

Differentiation of monocytes into macrophages induces the upregulation of histamine H₁ receptor

Massimo Triggiani, MD, PhD,^{a,b} Angelica Petraroli, MD, PhD,^a Stefania Loffredo, PhD,^a Annunziata Frattini, PhD,^a Francescopaolo Granata, MD,^a Paolo Morabito, PhD,^c Rosaria Ilaria Staiano, PhD,^{a,d} Agnese Secondo, PhD,^d Lucio Annunziato, MD,^d and Gianni Marone, MD^{a,b} *Naples, Italy*

Background: Histamine modulates several functions in human monocytes, macrophages, and dendritic cells. However, responses elicited by histamine differ depending on cell type, suggesting variable expression of histamine receptors in the monocyte/macrophage lineage.

Objective: We sought to examine whether the expression of H₁ receptors was regulated by cell differentiation of human monocytes into macrophages or dendritic cells.

Methods: Expression of H₁ receptor was evaluated by RT-PCR and western blot in monocytes, monocyte-derived macrophages (MDMs), monocyte-derived dendritic cells (DCs) and human lung macrophages (HLMs).

Results: Expression of H₁ receptor mRNA and protein was higher in HLMs and DCs than in monocytes. H₁ expression was approximately 15-fold and 4-fold higher in MDMs and HLMs, respectively, as compared with that seen in monocytes. H₁ receptor protein was undetectable in monocytes, whereas it was conspicuous in MDMs. Simultaneous analysis of H₂ and H₁ mRNA expression indicated that the H₂/H₁ ratio decreased from 202.7 ± 14.8 in monocytes to 2.2 ± 0.4 in MDM and 39.5 ± 5.0 in DCs. Incubation of monocytes with histamine neither affected intracellular Ca²⁺ concentrations nor influenced IL-8 production. In contrast, histamine rapidly induced a Ca²⁺ signal and stimulated IL-8 production in MDMs. Both effects were inhibited by H₁ blockade with levocetirizine, but not by H₂ blockade with ranitidine.

Conclusions: Differentiation of monocytes into macrophages or dendritic cells is associated with profound changes of histamine receptor expression. Upregulation of H₁ receptors confers on macrophages the capacity of being activated by histamine.

Clinical implications: Regulation of H₁ and H₂ receptor expression in the monocyte/macrophage lineage can be relevant to the pathogenesis of allergic inflammation. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Histamine, H₁ receptor, macrophage, dendritic cells, monocyte, IL-8

Histamine released from mast cells and basophils plays a central role in allergic and inflammatory reactions.¹ The acute effects of histamine, such as bronchoconstriction, vasodilation, and increased vascular permeability, are responsible for most of the symptoms of rhinitis, asthma, urticaria, and anaphylaxis.² In addition, histamine exerts immunomodulatory effects by influencing the functions of a number of immune cells (eg, T cells, dendritic cells, monocytes, and macrophages) that play a primary role in initiating and sustaining allergic inflammation.³ In particular, histamine influences the profile of proinflammatory and immunoregulatory cytokines produced by blood monocytes, tissue macrophages, and dendritic cells.^{4,5} For example, histamine induces the production of IL-10 and inhibits that of TNF-α and IL-12 from monocytes.⁶⁻⁸ Furthermore, histamine induces IL-6 and IL-10 production from human lung macrophages (HLMs).^{9,10} Finally, histamine induces cytokine and chemokine production^{11,12} and polarizes immature, but not mature, dendritic cells into T_H2 response-promoting cells.¹³ Interestingly, histamine induces TNF-α production from macrophages¹⁴ but not from monocytes⁶ or dendritic cells.¹¹ Although these observations indicate that histamine exerts important immunoregulatory effects, they also illustrate the heterogeneity of the responses of human immune cells to this mediator.

The biologic effects of histamine are mediated by activation of 4 distinct receptors (H₁, H₂, H₃, and H₄).^{3,15} However, several histamine-induced immunoregulatory responses are related to the engagement of H₁ and H₂ receptors.³⁻⁵ Indeed, the interaction of histamine with these 2 receptors elicits responses that are often divergent,

From ^athe Division of Clinical Immunology and Allergy, University of Naples Federico II; ^bthe Center for Basic and Clinical Immunology Research (CISI), Naples; ^cthe Division of Immunohematology and Transfusion Medicine, A. Cardarelli Hospital, Naples; and ^dthe Division of Pharmacology, Department of Neuroscience, University of Naples Federico II.

Supported by grants from the Ministero dell'Istruzione, dell'Università e della Ricerca (M.T. and G.M.); the Istituto Superiore di Sanità (AIDS Project 40D.57, G.M.); the Ministero della Salute "Alzheimer Project" (Rome, Italy; G.M. and L.A.); the Consiglio Nazionale delle Ricerche (Rome, Italy; G.M.); and UCB Pharma (Chemin du Foriest, Belgium; G.M.).

Disclosure of potential conflict of interest: G. Marone has received grant support from UCB Pharma. The rest of the authors have declared that they have no conflict of interest.

Received for publication February 15, 2006; revised July 26, 2006; accepted for publication September 22, 2006.

Reprint requests: Massimo Triggiani, MD, PhD, Division of Clinical Immunology and Allergy, University of Naples Federico II, 80131 Naples, Via S. Pansini 5, Italy. E-mail: triggiani@unina.it

0091-6749/\$32.00

© 2006 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2006.09.027

Abbreviations used

[Ca²⁺]_i: Intracellular calcium concentrations
 DC: Monocyte-derived dendritic cell
 Fura-2AM: Fura-2 acetoxymethyl ester
 HLM: Human lung macrophage
 HRP: Horseradish peroxidase
 MDM: Monocyte-derived macrophage
 MPS: Mononuclear phagocyte system
 PAF: Platelet-activating factor

with H₁ being predominantly stimulatory^{9,10} and H₂ being inhibitory in most cases.⁶⁻⁸ Thus the expression of histamine receptors might be a mechanism to regulate cell responsiveness to this mediator, which would explain the heterogeneity of histamine's effects on inflammatory and immune cells.

The H₁ receptor plays a crucial role in mediating both the acute and long-term effects of histamine in inflammation.¹⁶ Activation of the H₁ receptor generates signals through the Gq/11 family of G proteins that is functionally coupled with phospholipase C.¹⁷ Subsequent phospholipase C-catalyzed phospholipid breakdown generates 1,2-diacylglycerol and inositol 1,4,5 triphosphate, which induces an increase in intracellular Ca²⁺. Studies with cells transfected with the human H₁ receptor have shown that its function can be regulated by different mechanisms: (1) upregulation or downregulation of gene expression, (2) postranscriptional modifications leading to changes in the ligand affinity, and (3) homologous and heterologous desensitization.^{18,19} These observations strongly support the hypothesis that the H₁ receptor is a dynamic structure, the expression of which might influence responsiveness to target cells.

The differential responsiveness to histamine of human monocytes, macrophages, and dendritic cells led to the hypothesis that cell differentiation in the monocyte/macrophage lineage might induce changes in the expression of the H₁ receptor. These cells are components of the mononuclear phagocyte system (MPS), a widely distributed cellular network that has a primary role in innate immunity.²⁰ MPS cells are thought to derive from CD34⁺ bone marrow cells differentiating into monocytes. Monocytes are the predominant MPS cells in the blood, and they differentiate into macrophages or dendritic cells as they migrate within various organs and tissues.²¹ Upregulation of the H₁ receptor has been reported in the histiocytic lymphoma cell line U937 differentiated into macrophages by exposure to phorbol esters.¹⁴ However, it is not known whether changes in the H₁ receptor expression occur when normal blood monocytes differentiate into macrophages or dendritic cells and whether the inducible H₁ receptor is functionally active.

In this study we compared the expression of H₁ and H₂ receptors in peripheral blood monocytes, macrophages purified from human lung tissue (HLMs), monocyte-derived macrophages (MDMs), and monocyte-derived dendritic cells (DCs). Our results indicate that HLMs, MDMs, and DCs express larger amounts of the H₁ receptor than their precursor monocytes and that the expression

of the H₁ receptor is upregulated at both the mRNA and protein levels as monocytes differentiate into macrophages or dendritic cells. Differentiation of monocytes into macrophages also induces a dramatic downregulation of the H₂ receptors, whereas differentiation into dendritic cells has no significant influence on H₂ receptor expression. Overexpressed H₁ receptor is functionally active, and it mediates increased production of IL-8 in histamine-stimulated macrophages.

METHODS

Reagents

The following reagents were purchased: histamine dihydrochloride, ranitidine hydrochloride, Percoll, Histopaque-1077, L-glutamine, antibiotic-antimycotic solution (10,000 UI/mL penicillin, 10 mg/mL streptomycin, and 25 µg/mL amphotericin B), LPS (from *Escherichia coli* serotype 026:B6), Triton X-100, and BSA (Sigma, St Louis, Mo); RPMI 1640 and FCS (ICN, Costa Mesa, Calif); the specific fluorochrome-conjugated mAbs anti-CD1a, anti-CD11c, anti-CD14, anti-CD45, anti-CD83, and anti-HLA-DR (Becton Dickinson, San Jose, Calif); IgG₁ and anti-CD71 (Immunotech, Marseille, France); IgG₂ and anti-CD86 (Caltag, Burlingame, Calif); rabbit anti-human H₁ receptor (H-300) antibody and JAR (choriocarcinoma) cell lysate (Santa Cruz Biotechnology, Santa Cruz, Calif); horseradish peroxidase (HRP)-conjugated donkey anti-rabbit Ig antibody (Amersham Biosciences, Buckinghamshire, United Kingdom [UK]); and platelet-activating factor (PAF; Biomol, Plymouth Meeting, Pa). Levocetirizine was a gift from UCB Pharma (Chemin du Foriest, Belgium). All other reagents were from Carlo Erba (Milan, Italy).

Cell isolation, purification, and culture

The study protocol involving the use of human blood cells and lung tissue was approved by the Ethical Committee of the University of Naples Federico II, and informed consent was obtained from blood donors and patients undergoing thoracic surgery.

Monocytes were purified from buffy coats of healthy donors by means of negative immunomagnetic selection (Miltenyi Biotec, Bergisch Gladbach, Germany), as previously described.²² This procedure yields a population of monocytes with a purity of greater than 97%, as assessed by means of flow cytometric analysis of CD14.

MDMs were obtained by culturing purified monocytes for 7 to 10 days in RPMI containing 2 mmol/L glutamine, 1% antibiotic-antimycotic solution, and 20% FCS. After either 7 or 10 days of culture, the expression of CD14, CD86, CD11c, and HLA-DR was evaluated by means of flow cytometry, and β-glucuronidase activity was determined as previously described.¹⁰

DCs were generated as previously reported²³ by incubating purified monocytes for 7 days in RPMI 10% FCS supplemented with 50 ng/mL GM-CSF (PeproTech, London, UK) and 10 ng/mL IL-13 (PeproTech). Flow cytometric analysis showed that these cells were negative or low positive for CD14, highly positive for CD1a, and negative for CD83 expression.

HLMs were purified from the parenchyma of patients undergoing thoracic surgery, as previously reported.¹⁰ The purity of HLMs (>95%) was assessed by means of flow cytometric analysis of CD14/CD71/HLA-DR.

RT-PCR for histamine H₁ and H₂ receptors

Total RNA from monocytes, MDMs, DCs, and HLMs was extracted by using the SV 96 total RNA isolation system (Promega, Milan, Italy) and treated with RNase-free DNase I. Reverse transcription was performed as previously described.²² cDNA was then titrated for

β -actin message, and the expression of histamine H_1 and H_2 receptors was evaluated by means of PCR. For semiquantitative PCR, equivalent templates of cDNAs obtained from monocytes, HLMs, and DCs were amplified by using the H_1 -specific primers prepared as previously published.²⁴ The PCR products were separated on 2% agarose gel, stained with ethidium bromide, and visualized with the image analysis system ChemidocXRS (Bio-Rad Laboratories, Hercules, Calif).

Real-time quantitative PCR was performed on the iCycler (Bio-Rad) by using the PE SYBR Green PCR kit (Applied Biosystems, Foster City, Calif). Target-specific primers for β -actin and H_1 and H_2 receptors suitable for real-time PCR were designed by using Beacon Designer 3.0 software (Bio-Rad; β -actin forward: 5'-TGCGTGACATTAAGGAGAAG-3'; β -actin reverse: 5'-GCTCGTAGCTCTTCTCCA-3'; H_1 forward: 5'-CACACTGAACCCCTCATCT-3'; H_1 reverse: 5'-GGCCTTCGTCCTCTATTTCC-3'; H_2 forward: 5'-TCTGAGTCCAACGCC TCTC-3'; H_2 reverse: 5'-TTCCTGTTCCTGGGTCTC-3'). After an initial denaturation step at 95°C for 10 minutes, amplification was performed with 40 cycles of denaturation (95°C) for 30 seconds and annealing (60°C) for 1 minute. The PCR efficiency was examined by serially diluting the template cDNA, and the melting curve data were collected to check the PCR specificity. Each cDNA sample was analyzed in triplicate, and the corresponding no-RT mRNA sample was included as a negative control. The mRNA signal for the H_1 and H_2 receptors in each sample was normalized to that of the β -actin mRNA. The data were analyzed with iCycler iQ analysis software (Bio-Rad), and the changes in H_1 and H_2 receptor mRNA in MDMs, DCs, and HLMs were expressed as the ratio versus monocytes.

Western blot analysis of the H_1 receptor

Monocytes, MDMs cultured for 7 or 10 days, DCs, and HLMs were lysed in lysis buffer (20 mmol/L Tris [pH 7.5], 5 mM EDTA, 1 mmol/L phenylmethylsulfonyl fluoride, 2 mmol/L benzamidine, 10 μ g/mL leupeptin, 10 mmol/L NaF, 150 mmol/L NaCl, 1% Nonidet P-40, and 5% glycerol). Cell lysates were kept on ice for 20 minutes and then centrifuged (14,000 rpm, 4°C, 20 minutes). Protein extracts were diluted in lithium dodecylsulfate sample buffer containing 2.5% 2 β -mercaptoethanol. Samples were boiled for 5 minutes and stored at -80°C. Equal protein extracts (10-50 μ g per sample) were separated on 4% to 12% Bis-Tris gels (NuPAGE; Novex, Invitrogen, Milan, Italy) and transferred to a nitrocellulose membrane (Bio-Rad) together with a biotinylated protein ladder. After blocking for 2 hours in TBST (20 mmol/L Tris-HCl, 150 mmol/L NaCl, and 0.05% Tween-20 [pH 7.5]) containing 4% BSA, membranes were washed with TBST and then incubated (4°C overnight) with rabbit anti-human H_1 antibody. After washing, the membranes were incubated (22°C for 1 hour) with HRP-conjugated goat anti-rabbit IgG antibody together with HRP-conjugated anti-biotin antibody. Membrane-bound antibodies were visualized with the ECL Western blotting detection reagent (Amersham Biosciences) by using the ChemidocXRS analysis system.

Measurement of intracellular Ca^{2+} concentrations

Intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) were measured by means of single-cell computer-assisted video imaging.¹⁰ Briefly, monocytes and MDMs cultured on glass cover slips were loaded with 3 μ mol/L Fura-2 acetoxymethyl ester (Fura-2AM; Molecular Probes, Eugene, Ore) for 1 hour at room temperature in RPMI 1640 (pH 7.4). At the end of the Fura-2AM loading period, the cover slips were placed into a perfusion chamber (Medical System, Co., Greenvale, NY) mounted onto the stage of an inverted fluorescence microscope (Nikon Diaphot, Nikon, Torrance, Calif). The cells were stimulated with histamine or PAF by means of single-bolus fast injection. A 100-W Xenon lamp (Osram, Berlin, Germany)

with a computer-operated filter wheel bearing 340-nm and 380-nm interference filters illuminated the microscopic field light every 3 seconds, alternating the wavelengths at an interval of 500 ms. The light emitted by Fura-2AM-loaded cells was passed through a 400-nm dichroic mirror filtered at 510 nm and collected with an intensified camera (Photonic Science, Photonic Science, Robertsbridge, UK). Images were analyzed with a Magiscan image processor (Applied Imaging Ltd, Dukesway, UK) driven by the AUTOLAB software (RBR Altair, Florence, Italy). The ratios of fluorescence intensity were converted by the software into $[Ca^{2+}]_i$ by using a previously obtained calibration curve.¹⁰ For each experimental condition, 15 to 25 cells were analyzed.

IL-8 release

Monocytes and MDMs cultured for 10 days were incubated (37°C for 18 hours) with histamine or LPS. In selected experiments the cells were preincubated (37°C for 30 minutes) with levocetirizine (0.1-10 μ mol/L) or ranitidine (0.1-10 μ mol/L) and then stimulated (37°C for 18 hours) with histamine (1 μ mol/L). IL-8 release in the supernatant was measured in duplicate determinations by means of ELISA (Euro Clone, Devon, UK), and the results were normalized for the protein content of each well.¹⁰

Statistical analysis

The data are presented as the means $\pm$ SE of the indicated number of experiments. *P* values were determined by using the Student paired *t* test.²⁵

RESULTS

Expression of H_1 receptor mRNA and protein in monocytes, HLMs, and DCs

Initial experiments were performed to compare the expression of the H_1 receptor gene in monocytes, HLMs, and DCs. Fig 1 shows the RT-PCR amplification signals for the H_1 receptor and for the constitutive β -actin gene obtained in 1 experiment representative of 4. Small quantities of H_1 mRNA were detected in freshly isolated monocytes. Larger amounts of H_1 mRNA were consistently detected in HLMs and DCs (Fig 1, A). To evaluate whether the increased mRNA level for the H_1 receptor resulted in a larger production of the H_1 protein, we examined monocyte, HLM, and DC lysates by means of Western blotting with a polyclonal anti-human H_1 receptor antibody (Fig 1, B). JAR (choriocarcinoma) cells expressing the human H_1 receptor were used as positive controls. The anti- H_1 receptor antibody detected 1 major band at approximately 60 kd, which corresponds to the native form of the H_1 receptor.²⁶ The 60-kd band was clearly evident in HLMs and DCs but was consistently undetectable in blood monocytes, suggesting that the amount of H_1 receptor in monocytes is less than the detection limit of the technique. Additional bands of about 50 and 70 kd were evident in HLM and DC lysates, as well as in the JAR cells. Whether these bands represent proteolytic fragments or glycosylated forms of the H_1 receptor is still unknown. These results indicate that HLMs and DCs contain larger quantities of mRNA and protein for H_1 receptor than monocytes, suggesting that differentiation of macrophages or dendritic cells from monocytes is associated with enhanced H_1 gene transcription.

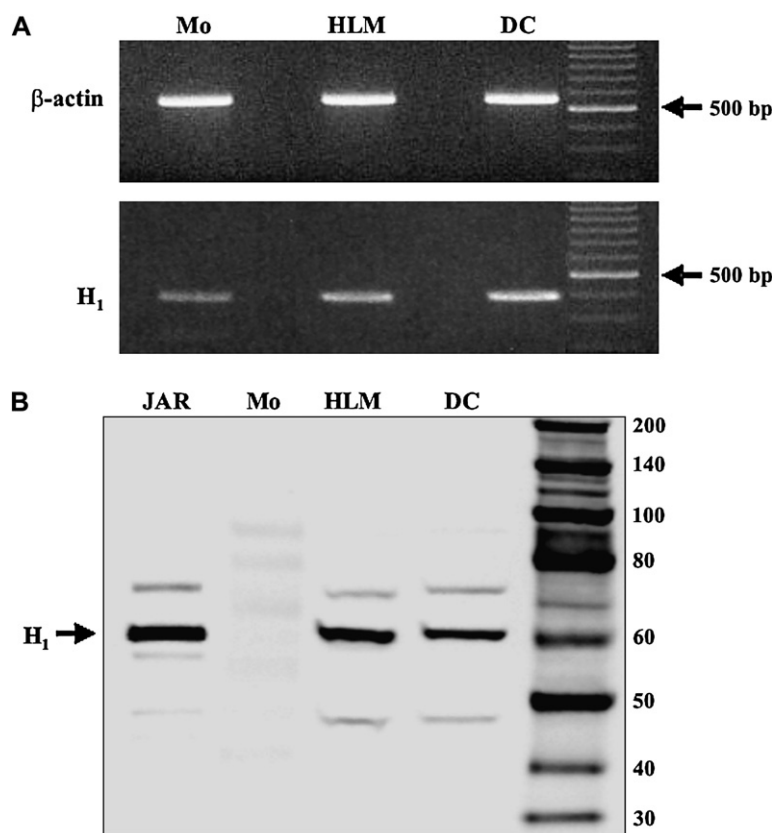


FIG 1. Expression of mRNA for H₁ receptor in monocytes, HLMs, and DCs. **A**, β -Actin and H₁ receptor RT-PCR amplification products from monocytes (Mo), HLMs, and DCs. **B**, Immunoblot with anti-human H₁ receptor of monocyte (Mo), HLM, and DC lysates (40 μ g per sample). JAR (choriocarcinoma) cells were used as a positive control. Data are representative of 4 experiments.

Differentiation of macrophages from monocytes *in vitro*

To test the hypothesis that differentiation of macrophages was associated with the upregulation of the H₁ receptor, we used an *in vitro* model to obtain MDMs. We initially verified that monocytes cultured with 20% FCS acquired features of mature macrophages. Fig 2 shows the flow cytometric analysis of freshly isolated monocytes and the corresponding preparation of MDMs obtained by means of *in vitro* culture for 10 days. MDMs displayed a homogeneous enhancement of the forward- and side-scatter signals compared with monocytes, which indicates an increase in cell size and granule content. In addition, MDMs consistently upregulated the expression of CD11c, CD86, and HLA-DR, which are surface markers of mature tissue macrophages.²⁷ Moreover, the cellular content of β -glucuronidase, which was 0.41 ± 0.03 U/mg of protein in monocytes, significantly increased after 7 days of culture (1.84 ± 0.05 U/mg, $P < .01$ vs monocytes) and reached a maximum after 10 days of culture (3.32 ± 0.16 U/mg, $P < .01$ vs monocytes). These results indicate that the monocytes incubated for 7 to 10 days with 20% FCS acquire the phenotypical and biochemical features of differentiated macrophages.

Upregulation of H₁ receptor expression in MDMs and HLMs

In the next group of experiments, we quantitated H₁ receptor mRNA by means of real-time PCR in MDMs differentiated for 7 or 10 days, in HLMs, and in the precursor monocytes. Fig 3, A, shows the results obtained in 4 different preparations in which the expression of H₁ receptor increased 4.9 ± 0.8 -fold in MDMs cultured for 7 days and 15.0 ± 2.9 -fold in MDMs cultured for 10 days compared with that seen in monocytes. Similarly, quantitative analysis of 4 different lung preparations also revealed that H₁ gene expression in HLMs was higher (3.9 ± 0.7 -fold) than that in monocytes. These data demonstrate that H₁ gene expression is significantly upregulated as monocytes differentiate into macrophages either *in vitro* or in the lung *in vivo*.

We next examined whether the enhanced H₁ gene expression in differentiated macrophages was translated into an increased production of H₁ receptor protein by means of Western blotting. Fig 3, B, shows the data obtained in 2 preparations of monocytes and MDMs. The 60-kD band corresponding to the H₁ receptor is clearly evident in MDMs cultured for 7 days and is further increased in MDMs cultured for 10 days. The H₁ receptor signal was not detected in any of the monocyte

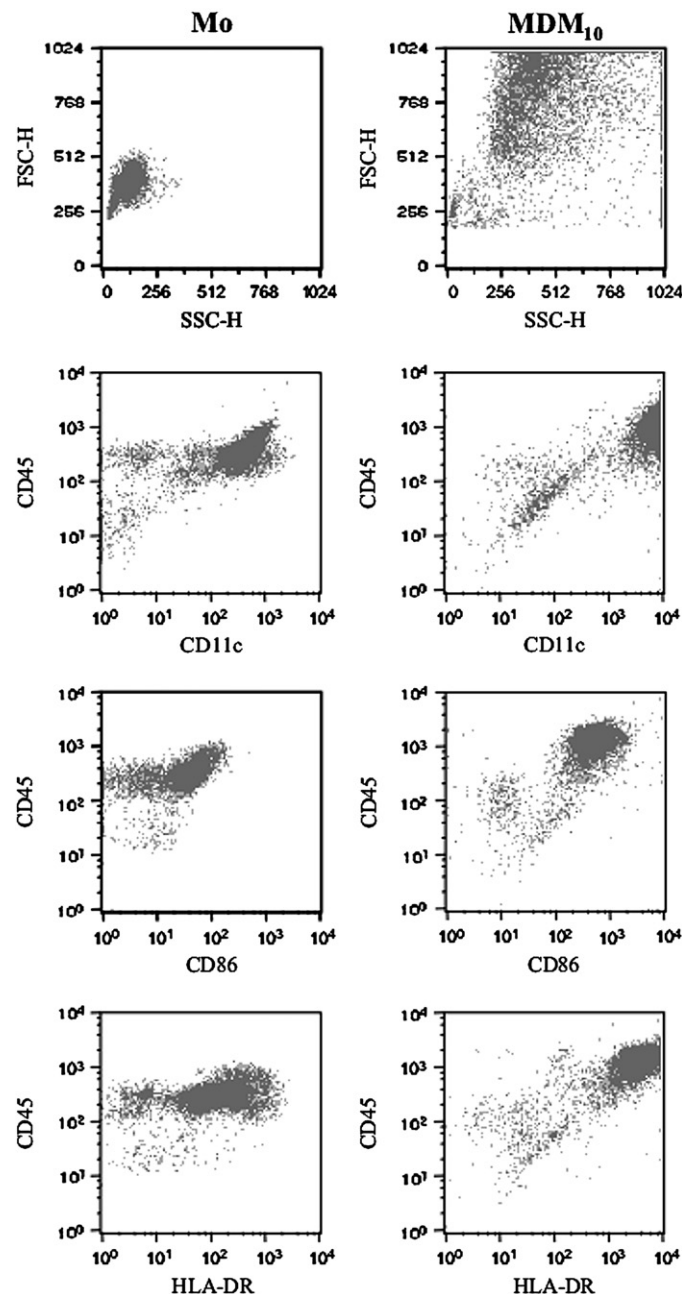


FIG 2. Phenotypic changes in human MDMs. FACS analysis of forward-scatter (*FSC-H*) and side-scatter (*SSC-H*) characteristics and surface expression of CD45, CD11c, CD86, and HLA-DR in freshly isolated blood monocytes (*Mo*) and MDMs cultured for 10 days (*MDM₁₀*). Data are representative of 4 separate experiments.

preparations, even when up to 50 μ g of protein extracts was loaded onto the gel. These results indicate that upregulation of H_1 gene expression in differentiated macrophages is associated with an increased synthesis of the H_1 receptor protein.

Relative expression of H_1 and H_2 receptors in MDMs and DCs

H_1 and H_2 receptors are coexpressed in most inflammatory cells.¹⁵ These receptors play distinct and often

divergent roles on cell activation.⁶⁻¹⁰ Therefore we examined simultaneously, by means of quantitative PCR, the changes in H_1 and H_2 mRNA expression occurring when monocytes differentiate into MDMs or DCs. In these experiments we used MDMs and DCs differentiated in parallel from monocytes obtained from the same donor. Fig 4, A, shows that the H_1 receptor mRNA significantly increases in MDMs (12.4 ± 0.81 -fold, $P < .01$) and, to a lesser extent, in DCs (5.77 ± 0.74 -fold, $P < .01$) compared with that seen in monocytes. In contrast, the mRNA for the H_2 receptor

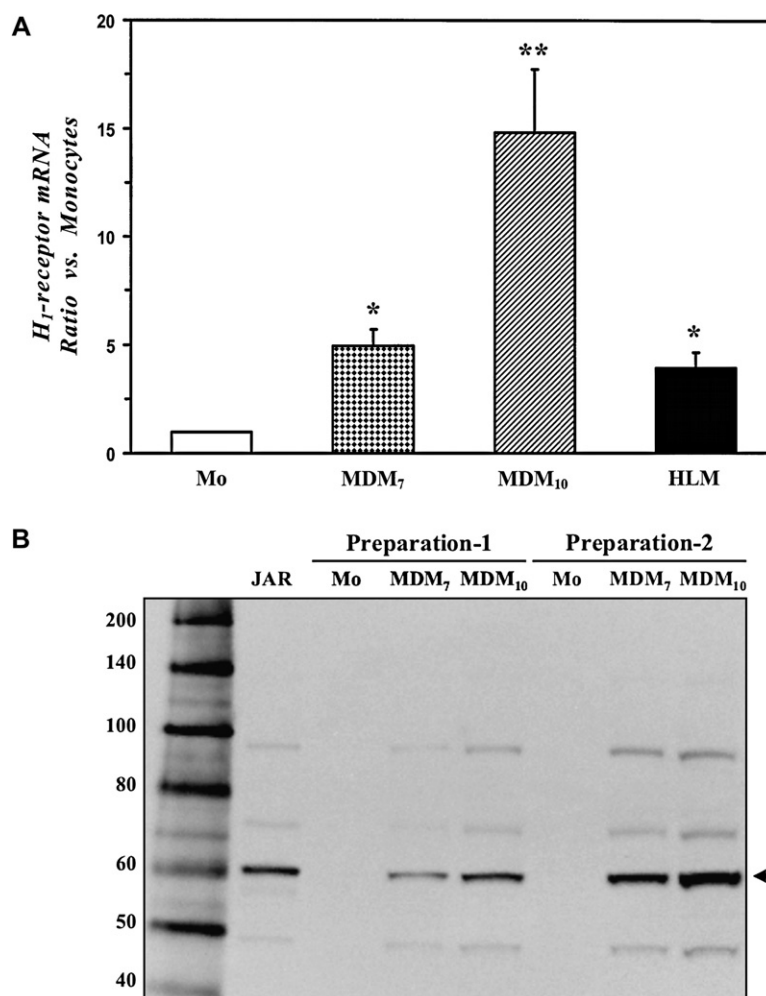


FIG 3. Upregulation of H₁ receptor in MDMs and HLMs. **A**, Quantitative RT-PCR of H₁ mRNA levels in monocytes (Mo), MDMs cultured for 7 days (MDM₇) or 10 days (MDM₁₀), and HLMs. Data are presented as the mean $\pm$ SE of 4 experiments. * P < .05 versus monocytes; ** P < .01 versus monocytes. **B**, Immunoblot with anti-human H₁ receptor of lysates of monocyte (Mo) and MDMs cultured for 7 days (MDM₇) or 10 days (MDM₁₀) (10 μ g per sample). Data are representative of 4 experiments. JAR, Choriocarcinoma cells.

was markedly reduced to 0.13 ± 0.03 (P < .01) when monocytes differentiated into MDMs, whereas it did not change when monocytes differentiated into DCs (Fig 4, B). These data revealed that the relative expression of H₂ versus H₁ receptor dramatically changed on differentiation of monocytes into MDMs (from 202.7 ± 14.8 to 2.2 ± 0.4) or into DCs (from 202.7 ± 14.8 to 39.5 ± 5.0 ; Fig 4, C). These results indicate that a switch from H₂ to H₁ receptors occurs when monocytes differentiate into macrophages, whereas just upregulation of H₁ receptors is detected when monocytes differentiate into DCs.

Effect of histamine on cytosolic Ca²⁺ concentrations in monocytes and MDMs

Fura-2AM single-cell microfluorometric experiments were performed by using a video-imaging technique to test the functional activity of the H₁ receptors in

monocytes and MDMs. Histamine failed to induce changes in [Ca²⁺]_i in monocytes (Fig 5). In contrast, PAF used as a positive control induced a significant increase of [Ca²⁺]_i (Fig 5). MDMs cultured for 10 days rapidly responded to histamine, generating an increase of [Ca²⁺]_i that was comparable with that induced by PAF (Fig 6, A). Data from 3 experiments revealed that histamine and PAF both induce significant [Ca²⁺]_i changes (basal: 126.0 ± 2.8 nmol/L; histamine: 173.8 ± 4.7 nmol/L, P < .01; PAF: 196.0 ± 6.1 nmol/L, P < .01).

To verify that the Ca²⁺ signal induced by histamine in MDMs cultured for 10 days was due to engagement of H₁ receptors, these cells were preincubated with the H₁ antagonist levocetirizine¹⁶ before the addition of histamine. Levocetirizine completely blocked the increase in Ca²⁺ signal induced by histamine (Fig 6, B and C), but it had no effect on the Ca²⁺ signal induced by PAF (Fig 6, B).

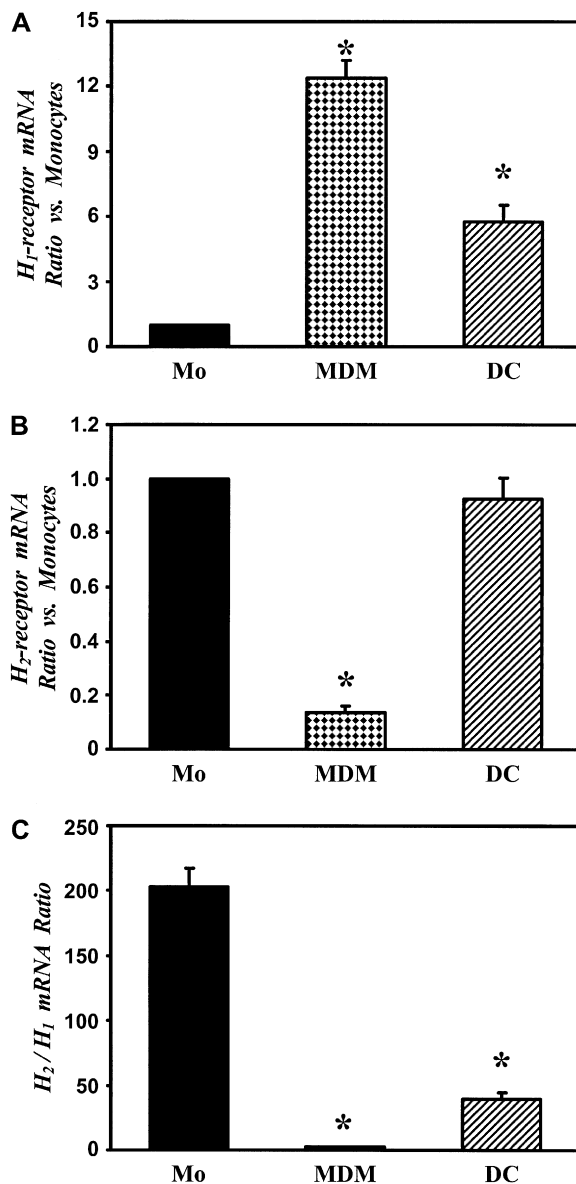


FIG 4. Relative expression of H_1 and H_2 receptors in monocytes (Mo), MDMs, and DCs. Quantitative RT-PCR of H_1 (A) and H_2 (B) receptor mRNA levels in monocytes, MDMs, and DCs are shown. Data are expressed as the ratio versus monocytes (Fig 4, A and B) or the H_2/H_1 ratio (C) and are presented as the mean $\pm$ SE of 4 experiments.

Histamine-induced IL-8 production in monocytes and MDMs

Previous studies have shown that histamine induces IL-8 release in endothelial cells and keratinocytes by activating the H_1 receptor.^{28,29} We therefore investigated IL-8 production in monocytes and macrophages to examine whether upregulation of the H_1 receptor increased responsiveness to histamine. Fig 7, A, shows that histamine did not induce production of IL-8 from monocytes. In contrast, histamine resulted in concentration-dependent release of IL-8 from MDMs. The release of IL-8 caused by

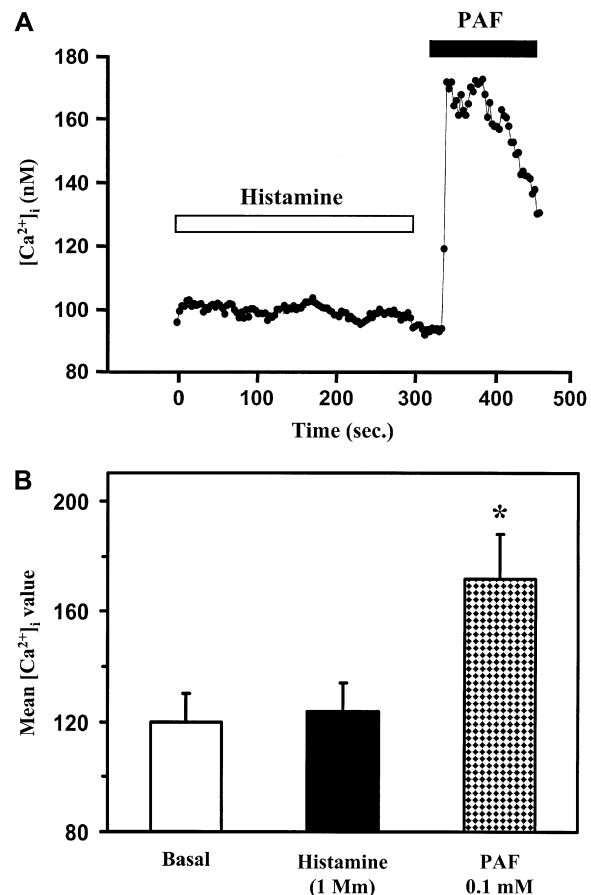


FIG 5. Effect of histamine on $[Ca^{2+}]_i$ in monocytes. **A**, Single-cell tracing of Fura-2AM-loaded monocytes incubated with histamine (1 μ mol/L) or PAF (0.1 μ mol/L). The trace is representative of 75 monocytes recorded in 3 different sessions. **B**, Mean $[Ca^{2+}]_i$ values $\pm$ SE of 3 preparations. * $P < .05$ versus basal value.

histamine from MDMs was not due to an increased capacity of macrophages to produce this cytokine because the release induced by LPS was similar in monocytes and MDMs (Fig 7, A). Histamine-induced production of IL-8 in MDMs was due to the increased expression of the H_1 receptor because levocetirizine inhibited this response in a concentration-dependent fashion (Fig 7, B). Incubation of MDMs with the H_2 antagonist ranitidine had no influence on the release of IL-8 induced by histamine (Fig 7, B).

DISCUSSION

Differentiation of monocytes into macrophages or dendritic cells *in vitro*, as well as maturation of macrophages in the lung *in vivo*, is associated with profound changes in the expression of histamine H_1 and H_2 receptors. Upregulation of H_1 receptor and downregulation of H_2 receptor occur when monocytes differentiate into macrophages, whereas upregulation of H_1 receptor without changes in H_2 receptor takes place when monocytes

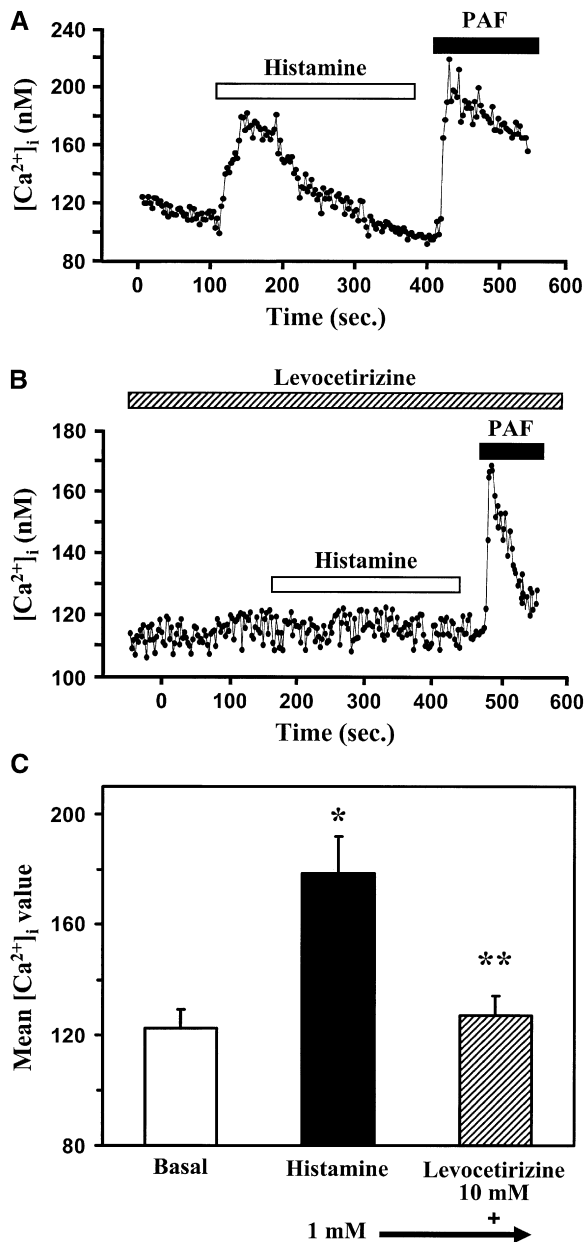


FIG 6. Effect of histamine on $[Ca^{2+}]_i$ in MDMs. Single-cell tracing of Fura-2AM-loaded MDMs incubated with histamine (1 μ mol/L) or PAF (0.1 μ mol/L; **A**) or preincubated with levocetirizine (10 μ mol/L) before the addition of stimuli (**B**) is shown. The traces are representative of 80 MDMs recorded in 3 different sessions. **C**, Mean $[Ca^{2+}]_i$ values $\pm$ SE of 3 preparations. * $P < .05$ versus basal value; ** $P < .05$ versus histamine.

differentiate into DCs. The upregulation of H_1 receptor confers on mature macrophages the capacity of being activated by histamine. These findings indicate that the inducible H_1 receptor is functionally active.

It is conceivable that the plasticity of histamine receptors in primary human cells might be a means to modulate cell responsiveness to histamine *in vivo*. In this study we report for the first time that H_1 receptor expression is increased in lung macrophages and dendritic cells compared

with that seen in blood monocytes (Fig 1). Macrophages and dendritic cells differentiate from monocytes as they migrate into peripheral tissues. Therefore we hypothesized that differentiation of the monocyte/macrophage lineage induced the upregulation of the H_1 receptor. We tested this hypothesis in a macrophage differentiation model *in vitro* that was verified on the basis of biochemical and phenotypic criteria. The increase in H_1 receptor expression in macrophages differentiated *in vitro* (MDMs) occurred at both the mRNA and protein levels, is evident after 7 days, and is maximal after 10 days.

A major strength of our study was that H_1 receptor regulation was evaluated at different levels: gene transcription, protein synthesis, intracellular signaling, and functional activity. Blood monocytes express relatively small quantities of H_1 mRNA (Figs 1, A, and 3, A), and H_1 receptor protein in the cell lysates was undetectable by means of Western blotting (Figs 1, B, and 3, B). These data suggest that the H_1 receptor is expressed at a very low level in monocytes and that the number of receptors displayed on these cells is too low to generate Ca^{2+} signals (Fig 5) and to activate cytokine production in response to histamine (Fig 7, A). However, monocytes also express relatively large quantities of the H_2 receptor, with a ratio of H_2/H_1 mRNA greater than 200 (Fig 4, C). The relative abundance of H_2 receptors compared with H_1 receptors is likely to make monocytes more prone to the inhibitory effects of histamine.⁶⁻⁸ Differentiation of monocytes into MDMs results in the simultaneous upregulation of H_1 receptors and downregulation of H_2 receptors (Figs 3 and 4). The change in the ratio of H_2/H_1 mRNA endows the macrophages with the capacity to generate Ca^{2+} signals and to produce IL-8 in response to histamine. However, the observation that these effects are blocked by levocetirizine and are not influenced by ranitidine suggests that upregulation of H_1 rather than downregulation of H_2 is responsible for histamine-induced activation of macrophages. Interestingly, differentiation of monocytes into DCs induces H_1 upregulation without changes of H_2 receptors (Fig 4). These data suggest that the expression of the H_1 and H_2 receptors is differentially regulated in the human MPS cells and that activation of distinct differentiation programs in monocytes (eg, macrophages vs dendritic cells) influences the profile of histamine receptors expressed on differentiated cells. A similar phenomenon has been described in naive T cells that upregulate H_1 or H_2 receptors when they differentiate into T_H1 or T_H2 cells, respectively.³⁰ In addition, a switch from H_2 to H_1 receptor expression has been reported in U937 cells incubated with phorbol esters¹⁴ or GM-CSF.³¹ Thus the expression of histamine H_1 and H_2 receptors is a dynamic process that is finely regulated by differentiation and maturation of inflammatory cells.

Histamine is released by mast cells primarily in peripheral tissues, such as the lung, the gastrointestinal tract, and the skin.¹ Evidence that monocytes upregulate the H_1 receptor when they differentiate into mature macrophages within tissues supports the hypothesis that these cells acquire responsiveness to histamine produced at sites of allergic inflammation. It has also been reported that human monocytes and macrophages express a histidine

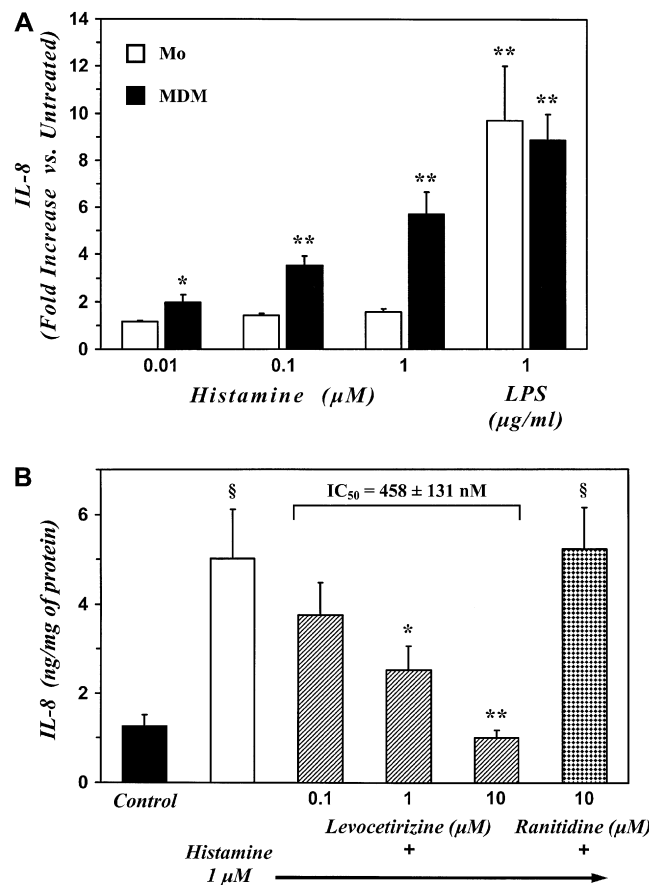


FIG 7. Effect of histamine on IL-8 release from monocytes (Mo) and MDMs. **A**, Cells were incubated (37°C for 18 hours) with histamine or LPS. Data are presented as the mean $\pm$ SE of 3 experiments. * P < .05 versus untreated; ** P < .01 versus untreated. **B**, MDMs were preincubated (37°C for 30 minutes) with levocetirizine or ranitidine and stimulated (37°C for 18 hours) with histamine. Data are presented as the mean $\pm$ SE of 3 experiments. § P < .01 versus control; * P < .05 versus histamine; ** P < .01 versus histamine. IC₅₀, Inhibitory concentration of 50%.

decarboxylase activity³² that can be upregulated *in vivo* during inflammation.³¹ Therefore monocytes and macrophages might be a potential source of histamine that could further enhance cell activation and cytokine production acting in an autocrine fashion.

Our results expand the profile of histamine's effect on human macrophages by showing that in addition to IL-10,⁹ IL-6,¹⁰ and TNF- α ,¹⁴ activation of H₁ receptors on these cells also induces IL-8 production. The ability of macrophages to respond to H₁ receptor activation by releasing proinflammatory cytokines and chemokines supports the potential role of these cells in bronchial asthma.^{5,27}

In our study macrophages purified *ex vivo* from the human lung have an expression profile of the H₁ receptor comparable with that of MDMs and show similar responsiveness to histamine in terms of cytokine production.¹⁰ The H₁ receptor inducible on macrophages differentiated *in vitro* has pharmacologic properties similar to that expressed on macrophages *in vivo*. In fact, levocetirizine displays an inhibitory concentration of 50% on histamine-induced production of IL-8 from MDMs

(approximately 450 nM) that is comparable with the inhibitory concentration of 50% (approximately 500 nM) reported to inhibit histamine-induced production of IL-6 from HLMs.³³

The observation that histamine receptor expression can be modulated in cells that are targets of histamine prompts the hypothesis that H₁ overexpression could be involved in the pathogenesis of such allergic disorders as rhinitis, asthma, urticaria, and anaphylaxis. *In vitro* and *in vivo* studies have shown that exposure to allergens,³⁴ T_H2 cytokines,^{35,36} and β_2 -adrenoceptor agonists³⁷ can modulate H₁ receptor expression on epithelial cells and airway smooth muscle cells. Studies are currently ongoing to investigate whether H₁ receptor is upregulated *in vivo* in airway macrophages of patients with bronchial asthma.

In conclusion, our results support the novel concept of the H₁ receptor plasticity in human inflammatory cells and suggest that the regulation of H₁ receptor expression can be relevant to the pathogenesis of inflammatory and allergic diseases.

We thank Jean Ann Gilder for text editing.

REFERENCES

- Marone G, Lichtenstein LM, Galli SJ. Mast cells and basophils. San Diego: Academic Press; 2000.
- Naclerio R. Clinical manifestations of the release of histamine and other inflammatory mediators. *J Allergy Clin Immunol* 1999;103(suppl):S382-5.
- Akdis CA, Blaser K. Histamine in the immune regulation of allergic inflammation. *J Allergy Clin Immunol* 2003;112:15-22.
- Jutel M, Watanabe T, Akdis M, Blaser K, Akdis CA. Immune regulation by histamine. *Curr Opin Immunol* 2002;14:735-40.
- Marone G, Granata F, Spadaro G, Genovese A, Triggiani M. The histamine-cytokine network in allergic inflammation. *J Allergy Clin Immunol* 2003;112(suppl):S83-8.
- Vannier E, Miller LC, Dinarello CA. Histamine suppresses gene expression and synthesis of tumor necrosis factor alpha via histamine H₂ receptors. *J Exp Med* 1991;174:281-4.
- Elenkov IJ, Webster E, Papanicolaou DA, Fleisher TA, Chrousos GP, Wilder RL. Histamine potently suppresses human IL-12 and stimulates IL-10 production via H₂ receptors. *J Immunol* 1998;161:2586-93.
- van der Pouw Kraan TC, Slijders A, Boeijs LC, de Groot ER, Alewijnse AE, Leurs R, et al. Histamine inhibits the production of interleukin-12 through interaction with H₂ receptors. *J Clin Invest* 1998;102:1866-73.
- Sirois J, Menard G, Moses AS, Bissonnette EY. Importance of histamine in the cytokine network in the lung through H₂ and H₃ receptors: stimulation of IL-10 production. *J Immunol* 2000;164:2964-70.
- Triggiani M, Gentile M, Secondo A, Granata F, Oriente A, Tagliabata M, et al. Histamine induces exocytosis and IL-6 production from human lung macrophages through interaction with H₁ receptors. *J Immunol* 2001;166:4083-91.
- Caron G, Delneste Y, Roelandts E, Duez C, Herbault N, Magistrelli G, et al. Histamine induces CD86 expression and chemokine production by human immature dendritic cells. *J Immunol* 2001;166:6000-6.
- Mazzoni A, Young HA, Spitzer JH, Visintin A, Segal DM. Histamine regulates cytokine production in maturing dendritic cells, resulting in altered T cell polarization. *J Clin Invest* 2001;108:1865-73.
- Caron G, Delneste Y, Roelandts E, Duez C, Bonnefoy JY, Pestel J, et al. Histamine polarizes human dendritic cells into T_H2 cell-promoting effector dendritic cells. *J Immunol* 2001;167:3682-6.
- Wang KY, Arima N, Higuchi S, Shimajiri S, Tanimoto A, Murata Y, et al. Switch of histamine receptor expression from H₂ to H₁ during differentiation of monocytes into macrophages. *FEBS Lett* 2000;473:345-8.
- Hill SJ, Ganellin CR, Timmerman H, Schwartz JC, Shankley NP, Young JM, et al. International Union of Pharmacology. XIII. Classification of histamine receptors. *Pharmacol Rev* 1997;49:253-78.
- Simons FE. Advances in H₁-antihistamines. *N Engl J Med* 2004;351:2203-17.
- Leurs R, Smit MJ, Timmerman H. Molecular pharmacological aspects of histamine receptors. *Pharmacol Ther* 1995;66:413-63.
- Kawakami N, Miyoshi K, Horio S, Fukui H. Beta(2)-adrenergic receptor-mediated histamine H(1) receptor down-regulation: another possible advantage of beta(2) agonists in asthmatic therapy. *J Pharmacol Sci* 2004;94:449-58.
- Smit MJ, Timmerman H, Hijzelendoorn JC, Fukui H, Leurs R. Regulation of the human histamine H₁ receptor stably expressed in Chinese hamster ovary cells. *Br J Pharmacol* 1996;117:1071-80.
- Hume DA, Ross IL, Himes SR, Sasmono RT, Wells CA, Ravasi T. The mononuclear phagocyte system revisited. *J Leukoc Biol* 2002;72:621-7.
- Fogg DK, Sibon C, Miled C, Jung S, Aucouturier P, Littman DR, et al. A clonogenic bone marrow progenitor specific for macrophages and dendritic cells. *Science* 2006;311:83-7.
- Triggiani M, Granata F, Oriente A, Gentile M, Petraroli A, Balestrieri B, et al. Secretory phospholipases A2 induce cytokine release from blood and synovial fluid monocytes. *Eur J Immunol* 2002;32:67-76.
- Allavena P, Piemonti L, Longoni D, Bernasconi S, Stoppacciaro A, Ruco L, et al. IL-10 prevents the differentiation of monocytes to dendritic cells but promotes their maturation to macrophages. *Eur J Immunol* 1998;28:359-69.
- Idzko M, la Sala A, Ferrari D, Panther E, Herouy Y, Dichmann S, et al. Expression and function of histamine receptors in human monocyte-derived dendritic cells. *J Allergy Clin Immunol* 2002;109:839-46.
- Snedecor GW. Statistical methods. Ames: Iowa State University Press; 1980.
- Ratnala VR, Swarts HG, Van Oostrum J, Leurs R, DeGroot HJ, Bakker RA, et al. Large-scale overproduction, functional purification and ligand affinities of the His-tagged human histamine H₁ receptor. *Eur J Biochem* 2004;271:2636-46.
- Viksman MY, Liu MC, Bickel CA, Schleimer RP, Bochner BS. Phenotypic analysis of alveolar macrophages and monocytes in allergic airway inflammation. I. Evidence for activation of alveolar macrophages, but not peripheral blood monocytes, in subjects with allergic rhinitis and asthma. *Am J Respir Crit Care Med* 1997;155:858-63.
- Jeannin P, Delneste Y, Gosset P, Molet S, Lassalle P, Hamid Q, et al. Histamine induces interleukin-8 secretion by endothelial cells. *Blood* 1994;84:2229-33.
- Kohda F, Koga T, Uchi H, Urabe K, Furue M. Histamine-induced IL-6 and IL-8 production are differentially modulated by IFN-gamma and IL-4 in human keratinocytes. *J Dermatol Sci* 2002;28:34-41.
- Jutel M, Watanabe T, Klunker S, Akdis M, Thomet OA, Malolepszy J, et al. Histamine regulates T-cell and antibody responses by differential expression of H₁ and H₂ receptors. *Nature* 2001;413:420-5.
- Murata Y, Tanimoto A, Wang KY, Tsutsui M, Sasaguri Y, De Corte F, et al. Granulocyte macrophage-colony stimulating factor increases the expression of histamine and histamine receptors in monocytes/macrophages in relation to arteriosclerosis. *Arterioscler Thromb Vasc Biol* 2005;25:430-5.
- Mirossay L, Chastre E, Callebort J, Launay JM, Housset B, Zimmer A, et al. Histamine H₂ receptors and histidine decarboxylase in normal and leukemic human monocytes and macrophages. *Am J Physiol Regul Integr Comp Physiol* 1994;267:R602-11.
- Triggiani M, Giannattasio G, Balestrieri B, Loffredo S, Forte V, Granata F. Phenotypic and functional heterogeneity of human lung macrophages. *Clin Exp Allergy Rev* 2004;4:129-34.
- Iriyoshi N, Takeuchi K, Yuta A, Ukai K, Sakakura Y. Increased expression of histamine H₁ receptor mRNA in allergic rhinitis. *Clin Exp Allergy* 1996;26:379-85.
- Wierzbicki T, Iqbal SM, Cuvelier SL, Awong G, Tibbles LA, Patel KD. IL-4 primes human endothelial cells for secondary responses to histamine. *J Leukoc Biol* 2003;74:420-7.
- Giustizieri ML, Albanesi C, Fluhr J, Gisondi P, Norgauer J, Girolomoni G. H₁ histamine receptor mediates inflammatory responses in human keratinocytes. *J Allergy Clin Immunol* 2004;114:1176-82.
- Mak JC, Roffel AF, Katsunuma T, Elzinga CR, Zaagsma J, Barnes PJ. Up-regulation of airway smooth muscle histamine H(1) receptor mRNA, protein, and function by beta(2)-adrenoceptor activation. *Mol Pharmacol* 2000;57:857-64.