

Histamine downregulates monocyte CCL2 production through the histamine H₄ receptor

Dorothea Dijkstra, MSc,^a Rob Leurs, PhD,^b Paul Chazot, PhD,^c Fiona C. Shenton, MSc,^c Holger Stark, PhD,^d Thomas Werfel, MD,^a and Ralf Gutzmer, MD^a *Hannover and Frankfurt/Main, Germany, Amsterdam, The Netherlands, and Durham, United Kingdom*

Background: The expression of the recently cloned histamine H₄ receptor (H₄R) by leukocytes suggests a role in immunomodulation.

Objective: The expression and function of the H₄R on human monocytes obtained from peripheral blood was investigated. **Methods:** H₄R expression was studied by using flow cytometry. Effects of H₄R stimulation on Ca²⁺ mobilization was determined fluorometrically, CCL2 production was determined by means of ELISA, intracellular CCL2 staining was measured with flow cytometry, and CCL2 mRNA was measured by using real-time quantitative LightCycler PCR. The relevance of CCL2 production was determined in chemotaxis transmigration assays.

Results: H₄R protein was expressed by monocytes and upregulated by IFN- γ . H₄R agonists (clobenpropit and 4-methylhistamine) induce a Ca²⁺ mobilization in monocytes, which could be blocked with the selective H₄R antagonist JNJ777120. Furthermore, H₄R agonists downregulated CCL2 protein production. This effect could also be blocked by JNJ777120. Supernatants of H₄R agonist-stimulated monocytes attracted less monocytes in transmigration assays. The downregulation of CCL2 production was regulated at different levels. First, the synthesis of CCL2 mRNA was significantly decreased. Second, intracellular staining suggested an inhibition of CCL2 secretion after stimulation with H₄R agonists.

Conclusion: Human monocytes express the H₄R, and its stimulation leads to a Ca²⁺ influx and an inhibition of CCL2 production, resulting in a reduction of monocyte recruitment.

Clinical implications: The H₄R could represent an important anti-inflammatory receptor on monocytes and could be an

interesting target for drug development. (*J Allergy Clin Immunol* ■■■■;■■■:■■■-■■■.)

Key words: Histamine, monocytes, H₄ receptor, CCL2

Histamine is an important mediator of allergic reactions. In the periphery it is released mainly by mast cells¹ and basophils.² The effects of histamine are diverse and mediated through 4 known G-protein-coupled receptors, the histamine H₁, H₂, H₃, and H₄ receptors (H₄Rs). The recently discovered and cloned histamine H₄R³⁻⁸ is expressed by leukocytes and is thought to have an immunomodulatory function. Several functions of the histamine H₄R expressed by different cell types have been found. In particular directly on chemotaxis of mast cells⁹ and eosinophils^{10,11} and indirectly, through leukotriene B₄ produced by mast cells, on neutrophil mobilization from the bone marrow.¹² Recently, we showed that monocyte-derived dendritic cells (DCs) are chemoattracted through the histamine H₄R, and stimulation of the H₄R led to suppression of IL-12p70 production.¹³ Furthermore, effects on T lymphocytes have also been observed. Agonist stimulation of the H₄R upregulates IL-16 release by CD8⁺ T lymphocytes.¹⁴ The importance of the H₄R in modulation of CD4⁺ T-lymphocyte activation and T_H2 responses in a murine model of asthma has been shown.¹⁵ These results indicate that the H₄R is an important modulator during DC-T-lymphocyte interactions. Because the monocyte (CD14⁺) fraction of PBMCs contain the direct precursors of DC- and skin-associated macrophages, we investigated the expression and function of the H₄R on monocytes. We demonstrate that the H₄R is expressed and upregulated by IFN- γ . In microarray experiments the mRNA expression of several cytokines and chemokines after incubation with the most specific H₄R agonist, 4-methylhistamine,¹⁶ was investigated (results not shown). The results indicated an inhibition of CCL2 mRNA and prompted us to investigate the effect of histamine on the expression of this chemokine in more detail because it plays an important role in allergic inflammation. In mice lacking CCL2, macrophage attraction to sites of inflammation was disturbed, and antigen-specific T_H1 cells were producing less IFN- γ .¹⁷ In mice lacking CCR2 (the receptor for CCL2), an enhanced airway T_H2 response was found (eg, eosinophilia, Ig subclass switching, and IL-5 production).¹⁸ In human subjects CCL2 production of keratinocytes was enhanced by the

From ^athe Department of Dermatology and Allergology, Hannover Medical University; ^bLeiden/Amsterdam Center for Drug Research, Department of Medicinal Chemistry, Vrije Universiteit Amsterdam; ^cDurham University, Centre for Integrative Neuroscience, School of Biological and Biomedical Sciences; and ^dInstitute for Pharmaceutical Chemistry, ZAFES, Johann Wolfgang Goethe-University, Frankfurt/Main.

Supported by grants from the Deutsche Forschungsgemeinschaft (DFG Gu434/5-1) and SFB 566A6 and Wellcome Trust (United Kingdom).

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication December 22, 2006; revised February 26, 2007; accepted for publication March 22, 2007.

Reprint requests: Dorothea Dijkstra, MSc, Department of Dermatology and Allergology, Hannover Medical University, Ricklinger Str. 5, D-30449, Hannover, Germany. E-mail: dijkstra.dorothea@mh-hannover.de.

0091-6749/\$32.00

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doi:10.1016/j.jaci.2007.03.024

Abbreviations used

DC:	Dendritic cell
GAPDH:	Reduced glyceraldehyde-phosphate dehydrogenase
H ₄ R:	Histamine H ₄ receptor
MMP:	Matrix metalloproteinase

T_H2 cytokine IL-13, which is found, for example, in lesions of atopic dermatitis.¹⁹ Furthermore, a polymorphism (−2518G) in the gene regulatory region of CCL2, which resulted in a higher expression of CCL2, was found associated to a more severe asthma phenotype in children.²⁰ Thus CCL2 is induced by and contributes to a T_H2 environment in allergic inflammation and leads to migration of cells found in allergic inflammation, such as monocytes, activated T lymphocytes, and natural killer cells, which express CCR2.²¹ Here we show that histamine H₄R stimulation results in the downregulation of spontaneous CCL2 production in monocytes. This could represent a negative feedback mechanism to avoid an overwhelming T_H2 environment in case of a persistent histamine release at sites of allergic inflammation and could contribute to the shift from T_H2 to T_H1 observed in the transition from acute to chronic allergic inflammation (eg, in atopic dermatitis).²²

METHODS**Monocyte enrichment and isolation**

Buffy coats from anonymous healthy blood donors obtained from the local blood bank were used. The buffy coats were disposed of by the blood bank after thrombocyte preparation. Total IgE levels, as well as levels IgE against common aeroallergens (sx-1 screening test), were measured (CAP System; Pharmacia, Freiburg, Germany) and were less than the normal limits of 100 kU/L and 0.35 kU/L, respectively. In case of increased IgE levels, the patient was excluded from the study. Human PBMCs were separated from the buffy coats by means of density gradient centrifugation on Lymphoprep (Fresenius Kabi Norge AS, Oslo, Norway).

PBMCs were then cultured in supplemented Iscove medium on flat-bottom plates (6-well plate: 1×10^7 cells per well for chemotaxis-supernatants; 24-well plate: 1×10^6 cells per well for ELISA or intracellular CCL2 measurements) for 1 hour (37°C, 5% CO₂, humidified atmosphere). Then the nonadherent cells were removed by washing with PBS. The adherent cells (enriched monocytes, purity ≥85%) were further cultured (and stimulated with H₄R agonists or other substances used in the H₄R regulation studies) in supplemented RPMI 1640 medium (6-well plate: 2 mL; 24-well plate: 400 μL; 96-well plate: 250 μL). Adherent cells were counted after detaching them with trypsin-EDTA (Pan Biotech GmbH, Aidenbach, Germany). The number of adherent cells was generally around 2.5×10^5 cells per well in a 24-well plate. The purity of the adherent monocytes was 85% minimum. This was controlled by using flow cytometry to determine CD14⁺ cells in each experiment. Contaminating cells were evaluated in selected experiments by means of flow cytometry and included minimal numbers of T lymphocytes (CD3⁺ cells, <0.5%), B lymphocytes (CD19, <0.5%), and natural killer cells (CD56, <3.0%), whereas granulocytes (CD16) were absent. Macrophages (CD206) and DCs (CD1a) represented around 10% of the nonmonocyte fraction. The viability of trypsinated cells (used for

the intracellular CCL2 staining and staining controls only) was tested in each experiment by staining with trypan blue and was greater than 95%. In experiments for quantitative PCR, we isolated monocytes by means of magnetic cell sorting (MACS; Monocyte isolation kit II; Miltenyi Biotec Inc, Bergisch Gladbach, Germany; monocyte purity ≥95%), adhered the cells (2×10^5 cells per well on a 96-well flat-bottom plate) for 1 hour (37°C, 5% CO₂, humidified atmosphere) in supplemented Iscove medium, and cultured them in supplemented RPMI medium.

Media

For the adherence of monocytes, Iscove medium supplemented with AB serum (5% vol/vol), nonessential salts (1% wt/vol), L-glutamine (1% wt/vol), penicillin/streptomycin (1% wt/vol), and gentamicin (0.5% wt/vol) was used. For culturing monocytes after adherence, we used RPMI 1640 medium supplemented with FCS (5% vol/vol), 12 mmol/L Hepes, L-glutamine (1% wt/vol), and penicillin/streptomycin (1% wt/vol; all media and supplements were from Biochrom AG, Berlin, Germany).

Chemicals

Clobenpropit was obtained from Sigma-Aldrich (Deisenhofen, Germany). 4-Methylhistamine was kindly provided by R. Leurs and synthesized in the Department of Pharmacochimistry of the Vrije Universiteit Amsterdam in The Netherlands, as described previously.¹⁶ JNJ7777120 was synthesized by H. Stark from the Johann Wolfgang Goethe University in Frankfurt/Main, Germany, as described previously.²³ GM6001²⁴ was obtained from Chemikon (Hampshire, United Kingdom).

The polyclonal rabbit anti-human H₄R antibody and its antigenic peptide were prepared by F. C. Shenton and P. Chazot from Durham University, United Kingdom, and was described previously.²⁵ The antibody recognizes the amino acid sequence (374-390) that is positioned in the C-terminal tail of the H₄R. Neutralizing anti-CCL2 was obtained from R&D Systems (Wiesbaden, Germany). CCL2 was obtained from TebuBio (Offenbach, Germany).

H₄R (amino acid 374-390) staining

Cells were stained according to the protocol and with the reagents provided by BD Biosciences (Heidelberg, Germany) for intracellular epitope-directed antibodies. In brief, cells were fixed and permeabilized. Subsequently, the cells were labeled with (1) H₄R antibody, (2) polyclonal rabbit isotype antibody, or (3) H₄R antibody preincubated with a 10-fold higher concentration of antigenic oligopeptide used to generate the antibody. This was followed by labeling with goat anti-rabbit antibody conjugated with biotin (Jackson ImmunoResearch Laboratories, Suffolk, England). Subsequently, staining was performed with fluorescein isothiocyanate-coupled streptavidin, and the H₄R positivity of the cells was quantified by means of flow cytometry (FACS Calibur; Becton Dickinson, Heidelberg, Germany).

Calcium mobilization assay

Monocytes were plated after isolation by means of magnetic cell sorting (Miltenyi Biotec, Inc) and adhered on flat-bottom 96-well plates (2.5×10^5 cells per well). Then they were labeled with FLUO-4 according to the manufacturer's description (Invitrogen GmbH, Karlsruhe, Germany). Calcium mobilization was measured in a FluoStar plate reader (BMG Lab Technologies GmbH, Jena, Germany). Fluorescence (excitation, 485 nm; emission, 520 nm) was measured per second for 50 seconds, and at a time of 5 seconds, the H₄R ligands histamine, clobenpropit, and 4-methylhistamine (10^{-5} mol/L) were added to the cells. A calcium ionophore (20 nmol/L A23187, Sigma) was used as a positive control. JNJ7777120 (10^{-5} mol/L) was added to respective wells 30 minutes before the measurements.

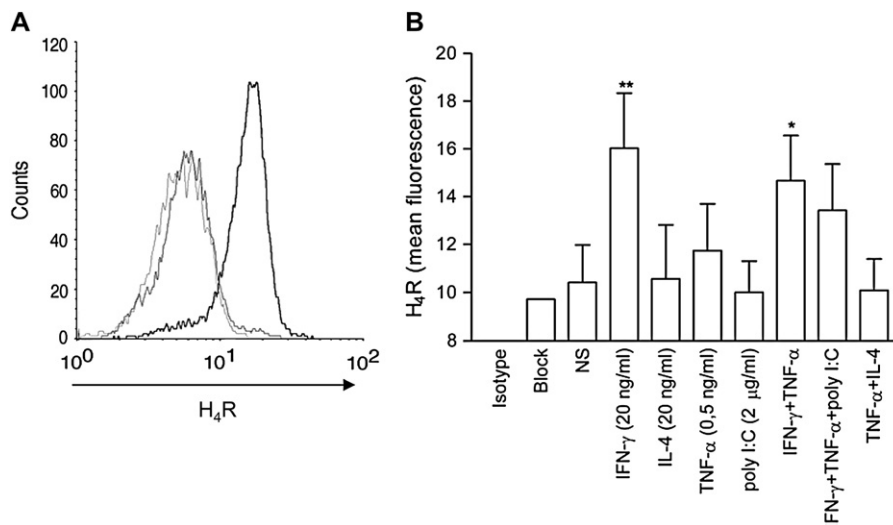


FIG 1. The H₄R is expressed by monocytes and upregulated by IFN- γ . **A**, Staining of monocytes with H₄R (solid, thick line), isotype (dotted line), and blocked H₄R antibody (solid, thin line). **B**, H₄R protein expression after 48 hours' incubation with different ligands. Averages and SDs of 3 experiments are shown (statistical test: ANOVA). NS, Nonstimulated cells.

CCL2 ELISA

CCL2 levels were investigated by using a CCL2 sandwich ELISA with a lower detection limit of 8 pg/mL and a highest detection limit of 3000 pg/mL. For the CCL2 ELISA (TebuBio), PBMCs were plated in supplemented Iscove medium on a 24-well plate (1×10^6 cells per well/250 μ L). Monocytes were allowed to adhere for 1 hour, as previously described, and subsequently washed vigorously with PBS. Adherent cells were incubated in RPMI with ligands at the indicated concentrations for 4, 24, and 48 hours; supernatants were taken; and CCL2 was determined according to the manufacturer's instructions. The plate was read at a wavelength of 405 nm on a FluoStar plate reader (BMG Lab Technologies, GmbH).

CCL2 intracellular staining

Adherent cells (from 1×10^6 PBMCs per well) were stimulated with 10^{-5} M H₄R ligands for 4 or 48 hours to measure amounts of intracellular CCL2. Cells were detached with trypsin-EDTA at 37°C, and the reactions were stopped with an equal volume of heat-inactivated FCS after 10 minutes. Then the cells were washed twice with PBS, and intracellular CCL2 was stained according to the protocol provided by BD Biosciences for intracellular staining. In brief, cells were fixed and permeabilized and then stained with the allophycocyanin-labeled anti-CCL2 antibody (50 ng/mL, IgG2B, clone 23002) or an allophycocyanin-labeled isotype control antibody (IgG2B) for 30 minutes at 4°C. The cells were washed, and CCL2 positivity was quantified by means of flow cytometry.

CCL2 quantitative PCR

Monocytes were isolated by means of magnetic cell sorting (Miltenyi Biotec, Inc) and then adhered on flat-bottom 96-well plates (2×10^5 cells/200 μ L), as previously described. After transfer to supplemented RPMI, monocytes were stimulated with ligands for 4 hours. Total RNA was isolated, according to the manufacturer's instructions, with the High Pure mRNA Isolation kit (Roche Molecular Biochemicals, Mannheim, Germany). The cDNA was synthesized by means of reverse transcription (Quantitect reverse transcription kit; Qiagen, Hilden, Germany). Real-time quantitative PCR was performed

with Quantitect primer assays for CCL2 (QT00212730) and reduced glyceraldehyde-phosphate dehydrogenase (GAPDH; QT00079247) by using SYBR Green, according to the manufacturer's instructions (Qiagen). A genomic DNA digestion was included in this kit.

The following settings in the LightCycler PCR were used. An initial activation step of 15 minutes at 95°C and a ramp of 20°C/s was followed by 3-step cycling (42 cycles): denaturation; 15 seconds at 94°C, annealing; 20 seconds at 55°C, extension; and 20 seconds at 72°C (all 3 with a ramp of 2°C/s). A melting curve was recorded by cooling the samples down to 60°C and then increasing the temperature to 95°C (ramp, 2°C/s). The amounts of CCL2 relative to the amount of the reference GAPDH in the same sample was calculated by using the relative quantification approach with the software provided by the manufacturer (Roche). Mathematically, the result for each sample is expressed as the ratio of CCL2 and GAPDH derived from the crossing points, which are obtained in the Lightcycler software (version 1.0, Roche). In addition, because the basal levels of CCL2 were different per patient, the data were normalized to the CCL2 level in the nonstimulated sample (100%). The values obtained for H₄R agonist-stimulated monocytes were then expressed as the percentage of the nonstimulated monocytes.

Chemotaxis assay

Chemotaxis of monocytes was measured over polycarbonate membranes with a 5- μ m pore diameter (Corning, Inc/Costar, NY). A 600- μ L supernatant from cultured adhered monocytes (with 10^{-5} mol/L stimuli and neutralizing anti-CCL2 antibody, as indicated in the text) was used as a chemoattractant in the lower chamber. The upper chamber with the membrane was filled with 250 μ L of medium with 10^6 isolated monocytes. Chemotaxis was allowed for 1.5 hours, and the number of transmigrated cells was counted after staining with trypan blue (Sigma-Aldrich) in a Burkert-Türk counting chamber. Controls were conducted as described in the text and figures.

Statistics

Paired *t* tests and ANOVA (the Bonferroni multiple comparisons test) were used, and a *P* value of less than .05 was regarded as significant (*P* < .05 was labeled with an asterisk, and *P* < .005 was labeled with a double

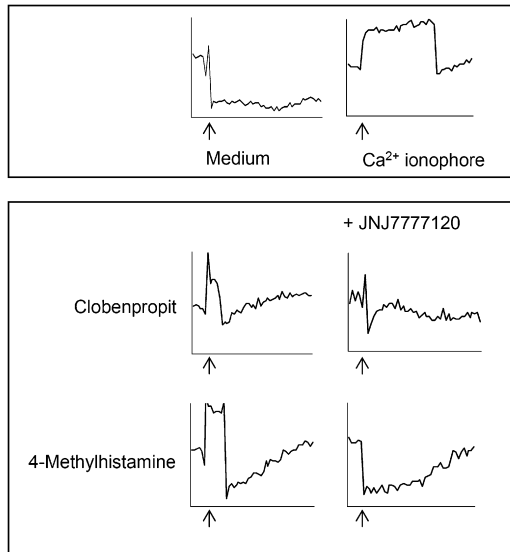


FIG 2. Histamine, 10^{-5} mol/L, induces calcium mobilization in monocytes through the H₄R. JNJ777120 at 10^{-5} mol/L blocks this effect. Injection of the ligands was at a time of 5 seconds (arrows), and FLUO-4 fluorescence was measured for 40 seconds. A representative of 3 experiments is shown.

asterisk). For the statistical analyses, the program GraphPad Prism, version 3.02 (GraphPad Software, Inc, San Diego, Calif), was used.

RESULTS

Expression of the H₄R in monocytes

Monocytes have been shown to express the H₄R at the mRNA level, as described by us¹³ and others.⁶ Here we showed H₄R protein expression on monocytes by means of flow cytometry after staining with a selective H₄R antibody directed against the C-terminal tail.²⁵ The specificity of the immunoreactivity was controlled by blocking the staining of the H₄R antibody with the respective peptide used to generate the antibody (Fig 1, A).

Upregulation of H₄R expression by IFN- γ

The regulation of H₄R protein expression was investigated after incubation with various ligands, such as IFN- γ (20 ng/mL), IL-4 (20 ng/mL), TNF- α (0.5 ng/mL), and the Toll-like receptor 3 ligand poly I:C (2 μ g/mL), as well as combinations of these. The H₄R expression was measured by means of antibody staining, followed by flow cytometry. We found that IFN- γ upregulated the H₄R significantly at the protein level (Fig 1, B). In contrast, IL-4, TNF- α , and poly I:C or combinations of these did not regulate H₄R protein expression.

Calcium influx

The H₄R agonists histamine, clobenpropit, and 4-methylhistamine¹⁶ were all able to stimulate calcium mobilization in monocytes. The effects caused by 10^{-5} mol/L clobenpropit and 4-methylhistamine were blocked by 10^{-5} mol/L JNJ777120,²⁶ the specific H₄R blocker (Fig 2).

H₄R agonists inhibit monocyte CCL2 production dose dependently

Monocytes synthesize CCL2 spontaneously.²⁷ Histamine, 4-methylhistamine, and clobenpropit as H₄R agonists dose-dependently and effectively inhibited CCL2 production with different inhibitory concentration of 50% values (4-methylhistamine > histamine > clobenpropit; Fig 3, A). The inhibition of CCL2 production by histamine and the H₄R agonists clobenpropit and 4-methylhistamine was already seen after 4 hours of incubation and was consistent after 24 and 48 hours of incubation (Fig 3, B). This effect was inhibited by the specific H₄R antagonist JNJ777120 at 10^{-5} mol/L. The amount of CCL2 produced by nonstimulated monocytes was increasing with time (Fig 3, C). In some experiments, also TNF- α was measured (results not shown). The cells did not spontaneously produce this inflammatory cytokine, demonstrating that the adherent cells were not activated during this isolation method.

Histamine inhibits CCL2 synthesis and secretion

We investigated the mechanism of CCL2 downregulation by the H₄R agonists. First, it was found that the mRNA synthesis for CCL2 was downregulated in monocytes incubated with H₄R agonists for 4 hours at 10^{-5} mol/L (Fig 4). Furthermore, when monocytes were incubated with the most selective H₄R ligand, 4-methylhistamine, for 4 hours at 10^{-5} mol/L, the intracellular level of CCL2, as determined by means of intracellular staining and flow cytometry, was higher than in nonstimulated cells (Fig 5). Second, after 48 hours of incubation, 10^{-5} mol/L of 4-methylhistamine inhibited intracellular CCL2 content compared with that seen in nonstimulated cells. The melting point of the amplified CCL2 was 74.7°C (see Fig E1 in the Online Repository at www.jacionline.org).

Matrix metalloproteinases (MMPs) 1, 3, or 8 are not responsible for the decrease of CCL2 levels

MMP1, MMP3, and MMP8 are known to degrade CCL2.²⁸ To test whether MMPs were responsible for the histamine-induced CCL2 decrease, we applied an appropriate inhibitor, GM6001.²⁴ This potent broad-spectrum MMP inhibitor was coincubated with the H₄R agonists. No change in the effect induced by H₄R agonists was seen (see Fig E2 in the Online Repository at www.jacionline.org) at GM6001 concentrations of 1 or 10 μ M. This indicates that the lower CCL2 level was not caused by a higher MMP secretion by the monocytes.

Monocyte chemotaxis is lower toward supernatants of H₄R-stimulated monocytes

The medium from H₄R-stimulated monocytes (48 hours) attracted less monocytes than medium from nonstimulated monocytes (Fig 6). This effect was caused by CCL2 in the supernatants because chemotaxis toward nonstimulated monocyte medium was inhibited by a

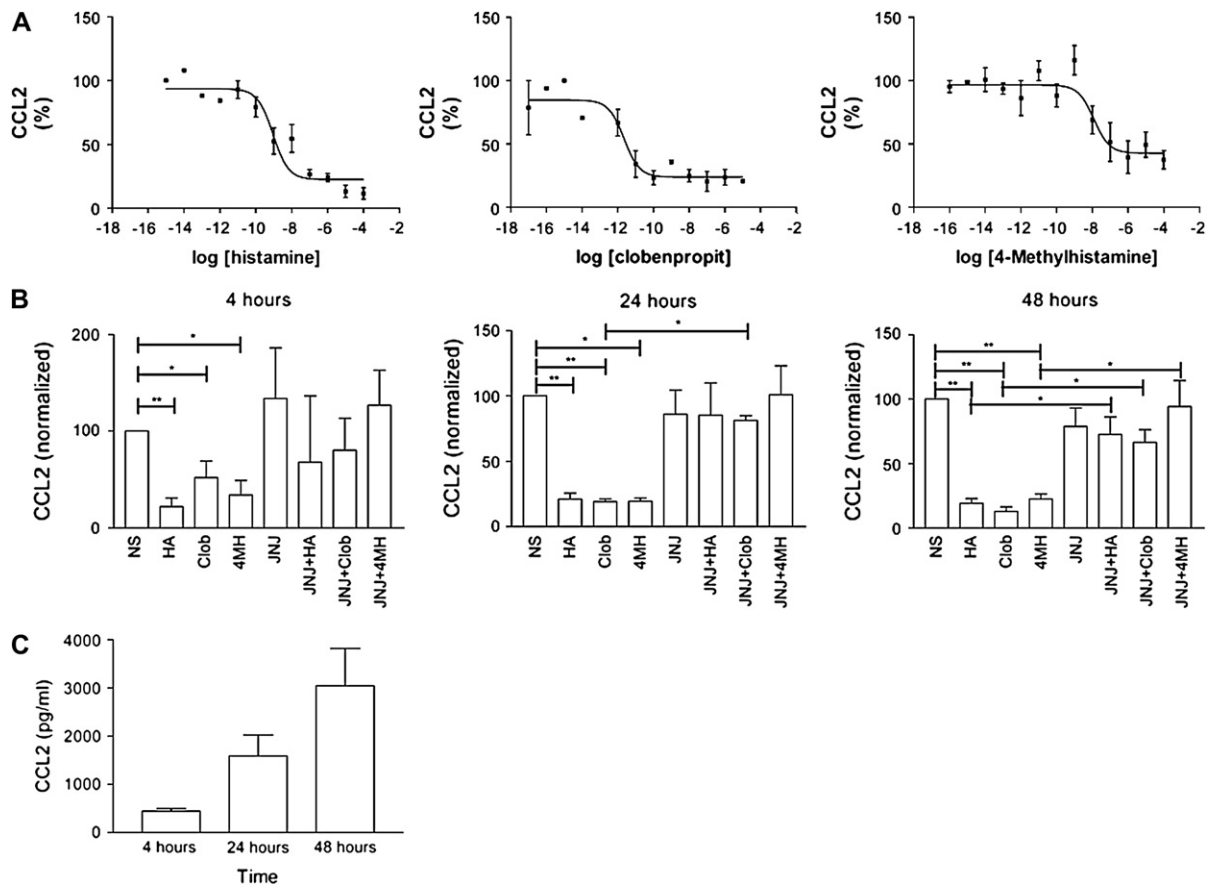


FIG 3. **A**, Dose dependency of CCL2 downregulation by histamine (HA), clobenpropit (Clob), and 4-methylhistamine (4-MH). **B**, Inhibition of CCL2 production is blocked by JNJ777120. **C**, Absolute CCL2 production at different incubation times. Data are normalized to nonstimulated (NS) values in Fig 3, A and B. Averages and SEMs (Fig 3, A), SDs (Fig 3, B), and SEMs (Fig 3, C) of at least 3 experiments are shown (statistical test: ANOVA).

neutralizing CCL2 antibody. Furthermore, addition of human recombinant CCL2 to the H₄R agonist-stimulated monocyte media could reinstate chemotaxis. As a control, fresh medium with histamine, clobenpropit, or 4-methylhistamine did not induce chemotaxis of monocytes.

DISCUSSION

Previously, it has been shown that the H₄R has important immunomodulatory effects on human T lymphocytes^{14,29} and human DCs¹³ *in vitro*. In a recent investigation using a murine model of allergic airway inflammation, an immunomodulatory role for the H₄R was also shown *in vivo*. H₄R knockout mice and mice treated with the H₄R inhibitor JNJ777120 had decreased T-lymphocyte function, which was attributed to a decreased cytokine and chemokine production by DCs.¹⁵ The monocyte cell fraction that contains the precursor cells for DCs and skin-associated macrophages has not been investigated thus far and was addressed in this study.

Here we show for the first time that human monocytes express the H₄R at the protein level, taking advantage of

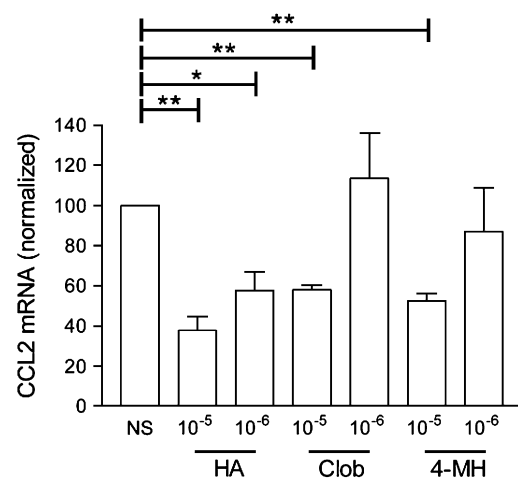


FIG 4. CCL2 mRNA synthesis is downregulated through histamine (HA) and the H₄R agonists clobenpropit (Clob) and 4-methylhistamine (4-MH). Averages and SEMs of 6 experiments are shown (statistical test: ANOVA).

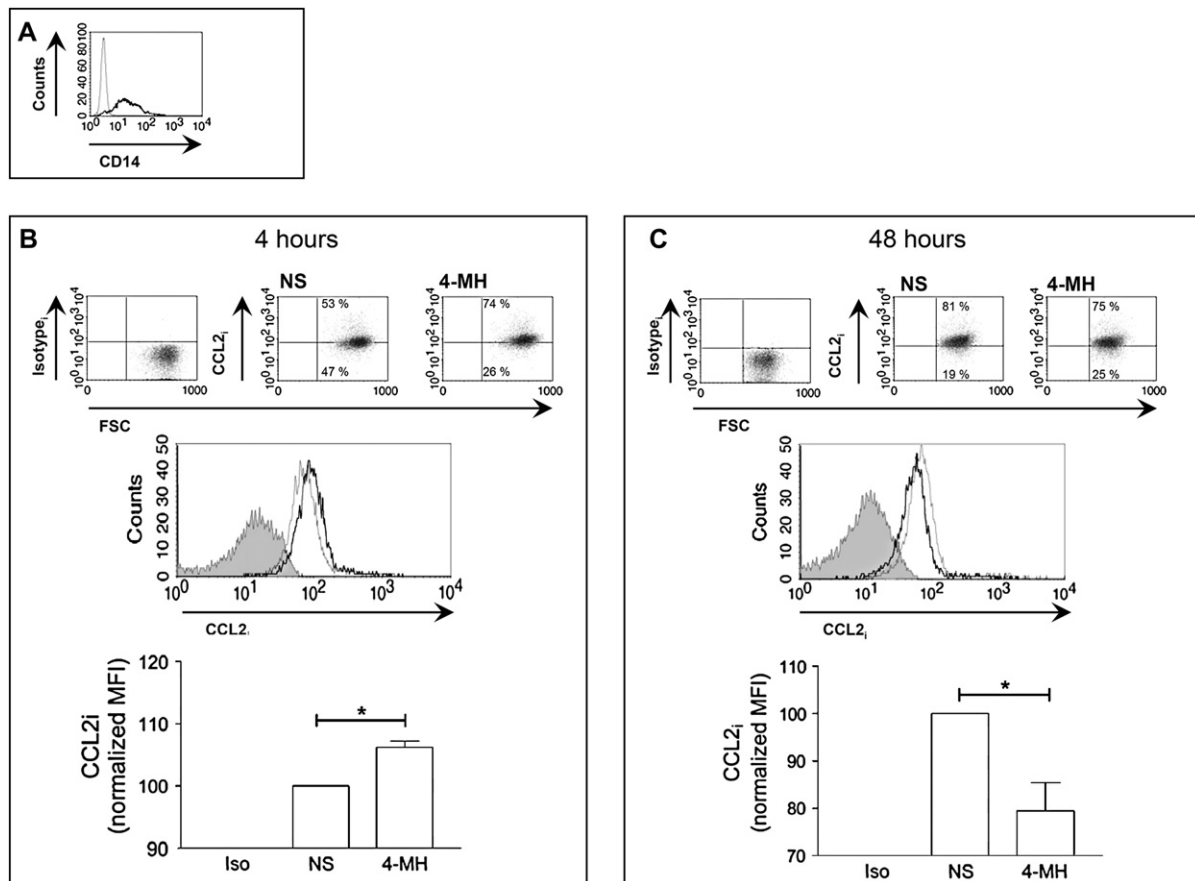


FIG 5. CD14 staining to determine the monocyte fraction within adherent cells (A). Effect of 4-methylhistamine (4-MH, thick solid line) on intracellular CCL2 after 4 hours (B) and 48 hours (C) of incubation. Thin solid line, Nonstimulated cells (NS); filled section, isotype. A representative experiment and averages of normalized mean intracellular CCL2 fluorescence values and SEMs of 5 experiments are shown (statistical test: paired *t* test).

the newly developed anti-human H₄R antibody.²⁵ The expression of H₄R mRNA by monocytes has already been shown by our group¹³ and another group,⁶ but thus far, no function for the H₄R expressed by monocytes has been reported. Here we show that monocytes express H₄R protein and that IFN- γ upregulates the receptor. It has been shown previously that stimulation of the H₄R leads to a calcium mobilization in cells.^{4,6,8,9} Also, in human monocytes H₄R agonists induced calcium mobilization, which was inhibited when the cells were coincubated with the H₄R-specific antagonist JNJ777120.²³ Furthermore, we show that monocytes, on stimulation with the H₄R-specific agonists clobenpropit and 4-methylhistamine and the H₄R-nonspecific agonist histamine, downregulate CCL2 production dose dependently. JNJ777120 was able to fully block this downregulation.

We found 2 possible mechanisms to explain the H₄R-mediated decrease in CCL2 levels produced by monocytes. First, an inhibition of synthesis was demonstrated by decreased CCL2 mRNA levels (4 hours of incubation), leading to decreased intracellular CCL2 levels (48 hours of incubation) when cells were incubated with H₄R

agonist compared with levels seen in nonstimulated cells. Second, intracellular CCL2 protein levels were increased after 4 hours of incubation with 4-methylhistamine compared with levels in the nonstimulated control cells, demonstrating an inhibition of CCL2 secretion after H₄R agonist binding. In contrast, no evidence was found for CCL2 degradation by MMPs, which were previously shown to potentially degrade CCL2.²¹

Histamine has not been shown previously to modulate the secretion of soluble mediators, although it is able to inhibit the production of IL-12p70 by monocytes and monocyte-derived DCs through the H₂R and H₄R,^{13,30} as well as of TNF- α by macrophages and mast cells through the H₂R and H₃R.^{31,32} The possible downregulation of secretion of these mediators, however, has not been investigated, and the decreased cytokine levels were explained by a downregulation of synthesis. In this study we provide evidence that CCL2 synthesis and secretion is indeed downregulated.

The supernatants of monocytes stimulated with H₄R agonists were used as chemoattractants in Boyden chamber assays. We observed significantly lower monocyte

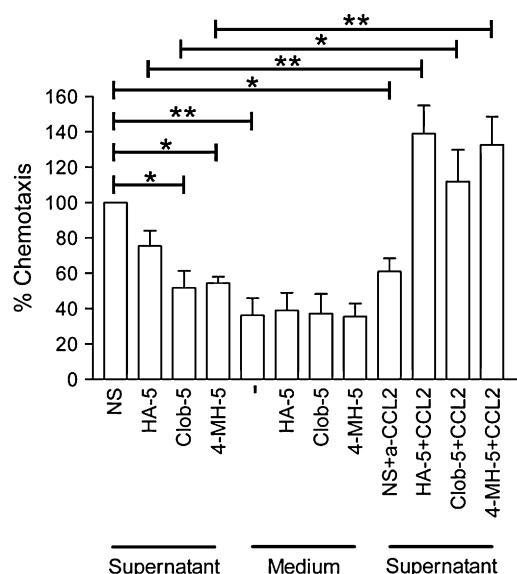


FIG 6. The supernatants of H₄R agonist-stimulated monocytes induce less chemotaxis of monocytes. Values are normalized to chemotaxis induced by supernatants from nonstimulated monocytes (NS). Histamine (HA), clobenpropit (Clob), and 4-methylhistamine (4-MH; all 10⁻⁵ mol/L) and CCL2-neutralizing antibody (a-CCL2) are shown. Averages and SEMs of 4 experiments are shown (statistical test: ANOVA).

migration levels into supernatants from cells incubated with the H₄R agonists clobenpropit and 4-methylhistamine compared with that of nonstimulated monocytes. The migration of monocytes to supernatants from nonstimulated monocytes could be blocked close to media control levels by a CCL2-neutralizing antibody, which demonstrated the importance of CCL2 as a monocyte chemoattractant. The ligands themselves did not stimulate chemotaxis of freshly isolated monocytes, as was previously shown for monocyte-derived DCs,¹³ eosinophils,^{10,11} and mast cells.⁹

In summary, we showed, for the first time, that the H₄R protein is expressed by monocytes and that the H₄R is functional. This is demonstrated by the induction of a Ca²⁺ influx and the downregulation of CCL2. This extends our understanding of the immunologic role of the H₄R and the therapeutic potential of H₄R antagonists in inflammatory disorders.

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