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Histamine H4 Receptor Stimulation Suppresses IL-12p70 Production and Mediates Chemotaxis in Human Monocyte-Derived Dendritic Cells[1]

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There is increasing evidence that histamine as an important mediator of immediate type allergic reactions also effects professional APCs. Recent reports showed effects of histamine on human monocyte-derived dendritic cells (MoDC) mediated primarily via histamine H1 receptors (H1R) and H2R. We show here that MoDC also express H3R and H4R at the mRNA and protein level. mRNA of the H3R is down-regulated and mRNA of the H4R is up-regulated during the differentiation from monocytes to MoDC. H4R or H2R stimulation suppressed IL-12p70 production in MoDC. Induction of cAMP was necessary for IL-12p70 inhibition mediated via the H2R. In contrast, H4R stimulation did not affect cAMP production but induced the transcription factor AP-1, and U0126, an inhibitor of AP-1 transactivation and MEK, rescued H4R mediated IL-12p70 suppression. Moreover, MoDC responded to a H4R agonist (and also to a H2R agonist) with increased F-actin polymerization and migration in modified Boyden chamber assays, suggesting a chemotactic effect of histamine via the H2R and the H4R. Thus, H4R stimulation on MoDC results in immunomodulatory and chemotactic effects. Histamine induces chemotaxis and IL-12p70 suppression via different receptors using different signaling pathways, which might be important for the pathogenesis of and therapeutic interventions in allergic diseases. *The Journal of Immunology,* **2005, 174: 5224–5232.**

Dendritic cells (DC)[3] play a key role in adaptive immunity, because they represent the most potent APC and are able to activate naive T cells (for review, see Ref. 1). They reside in an immature state in many nonlymphoid tissues which are under high pathogen exposure like skin or airways mucosa as sentinels of the immune system. In this immature state, DC are specialized on Ag uptake and presentation. After Ag capture, DC migrate to the draining lymph nodes, where they encounter T cells. During their migration DC convert from an Ag-capturing to an Ag-presenting mode, that is termed "maturation". While DC mature, the surrounding microenvironment has a great impact on their functional capabilities, especially regarding their T cell stimulatory capacities. Maturation in the presence of IFN-γ leads to DC that produce high levels of IL-12 and prime Th1 cells, whereas the presence of PGE_2 generates DC that assign Th2 cells (2, 3). The presence of IL-10 is thought to lead to DC that induce anergic or regulatory T cells (2, 4). Histamine is another factor that is likely to get in contact with DC, e.g., in the skin during hypersensitivity reactions. The effects of histamine are mediated via at least four different G protein-coupled receptors, H1R, H2R, H3R, and H4R (5, 6). Recent studies demonstrated the expression of H1R and H2R and effects of histamine on cells of the adaptive immune system, in particular, T cells and DC (7). In human and murine T cells, H1R signaling appears to trigger Th1-responses, whereas H2R signaling suppresses both Th1- and Th2-type responses (8, 9).

The expression of H1R and H2R on human monocyte-derived DC (MoDC) has also recently been reported (10–13), and stimulation of MoDC with histamine resulted in induction of CD86 surface expression (14), suppression of IL-12 production (10–13), and polarization of DC into Th2 cell promoting cells (10, 13).

Effects of the H3R and the recently cloned H4R (15–21) on cells of the adaptive immune system are less clear. H3R is primarily expressed in the CNS whereas H4R is primarily expressed on peripheral tissues such as spleen, thymus, colon, peripheral blood leukocytes, and bone marrow (15, 20, 22).

On human MoDC, results with regard to the expression and function of the H3R are controversial. Whereas some studies were able to detect the receptor mRNA by RT-PCR (12, 13), others found only a faint (22) or no signal (10). Functional analyses in these studies were also controversial, describing no effect of the H3R antagonist thioperamide on histamine-induced IL-12p70 suppression (10, 13) or suppression of IL-12p70 by the H3R agonist R-α-methylhistamine (R-α-MeH) (12). However, the compounds that were used to address the H3R also bind to the H4R and, therefore, do not allow the discrimination of the two receptors (6).

The expression of the H4R on human MoDC has been described in a number of studies (16–19, 21, 22), but functional data are lacking.

Here, we reanalyzed H3R and H4R expression on human MoDC. Furthermore, we studied immunologic and chemotactic

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[3] Abbreviations used in this paper: DC, dendritic cell; MoDC, monocyte-derived DC; NBD, nitrobenzoxadiazole; R-α-MeH, R-α-methylhistamine; Rp-8-Br-cAMP, adenosine 3′,5′-cyclic monophosphorothioate 8-bromo, Rp-isomer; HR, histamine receptor; RT, reverse transcriptase.

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effects and signal transduction pathways on human MoDC by using the stimuli mentioned above and by using additional substances that allow the dissection of H3R and H4R signaling, i.e., clobenpropit, an antagonist of the H3R and an agonist of the H4R, allowing the dissection of H3R and H4R function (6, 17, 20, 22), ciproxifan, an antagonist of the H3R (23–25), and JNJ7777120, an antagonist of the H4R (24, 26, 27).

Materials and Methods

Preparation and FACS analysis of MoDC

Human MoDC were differentiated from monocytes as described previously (11, 28). In brief, monocytes were isolated from PBMC of leukocyte-enriched buffy coats by means of plastic adherence and removal of non-adherent cells by vigorous washing five times with PBS. The remaining adherent cells (monocytes) were cultured in Iscove's medium supplemented with 4% heat inactivated human serum, 250 U/ml IL-4 (R&D Systems), and 1000 U/ml GM-CSF (Novartis Pharma). On day 7, nonadherent cells (defined as immature MoDC) were harvested and analyzed by double color flow cytometry for contaminating $CD3^+$ T cells, $CD20^+$ B cells, $CD56^+$ NK cells, or $CD16^+$ granulocytes as described previously (11). Of these MoDC preparations, all cells expressed the myeloid marker CD11c, >90% of cells expressed HLA-DR and CD86, and after stimulation with TNFα and PGE_2 >80% expressed CD83. In brief, cells (1×10^5) were washed and resuspended in PBS containing 0.2% gelatin, 20 mM sodium-azide, and 10 μg/ml heat-aggregated human IgG (Sigma-Aldrich). Subsequently, cells were incubated with fluorescent-labeled Abs on ice for 30 min (HLA-DR-FITC, CD3-FITC, CD16-PE, CD20-FITC, CD56-PE, CD86-PE, or isotype-matched controls; Immunotech). Stained cells were analyzed by flow cytometry (FACScan; BD Biosciences). Only preparations with <5% contaminating T, B, NK cells, or granulocytes were used in subsequent experiments (for RT-PCR experiments the purity was required to be <2% contaminating cells).

Histamine receptor (HR) ligands and stimulation of MoDC

A variety of histamine receptor ligands was used for the evaluation of expression and function of histamine receptors on MoDC. If not otherwise stated, they were used at a concentration of 10^{-5} M. Name, specificity, and source are listed in the following: histamine (agonist for all receptors; Sigma-Aldrich), betahistine (H1R agonist; Sigma-Aldrich), dimaprit (H2R agonist; Sigma-Aldrich), imetit (H3R and, less efficient, H4R agonist; Sigma-Aldrich), R-α-MeH (H3R > H4R agonist; Sigma-Aldrich), clobenpropit (H3R antagonist, H4R agonist; Calbiochem), cimetidine (H2R antagonist; Sigma-Aldrich), thioperamide (H3R antagonist and, less efficient, H4R antagonist; BioTrend). The histamine H3R antagonist ciproxifan and the H4R antagonist JNJ7777120 were synthesized as stated previously (24, 25).

To assess signal transduction pathways, MoDC were incubated for 2 h before the second stimulus with 10 μM U0126 (Calbiochem; inhibitor for the transcription factor AP-1 and MEK1/2) (29), 10 μM SB202474 (Calbiochem, negative control for U0126), or 50 μM adenosine 3′,5′-cyclic monophosphorothioate 8-bromo, Rp-isomer (Rp-8-Br-cAMP; Calbiochem, inhibitor of cAMP).

FACS analysis of HR surface expression

The assessment of cell surface expression of HR was performed following a published protocol (30). In brief, cells were incubated for 30 min on ice with BODIPY FL-histamine (10^{-5} M; Molecular Probes), a histamine labeled with a fluorescent dye that can be detected by flow cytometry in the FL-1 channel. To demonstrate specific binding, cells were preincubated for 5 min with histamine- (5×10^{-3} M) or HR-specific ligands (5×10^{-4} M) alone or in combination or with buffer.

mRNA isolation, reverse transcription, and LightCycler PCR

mRNA was isolated from 1×10^5 MoDC using a mRNA isolation kit (Roche) according to the supplier′s instructions. For RT-PCR analysis, RNA was subjected to first-strand cDNA synthesis using $oligo(dT)_{15}$ for full-length cDNA synthesis. The reverse transcriptase (RT) reaction mixture contained final concentrations of 50 U Expand-RT (Roche), 10 mM DTT, 1× first-strand RT buffer for Expand-RT, 0.5 mM of each dNTP (Roche), RNase inhibitor (Invitrogen Life Technologies), and 80 pmol $oligo(dT)_{15}$ (Roche). To control for genomic DNA contamination, cDNA synthesis was performed in the absence of RT.

H3R and H4R amplifications were performed with LightCycler primer sets from LC Research according to the instructions of the manufacturer. Real-time fluorescence PCR was performed using the LightCycler (Roche) as described previously (28) with an initial denaturation step at 95°C for 10 min, amplification with 35 cycles of denaturation (95°C, 10 s), annealing (68°C, 10 s), and extension (72°C, 16 s). After amplification had been completed, a final melting curve was recorded by cooling the samples to 65°C at 20°C per second and then increasing the temperature to 95°C at 0.2°C per second. Fluorescence was measured continuously during the slow temperature rise to monitor the dissociation of the PCR product. The fluorescence signal was plotted in real-time against the temperature to produce melting curves of each sample. Melting curves were then converted to melting peaks by plotting the negative derivative of the fluorescence with respect to temperature against temperature (−dF/dT vs T). Thus, each specific PCR product generates a specific signal and, therefore, a product-specific melting peak.

PCR products were subjected to electrophoresis on a 2% agarose gel, stained with ethidium bromide, visualized, and photographed under UV illumination.

Real-time monitoring of amplification after each annealing period allows quantification of the samples during log-linear phase of the PCR. For quantitative analyses, the LightCycler relative quantification software (Roche) was used that is designed to determine exact efficiency corrected relative nucleic acid concentration, normalized to a calibrator sample. Amplification of the β-actin gene was performed in parallel to amplification of H3R and H4R as previously reported (11, 31).

Measurement of cAMP

A total of 1×10^5 MoDC were incubated at 37°C in PBS containing 0.4% human albumin and 2 mM 3-isobutyl-1-methyl-xanthine (Sigma-Aldrich). After 10 min, histamine (10^{-4} M) or receptor-specific agonists were added. In some experiments, the H2R antagonist cimetidine (10^{-4} M) or the H3R/H4R antagonist thioperamide (10^{-4} M) were added 10 min before histamine was added. The reaction was stopped 5 min later with 1/5 volume 0.5 N HCl. cAMP levels were determined with a commercially available cAMP assay kit according to the manufacturer instructions (R&D Systems). To assess the effect of HR on forskolin stimulated cells, receptor-specific ligands were added 10 min before forskolin (10 μM; Sigma-Aldrich).

Assessment of IL-12p70 and IL-10 production

For assessment of IL-12p70, 1×10^5 immature MoDC were treated with histamine or receptor-specific ligands for 24 h. Then, polyinosinic-polycytidylic acid (polyIC, 10 μg/ml, Sigma-Aldrich) as a well known inductor of IL-12p70 was added. Supernatants were harvested 24 h later and analyzed for IL-12p70 content using a commercially available ELISA (BioCarta Europe).

For assessment of IL-10, 1×10^5 immature MoDC were treated with histamine or receptor-specific ligands or (as positive control) with polyIC for 48 h. Histamine or clobenpropit were also used in combination with polyIC to assess a possible inhibition or synergistic effect. Supernatants were analyzed for IL-10 content using a commercially available ELISA (BioCarta Europe).

Western blotting

A total of 1×10^6 MoDC were stimulated for 15 or 30 min as indicated and homogenized in PhosphoSafe extraction buffer (Novagen). After cell lysis, the samples were centrifuged at $3000 \times g$ for 10 min (4°C), and the protein concentration in the supernatants was determined with a protein assay (Bio-Rad). A total of 250 μg of protein per sample were separated on a 4–15% SDS-polyacrylamide gel under reducing conditions and transferred onto a nitrocellulose membrane for (semidry system, 30 min, 20 V, room temperature). A mouse-anti-human phospho-p44/42-ERK1/2 Ab (New England Biolabs, Frankfurt, Germany) was used to detect phosphorylated ERK1/2, followed by an HRP-conjugated horse-anti-mouse secondary Ab (New England Biolabs). Immunoreactivity was detected using a chemiluminescence kit according to the instructions of the manufacturer (Pierce). As positive control, PMA was used (10 ng/ml, Sigma-Aldrich).

ERK1/2 ELISA

A commercially available ELISA kit was used to determine ERK1/2 phosphorylation in MoDC (Human Phospho-ERK1/ERK2 Duo Set; R&D Systems). A total of 1×10^6 MoDC were stimulated for 15 min as indicated and processed exactly as recommended by the manufacturer of the ELISA kit.

EMSA

EMSA was used to detect specific binding of the transcription factor AP-1 to its specific DNA consensus sequence. Nuclear extraction was performed using NE-PER Nuclear and Cytoplasmic Extraction Reagents according to the manufacturer's instructions (Pierce). Protein content of nuclear extracts was determined with a protein assay (Bio-Rad).

Nuclear protein-DNA binding reactions were conducted for 20 min at room temperature in a 20-μl volume containing 10 mM Tris, 50 mM KCl, 1 mM DTT, 2.5% glycerol, 5 mM $MgCl_2$, 1 μg/μl polyIC, 1% Nonidet P-40, (all the reagents are included in the LightShift Chemiluminescent EMSA kit; Pierce), 100 fM biotin-labeled probe of the double-stranded AP-1 consensus sequence (TIB MOLBIOL) containing a binding site for Jun/Fos heterodimeric complexes (5′-CGC TTG ATG ACT CAG CCG GCA-3′), and 5 μg of nuclear protein. Binding reactions were analyzed using 8% native PAGE. After blotting to a nylon membrane, labeled oligonucleotides were detected with the LightShift Chemiluminescent EMSA kit following the instructions of the manufacturer (Pierce).

The binding specificity was confirmed by adding to the nuclear protein-DNA binding reaction either an excess of unlabeled AP-1 oligonucleotides (20 pM) or a biotin-labeled mutated AP-1 oligonucleotide instead of the correct oligonucleotide (100 fM, 5′-CGC TTG ATG ACT TGG CCG GAA-3′, the mutated bases are underlined).

Assessment of F-actin polymerization

Nitrobenzoxadiazole (NBD)-phallacidin (Molecular Probes) staining of MoDC was conducted by modification of the method described by Howard and Meyer (28, 32). Briefly, cells were resuspended at a concentration of 2×10^6 cells/ml in PBS-buffer lacking Ca^{2+} and stimulated with the indicated stimuli at room temperature. Following stimulation, cells were fixed using 3.7% formaldehyde for 60 min. Lysophosphatidylcholine (20 μg/ml; Sigma-Aldrich) and 3.7×10^{-7} M NBD-phallacidin were added to the sample and incubated for a period of 60 min in the dark. NBD-phallacidin-stained cells were analyzed on a BD Biosciences FACScan with a linear fluorescence channel (FL-1) where the fluorescence is proportional to F-actin content (32). Relative F-actin content is expressed as the ratio of the mean channel fluorescence (integrated fluorescence) between stimulated and nonstimulated cells (28).

Boyden chamber chemotaxis assays

Chemotactic activity of MoDC was determined using a modified Boyden chamber technique as described previously (28). In brief, Boyden chambers (Nucleopore) were filled with stimuli (histamine or HR agonists) and covered with polycarbonate filters (pore size 8 μm; Nucleopore). One hundred microliters of a MoDC suspension (1×10^6 cells/ml) were added to each chamber. After incubation for 90 min at 37°C, migrated cells in the lower part of the Boyden chambers were lysed by adding 0.1% Triton X-100, and β-glucoronidase activity in the lysates was determined photometrically using *p*-nitrophenyl β-D-glucuronide as a substrate (all from Sigma-Aldrich). Fluorescence readings were performed with the EAR400AT reader from SLT/TECAN Labinstruments. 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 MoDC.

Statistical analysis

Statistical analyses were performed using the paired *t* test.

Results

Expression of H3R and H4R on MoDC

mRNA of H3R and H4R could be detected in MoDC by RT-PCR. cDNA was amplified with a LightCycler System using primers specific for the H3R and H4R. The PCR was analyzed by LightCycler melting curve analysis and yielded melting peaks for H3R at 91.2°C and for H4R at 80.8°C, whereas the negative control showed no peak (Fig. 1, *a* and *b*). Agarose gel electrophoresis revealed one sharply demarcated band for H3R amplicons (Fig. 1*c*) and H4R amplicons (Fig. 1*d*).

Binding of BODIPY FL-histamine was detected on MoDC (Fig. 2). This binding was in part specific for H3R and H4R because it could be partly blocked by preincubation with H3R/H4R ligands as assessed by flow cytometry (Fig. 2). Preincubation of MoDC with unlabeled histamine or a mixture of ligands to the four HRs

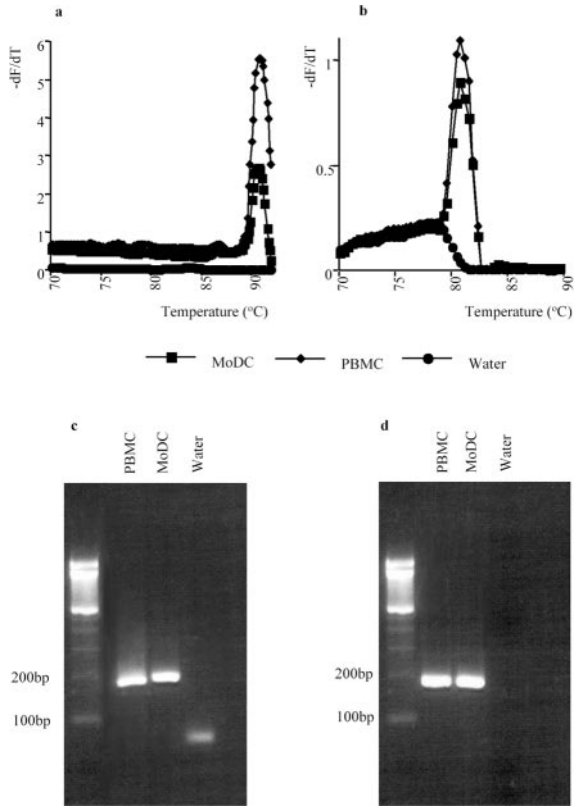

FIGURE 1. H3R and H4R are expressed on MoDC at the mRNA level. cDNA derived from human PBMC and MoDC were amplified by real time LightCycler PCR. Analysis of amplification products by melting curve analysis revealed melting peaks at 91.2°C for the H3R (*a*) and 80.8°C for the H4R (*b*). Agarose gel electrophoresis demonstrated one sharp band of the same size in both MoDC and PBMC for H3R (*c*) and H4R (*d*). (PBMC, positive control; Water, negative control).

reduced binding of BODIPY FL-histamine to a similar extent (Fig. 2).

In addition to this indirect staining protocol, we also tried to detect the H3R and H4R by Western blotting and FACS analysis using commercially available polyclonal rabbit Abs. However, these Abs were only characterized by ELISA with the immunogenic peptide used to vaccinate rabbits and not tested for Western blot or flow cytometry. By using positive control protein extracts and cells, we were unable to obtain bands of the predicted sizes in Western blot experiments and to obtain specific staining in flow cytometric experiments (data not shown). Thus, we were unable to use these Abs for the evaluation of H3R and H4R protein expression on MoDC.

Quantitative real time LightCycler RT-PCR was used to investigate the regulation of H3R and H4R expression along the differentiation from monocytes to DC and upon maturation of DC with LPS. Monocytes and immature and LPS-matured MoDC expressed both H3R and H4R mRNA (Fig. 3). Monocytes cultured in IL-4 and GM-CSF containing medium significantly down-regulated H3R mRNA (Fig. 3*a*) and up-regulated H4R mRNA (Fig. 3*b*). The maturation of immature MoDC with LPS had no significant effect on H3R and H4R mRNA expression (Fig. 3).

cAMP formation is not affected by H3R/H4R signaling in MoDC

Histamine is well known to induce cAMP formation via H2R (33), and it has been argued that cAMP production may be inhibited by H3R signaling (34) or H4R signaling (16, 18). Histamine induced cAMP production in MoDC via the H2R (Fig. 4*a*). However, HR

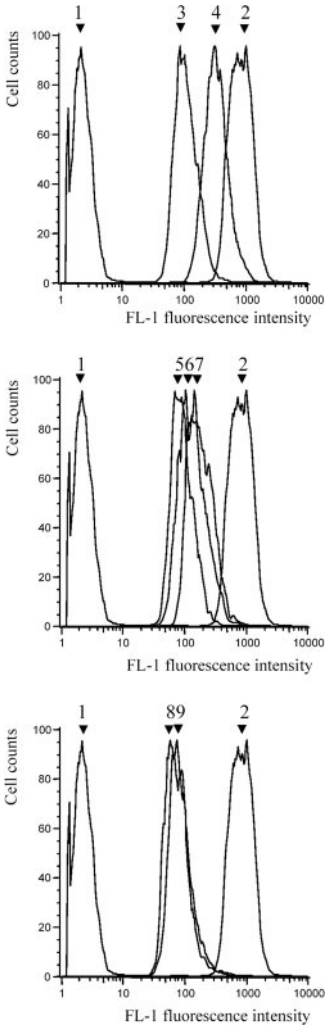

FIGURE 2. H3R and H4R are expressed at the protein level. Cell surface expression of H3R and H4R on MoDC was assessed by flow cytometry by staining of cells with BODIPY FL-histamine (that can be detected in the FL-1 channel). MoDC were either not stained (1), stained with BODIPY FL-histamine (2), or stained with BODIPY FL-histamine after preincubation with betahistine to block the H1R (3), dimaprit to block the H2R (4), imetit to block the H3R and H4R (5), clobenpropit to block the H3R and H4R (6), or R-α-MeH to block the H3R and H4R (7). Preincubation with unlabeled histamine (8) or the combination of betahistine, dimaprit, R-α-MeH, and clobenpropit (9) resulted in the same reduction of BODIPY FL-histamine binding. Representative results from one of three independent experiments are shown.

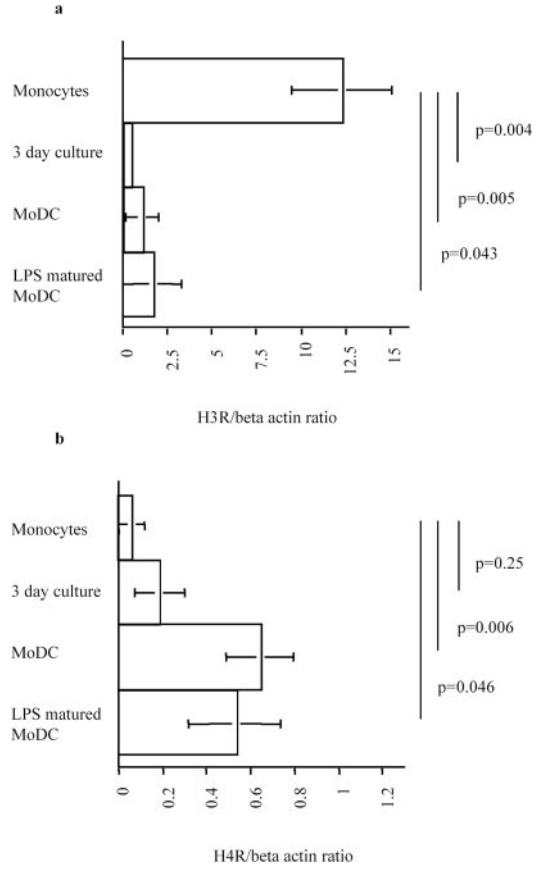

FIGURE 3. The mRNA for the H3R is down-regulated and the mRNA for the H4R is up-regulated during the differentiation from monocytes to MoDC. RT-PCR was performed with monocytes, monocytes cultured for 3 or 6 days (immature MoDC) in medium containing GM-CSF and IL-4, and MoDC stimulated for 48 h with LPS. Real-time LightCycler PCR was performed for H3R, H4R, and β-actin. The relative amounts of H3R signal and H4R signal were determined according to an internal standard as indicated in *Materials and Methods* and referred to the β-actin amounts. Results of six independent experiments (mean ± SEM) are shown for H3R (*a*) and H4R (*b*).

ligands to the H3R and H4R neither induced the cAMP production nor affected the cAMP production induced by the well-known cAMP inductor forskolin (Fig. 4*b*).

Effects on IL-12p70 and IL-10 production

IL-12p70 is produced by APCs and is in particular important for the elucidation of a Th1-type immune response, whereas the lack of IL-12p70 has been associated with Th2-type immune responses (2). Histamine has been shown to decrease the production of IL-12p70 of monocytes (35, 36) and MoDC (10–13) via the H2R. We show here that histamine also blocked IL-12p70 production of polyIC stimulated MoDC via the H4R as shown directly by the H4R agonist clobenpropit and indirectly by the H3R/H4R antagonist thioperamide (Fig. 5*a*). This effect was dose dependent (Fig. 5*b*). Additional experiments using ciproxifan as H3R antagonist and JNJ7777120 as H4R antagonist clearly demonstrated that the clobenpropit-induced IL-12p70 suppression was due to H4R stimulation and not due to H3R blockade (Fig. 5*c*).

To evaluate downstream signal transduction, two key pathways known for their role in IL-12p70 regulation were investigated. For H2R signaling, the cAMP pathway was suspected, because cAMP is induced via the H2R (see above) and suppression of IL-12p70 has been reported for a variety of cAMP-inducing G-protein-coupled receptors (37). Indeed, by blocking cAMP production using the specific inhibitor Rp-8-Br-cAMP, the IL-12p70 suppression via the H2R-mediated response but not via the H4R-mediated signaling was neutralized (Fig. 6*a*). For suppression of IL-12p70 production, the MAPK pathway was suspected (31), because the H4R is a $G\alpha_{i/o}$-coupled receptor (7). Such receptors have been reported to activate MAPK pathways, and activation of MAPK pathways has been reported to suppress IL-12p70 production (31). Indeed, preincubation of MoDC with U0126, an inhibitor of MEK 1/2 that blocks the phosphorylation of ERK1/2, rescued IL-12p70 suppression via the H4R-mediated signaling but not via the H2R-mediated response (Fig. 6*b*).

In contrast to IL-12p70 production, we did not observe a consistent effect of histamine or clobenpropit on IL-10 production of MoDC. PolyIC stimulation resulted in an up-regulation of IL-10

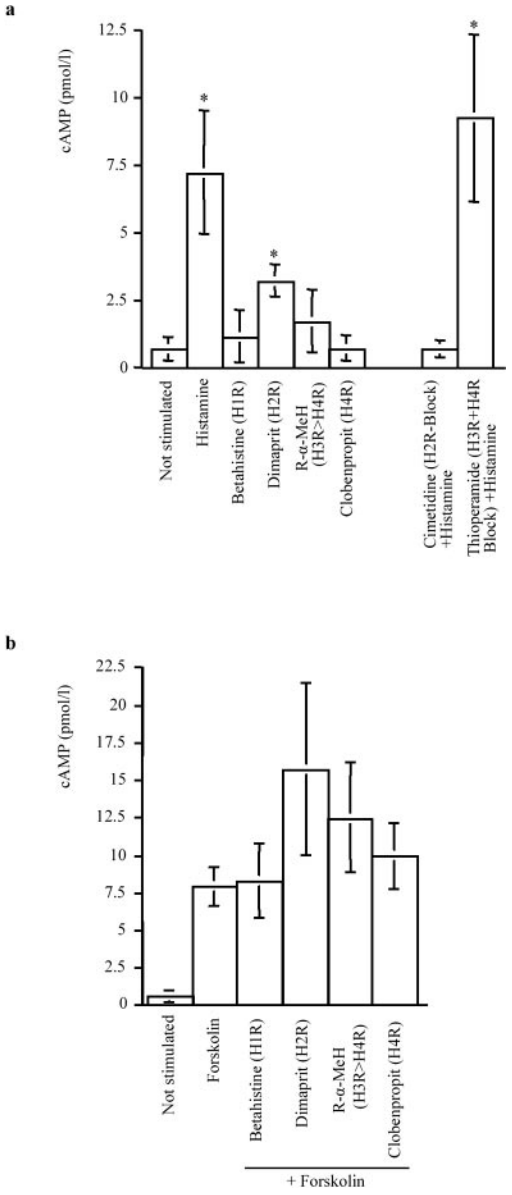

FIGURE 4. Histamine induces cAMP production in MoDC via the H2R. Significant cAMP production could be induced in MoDC by stimulation with histamine and the H2R agonist dimaprit. This histamine effect could be completely blocked by preincubation of MoDC with the H2R antagonist cimetidine. There were no significant effects of agonists or antagonists to the H3R and H4R (*a*; mean ± SEM of five independent experiments). Forskolin as known cAMP inductor significantly induced cAMP production in MoDC (*b*; $p = 0.005$ as compared with not stimulated MoDC). Combined stimulation of MoDC with forskolin and HR agonists did not significantly increase cAMP levels as compared with forskolin alone (*b*; mean ± SEM of six independent experiments).

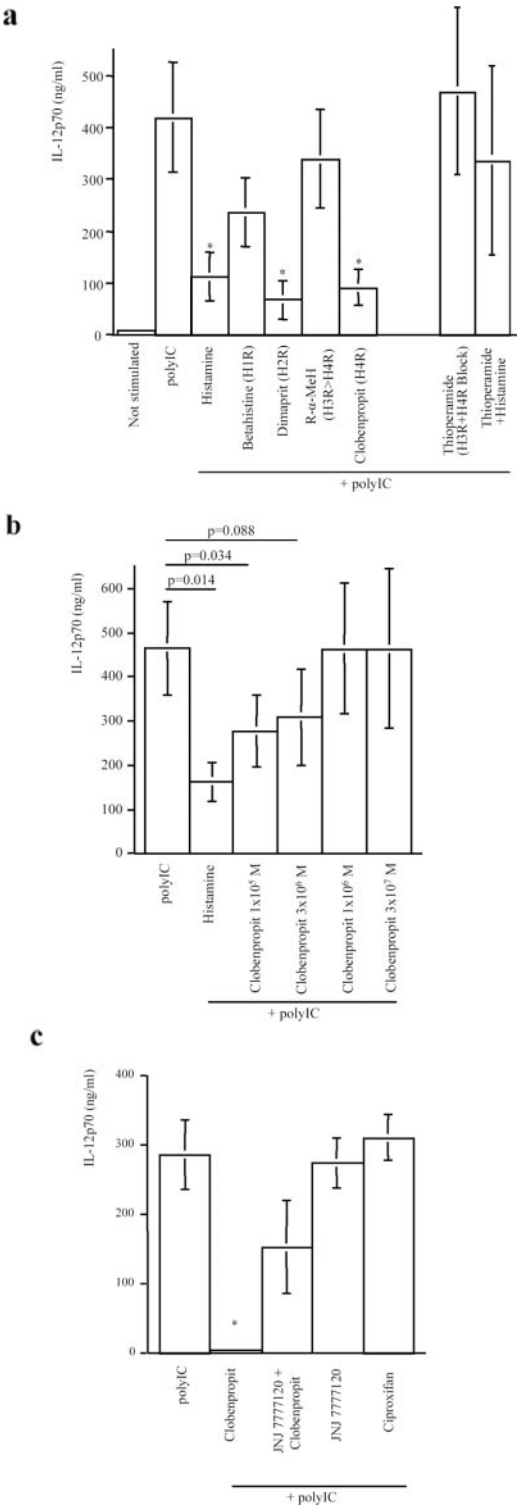

FIGURE 5. Histamine suppresses the IL-12p70 production of MoDC via the H2R and the H4R. MoDC were stimulated for 24 h with histamine or HR ligands; next, polyIC was added for another 24 h to induce IL-12p70 production. Supernatants were assayed for IL-12p70 concentrations by ELISA. Mean ± SEM of six independent experiments (*a* and *b*) and four independent experiments (*c*) are shown; ∗, significant differences as compared with MoDC stimulated with polyIC alone ($p < 0.05$). *a*, Histamine, the H2R agonist dimaprit and the H4R agonist clobenpropit significantly inhibited IL-12p70 production. *a*, The H3R/H4R antagonist thioperamide rescued IL-12p70 production inhibited by histamine. *b*, The effect of clobenpropit on IL-12p70 suppression was dose dependent. *c*, The effect of clobenpropit was inhibited by the H4R antagonist JNJ7777120, whereas the H3R antagonist ciproxifan had no effect.

that was not inhibited or increased by the addition of histamine or clobenpropit (data not shown).

H4R signaling does not phosphorylate ERK1/2 but stimulates DNA binding of the AP-1 transcription factor

Because U0126 is known to inhibit ERK1/2 phosphorylation by blocking MEK1/2 (29), we next investigated whether the H4R is able to induce ERK1/2 phosphorylation. However, we were unable to detect ERK1/2 phosphorylation by stimulation with histamine or clobenpropit using an ELISA (Fig. 7*a*) and Western blot (Fig.

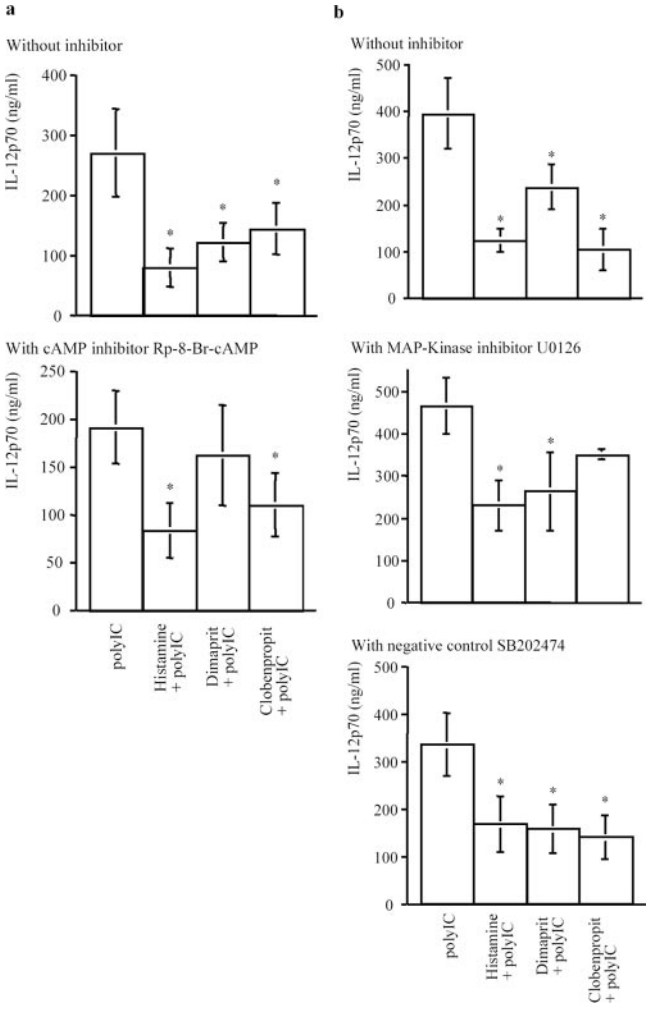

FIGURE 6. Histamine-induced IL-12p70 suppression is mediated via different downstream signaling pathways. Preincubation of MoDC with the cAMP inhibitor Rp-8-Br-cAMP rescued IL-12p70 suppression mediated via the H2R agonist dimaprit but not via the H4R agonist clobenpropit (*a*; mean ± SEM of nine independent experiments; ∗, $p < 0.05$). Preincubation of MoDC with the MEK and AP-1 inhibitor U0126 rescued clobenpropit-mediated IL-12p70 suppression but not the inhibitory effect of dimaprit (*b*; mean ± SEM of nine independent experiments; ∗, $p < 0.05$). SB202474 was used as negative control and had no significant effects on IL-12p70 suppression by histamine and HR agonists (*b*; mean ± SEM of seven independent experiments; ∗, $p < 0.05$).

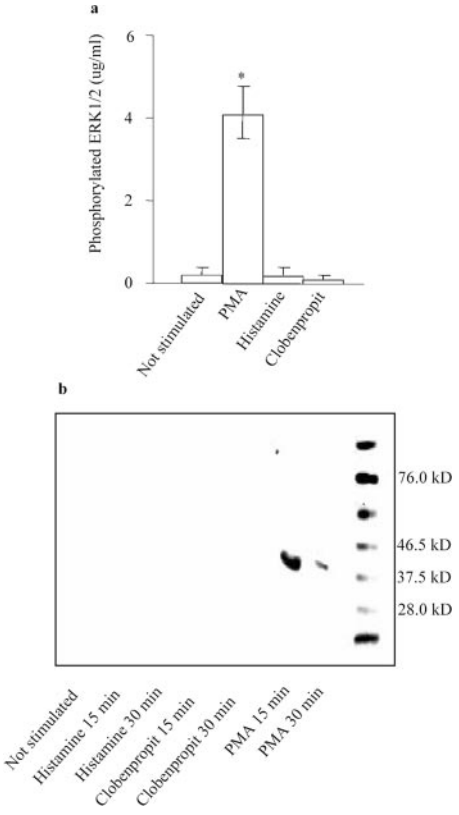

FIGURE 7. Histamine is unable to induce ERK1/2 phosphorylation in MoDC. Phosphorylation of ERK1/2 was studied using an ELISA(*a*; mean ± SEM of six independent experiments) and Western blot (*b*; one representative of three independent experiments). A total of 1×10^6 MoDC were stimulated for 15 min (ELISA) and 15 or 30 min (Western blot) with the indicated stimuli. No phosphorylation of ERK1/2 was detected in MoDC stimulated with histamine or clobenpropit under conditions PMA as positive control was able to induce ERK1/2 phosphorylation.

7*b*). PMA as positive control induced ERK1/2 phosphorylation in both assays.

Because U0126 has also been defined as inhibitor of the AP-1 transcription factor, we next studied the induction of AP-1 by H4R signaling as a downstream event of different MAPK pathways. Nuclear protein extracts were obtained from MoDC stimulated with histamine, HR-specific ligands, and PMA as positive control and used in EMSA experiments with oligonucleotides containing the AP-1 binding sequence. Histamine, clobenpropit, and PMA, but not agonists for the H1R, H2R, and H3R induced AP-1 transcription factor binding to the oligonucleotides (Fig. 8*a*). AP-1 induction by clobenpropit could be blocked by the H4R antagonist JNJ7777120, demonstrating that AP-1 is induced by H4R stimulation (Fig. 8*b*). Controls to ensure specificity of the EMSA are shown in Fig. 8*c*. Thus, histamine appears to induce the AP-1 transcription factor complex independent of ERK1/2 phosphorylation.

Induction of F-actin polymerization and chemotaxis via HR

The effect of histamine on the cytoskeleton of MoDC as an indirect parameter for induction of cell migration was studied by looking at F-actin polymerization. Histamine induced F-actin polymerization in MoDC. This effect could also be induced by H2R agonist dimaprit, the H3R agonist R-α-MeH, the H4R agonist clobenpropit, but not with the H1R agonist betahistine (Fig. 9*a*). F-actin polymerization induced by clobenpropit could be blocked by the H4R antagonist JNJ7777120, whereas blockade of the H3R by ciproxifan had no effect (Fig. 9*b*).

Furthermore, cell migration of MoDC induced by histamine was studied using Boyden chambers (Fig. 10). MoDC migrated toward a gradient of histamine, the H2R agonist dimaprit, and the H4R agonist clobenpropit. For the H3R agonist R-α-MeH, statistical analysis of five experiments revealed only a trend, whereas a gradient of the H1R agonist betahistine had no effect.

Discussion

In this study, we show that the H3R and the H4R are expressed at the mRNA and protein level by human MoDC. H4R mRNA is up-regulated while H3R mRNA is down-regulated in MoDC as compared with monocytes. A number of previous studies demonstrated H4R mRNA expression in MoDC but did not provide functional data (16–22). Comparing monocytes and MoDC, MoDC appear to express higher levels of H4R mRNA also in another study (22).

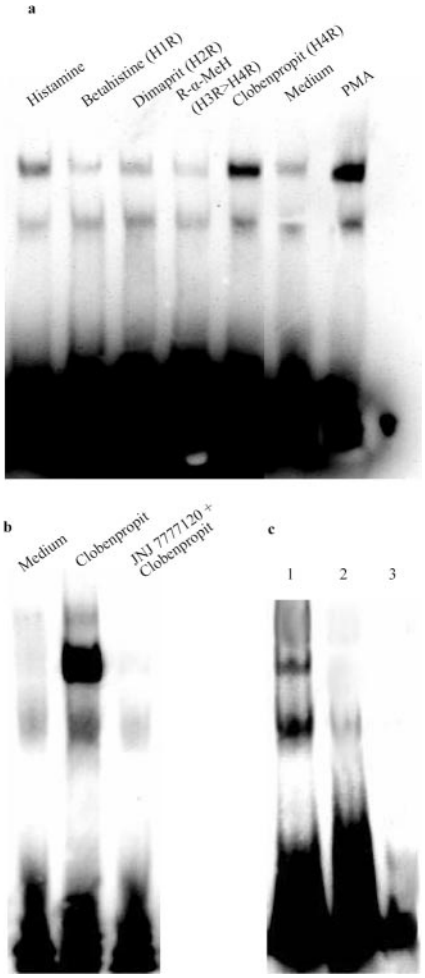

FIGURE 8. Histamine induces the AP-1 transcription factor via the H4R. MoDC were stimulated with histamine or HR ligands for 15 min, and nuclear extracts were obtained and analyzed for the presence of AP-1 transcription factor formation by EMSA. *a*, Clobenpropit and histamine induced AP-1 as demonstrated by the binding to labeled double-stranded oligonucleotides containing the AP-1 binding site. *b*, Clobenpropit-induced DNA binding of AP-1 was completely inhibited by the H4R antagonist JNJ7777120. As controls for the specificity of the EMSA, nuclear extracts from PMA stimulated cells were either subjected to the regular binding reaction (*c*, *lane 1*), or an excess of unlabeled oligonucleotides containing the AP-1 binding site was added to the binding reaction for competition with the labeled oligonucleotides (*lane 2*), or the AP-1 oligonucleotide was replaced by a mutated nucleotide that misses binding activity (*lane 3*). One representative experiment of four is shown.

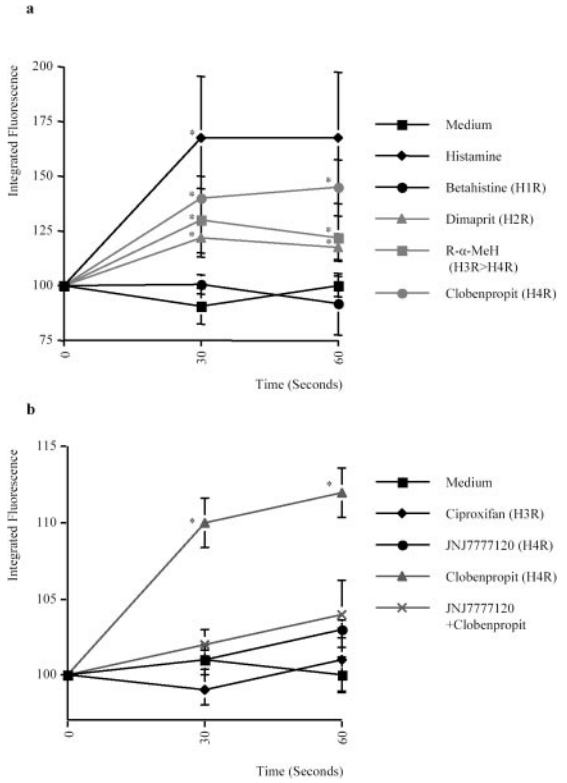

FIGURE 9. Histamine induces F-actin polymerization in MoDC via H2R, H3R, and H4R. MoDC were stimulated with histamine and specific HR ligands or medium (as negative control) for 30 and 60 s. Following stimulation, cells were fixed, stained with NBD-phallacidin, and FL-1 fluorescence was detected as measure for F-actin content as described in *Materials and Methods*. Relative F-actin content is expressed as the ratio of the mean channel fluorescence (integrated fluorescence) between stimulated and nonstimulated cells. Significant F-actin polymerization was induced by histamine, the H2R agonist dimaprit and the H3R agonist R-α-MeH and the H4R agonist clobenpropit, but not by the H1R agonist betahistine (*a*; mean ± SEM of seven independent experiments; *, $p < 0.05$ as compared with the medium control). The effect of clobenpropit was inhibited by the H4R antagonist JNJ7777120, whereas the H3R antagonist ciproxifan had no effect (*b*; mean ± SEM of five independent experiments; *, $p < 0.05$ as compared with the medium control).

Studies investigating H3R mRNA expression in MoDC are controversial; some detected mRNA signal (12, 13), whereas others found only a faint (22) or no signal (10). This might be due to the down-regulation of H3R mRNA during the differentiation from monocytes to MoDC observed by us and can be explained by different culture conditions.

To elucidate effects of the H3R and H4R on cytokine production, signal transduction and chemotaxis in human MoDC, we compared clobenpropit as H3R antagonist and H4R agonist to other HR agonists and antagonist (6, 17, 20, 22). R-α-MeH served as agonist for the H3R which stimulates less efficiently also the H4R (6).

Our results show a down-regulation of polyIC-induced IL-12p70 production by clobenpropit but not R-α-MeH in MoDC, pointing toward a role of the H4R and against the H3R in histamine-induced IL-12p70 suppression. Previous studies were controversial at this point; Idzko et al. (12) found a suppression of IL-12p70 production by R-α-MeH, whereas Mazzoni et al. (13) and Caron et al. (10) were unable to reduce histamine-induced IL-12p70 production with the H3R and (less efficient) H4R antagonist thioperamide. In our experiments, histamine-induced IL-12p70 suppression was rescued by thioperamide pretreatment of MoDC (Fig. 5*a*). These differences might again be explained by different culture conditions used in different studies. However, no previous study addressed H4R signaling with an appropriate ligand.

IL-12p70 suppression via the H2R observed in our study is in agreement with previous studies (10, 12, 13). The ability of the $G\alpha_s$-coupled H2R to induce cAMP in monocytes and MoDC has been described previously (11, 12, 35, 36). Many of such $G\alpha_s$-coupled, cAMP-inducing receptors have been reported to block IL-12p70 (reviewed in Ref. 37). By inhibiting cAMP production with a specific inhibitor, we could rescue IL-12p70 suppression via the H2R but not via the H4R, providing direct evidence for the role of cAMP in H2R mediated IL-12p70 suppression.

Pathways recruited by the H4R are less clear. The H4R as a $G\alpha_{i/o}$-coupled receptor has been reported to inhibit forskolin-induced cAMP formation in cells transfected with the H4R and a

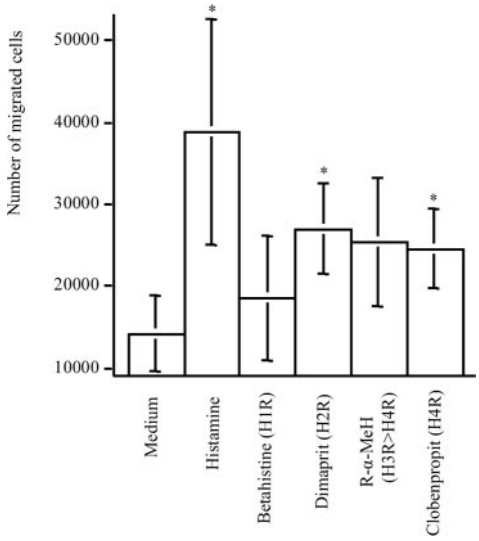

FIGURE 10. Histamine induces chemotaxis in MoDC via H2R and H4R. A total of 1×10^5 MoDC were exposed to a gradient of histamine or HR-specific agonists in Boyden chambers. Medium served as background control. Migrated cells in the lower part of the Boyden chambers were lysed, and glucoronidase activity in the lysates was determined in a photometrical assay as described in *Materials and Methods*. MoDC showed a significant migration toward a gradient of histamine, H2R agonist dimaprit, and H4R agonist clobenpropit. The H1R agonist betahistine had no significant effect; the H3R agonist R-α-MeH revealed a trend ($p = 0.108$) (mean ± SEM of five independent experiments; *, $p < 0.05$ as compared with the medium control).

cAMP responding reporter construct (16, 18, 20, 21); however, the cAMP inhibitory effect was low as compared with that of the H3R. Another study could not alter cAMP levels in H4R transfected cells, but instead increased calcium mobilization when the cells were cotransfected with $G\alpha_{q/i1/2}$, $G\alpha_{q/i3}$, or $G_{\alpha 16}$ proteins (17). Induction of calcium mobilization from intracellular calcium stores via H4R signaling was also demonstrated in murine mast cells (38). In a previous study, we were unable to induce Ca^{2+} influx by histamine in MoDC (11). Mazzoni et al. (13) were able to detect Ca^{2+} influx in MoDC mediated via the H1R, whereas Idzko et al. (12) reported the induction of Ca^{2+} influx by R-α-MeH and betahistine, suggesting a role for H1R and H3R signaling.

Because we could neither induce Ca^{2+} influx by histamine (11) nor influence cAMP formation via H4R stimulation (Fig. 4) in MoDC, we decided to investigate the MAPK pathway as a potential pathway induced by the H4R that is able to block IL-12p70 production (31). U0126, an inhibitor of AP-1 transactivation thought to act specifically at the level of the MEK1/2, preventing the phosphorylation of ERK1/2, rescued H4R-induced but not H2R-induced IL-12p70 suppression. To demonstrate directly the phosphorylation of ERK1/2 via histamine in MoDC, we used Western blot and ELISA. Surprisingly, we were unable to detect phosphorylation of ERK1/2 in MoDC stimulated with histamine or clobenpropit under conditions where PMA phosphorylated ERK1/2 (Fig. 7). This is in contrast to the observation of Morse et al. (17) that histamine stimulates ERK1/2 phosphorylation in HEK-293 cells transfected with the H4R. Because U0126 was originally selected for its ability to functionally antagonize AP-1-driven gene activation and can also bind (with less efficiency) to other MAPK members such as mitogen-activated protein kinase kinase 3/6 (29) and has been shown to block other kinases such as p70 S6 (39), we next looked for H4R-induced AP-1 activation as a downstream event of MAPK signaling. In EMSA, histamine and clobenpropit but not agonists to the H1R, H2R, and H3R were able to induce AP-1. Thus, we conclude that H4R signaling is able to induce the transcription factor AP-1 without phosphorylation of ERK1/2. AP-1, a heterodimer consisting of c-Fos and c-Jun, is a downstream target for MAPK pathways, i.e., ERK1/2, JNK, and p38 MAPK (40). AP-1 activation has been previously related to the inhibition of IL-12p70 in different studies. First, macrophages from c-*fos* knockout mice produced significantly more IL-12p70 as compared with macrophages from wild type mice (41). Second, transfection of RAW264.7 cells with a dominant-negative mutant of AP-1 dramatically enhanced IL-12p40 production (40). Our findings are further supported by a recent study showing that histamine enhances AP-1 DNA binding in keratinocytes (42). Thus, histamine suppresses IL-12p70 by acting in parallel on H2R and H4R via different intracellular signaling pathways.

Induction of F-actin polymerization and chemotaxis via the H2R and the H4R was observed in our study. In addition, stimulation of the H3R with R-α-MeH revealed a significant F-actin polymerization but only a positive trend for chemotaxis. However, because R-α-MeH stimulates also the H4R (6) it is not clear if the F-actin polymerization induced by R-α-MeH in our study and in the study by Idzko (12) reflects stimulation of the H4R rather than the H3R. H4R-induced chemotaxis or cytoskeletal changes have been described recently in other cell types such as eosinophils (43–45) and mast cells (38). A recent study also showed that the CC chemokine CCL-16 mediates chemotaxis via the H4R in eosinophils (46). Thus, histamine might play a role in the accumulation of DC and other cell types in allergic diseases such as atopic dermatitis or contact dermatitis.

In summary, we observed chemotactic and immunomodulatory effects of the H4R on human MoDC. Furthermore, H4R signaling but not stimulation of other HR led to activation of the AP-1 transcription factor. Effects of the H3R are less clear, because the agonists and antagonists available to us also bind to the H4R. Studies need to be performed using more selective H3R ligands to clarify the role of the H3R in human DC.

It is tempting to speculate about the implications of H4R effects on DC for the treatment of allergic diseases where histamine is thought to play a role. For example, in the acute phase of atopic dermatitis, DC and T cells accumulate in the skin in a Th2 type cytokine milieu. Histamine could play a role in attracting DC and suppression of DC produced IL-12p70, thereby contributing to the Th2 cytokine milieu. Therapeutic intervention targeting the H1R (by so-called anti-histamines) and H2R might be insufficient to control this situation because H4R activation can still lead to IL-12p70 suppression and chemotaxis. Further studies are needed to investigate the interactions of histamine in allergic diseases where DC are present under special consideration of the H4R.

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Disclosures

The authors have no financial conflict of interest.

References

1. Banchereau, J., and R. M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature 392:245*.
2. Kalinski, P., C. M. Hilkens, E. A. Wierenga, and M. L. Kapsenberg. 1999. T-cell priming by type-1 and type-2 polarized dendritic cells: the concept of a third signal. *Immunol. Today 20:561*.
3. Vieira, P. L., E. C. de Jong, E. A. Wierenga, M. L. Kapsenberg, and P. Kalinski. 2000. Development of Th1-inducing capacity in myeloid dendritic cells requires environmental instruction. *J. Immunol. 164:4507*.

4. Steinbrink, K., M. Wolfl, H. Jonuleit, J. Knop, and A. H. Enk. 1997. Induction of tolerance by IL-10-treated dendritic cells. *J. Immunol. 159:4772*.
5. Hill, S. J., C. R. Ganellin, H. Timmerman, J. C. Schwartz, N. P. Shankley, J. M. Young, W. Schunack, R. Levi, and H. L. Haas. 1997. International Union of Pharmacology. XIII. Classification of histamine receptors. *Pharmacol. Rev. 49:253*.
6. Hough, L. B. 2001. Genomics meets histamine receptors: new subtypes, new receptors. *Mol. Pharmacol. 59:415*.
7. Akdis, C. A., and K. Blaser. 2003. Histamine in the immune regulation of allergic inflammation. *J. Allergy Clin. Immunol. 112:15*.
8. Jutel, M., T. Watanabe, S. Klunker, M. Akdis, O. A. Thomet, J. Malolepszy, T. Zak-Nejmark, R. Koga, T. Kobayashi, K. Blaser, and C. A. Akdis. 2001. Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. *Nature 413:420*.
9. Lagier, B., B. Lebel, J. Bousquet, and J. Pene. 1997. Different modulation by histamine of IL-4 and interferon-γ (IFN-γ) release according to the phenotype of human Th0, Th1 and Th2 clones. *Clin. Exp. Immunol. 108:545*.
10. Caron, G., Y. Delneste, E. Roelandts, C. Duez, J. Y. Bonnefoy, J. Pestel, and P. Jeannin. 2001. Histamine polarizes human dendritic cells into Th2 cell-promoting effector dendritic cells. *J. Immunol. 167:3682*.
11. Gutzmer, R., K. Langer, M. Lisewski, S. Mommert, D. Rieckborn, A. Kapp, and T. Werfel. 2002. Expression and function of histamine receptors 1 and 2 on human monocyte-derived dendritic cells. *J. Allergy Clin. Immunol. 109:524*.
12. Idzko, M., A. la Sala, D. Ferrari, E. Panther, Y. Herouy, S. Dichmann, M. Mockenhaupt, F. Di Virgilio, G. Girolomoni, and J. Norgauer. 2002. Expression and function of histamine receptors in human monocyte-derived dendritic cells. *J. Allergy Clin. Immunol. 109:839*.
13. Mazzoni, A., H. A. Young, J. H. Spitzer, A. Visintin, and D. M. Segal. 2001. Histamine regulates cytokine production in maturing dendritic cells, resulting in altered T cell polarization. *J. Clin. Invest. 108:1865*.
14. Caron, G., Y. Delneste, E. Roelandts, C. Duez, N. Herbault, G. Magistrelli, J. Y. Bonnefoy, J. Pestel, and P. Jeannin. 2001. Histamine induces CD86 expression and chemokine production by human immature dendritic cells. *J. Immunol. 166:6000*.
15. Coge, F., S. P. Guenin, H. Rique, J. A. Boutin, and J. P. Galizzi. 2001. Structure and expression of the human histamine H4-receptor gene. *Biochem. Biophys. Res. Commun. 284:301*.
16. Liu, C., X. Ma, X. Jiang, S. J. Wilson, C. L. Hofstra, J. Blevitt, J. Pyati, X. Li, W. Chai, N. Carruthers, and T. W. Lovenberg. 2001. Cloning and pharmacological characterization of a fourth histamine receptor (H(4)) expressed in bone marrow. *Mol. Pharmacol. 59:420*.
17. Morse, K. L., J. Behan, T. M. Laz, R. E. West, Jr., S. A. Greenfeder, J. C. Anthes, S. Umland, Y. Wan, R. W. Hipkin, W. Gonsiorek, et al. 2001. Cloning and characterization of a novel human histamine receptor. *J. Pharmacol. Exp. Ther. 296:1058*.
18. Nakamura, T., H. Itadani, Y. Hidaka, M. Ohta, and K. Tanaka. 2000. Molecular cloning and characterization of a new human histamine receptor, HH4R. *Biochem. Biophys. Res. Commun. 279:615*.
19. Nguyen, T., D. A. Shapiro, S. R. George, V. Setola, D. K. Lee, R. Cheng, L. Rauser, S. P. Lee, K. R. Lynch, B. L. Roth, and B. F. O'Dowd. 2001. Discovery of a novel member of the histamine receptor family. *Mol. Pharmacol. 59:427*.
20. Oda, T., N. Morikawa, Y. Saito, Y. Masuho, and S. Matsumoto. 2000. Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. *J. Biol. Chem. 275:36781*.
21. Zhu, Y., D. Michalovich, H. Wu, K. B. Tan, G. M. Dytko, I. J. Mannan, R. Boyce, J. Alston, L. A. Tierney, X. Li, et al. 2001. Cloning, expression, and pharmacological characterization of a novel human histamine receptor. *Mol. Pharmacol. 59:434*.
22. Gantner, F., K. Sakai, M. W. Tusche, W. W. Cruikshank, D. M. Center, and K. B. Bacon. 2002. Histamine h(4) and h(2) receptors control histamine-induced interleukin-16 release from human $CD8^+$ T cells. *J. Pharmacol. Exp. Ther. 303:300*.
23. Pillot, C., A. Heron, J. C. Schwartz, and J. M. Arrang. 2003. Ciproxifan, a histamine H3-receptor antagonist/inverse agonist, modulates the effects of methamphetamine on neuropeptide mRNA expression in rat striatum. *Eur. J. Neurosci. 17:307*.
24. Stark, H. 2003. Recent advances in histamine H3/H4 receptor ligands. *Expert Opin. Ther. Patents 13:851*.
25. Stark, H., M. Kathmann, E. Schlicker, W. Schunack, B. Schlegel, and W. Sippl. 2004. Medicinal chemical and pharmacological aspects of imidazole-containing histamine H3 receptor antagonists. *Mini Rev. Med. Chem. 4:965*.
26. Jablonowski, J. A., C. A. Grice, W. Chai, C. A. Dvorak, J. D. Venable, A. K. Kwok, K. S. Ly, J. Wei, S. M. Baker, P. J. Desai, et al. 2003. The first potent and selective non-imidazole human histamine H4 receptor antagonists. *J. Med. Chem. 46:3957*.
27. Thurmond, R. L., P. J. Desai, P. J. Dunford, W. P. Fung-Leung, C. L. Hofstra, W. Jiang, S. Nguyen, J. P. Riley, S. Sun, K. N. Williams, et al. 2004. A potent and selective histamine H4 receptor antagonist with anti-inflammatory properties. *J. Pharmacol. Exp. Ther. 309:404*.
28. Gutzmer, R., K. Langer, S. Mommert, M. Wittmann, A. Kapp, and T. Werfel. 2003. Human dendritic cells express the IL-18R and are chemoattracted to IL-18. *J. Immunol. 171:6363*.
29. Favata, M. F., K. Y. Horiuchi, E. J. Manos, A. J. Daulerio, D. A. Stradley, W. S. Feeser, D. E. Van Dyk, W. J. Pitts, R. A. Earl, F. Hobbs, et al. 1998. Identification of a novel inhibitor of mitogen-activated protein kinase kinase. *J. Biol. Chem. 273:18623*.
30. Harris, J. N., and I. V. Hutchinson. 1994. A novel fluorescein-histamine reagent to quantitate histamine receptor expression on leucocytes. *Int. J. Immunopharmacol. 16:633.*
31. Wittmann, M., P. Kienlin, S. Mommert, A. Kapp, and T. Werfel. 2002. Suppression of IL-12 production by sCD40L—evidence for involvement of the p44/42 MAPK pathway. *J. Immunol. 168:3793*.
32. Howard, T. H., and W. H. Meyer. 1984. Chemotactic peptide modulation of actin assembly and locomotion in neutrophils. *J. Cell Biol. 98:1265.*
33. Leurs, R., M. J. Smit, and H. Timmerman. 1995. Molecular pharmacological aspects of histamine receptors. *Pharmacol. Ther. 66:413*.
34. Lovenberg, T. W., B. L. Roland, S. J. Wilson, X. Jiang, J. Pyati, A. Huvar, M. R. Jackson, and M. G. Erlander. 1999. Cloning and functional expression of the human histamine H3 receptor. *Mol. Pharmacol. 55:1101*.
35. Elenkov, I. J., E. Webster, D. A. Papanicolaou, T. A. Fleisher, G. P. Chrousos, and R. L. Wilder. 1998. Histamine potently suppresses human IL-12 and stimulates IL-10 production via H2 receptors. *J. Immunol. 161:2586*.
36. van der Pouw Kraan, T. C., A. Snijders, L. C. Boeije, E. R. de Groot, A. E. Alewijnse, R. Leurs, and L. A. Aarden. 1998. Histamine inhibits the production of interleukin-12 through interaction with H2 receptors. *J. Clin. Invest. 102:1866*.
37. Braun, M. C., and B. L. Kelsall. 2001. Regulation of interleukin-12 production by G-protein-coupled receptors. *Microbes Infect. 3:99*.
38. Hofstra, C. L., P. J. Desai, R. L. Thurmond, and W. P. Fung-Leung. 2003. Histamine H4 receptor mediates chemotaxis and calcium mobilization of mast cells. *J. Pharmacol. Exp. Ther. 305:1212*.
39. Fukazawa, H., and Y. Uehara. 2000. U0126 reverses Ki-ras-mediated transformation by blocking both mitogen-activated protein kinase and p70 S6 kinase pathways. *Cancer Res. 60:2104*.
40. Mitsuhashi, M., J. Liu, S. Cao, X. Shi, and X. Ma. 2004. Regulation of interleukin-12 gene expression and its anti-tumor activities by prostaglandin E_2 derived from mammary carcinomas. *J. Leukocyte Biol. 16:322*.
41. Roy, S., R. Charboneau, K. Cain, S. DeTurris, D. Melnyk, and R. A. Barke. 1999. Deficiency of the transcription factor c-*fos* increases lipopolysaccharide-induced macrophage interleukin 12 production. *Surgery 126:239*.
42. Kanda, N., and S. Watanabe. 2004. Histamine enhances the production of granulocyte-macrophage colony-stimulating factor via protein kinase Cα and extracellular signal-regulated kinase in human keratinocytes. *J. Invest. Dermatol. 122:863*.
43. Buckland, K. F., T. J. Williams, and D. M. Conroy. 2003. Histamine induces cytoskeletal changes in human eosinophils via the H(4) receptor. *Br. J. Pharmacol. 140:1117*.
44. Ling, P., K. Ngo, S. Nguyen, R. L. Thurmond, J. P. Edwards, L. Karlsson, and W. P. Fung-Leung. 2004. Histamine H4 receptor mediates eosinophil chemotaxis with cell shape change and adhesion molecule upregulation. *Br. J. Pharmacol. 142:161*.
45. O'Reilly, M., R. Alpert, S. Jenkinson, R. P. Gladue, S. Foo, S. Trim, B. Peter, M. Trevethick, and M. Fidock. 2002. Identification of a histamine H4 receptor on human eosinophils–role in eosinophil chemotaxis. *J. Recept. Signal Transduct. Res. 22:431*.
46. Nakayama, T., Y. Kato, K. Hieshima, D. Nagakubo, Y. Kunori, T. Fujisawa, and O. Yoshie. 2004. Liver-expressed chemokine/CC chemokine ligand 16 attracts eosinophils by interacting with histamine H4 receptor. *J. Immunol. 173:2078*.