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Histamine H2 receptor stimulation up-regulates Th2 chemokine CCL17 production in human M2a macrophages

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Capsule summary:

Stimulation of the histamine H2 receptor significantly increased CCL17 production in human monocyte derived M2a macrophages. These results may be helpful to identify novel therapeutical target structures in diseases driven at least in part by histamine.

Key words:

M2 macrophages; histamine H2 receptor; CCL17

Abbreviations used:

H2R: Histamine H2 receptor, DCs: dendritic cells, AD: atopic dermatitis

To the editor

The pathogenesis of atopic dermatitis (AD) in acute and chronic inflammatory situations is characterized by a marked infiltration of myeloid derived dendritic cells, T cells and eosinophils in the epidermal and dermal compartments as well as by increased amounts of cytokines or chemokines which are crucial for the cellular crosstalk. In the onset of acute AD an increased production of the Th2 cytokines IL-4 and IL-13 by early skin-infiltrating T cells, mast cells or basophils takes place ¹. In consequence to the pathogenic and tissue derived changes, monocytes differentiate into macrophages and respond to environmental signals with polarization into distinct functional phenotypes. The M2a macrophage phenotype is induced by a Th2-biased immune response in the presence of IL-4 or IL-13 and characterized by secreting a specific panel of chemokines ^{E1,E2}, among them CCL17 (formerly known as Thymus- and Activation Regulated Chemokine, TARC) and CCL22 (formerly known as Macrophage Derived Chemokine, MDC) which are highly expressed in dermal dendritic cells (DCs) in AD skin ². CCL17 was identified as one of the most sensitive biomarkers for AD, since its expression in the epidermis as well as CCL17 serum levels of AD patients correlate with disease severity ^{E3-E6}. Histamine is found in lesional skin of AD ³ and has both pro-inflammatory and anti-inflammatory effects, depending on the expression profile of the four histamine receptors (HR) on the cells taking part in the immunologic responses. Targeting the H1R induces various pro-inflammatory and cell-activation responses which might foster allergic diseases ^{E7,E8}. The H2R has a similar expression pattern on immune cells compared to the H1R but opposed to the H1R, stimulation of the H2R attenuates a variety of functions on allergen specific T cells ⁴, DCs ⁵ and basophils ⁶. Regarding the H4R, various immunomodulatory effects mediated on DCs ⁷, T cells ⁸, NK cells ^{E9,E10} and keratinocytes ^{E11} have been described.

The aim of this study was to evaluate the role of histamine on chemokine production, in particular of CCL17 and CCL22, in human macrophages and in monocytes.

Fully differentiated human M2 macrophages were stimulated with IL-4 or IL-13 for different time periods (see additional information in the methods section in this article's Online Repository at www.jacionline.org). The up-regulation of CCL17 mRNA expression and protein production by IL-4 or IL-13 was time-dependent and showed the highest expression levels after 48 h (data not shown).

We assessed effects of histamine and agonists specific for the H1R, H2R and H4R on IL-4 or IL-13 activated M2a macrophages. Interestingly, we observed a significant up-regulation of the IL-4 or IL-13 induced expression of CCL17 at mRNA and protein level by histamine, by amthamine (specific H2R agonist) or by 4-methylhistamine (4-MH) (H2R and H4R agonist) indicating a critical role of the H2R for this effect. In the presence of the selective H2R antagonist ranitidine the effects of histamine, of amthamine and of 4-MH on CCL17 expression were reversed confirming a functional role of the H2R in this reaction (Fig 1, A, B, C, E and F and Fig 2, A-F). In contrast, pre-incubation with the selective H4R antagonist JNJ7777120 did not reverse the histamine or the 4-MH induced up-regulation of CCL17 (Fig 1, D and G). The H2R mediated up-regulation of CCL17 was observed to a lesser extent in IL-4 or IL-13 activated human monocytes (Fig E4, A and B) but not in: (i) resting M2 macrophages, (ii) in human IL-10 activated M2c or (iii) in classically activated M1 macrophages (data not shown). H1R or H4R agonists did not influence the CCL17 expression (Fig 1, A and B and Fig 2, G and H). The up-regulation of CCL17 production via the H2R in IL-4 activated human M2a macrophages occurred in a dose- and time-dependent manner (Fig. E3, A-C).

Comparing basal and IL-4 or IL-13 induced production of CCL17 in M2 macrophages obtained from healthy donors versus cells obtained from AD patients, we observed almost similar CCL17 expression levels in both groups induced by IL-4 but reduced expression of CCL17 induced by IL-13 in AD patients. The H2R mediated upregulation of CCL17 was

observable in cells from patients with AD to a lesser extent (see Fig E1, B and D in this article's Online Repository at www.jacionline.org).

Patients with AD have a background of a general systemic Th2 polarization. Therefore, we speculate that cells from AD patients are adapted to an inflammatory microenvironment leading to unresponsiveness to further stimulation.

Analyzing the characteristics of the different histamine receptors, the H2R is usually considered rather as a receptor mediating immune-suppressive and pro-tolerogenic functions. The H2R was shown to reduce TLR induced expression of cytokines/chemokines in monocytes and DCs^{5,E12,E13} as well as to induce tolerance by suppressing proliferation of allergen-stimulated T cells and increasing of IL-10 production^{E14} or by suppressing basophil activation and mediator release⁶.

Our data challenge this role of the H2R, since the pro-inflammatory Th2 related chemokine CCL17 was upregulated via H2R in M2a macrophages and in monocytes pointing towards a dual role of this receptor.

In contrast to the studies of McILroy et al.⁹ in immature monocyte-derived DCs, we did not observe that the elevated expression of CCL17 in response to histamine is accompanied by an up-regulation of CCL22 in M2a macrophages (see Fig E2, A and B in this article's Online Repository at www.jacionline.org) or monocytes (Fig E4, C and D). This supports the hypothesis that the physiology between CCL17 and CCL22 is at least partly non-redundant^{E15}.

In accordance with the observation in human M2a macrophages, we also detected increased expression of CCL17 upon stimulation with IL-4 in bone marrow-derived macrophages obtained from BALB/c mice. H2R stimulation also increased the IL-4 induced CCL17 production in these murine cells (data not shown).

In conclusion, we describe a novel function for the H2R in M2a macrophages and monocytes promoting a Th2 polarized response by up-regulating the production of CCL17 as key

121 chemokine in AD which may lead to enhanced recruitment of CCR4 expressing Th2 cells,
122 Tregs and CLA⁺ T cells to the site of inflammation. These results may be helpful to identify
123 novel therapeutic target structures in allergic and inflammatory diseases driven at least in part
124 by histamine.
125

Figure legends

Figure 1

Stimulation via H2R significantly up-regulates CCL17 mRNA expression and protein production in IL-4 activated human M2a macrophages.

A, B, IL-4 (20 ng/ml) activated M2a macrophages were stimulated with histamine, 2-pyridylethylamine (H1R agonist), amthamine (H2R agonist), 4-MH (H2R/H4R agonist) and ST-1006 (H4R agonist) for 24 h. C, E, F, The up-regulation of CCL17 mRNA expression in response to histamine, amthamine and 4-MH was significantly blocked by pre-incubation with the selective H2R antagonist ranitidine 30 minutes before stimulation with receptor agonists. D and G, The selective H4R antagonist JNJ7777120 had no effect. The concentration of 10 μ M was used for all ligands. CCL17 mRNA expression was detected by quantitative PCR. CCL17 protein concentrations were analyzed by ELISA. The mRNA- and protein expression were normalized to the IL-4 stimulated samples (IL-4 stimulated M2a macrophages produced in median 470 pg/ml CCL17, ranging from 97 to 770 pg/ml). Significant differences, as determined by the Wilcoxon signed rank test, are indicated as follows: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; medians are shown in the graphs. NS = non stimulated, Hist = histamine, 2-Pyrid = 2-pyridylethylamine, Amth = amthamine, 4-MH = 4-methylhistamine, Ran = ranitidine, JNJ = JNJ7777120.

Figure 2

Stimulation via H2R significantly up-regulates CCL17 mRNA expression and protein production in IL-13 activated human M2a macrophages.

IL-13 (15 ng/ml) activated M2a macrophages were stimulated with histamine, 2-pyridylethylamine (H1R agonist), amthamine (H2R agonist), 4-MH (H2R/H4R agonist) and ST-1006 (H4R agonist). The up-regulation of CCL17 mRNA expression and protein production in response to histamine, amthamine and 4-MH was significantly blocked by pre-

incubation with the selective H2R antagonist ranitidine. The concentration of 10 μ M was used for all ligands. A-C, CCL17 mRNA expression was detected by quantitative PCR. D-F, CCL17 protein concentrations were analyzed by ELISA. The mRNA- and protein expression were normalized to the IL-13 stimulated samples (IL-13 stimulated M2a macrophages produced in median 216 pg/ml CCL17, ranging from 5.5 to 549 pg/ml). G, H, Stimulation of the H1R or H4R shows no effect. Significant differences, as determined by the Wilcoxon signed rank test, are indicated as follows: * $p < 0.05$; ** $p < 0.01$; medians are shown in the graphs. NS = non stimulated, Hist = histamine, 2-Pyrid = 2-pyridylethylamine, Amth = amthamine, 4-MH = 4-methylhistamine, Ran = ranitidine, JNJ = JNJ7777120.

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.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

SMo is the primary author and analyzed the histamine receptor expression on human macrophages, conducted data collection, study design and analysis, interpretation of the data and writing the first draft of the manuscript. KGr performed a part of the experiments, generating and stimulating the macrophages and performing ELISA and qPCR. KRo performed experiments with murine BMDMs. KScha, TWi and RGu made significant contributions to the conception and design of the study. TWe made substantial contributions

182 to the conception, design of the study and interpretation of the data. All authors reviewed,
183 revised and approved the manuscript for publication.

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210 prostaglandin E up-regulate the production of Th2-attracting chemokines (CCL17 and
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Fig. 1

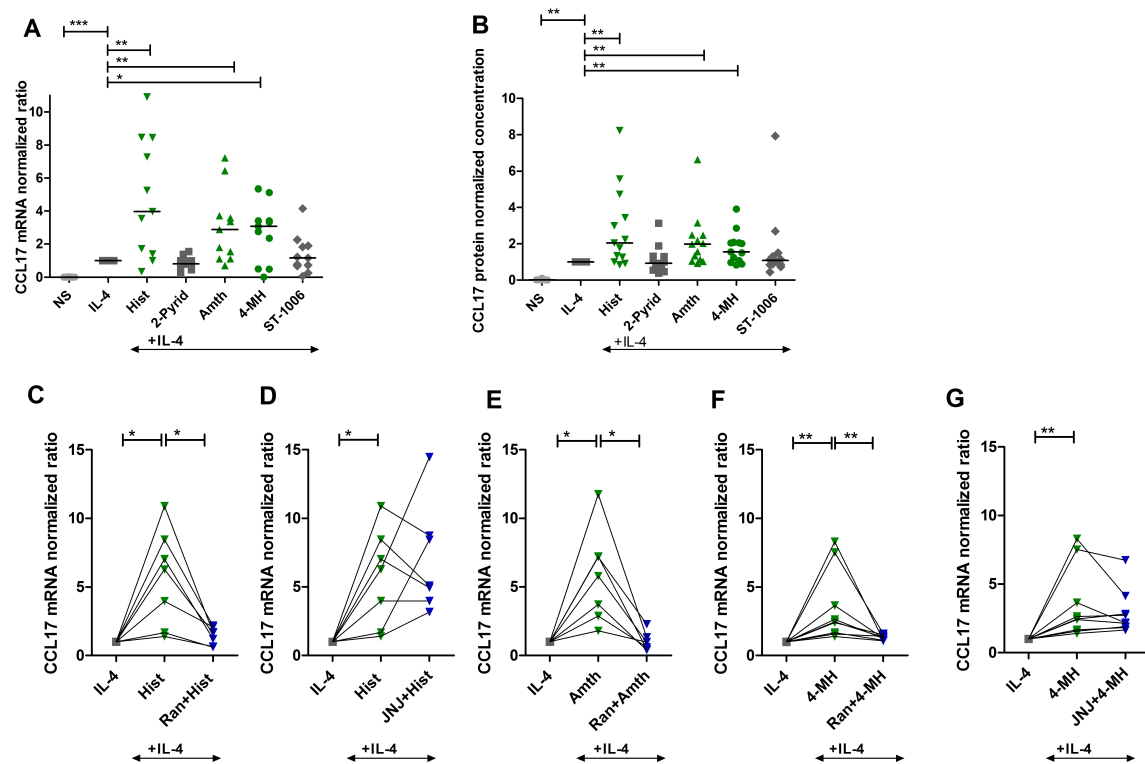
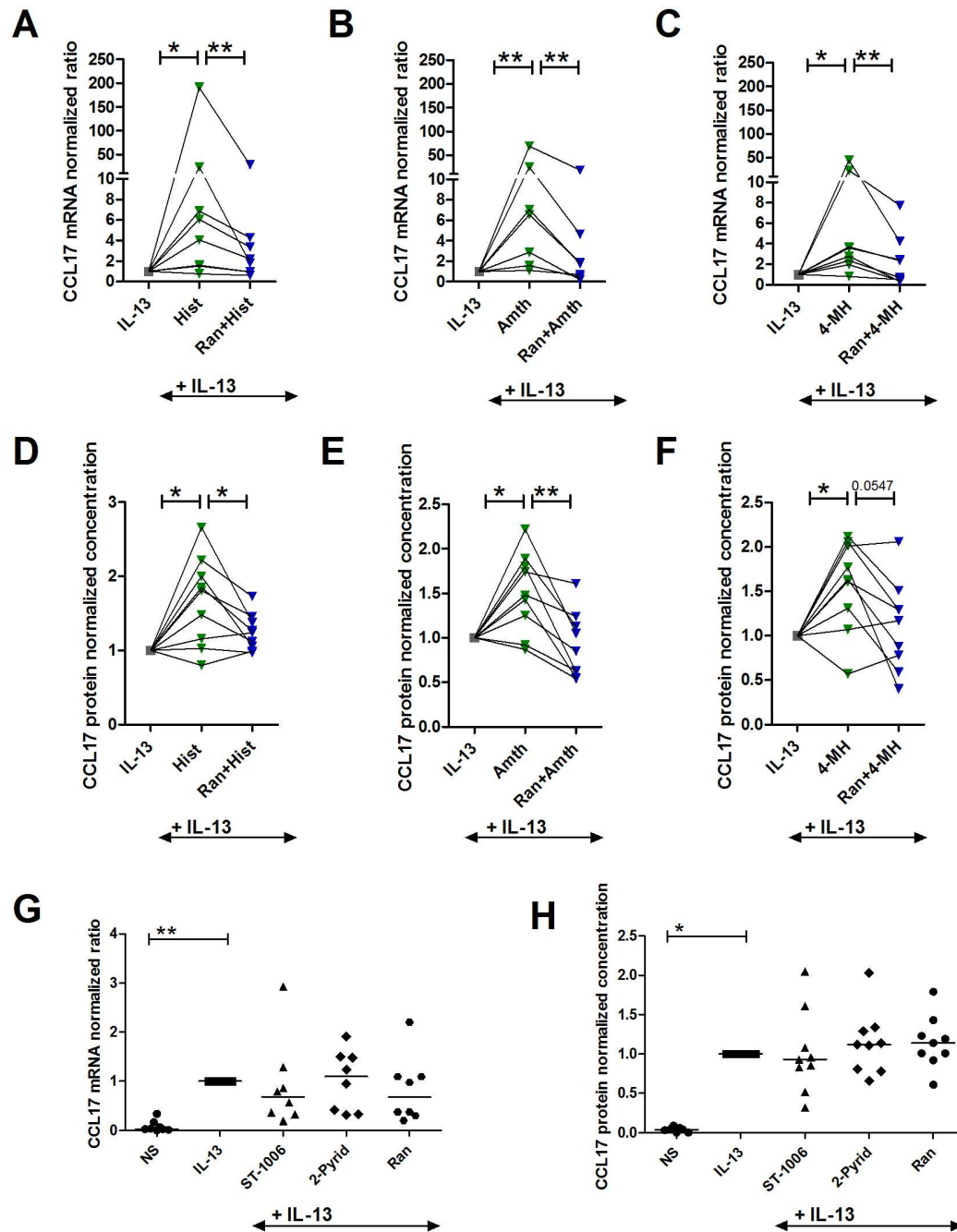


Fig. 2



Online Repository

Materials and Methods

Differentiation of M2 macrophages

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 peripheral blood mononuclear cells (PBMCs). Peripheral blood samples were taken from patients with extrinsic atopic dermatitis (AD). PBMCs were separated by density gradient centrifugation on lymphoprep (Fresenius Kabi Norge AS, Oslo, Norway). With a seeding density of 1×10^6 cells per well, PBMCs were plated in a 24 well plate in Iscoves Medium supplemented with AB serum (2,5 % v/v). To attach the monocytes, cells were incubated for 1.5 h at 5% CO₂ and 37°C. Non adherent cells were removed by vigorously washing of adherent cells three times with PBS. An appropriate amount of RPMI 1640, supplemented with 2 mM l-glutamine, 100 mg/ml penicillin/streptomycin, 12 mM Hepes, and 5% v/v FCS (PAN-Biotech, Aidenbach, Germany; all other media components from Biochrom, Berlin, Germany) and 10 ng/ml macrophage colony-stimulating factor (M-CSF) (R&D, San Diego, CA, USA) was added. Cells were incubated for 5 days at 37°C and 5% CO₂ without medium change. At day 5 another 50% by volume of fresh medium was added. At day 8 the medium was completely changed and at day 10 the fully differentiated M2 macrophages were activated with IL-4 (20 ng/ml) (R&D Systems) or IL-13 (15 ng/ml) (R&D Systems). Monocytes were generated from PBMCs obtained from the same source material used for isolation and differentiation of macrophages. The cells were allowed to attach at the culture dishes. After 2 h non adherent cells were removed and the adherent monocytes were cultured overnight without GM-CSF or M-CSF. Then the cells were stimulated comparable to the M2 macrophages.

Histamine receptor ligands

The following histamine receptor ligands were used in this study: Histamine (Alk-Scherax, Wedel, Germany) as agonist for all histamine receptors; 2-pyridylethylamine (Tocris Bioscience, Bristol, UK) as selective H1R agonist; amthamine (Tocris Bioscience, Bristol, UK) as selective H2R agonist; 4-methylhistamine (4-MH) as H2R/H4R agonist (Tocris Bioscience, Bristol, UK); ST-1006 (Institute of Pharmaceutical and Medicinal Chemistry, Heinrich Heine University, Germany) as H4R agonist^{E16}; ranitidine (Tocris Bioscience, Bristol, UK) as selective H2R antagonist; JNJ7777120 (Sigma Aldrich, Deisenhofen, Germany) as selective H4R antagonist. All histamine receptor ligands were used at a concentration of 10 μ M.

Stimulation of fully differentiated M2 macrophages

For assessment of chemokine production, fully differentiated M2 macrophages were stimulated with IL-4 (20 ng/ml) (R&D Systems) or IL-13 (15 ng/ml) (R&D Systems) for 24 h. IL-4 and IL-13 activated M2a macrophages were treated with histamine or histamine receptor specific ligands (10 μ M) for additional 24 h. To show the dose dependency M2a macrophages were stimulated in the range 0.1 μ M to 100 μ M with agonists as indicated.

Real time quantitative LightCycler RT-PCR

Total RNA was isolated 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 CCL17 (QT00096866), CCL22 (QT00089817) and RPS 20 (ribosomal protein S20) (QT00079247) using SYBR[®] Green according to the manufacturer's instructions (Qiagen, Hilden, Germany) using the LightCycler 480. The amount of the target mRNA relative to the amount of the reference gene, ribosomal protein S20 (rps 20), mRNA in the same sample was calculated using the comparative Ct method also known as the $[\Delta\Delta Ct]$ method provided by the Software LC 480 (Roche Molecular Biochemicals). The Ct

values of both the calibrator and the samples of interest are normalized to the appropriate endogenous reference gene rps 20.

ELISA

1×10^6 fully differentiated monocytes, M2 macrophages, M2c macrophages or M1 macrophages per well were activated with IL-4, IL-13, IL-10 or IFN- γ + LPS respectively for 24 h and either non stimulated or stimulated with histamine or the specific H1R, H2R and H4R agonists for 24 h (10 μ M). For blocking experiments cells were treated with the H2R antagonist ranitidine or the H4R antagonist JNJ7777120 30 minutes before stimulation with receptor agonists. RNA was stabilized in lysis buffer and cell-free supernatants were taken after 24 h from this time point. The chemokine concentrations of CCL17 and CCL22 were analyzed using commercially available ELISAs. The respective ELISAs were performed according to the manufacturer's instructions (R&D Systems).

Statistics

For statistical analyses, the software GraphPad Prism Version 5.0 was used. Wilcoxon matched pairs test was performed and the median is shown in the graph. 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 ***).

Supplementary Figure E1

The spontaneous secretion of CCL17 and the IL-4 induced CCL17 production in M2 macrophages are almost similar in cells obtained from healthy donors compared to cells obtained from AD patients. The IL-13 induced CCL17 secretion is attenuated in M2 macrophages obtained from AD patients as compared to healthy controls. Stimulation via H2R up-regulates CCL17 protein production in IL-4 and IL-13 activated human M2a macrophages obtained from AD patients.

A and C, Fully differentiated M2 macrophages obtained from healthy controls or AD patients were stimulated with IL-4 (20 ng/ml) or IL-13 (15 ng/ml) for 48 h or left unstimulated. CCL17 protein production was analyzed by ELISA. B and D, IL-4 or IL-13 activated M2a macrophages obtained from AD patients were stimulated with histamine, amthamine (H2R agonist) and 4-MH (H2R/H4R agonist) for 24 h. The concentration of 10 μ M was used for all ligands. CCL17 protein concentrations were analyzed by ELISA. The protein expression was normalized to the IL-4 or IL-13 stimulated samples (IL-4 or IL-13 stimulated M2a macrophages obtained from AD patients produced in median 372 pg/ml or 38.6 pg/ml CCL17, ranging from 7.5 to 2194 pg/ml or 10.4 to 612.2 pg/ml respectively. Significant differences, as determined by the Wilcoxon signed rank test, are indicated as follows: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; medians are shown in the graphs. NS = non stimulated, Hist = histamine, Amth = amthamine, 4-MH = 4-methylhistamine, AD = atopic dermatitis, NS = non stimulated.

Supplementary Figure E2

Stimulation with histamine or with selective histamine receptor agonists has no effect on protein production of CCL22 in IL-4 or IL-13 activated human M2a macrophages.

Fully differentiated M2 macrophages were activated with IL-4 (20 ng/ml) or IL-13 (15 ng/ml) for 24h. M2a macrophages were stimulated with histamine, amthamine (H2R agonist), 4-MH

(H2R/H4R agonist) and ST-1006 (H4R agonist) for 24 h. The concentration of 10 μ M was used for all agonists. A, CCL22 protein concentrations in IL-4 activated M2a macrophages. B, CCL22 protein concentrations in IL-13 activated M2a macrophages. Protein concentrations were analyzed by ELISA. The protein expression was normalized to the IL-4 or IL-13 stimulated samples (IL-4 stimulated M2a macrophages produced in median 167117 pg/ml CCL22, ranging from 89268 to 294926 pg/ml). (IL-13 stimulated M2a macrophages produced in median 115200 pg/ml CCL22, ranging from 16960 to 1312000 pg/ml). Medians are indicated in the graphs. NS = non stimulated, Hist = histamine, 2-Pyrid = 2-pyridylethylamine, Amth. = amthamine, 4-MH = 4-methylhistamine.

Supplementary Figure E3

Stimulation via H2R significantly up-regulates CCL17 mRNA expression and protein production in IL-4 activated human M2a macrophages in a dose- and time-dependent manner. The highest expression of CCL17 was found when the cells were stimulated for 24 h with a concentration of 10 μ M of the HR ligands.

A, B, M2a macrophages were stimulated for different time periods with histamine, amthamine and 4-MH as indicated. C, M2a macrophages were stimulated with different concentrations of histamine, amthamine and 4-MH as indicated for 24 h. A and C, CCL17 mRNA expression was detected by quantitative PCR. B, CCL17 protein concentrations were analyzed by ELISA. The mRNA- and protein expression were normalized to the IL-4 stimulated samples (IL-4 stimulated M2a macrophages produced in median 470 pg/ml CCL17, ranging from 97 to 770 pg/ml). Significant differences, as determined by the Wilcoxon signed rank test, are indicated as follows: * $p < 0.05$; ** $p < 0.01$; medians are shown in the graphs. NS = non stimulated, Hist = histamine, Amth = amthamine, 4-MH = 4-methylhistamine,

Supplementary Figure E4**Stimulation via H2R significantly up-regulates CCL17 but not CCL22 protein production in IL-4 or IL-13 activated human monocytes.**

IL-4 (20 ng/ml) or IL-13 (15 ng/ml) activated monocytes were stimulated with histamine, amthamine (H2R agonist), 4-MH (H2R/H4R agonist) and ST-1006 (H4R agonist) for 24 h. A and B, CCL17 production was assessed by ELISA. (IL-4 or IL-13 stimulated M2a macrophages produced in median 303 pg/ml or 120 pg/ml CCL17, ranging from 147 to 655 pg/ml or from 34 to 771 pg/ml). C and D, CCL22 production was assessed by ELISA. (IL-4 or IL-13 stimulated M2a macrophages produced in median 29790 pg/ml or 23310 pg/ml CCL22, ranging from 6576 to 116900 pg/ml or from 5237 to 104700 pg/ml). The concentration of 10 μ M was used for all ligands. Significant differences, as determined by the Wilcoxon signed rank test, are indicated as follows: * $p < 0.05$; medians are shown in the graphs. NS = non stimulated, Hist = histamine, Amth = amthamine, 4-MH = 4-methylhistamine.

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Fig. E2

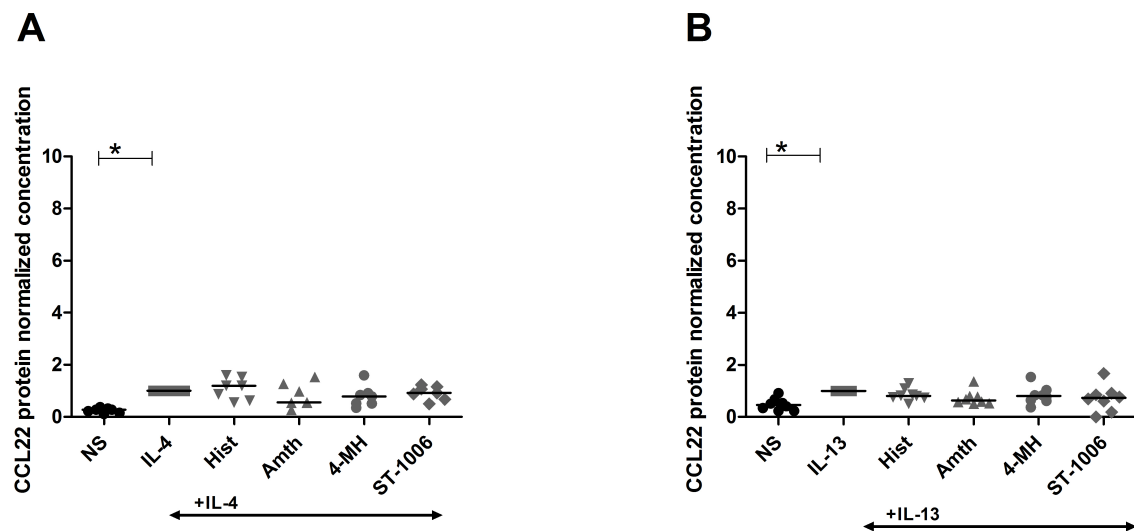


Fig. E3

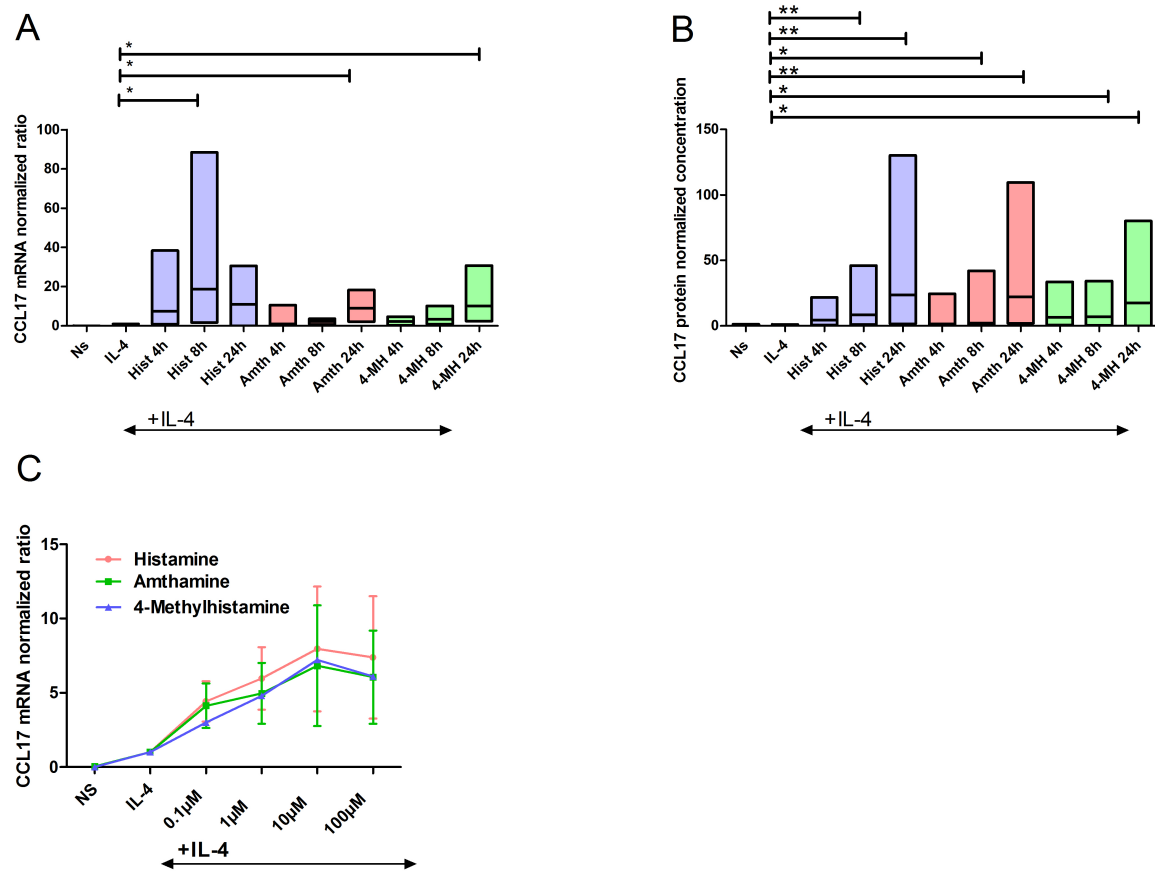


Fig. E4

