more sensitive than the test involving histamine release (25, 26), the release of sulfidoleukotrienes was determined as a measure of degranulation in our study. The release of sulfidoleukotrienes from cells of different allergic patients was suppressed when histamine or the H4R-agonist were co-incubated with the activating stimuli C5a, FMLP and PAF (Fig. 4A). Incubation of the cells with the anti-FcεRI antibody enhanced the sulfidoleukotriene liberation which could be suppressed by co-incubation with histamine and the H4R-agonist (Fig. 4B). In contrast, the basal release of sulfidoleukotrienes was unchanged in the presence of histamine or the H4R-agonist (Fig. 4C).

The release of sulfidoleukotrienes after hymenoptera venom challenge of IL-3 primed leukocytes, obtained from bee or wasp venom sensitized patients, is suppressed by histamine or the H4R-agonist ST-1006.

To investigate whether our *in vitro* findings also apply for patho-physiological conditions we assessed sulfidoleukotriene release in patients with a convincing history of allergic reactions to bee or wasp stings. All subjects included showed a release > 150 pg/ml of sulfidoleukotrienes from leukocytes which had been activated by bee or wasp venom. Incubation of the IL-3 primed leukocytes with bee

or wasp venom in the presence of histamine or the H4R-agonist ST-1006 for 40 min at 37 °C significantly lowered the hymenoptera venom induced liberation of sulfidoleukotrienes (Fig. 5A and B). To identify the histamine receptor (s) responsible for the suppression of sulfidoleukotriene release, the effects of the selective H1R-agonist 2-pyridylethylamine and the H2R-agonist amthamine were examined in comparison to histamine and the H4R-agonist ST-1006 in patients with wasp venom allergy. We observed a trend of inhibition of the sulfidoleukotriene release via the H2R beyond the significant inhibitory effect of H4R (Fig. 6A). The inhibitory effect via the H2R showed no clear dose dependency whereas the H4R-agonist was convincingly more effective as compared to the H2R-agonist and inhibited the wasp venom induced release of sulfidoleukotrienes in a dose dependent manner which was significant for the concentration of 10 μ M and 100 μ M (Fig. 6B). Furthermore, we found a time dependent reduction of the wasp venom induced leukotriene release via the H2R and H4R. The inhibitory effect was significant for all selected time points via the H2R and H4R. The strongest effect was observed for the H4R-agonist when the agonist and the wasp venom were added simultaneously (0 min) to the cells and for the H2R-agonist when the cells were pre-incubated for 10 min before the wasp venom was added. (Fig. 6C). The inhibitory effect via the H4R was stronger as compared to the H2R (Fig. 6C).

Discussion

Human basophils are rare blood leukocytes originating from CD34+ progenitors. Besides mast cells which are considered the main effector cells in allergic diseases, a potential role for basophils in contributing to the allergic reaction has been suggested. Since peripheral blood basophils are more easily to isolate from peripheral blood or to study in whole blood samples than their tissue resident counterparts, we intended to investigate the expression of the histamine receptors, in particular that of the H4R, on isolated basophils and to assess a functional role of histamine on basophils during the process of activation. We used dextran isolation of leukocytes, which has been shown to

provide the lowest level of spontaneous mediator release compared to other methods, and applied two approved techniques to evaluate activation of basophils.

In the present study we show that isolated human basophils express the H1R, the H2R and the H4R but not the H3R at mRNA level. Interestingly we showed that human basophils express higher H4R mRNA levels as compared to the H1R- and H2R mRNA levels. This is consistent with a previous study from Jemima et al., demonstrating higher expression levels of the H4R compared to the H1R on human mast cells, whereas both receptors were equally expressed on human monocytes or T cells (27). It is noteworthy that both, basophils and mast cells, are characterized by the release of pre-formed mediators in terms of activation and high expression of the FcεRI. The novel finding of the unexpected high expression levels of the H4R on both cell types suggests a dominant role for the H4R on basophils and mast cells. Indeed, here we could demonstrate that human basophils respond to histamine and the H4R-agonist with active migration, which could be inhibited by pre-incubation of the cells with the selective H4R-antagonist JNJ7777120. The H4R-mediated chemotaxis of mast cells and eosinophils has been shown previously (8, 16). Beyond several known mediators, such as C5a, FMLP or eotaxin which guide basophils to sites of inflammation, we could show here for the first time that histamine as well as the H4R-agonist ST-1006 induce chemotaxis in human basophils in the same way as compared to the positive control FMLP. As histamine is rapidly released from basophils or mast cells after allergen-induced aggregation of the FcεRI it might be one of the first signals leading to migration of basophils into the skin. Indeed bite lesions by *Cimex lectularis* and Scabies showed basophil accumulation which might be in part attributed to histamine (28).

Since life threatening anaphylactic reactions to bee or wasp stings occur in 0,8-5% of the general population (29), we focussed in the main part of the study on the role of histamine and the H4R in this scenario, using tools which are used in the daily routine diagnostic of insect venom allergy. We could show the capability of histamine and the H4R-agonist ST-1006 to suppress the FcεRI-induced expression of the activation markers CD63 and CD203c on the cell surface of basophils in a dose and

time dependent manner by means of flow cytometry. Moreover, when using leukotriene secretion as an indicator of basophil degranulation we could demonstrate that the synthesis and release of sulfidoleukotrienes from effector cells after activation with different stimuli or by FcεRI cross-linking were markedly reduced by selective stimulation with histamine and the H4R-agonist ST-1006 at the same time period. In addition we showed that the stimulation with histamine or the H4R-agonist ST-1006 alone did not alter the basal degranulation in human basophils. These observations are consistent with Hofstra et al (8) who showed that also murine bone marrow mast cells were not affected by stimulation with histamine alone in terms of leukotriene and prostaglandin release.

Several immunologic effects of histamine during basophil or mast cell activation in both *in vitro* assays and in the course of allergen immunotherapy were described in the literature (17, 18, 30). Former studies provided evidence that histamine is able to inhibit its own release in human leukocytes or mast cells (17, 30). It was shown in one of these early studies that histamine and different H2R-agonists inhibit the histamine release from human leukocytes with high potency. This effect was specifically blocked by an H2R-antagonist indicating that the H2R is involved (17). We could show the suppression of allergen-induced release of sulfidoleukotrienes from human basophils in the presence of histamine, the selective H2R-agonist amthamine or the selective H4R-agonist ST-1006, confirming these early observations with regard to the H2R and expanding them to the H4R.

Another report published in the 1990s points to a possible regulatory role of the H4R in FcεRI-dependent processes. In this study, it was shown that anaphylactic histamine release from rat peritoneal mast cells was inhibited by treatment with the H2R-agonist dimaprit. In contrast to the study with human leukocytes (17) the H2R-antagonist cimetidine did not affect the decreased release of histamine from rat peritoneal mast cells. Interestingly, the reduced release could be dose-dependently restored by thioperamide (30). Thioperamide is now described as a selective H3/4R-antagonist (31). Since the H4R was not yet discovered at the time of their observation, the authors concluded that H3R-like receptors were responsible for the negative feed-back regulation of

histamine (30). Based on our current findings that indeed the H4R plays a role in FcεRI-dependent processes by inhibiting basophil activation and degranulation, we can assume that the suppressive effect on the histamine release in their study was at least in part attributable to the H4R.

Immuno-silencing of activated basophils via the H2R was also observed by Novak et al. (18). In their newly published work, they observed a rapid up-regulation of the H2R and H4R mRNA expression on basophils from patients undergoing hymenoptera venom immunotherapy (VIT). In this context it is rather interesting that we provide evidence for higher mRNA expression levels of the H4R as compared to the H2R in human basophils from healthy donors. Moreover, the authors showed that the H2R attenuated the FcεRI-induced activation and release of mediators including histamine and sulfidoleukotrienes in a dose dependent manner (18). In accordance with their findings we could confirm the H2R-induced suppression of leukotriene release from human basophils. As opposed to the study of Novak et al. we investigated this issue in patho-physiological conditions by stimulating the H2R and also the H4R on human basophils from wasp venom allergic patients with the specific allergens. Interestingly, we observed that the H4R-agonist showed a clear dose dependent allergen specific desensitization and was significantly more effective as compared to the H2R-agonist. Therefore it is tempting to speculate that the H4R might have a decisive function in the protective immunological mechanisms which lead to an unresponsiveness to hymenoptera venom allergen in VIT. Novak et al. supposed a prominent role of the adenylyl cyclase mediated second messenger cAMP pathway as the main signalling event for the self-inactivation of human basophils (18). Since the H2R is coupled to the activation of cAMP signalling events, their assumption was further substantiated by the fact that the direct cAMP inducer forskolin had an even stronger and in combination with the H2R-agonist an additive effect on basophil inactivation (18). Based on our results also other signalling events might be relevant in the induction of basophil tolerance to allergens via the H4R. Previous studies showed that activation of the H4R can lead to an inhibition of adenylyl cyclase thereby inhibiting the cAMP pathway as well as activating of MAP kinases, phospholipase C or AP-1 (32,11,15). The way in which these different G protein coupled signalling

pathways might contribute to basophil inactivation should be investigated in further studies. In addition to the work of Hofstra et al. (8), two recently published studies characterized the function of the H4R on mast cells (19, 27). On murine bone marrow derived mast cells it was shown that the H4R mRNA expression increased during the maturation of the cells and thereby paralleled the FcεRI surface expression kinetics. In contrast to our work, stimulation of the H4R was performed using an H4R-antagonist instead of an H4R-agonist. Application of the H4R-antagonist inhibited mast cell degranulation and mediator release only when it was present during IgE sensitization phase. In this study, the H4R-antagonist showed no effect during the antigen challenge phase of murine mast cells (19). As opposed to the work with murine mast cells we showed on human basophils that the H2R and H4R exert suppressive effects on sulfidoleukotriene release during challenge with hymenoptera venom allergen in sensitized patients. In the second work the authors investigated the effect of histamine or the H4R-agonist 4-methylhistamine on human mast cell degranulation and observed a direct effect of these compounds. The study demonstrated that stimulation of the H4R on human mast cells triggers the release of cysteinyl leukotrienes similar to the IgE-induced activation (27). This contradicts the findings of Hofstra et al. (8) regarding murine mast cells as well as our findings regarding human basophils where treatment with histamine or the H4R-agonist ST-1006 alone showed no effect.

The differences of the H4R mediated effects on basophils or mast cells discussed in this study may be explained by (i) the cell types used i.e. human versus murine cells, mast cells versus basophils (ii) the time course of the stimulation profile or (iii) the various H4R-ligands which were used.

Taken together, we showed basophil silencing after activation when histamine or the H4R-agonist ST-1006 was added, thus far preventing an excessive reaction by initiating a negative feedback loop.

In this context our data supply for the first time the proof of a substantial role of the H4R as an intrinsic self-termination mechanism for IgE-dependent basophil activation.

The IgE-dependent release of pro-inflammatory mediators from basophils and mast cells represents a key event in allergic reactions. Therefore it has to be discussed in which way the H4R might be a target for future therapeutic interventions.

Author contributions

SM is the primary author and conducted data collection, analysis, interpretation of the data and writing the first draft of the manuscript. SK and MG performed the chemotaxis experiments and the basophil activation tests. BE-V made contributions to the interpretation of the data. HS provided the H4R-agonist ST-1006. RG, TW and UR made significant and substantial contributions to the conception, design and interpretation of the data. All authors reviewed, revised and approved the manuscript for publication.

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Conflict of interest

The authors declare no conflict of interest

Figure legends

Figure 1

Isolated human basophils express the histamine receptors H1R, H2R and H4R at mRNA level. (A) Representative LightCycler real-time PCR melting peaks of the target specific PCR products are depicted. GAPDH=glyceraldehyde-3-phosphate dehydrogenase, HXR=histamine receptor.

(B) The H4R mRNA is higher expressed compared to H1R or H2R mRNA in human basophils. Relative

expression levels of the histamine receptor mRNA were assessed by real time PCR and calculated by the $[\Delta] [\Delta] C_t$ method. The values of both the calibrator and the samples were normalized to the housekeeping gene GAPDH and expressed as fold induction (n=5-9 experiments; ** p < 0.01).

Figure 2

Histamine or the H4R-agonist ST-1006 induces basophil chemotaxis. (A) FMLP was used as positive control and phosphate-buffered saline served as negative control (n=13 experiments; *** p < 0.001). (B) The histamine-induced (n=8 experiments) and (C) H4R-agonist-induced (n=10 experiments) migration could be blocked by pre-incubation with the selective H4R-antagonist JNJ7777120 (** p < 0.01). The chemotactic index is presented which is defined as the ratio of FMLP, histamine or H4R-agonist attracted basophils divided by the number of basophils which were migrated spontaneously in response to the negative control (PBS without stimuli). NS=not stimulated, FMLP=Formyl-Methionyl-Leucyl-Phenylalanine, JNJ=JNJ7777120.

Figure 3

Pre-incubation of IL-3 primed basophils with histamine or the H4R-agonist ST-1006 suppresses anti-IgE antibody-induced surface expression of CD63 and CD203c. (A, G) The histamine and (B, H) the H4R-agonist ST-1006 mediated suppression of CD63 and CD203c were restored by pre-incubation of the cells with the selective H4R-antagonist JNJ7777120 (n=8 experiments; * p < 0.05; ** p < 0.01). (C, D) The basal expression levels of CD63 and (I, J) CD203c were not influenced by histamine and ST-1006 (n=8 experiments). (C, I) % gated CD63 and CD203c surface expression and (D, J) geo mean of CD63 or CD203c expressing basophils were analysed by flow cytometry. (E, F) The suppression of anti-IgE antibody-induced surface expression of CD63 and (K, L) CD203c by histamine and the H4R-agonist occurs in a concentration and time dependent manner. (E, K) The IL-3 primed basophils were pre-incubated with different concentrations (0,1 μ M-100 μ M) of histamine or the H4R-agonist ST-1006 for 15 min. The mean $\pm$ SEM value of the % CD63/CD203c (gated) surface expression of anti-

IgE antibody activated basophils and basophils after pre-incubation with increasing amounts of histamine or the H4R-agonist are depicted (n=5 experiments). (F, L) The IL-3 primed basophils were pre-stimulated at different time points (0, 5, 10, 15 min) with histamine or the H4R-agonist ST-1006. After the different pre-incubation times all approaches were activated with the anti-IgE antibody for 20 min. The mean $\pm$ SEM value of the % CD63/CD203c (gated) surface expression of anti-IgE antibody activated basophils and basophils after incubation with histamine or the H4R-agonist at different time points are depicted (n=5 experiments). NS=not stimulated, JNJ=JNJ7777120.

Figure 4

The release of sulfidoleukotrienes triggered by activating stimuli is suppressed by histamine or the H4R-agonist ST-1006. (A) The release of sulfidoleukotrienes triggered by activating stimuli C5a, FMLP and PAF was suppressed when the leukocytes from different allergic patients were co-incubated for 40 min with histamine or the H4R-agonist ST-1006. The activating stimuli C5a, FMLP, and PAF were mixed and given simultaneously with histamine or ST-1006 to the cells from different donors (n=13 experiments; *p < 0.05; **p < 0.01). (B) The release of sulfidoleukotrienes triggered by an anti-IgE receptor (FcεRI) antibody was significantly suppressed when the leukocytes from different allergic patients were co-incubated for 40 min with histamine or the H4R-agonist ST-1006. The anti-IgE receptor (FcεRI) antibody was given simultaneously with histamine or ST-1006 to the cells (n=13 experiments; **p < 0.01; ***p < 0.001). (C) No influence on the basal release of sulfidoleukotrienes was seen when the cells were incubated only with histamine or the H4R-agonist ST-1006 for 40 min (n=10 experiments). FMLP=Formyl-Methionyl-Leucyl-Phenylalanine, PAF=platelet-activating-factor.

Figure 5

(A) The release of sulfidoleukotrienes triggered by bee venom is suppressed when the leukocytes of bee venom sensitized patients are co-incubated for 40 min with histamine or the H4R-agonist ST-1006 (n=15 experiments; * $p < 0.05$; ** $p < 0.01$). (B) The release of sulfidoleukotrienes triggered by wasp venom is suppressed when the leukocytes of wasp venom sensitized patients are co-incubated for 40 min with histamine or the H4R agonist ST-1006 (n=15 experiments; * $p < 0.05$; ** $p < 0.01$).

Figure 6

(A) The suppression of the release of sulfidoleukotrienes triggered by wasp venom is mediated via the H2R and H4R. The effect is more pronounced when the cells are co-incubated for 40 min with the H4R-agonist ST-1006 as compared to the co-incubation with the H2R-agonist amthamine. The H1R-agonist 2-pyridylethylamine has no effect on the release of sulfidoleukotrienes (n=8 experiments; ** $p < 0.01$).

(B) The suppression of the release of sulfidoleukotrienes triggered by wasp venom occurs in a concentration dependent manner via the H4R which is significant for the concentration of 10 μM and 100 μM . The H4R-agonist is more effective as compared to the H2R-agonist. The cells were stimulated with different concentrations (0,1 μM -100 μM) of the H2R-agonist amthamine or the H4R-agonist ST-1006. The mean $\pm$ SEM value of sulfidoleukotrienes in pg/ml is depicted. (n=7 experiments; * $p < 0.05$). (C) The suppression of the release of sulfidoleukotrienes triggered by wasp venom occurs in a time dependent manner via the H2R and H4R. The H4R-agonist is more effective as compared to the H2R-agonist. The cells were pre-stimulated at different time points (0, 5, 10 min) with the H2R-agonist amthamine (10 μM) or the H4R-agonist ST-1006 (10 μM). After the different pre-incubation times all approaches were activated with wasp venom for further 40 min. The mean $\pm$ SEM value of sulfidoleukotrienes in pg/ml is depicted. (n=9 experiments; * $p < 0.05$, ** $p < 0.01$).

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FIG 1.

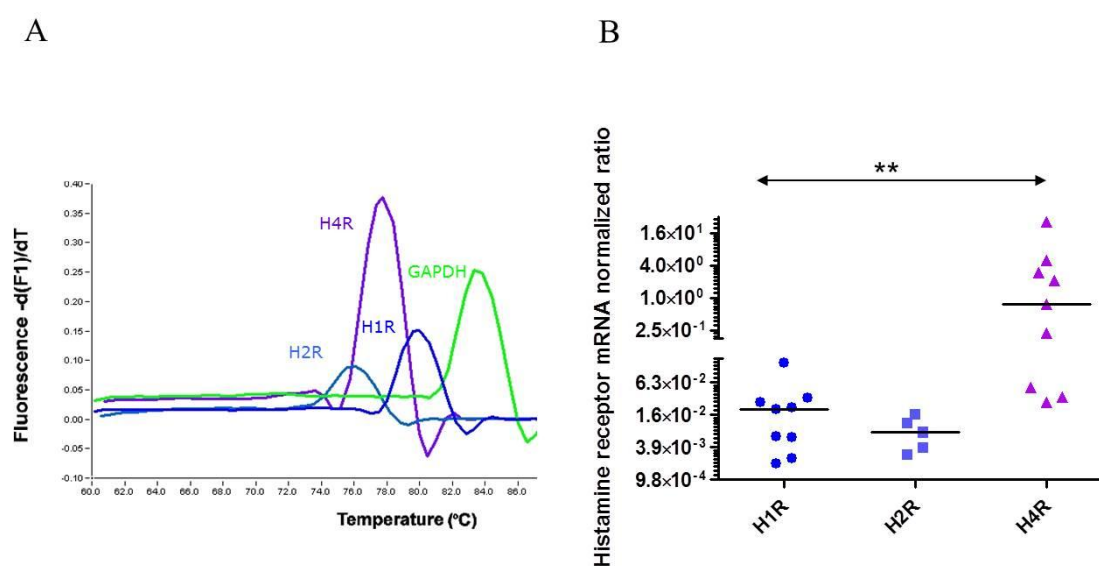


FIG 2.

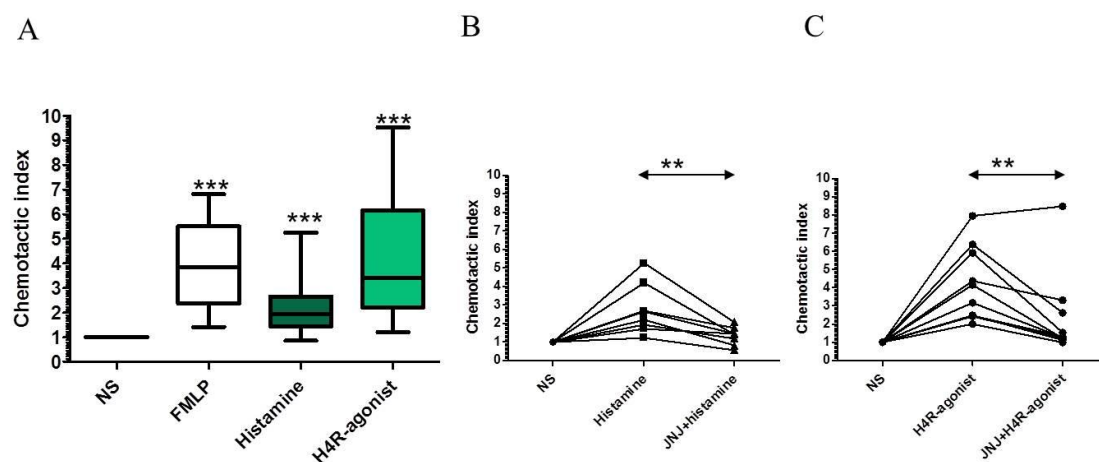


FIG 3.

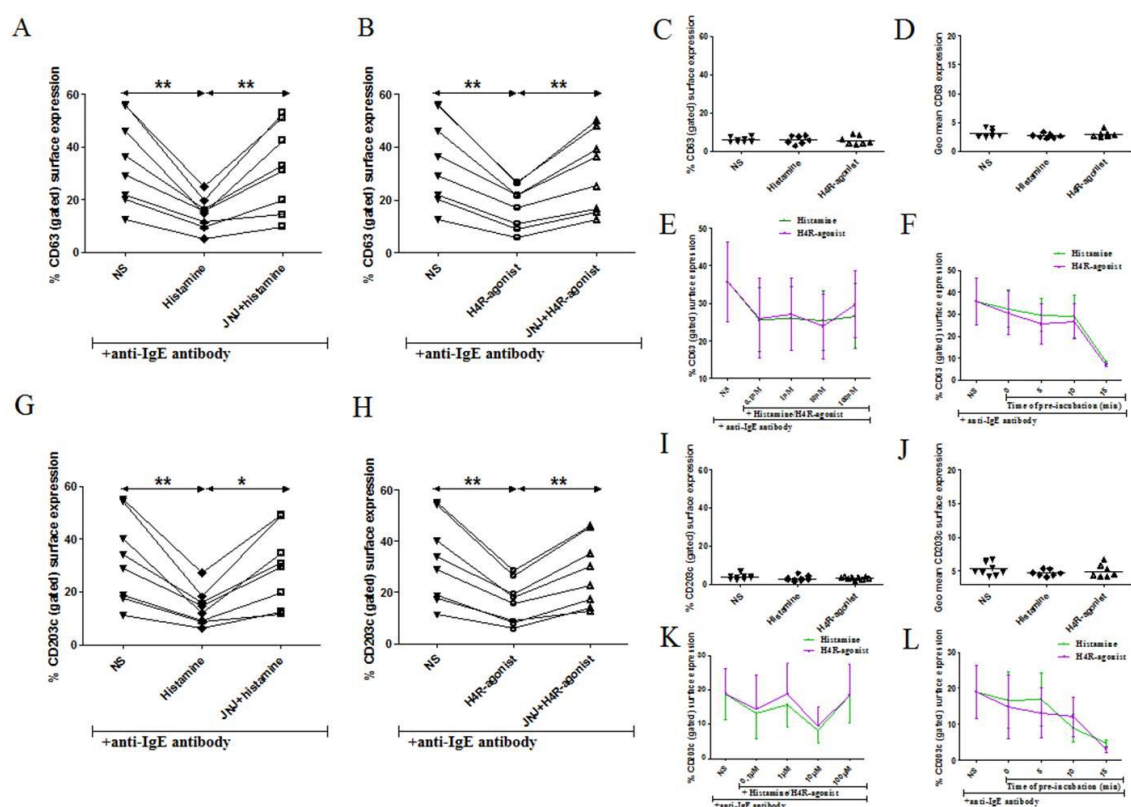


FIG 4.

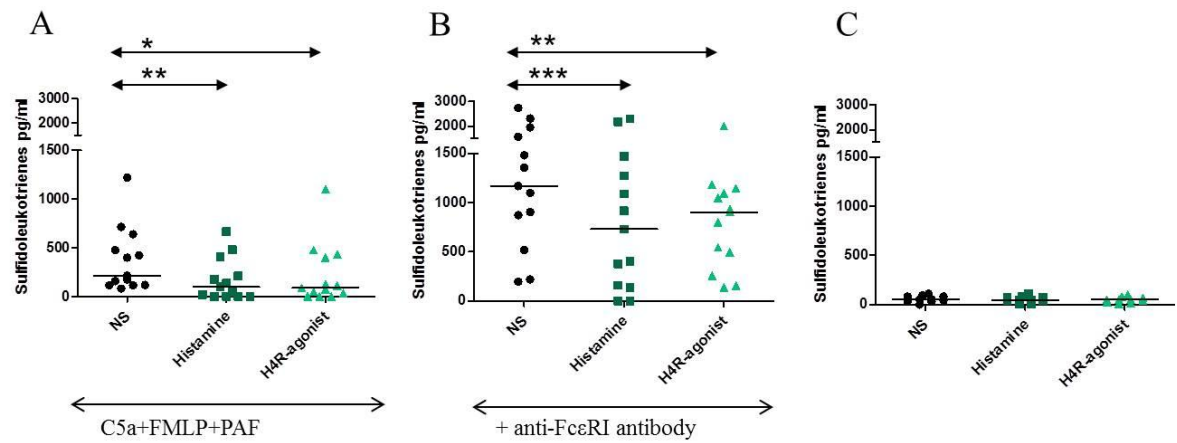


FIG 5.

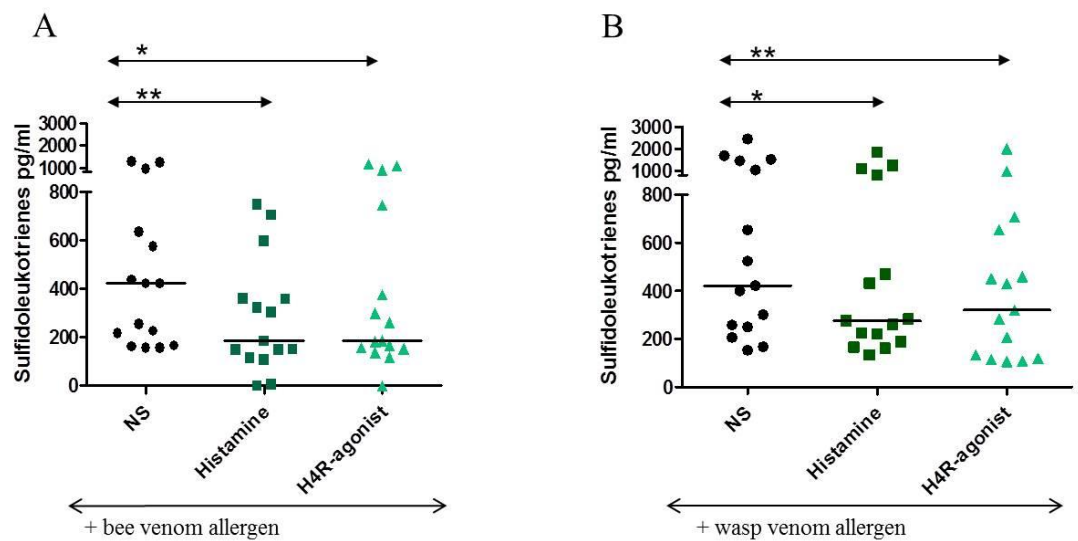


FIG 6.

