Histamine Downregulates the Th1-Associated Chemokine IP-10 in Monocytes and Myeloid Dendritic Cells

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Key Words

CXCL10 · Histamine · Histamine receptors · IP-10 · Monocytes · Myeloid dendritic cells

Abstract

Background: Histamine is an important mediator of allergic diseases. It modulates the cytokine expression of various subtypes of antigen-presenting cells by four known receptors, H₁R–H₄R. The effects of histamine on myeloid dendritic cells (mDC) are unclear. **Methods:** Monocytes and mDC were isolated from human PBMC. Histamine receptor expression was evaluated by real-time PCR. Cells were stimulated with histamine and histamine receptor ligands, and restimulated with polyinosinic-polycytidylic acid (poly I:C), and supernatants were analyzed by protein array and ELISA. **Results:** Monocytes and mDC express H₁R and H₂R without significant differences between the two cell types, whereas H₄R mRNA was significantly higher in mDC compared with monocytes and H₃R mRNA was not detected in any cell type. Prestimulation with histamine caused a significant decrease in poly I:C-induced expression of interferon-γ-induced protein (IP-10) in mDC and monocytes. Stimulation with specific H₁R, H₂R and H₄R agonists and antagonists showed that the observed ef-

fect was mediated via H₂R and H₄R in monocytes and mDC. **Conclusion:** Monocytes and mDC have similar histamine receptor repertoires with regard to H₁R, H₂R and H₃R, but H₄R expression is higher on mDC. Histamine stimulation shows similar functional effects on both cell types, i.e., downregulation of TLR3-induced IP-10 production. This might be a new mechanism how histamine fosters a Th2 milieu.

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Introduction

Histamine is a major mediator in allergic diseases and inflammatory processes, and it is involved in several physiological functions, e.g. differentiation and proliferation of cells, embryonic development, hematopoiesis and wound healing [1]. It plays an important role in acute inflammatory and immediate-type hypersensitivity responses as well as in chronic inflammation and delayed-type allergic reactions. Histamine concentrations are increased at sites of allergic reactions and inflammation, such as allergic asthma, atopic dermatitis (AD) or allergic contact dermatitis [2]. T helper cells are important for the initiation and regulation of immune responses. They can be divided into Th1 and

Th2 cells, which are characterized by the production of IFN- γ and interleukin (IL)-4, respectively. Th1 cells play a role in delayed-type hypersensitivity and cytotoxic responses, whereas Th2 cells are associated with the regulation of allergic diseases by activating B cells and by inducing the production of IgE and IgG [3]. Th1 and Th2 cells show different histamine receptor (HR) expression patterns and histamine interferes with the Th1/Th2 balance, e.g. via a reduction in Th1 cell proliferation and an enhanced secretion of IL-10 in T cells. Histamine also modulates the development of allergies by upregulation of Th2 cell proliferation and increased production of Th2 cytokines IL-4, IL-5, IL-10 and IL-13 [3, 4]. Furthermore, histamine influences the Th1/Th2 balance indirectly by acting on dendritic cells (DC), e.g. by blocking the production of the Th1-priming cytokine IL-12 in DC [5, 6]. As the most potent antigen-presenting cells (APC), DC play an important role in the human immune system and can be found in nearly all peripheral tissues [7]. They represent an important link between adaptive and innate immune responses and are involved in inflammatory and allergic disorders, e.g. allergic airway inflammation [8], psoriasis [9] or rheumatoid arthritis [10]. The main function of these cells is their interaction with T cells: DC trigger the proliferation of specific T cells and they influence the type of T cell responses [11]. Various subtypes of DC are known. One of the main subsets are myeloid DC (mDC), characterized by CD11c⁺ and CD123⁻, which pick up antigens in the periphery and trigger immunity after migration to lymphoid organs. Depending on the antigen and other factors, mDC are capable to induce a Th1 or a Th2 cytokine pattern [12].

Whereas effects of histamine on monocytes and monocyte-derived DC (MoDC) have been investigated in previous studies [5, 13], there are no data on the effects of histamine on human mDC. Therefore, we compared histamine receptor expression and function between primary mDC and monocytes. First, we studied the expression of the four histamine receptors at mRNA level and showed that mDC and monocytes express similar repertoires for H₁R, H₂R and H₃R, whereas H₄R expression is higher on mDC compared with monocytes. Next, we performed a protein array to evaluate functional effects of histamine on the expression of cytokines and chemokines. Finally, we analyzed the newly observed downregulation of the Th1-associated chemokine IP-10 by histamine in more detail. We observed similar functional effects towards histamine stimulation with regard to IP-10 regulation. Thus, our data show the similarity of monocytes and mDC with regard to histamine as well as a new mechanism that fosters a Th2 milieu induced by histamine.

Materials and Methods

Isolation and Culture of Peripheral Blood Mononuclear Cells, Monocytes and mDC

Peripheral blood mononuclear cells (PBMC) were isolated from buffy coats from anonymous healthy donors obtained from the local blood bank. PBMC were separated by density centrifugation on Lymphoprep (Fresenius Kabi Norge AS, Oslo, Norway) and erythrocytes were removed by Gey's lysis. Monocytes and mDC [characterized by CD1 (BDCA-1)⁺ and CD141 (BDCA-3)⁺; CD11c^{high}, CD123^{low}, CD32⁺, CD64⁺ and Fc ϵ R1⁺] were isolated from PBMC by negative isolation using magnetic cell sorting (MACS; Miltenyi Biotech Inc., Bergisch-Gladbach, Germany). 1×10^8 PBMC were used per isolation and the procedure was executed on ice or in the fridge. Cells were washed with MACS buffer and incubated with FcR blocking reagent and mDC antibody-biotin cocktail for 10 min. After centrifugation and two washing steps with MACS buffer, cells were subsequently incubated with anti-biotin MicroBeads for 15 min and separated by magnetic field. The unlabeled cells passing the column contained the desired mDC. Isolated mDC had a purity of at least 90% based on the expression of CD1, CD11c and CD141, and a lack of expression of CD3, CD14, CD16 and CD19 (fig. 2b). Monocytes and mDC were cultured for the stimulation time in RPMI 1640 medium supplemented with 12 mM HEPES, 2 mM L-glutamine, 100 mg/ml penicillin/streptomycin, 5% v/v fetal calf serum (PAN-Biotech, all other components from Biochrom, Berlin, Germany) at 37°C in a humidified atmosphere containing 5% CO₂.

Proteome Analysis

Isolated mDC (5×10^4) were pretreated with 10^{-5} M histamine (agonist for all histamine receptors; Alk-Schering, Wedel, Germany) and stimulated with 10^{-5} M polyinosinic-polycytidylic acid (poly I:C; Sigma-Aldrich, St. Louis, Mo., USA). Supernatants were collected after 24 h and five different independent experiments were pooled. This supernatant pool was analyzed with the Proteome Profiler™ human cytokine array panel A (R&D Systems, Minneapolis, Minn., USA). The probes were incubated for 1 h with a detection antibody cocktail and then the nitrocellulose membrane, containing 36 different anti-cytokine or anti-chemokine antibodies printed in duplicate, was incubated with 500 μ l of the supernatants overnight at 4°C. Immunoreactivity was detected by chemiluminescence according to the manufacturer's instructions (Pierce, Thermo Fisher Scientific, Rockford, Ill., USA) and the intensity of the dot plots was evaluated by densitometric analysis with ChemImager Software (Genetic Technologies, Miami, Fla., USA).

mRNA Isolation, Reverse Transcription and Quantitative Real-Time PCR

Monocytes and mDC were pretreated with histamine overnight and subsequently stimulated with 10^{-5} M poly I:C. Cells were harvested and total RNA was isolated using a Quick-RNA™ MiniPrep kit including additional DNase digestion with DNase I (both ZymoResearch, Orange, Calif., USA). Reverse transcription was performed using a Quantitect reverse transcription kit (Qiagen, Hilden, Germany). Quantification of IP-10, the four histamine receptors and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was performed on a LightCycler (Roche Molecular Biochemicals, Risch, Switzerland) using SYBR Green with QuantiTect primer assays (IP-10: QT01003065; H₁R: QT00199857,

H₃R: QT00210861, and GAPDH: QT01192646) according to the manufacturer's protocol (Qiagen) or for H₂R and H₄R with designed primers (H₂R: 5'-TACCAGCTGTCCTGCAAGTG-3' and 5'-CCCCAGGTGGATAGACAGAA-3', and H₄R: 5'-TGCTAG-GAAATGCTTTGGTC-3' and 5'-GCGTGTGAGGGATGTACAAA-3') [14] synthesized by Tib Molbiol (Berlin, Germany), under standard cyler conditions (annealing temperature 55°C). Melting curve analysis was performed from 60 to 90°C with a ramp rate of 20°C/s. For visualization of the amplified PCR products, agarose gel electrophoresis (2% agarose; Roth, Karlsruhe, Germany) was performed. Relative gene expression data were analyzed using an external calibrator for calculation and using the Relative Quantification Software (Roche Molecular Biochemicals).

Enzyme-Linked Immunosorbent Assay

Protein expression of IP-10 was analyzed after 24 h by ELISA. 0.5 to 1 × 10⁵ mDC and 3 × 10⁵ monocytes were seeded per well in 96-well plates and were preincubated overnight with histamine (Alk-Scherax) or one of the histamine receptor agonists (H₁R agonist 2-pyridylethylamine and H₂R agonist amthamine; both Tocris Bioscience, Bristol, UK; the H₄R agonist ST1006 was synthesized by Holger Stark, Goethe University, Frankfurt am Main, Germany, as published previously [15]). Afterwards cells were stimulated for 24 h with 10⁻⁵ M poly I:C, then cell-free supernatants were harvested and analyzed for IP-10 content using a commercially available ELISA according to the manufacturer's protocol (IP-10: R&D Systems). The plate was read at a wavelength of 450 nm on a Fluo-Star plate reader (BMG Lab Technologies GmbH, Jena, Germany). For blocking experiments, cells were pretreated for 30 min with one of the three different antagonists before histamine was added (H₁R antagonist levocetirizine; UCB, Anderlecht/Brüssel, Belgium; H₂R antagonist ranitidine; Biomol, Hamburg, Germany, and H₄R antagonist JNJ777120; Sigma Aldrich).

Statistical Analysis

Data are expressed as means ± SEM. A Wilcoxon matched pair test (fig. 1 and 3a mDC) or paired t test (fig. 3–6) was applied to determine statistically significant differences; a value of *p* < 0.05 was considered statistically significant. GraphPad Prism® (version 5; GraphPad Software, San Diego, Calif., USA) was used for statistical analysis.

Results

H₁R, H₂R and H₄R Expression on mDC and Monocytes

First we analyzed the expression of the four different HR in monocytes and mDC. Real-time PCR was performed and the amplified products were analyzed by LightCycler melting curves and agarose gel electrophoresis, where sharp bands at the expected sizes could be demonstrated (fig. 1a). The majority of monocytes expressed more H₁R receptor compared with mDC, but the experiments were inconsistent and the difference not statistically significant (fig. 1b). H₂R mRNA was detected at similar amounts and no mRNA for H₃R was detected in both

cell types. With respect to H₄R, we observed a significantly higher expression pattern on mDC compared with monocytes (fig. 1b).

Effects of Histamine on Chemokine and Cytokine Expression on mDC

To assess the effects of histamine on mDC, we performed a proteome array. We stimulated mDC with poly I:C or with poly I:C and histamine. We pooled the cell-free supernatants from five different cell preparations. Proteome analysis revealed the regulation of different cytokines and chemokines and we observed upregulation of the poly I:C-induced CXCL1 expression (poly I:C + histamine results in 120% densitometry compared to stimulation with poly I:C only) and a downregulation of poly I:C-induced expression of TNF-α (76%), CCL1 (78%), IL-6 (46%) and IP-10 (63%) following preincubation with histamine by densitometry (fig. 2a).

Inhibitory Effect of Histamine on Poly I:C-Induced Expression of IP-10

To confirm our observations from the protein array and to gain a deeper insight, we analyzed the effect of different histamine concentrations (10⁻⁵–10⁻⁸ M). As shown in figure 3a in mDC, histamine concentrations from 10⁻⁶ to 10⁻⁸ M led to a significant downregulation of IP-10. Also, in monocytes, it was possible to inhibit the production of IP-10 by histamine concentrations from 10⁻⁵ to 10⁻⁷ M (fig. 3a).

To analyze the time dependence of the poly I:C-induced IP-10 expression, monocytes were preincubated with histamine and incubated for different time periods with poly I:C. IP-10 mRNA was detected after 2, 4, 6 and 8 h and maximal mRNA expression was reached after 4 h. Subsequently, we analyzed the IP-10 protein in supernatants between 2 and 24 h. Protein expression peaked at 24 h. At all measured time points, we could observe a significant downregulation of IP-10 by histamine at the protein level, while at the mRNA level a difference was only detected for the first three time points between 2 and 6 h (fig. 3b).

Effect of H₂R and H₄R on Poly I:C-Induced Expression of IP-10 on Monocytes and mDC

To analyze which receptor is at the functional level responsible for the observed effects of histamine, we performed experiments with specific agonists and antagonists. We demonstrated that amthamine (H₂R agonist) and ST1006 (H₄R agonist) mimic the histamine effect on IP-10 expression in both monocytes and mDC (fig. 4). To verify these observations, we incubated the cells for

Fig. 1. mDC and monocytes express the histamine receptors H₁R, H₂R and H₄R at mRNA level. **a** Representative real-time LightCycler PCR melting peaks and agarose gel bands with the correct size of the amplified PCR products (H₁R = 116 bp, H₂R = 239 bp, H₃R = 150 bp, H₄R = 130 bp and GAPDH = 119 bp) of histamine receptors and GAPDH of 7 independent experiments are presented. As positive control for H₃R amplification H₃R-transfected HEK cells were used. **b** Both cell types were iso-

lated from PBMC and mRNA expression for H₁R, H₂R, H₄R and GAPDH was analyzed by LightCycler PCR. The relative amounts of the different receptors in monocytes and mDC were determined to a calibrator and normalized to GAPDH. mDC expressed less H₁R mRNA, equal amounts of H₂R mRNA and significantly more H₄R mRNA compared to monocytes. Results of 9 (H₁R and H₄R) or 12 (H₂R) independent experiments are shown. * p < 0.05 (Wilcoxon matched pair test).

Fig. 2. Histamine downregulates the expression of IP-10 in mDC. **a** mDC were cultured overnight in the presence or absence of 10^{-5} M histamine and then stimulated with 10^{-5} M poly I:C for 24 h. Supernatants of five different cell stimulations were pooled. The expression of 36 different chemokines and cytokines in this pool was analyzed by protein array. The dot plots show down-regulation of IP-10 in supernatants from cells stimulated with

poly I:C and histamine compared with samples stimulated with poly I:C only. In addition to IP-10, we also detected effects on other mediators such as TNF- α , CXCL1, CCL1 and IL-6. **b** Representative dot plot of purity of mDC is shown in comparison to isotype staining. mDC were gated by cell surface staining of CD1, CD11c and CD141, and for lineage staining CD3, CD14, CD16 and CD19 were used.

30 min with specific receptor antagonists before they were stimulated with histamine. In monocytes, blockade of H₂R with ranitidine as well as of H₄R with JNJ7777120 abolished the histamine effect. However, in mDC the histamine effect could be reversed only by blocking H₄R with JNJ7777120 (fig. 5). As both cell types also express H₁R, we performed experiments with a specific H₁R agonist and antagonist. With these ligands, no effects were observed in both monocytes and mDC (fig. 6). Taken together, the results with agonists and antagonists showed that IP-10 expression is regulated via H₂R and H₄R stimulation in these two cell types.

Discussion

In the immune system, histamine plays a role as an important mediator of inflammation and allergic diseases, and the development of some infections and allergic reactions is associated with a substantial production of histamine. It is also known that histamine modulates the function and activity of different cells of the immune system, including T lymphocytes and APC. Therefore, we focused our study on the expression pattern of histamine receptors

and histamine effects on mDC, a major subpopulation of APC. Monocytes and mDC are human blood cell subpopulations and have the same myeloid descent from bone marrow stem cells, so they are related with each other [16]. Since mDC represent a rather small fraction of around 0.5% of PBMC [17], most previous investigations used in vitro generated MoDC as a model to determine the effects of histamine on the expression and function on mDC [5, 18–20]. MoDC have differentiated from monocytes in vitro using a cytokine cocktail, most commonly IL-4 and granulocyte/macrophage colony-stimulating factor, and incubation results in so-called immature DC with high antigen-processing capacity and poor capability for T cell activation [21, 22]. To eliminate possible effects of this in vitro generation and to get a more realistic view of the natural conditions, we isolated mDC directly from human blood by negative magnetic separation from PBMC. These cells represent the natural mDC population and were compared in our experiments to monocytes from the same donors. In earlier studies, other subtypes of DC and monocytes expressed the histamine receptors H₁R, H₂R and H₄R [23]. The results of our present study demonstrated that mDC and monocytes also expressed three different histamine receptors at mRNA level (H₁R, H₂R and H₄R). Comparing monocytes and

Fig. 3. The expression of IP-10 occurs in a concentration- and time-dependent manner. ns = Nonstimulated. **a** Monocytes and mDC were pretreated overnight with different concentrations of histamine (Hista; 10^{-5} – 10^{-8} M) and then stimulated for 24 h with 10^{-5} M poly I:C. The content of IP-10 in supernatants was detected by ELISA (means and SEM of 6 independent experiments). Histamine significantly and dose-dependently decreased IP-10 release from human monocytes (mean $\pm$ SEM at 24 h of poly I:C = $2,119 \pm 570$ pg/ml, range 606–4,211 pg/ml) and mDC (mean $\pm$ SEM at 24 h of poly I:C = $4,302 \pm 1,127$ pg/ml, range 1,509–9,142 pg/ml) in response to poly I:C. **b** Monocytes were pretreated with 10^{-5} M histamine overnight and then stimulated for different time points with 10^{-5} M poly I:C. mRNA expression was detected by RT-PCR and peaked after 4 h (mean $\pm$ SEM at 4 h of poly I:C = 257 ± 120 relative mRNA expression, range 6–714; mean $\pm$ SEM at 4 h of poly I:C + 10^{-5} M histamine = 122 ± 89 relative mRNA expression, range 3–552). Expression of IP-10 at protein level was detected in supernatants by ELISA (means and SEM of 6 independent experiments are shown) and peaked after 24 h (mean $\pm$ SEM at 24 h of poly I:C = 826 ± 155 pg/ml, range 425–1,431 pg/ml). * $p < 0.05$, ** $p < 0.01$ poly I:C vs. poly I:C + histamine.

Fig. 4. Different histamine (Hista) receptor agonists mimic the histamine effect of downregulating IP-10 protein production in monocytes (**a**) and mDC (**b**). Monocytes and mDC were pretreated overnight with 10^{-5} M histamine or a specific histamine receptor agonist and then stimulated for 24 h with 10^{-5} M poly I:C. The content of IP-10 in supernatants was detected by ELISA [means and SEM of 8 (mDC) or 10 (monocyte) independent experiments are shown]. **a** In monocytes, both the H_2R agonist amthamine (Amtha) and the H_4R agonist ST1006 mimics the histamine effect on the IP-10 expression (mean $\pm$ SEM at 24 h of poly I:C = $2,273 \pm 449$ pg/ml, range 738–5,038 pg/ml). **b** In mDC, H_2R (Amtha) and H_4R (ST1006) agonists imitate the histamine effect of reduced IP-10 protein expression (mean $\pm$ SEM at 24 h of poly I:C = $2,710 \pm 715$ pg/ml, range 682–6,376 pg/ml). * $p < 0.05$, ** $p < 0.01$. ns = Nonstimulated.

Fig. 5. Different histamine receptor antagonists blocked the downregulation of IP-10 protein by histamine (Hista) in monocytes (**a**) and mDC (**b**). Monocytes and mDC were pretreated for 30 min with 10^{-5} M of the specific antagonist and subsequently incubated overnight with 10^{-5} M histamine. The next day, cells were stimulated for 24 h with 10^{-5} M poly I:C. The content of IP-10 in supernatants was detected by ELISA (mean and SEM of 7 independent experiments are shown). ns = Nonstimulated. **a** In monocytes, both antagonists for H_2R (ranitidine) and for H_4R (JNJ777120) reverse the histamine effect of reduced IP-10 protein expression (mean $\pm$ SEM at 24 h of poly I:C = $2,565 \pm 613$ pg/ml, range 738–5,038 pg/ml). **b** In mDC, only the H_4R antagonist JNJ777120 can reverse the effect of histamine (mean $\pm$ SEM at 24 h of poly I:C = $2,180 \pm 746$ pg/ml, range 599–6,144 pg/ml). * $p < 0.05$, ** $p < 0.01$.

Fig. 6. H_1R is not involved in the regulation of poly I:C-induced IP-10 expression. Monocytes (**a**) and mDC (**b**) were treated in the presence or absence of the H_1R antagonist levocetirizine (Levo) with histamine (Hista) or the H_1R -specific agonist 2-pyridylethylamine (2-Pyr) overnight. Then cells were stimulated for 24 h with 10^{-5} M poly I:C. The content of IP-10 in supernatants was detected by ELISA (mean and SEM of 8–13 independent experiments are shown). The results show no significant involvement of H_1R in monocytes (mean $\pm$ SEM at 24 h of poly I:C = $2,893 \pm 573$ pg/ml, range 283–5,594 pg/ml) and mDC (mean $\pm$ SEM at 24 h of poly I:C = $1,789 \pm 549$ pg/ml, range 305–4,508 pg/ml; * $p < 0.05$, ** $p < 0.01$). ns = Nonstimulated.

mDC, in most donors, monocytes expressed more H_1R than mDC. The amounts of H_2R were not different between the two cell types. This is consistent with our previous finding that compared to monocytes MoDC downregulate H_1R mRNA but not H_2R mRNA [13]. In contrast, we were unable to detect H_3R mRNA in both cell types, although other studies reported H_3R expression on monocytes and MoDC [5, 18]. However, H_3R is a presynaptic autoreceptor which is mainly present in the peripheral and central nervous system. The expression of H_4R mRNA was significantly high-

er in mDC than monocytes. This is consistent with our previous observation that compared to monocytes in vitro MoDC upregulate H_4R during their differentiation [5].

Histamine can modulate T helper cell responses, influence antigen presentation capacity and enhance proinflammatory cytokine production [6]. Histamine is a factor for DC polarization but not for DC maturation [20, 24], and mediates its influence on the Th1/Th2 balance through several effects on different cell types and through involvement of different histamine receptors. DC are of-

ten located in areas with high amounts of histamine [25]. Various mechanisms have been described how DC can influence Th1/Th2 polarization. One mechanism is the production of cytokines, such as IL-12, priming a Th1 response. In case IL-12 production is blocked, e.g. by histamine, DC prime a Th2 response [5, 26, 27]. McIlroy et al. [20] showed also an influence of histamine on the Th1/Th2 balance by an upregulation of the Th2 attractants CCL17 and CCL22 and the downregulation of IFN- γ -induced IP-10 in immature human MoDC through involvement of H₂R. In other cell types, we were able to show that H₂R and H₄R stimulation led to downregulation of cytokine and chemokine production, e.g. of TNF- α , CXCL8 and IFN- α in plasmacytoid DC and of IL-27 in monocytes [28, 29]. van der Pouw Kraan et al. [30] demonstrated that histamine inhibits the production of IL-12 via stimulation of H₂R in monocytes. Also, in whole blood cultures, histamine dose-dependently increases IL-10 expression and significantly decreases IL-12 after lipopolysaccharide treatment via H₂R stimulation [31]. Another mechanism for the influence of Th1/Th2 polarization is the DC type. Subtypes of DC (DC1 and DC2) have been characterized that stimulate either a Th1 or Th2 response, respectively [32]. DC1 have a high expression of H₁R and histamine acts as a stimulus for increased antigen presentation capacity, enhanced production of proinflammatory cytokines and Th1 priming activity. In DC2, histamine plays a suppressive role on antigen-presenting ability, increases IL-10 production and induces Th2 cells via H₂R [6]. Taken together, histamine is a potent mediator that primes DC to induce Th2 polarization [24, 32].

To evaluate the functional role of histamine on mDC, we used a protein array to study the effects of histamine on cytokine and chemokine expression on mDC. We observed a regulation of the expression of CXCL1, TNF- α , CCL1, IL-6 and IP-10 through histamine. The expression of the C-X-C motif chemokine 10 (CXCL10), also called IP-10, is associated with inflammation. This chemokine is released from different cell types, e.g. monocytes, DC, endothelial and epithelial cells and keratinocytes, in response to IFN- γ [33]. CXCL chemokines primarily attract Th1 lymphocytes and neutrophils, and CXC receptors are found predominantly on Th1 lymphocytes [34]. Twenty to 40% of circulating Th1 lymphocytes express CXC receptors, whereas Th2 lymphocytes express predominately CC chemokine receptors [35]. Due to the important role of IP-10 in inflammation and as a key chemokine for the recruitment of Th1 lymphocytes into tissue and the association of IP-10 with inflammatory and allergic diseases,

including allergic contact dermatitis, allergic pulmonary inflammation and AD [36–39], we chose IP-10 as a target for further investigations. For the first time, we describe an influence of histamine on poly I:C-induced expression of IP-10 in natural untouched mDC. Our results fit well in the picture that histamine exerts anti-inflammatory effects and promotes a Th2 milieu via H₂R and H₄R.

H₂R and H₄R might be new therapeutic targets also in allergic diseases such as AD. Potential antagonists of H₂R and H₄R mediate their therapeutic potential through alleviation of Th1 responses by targeting the expression of relevant cytokines such as IP-10 or IL-12. Thus, H₂R and H₄R antagonists might be useful for the treatment of allergic and inflammatory disorders with Th2 predominance, such as AD.

Since IP-10 is a Th1 chemokine and plays an important role in numerous diseases, first studies evaluating IP-10 as therapeutic target are in progress. Fife et al. [40] showed that the application of an anti-IP-10 antibody resulted in a decreased clinical and histological manifestation of experimental autoimmune encephalomyelitis. Blocking of IP-10 and CXCR3 reduced inflammatory colitis in mice [41].

In summary, our study demonstrates that mDC as another APC subpopulation express H₁R, H₂R and H₄R. In addition, the histamine-induced downregulation of IP-10 via H₂R and H₄R might affect the regulation of the Th1/Th2 balance. These results improve the understanding of immunological functions of histamine and suggest that specific receptor antagonists might be candidates for pharmacological treatment to inhibit the Th2 shift in inflammatory and allergic disorders.

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References

- 1 Akdis CA, Jutel M, Akdis M: Regulatory effects of histamine and histamine receptor expression in human allergic immune responses. *Chem Immunol Allergy* 2008;94:67–82.
- 2 Gutzmer R, Gschwandtner M, Rossbach K, Mommert S, Werfel T, Kietzmann M, Baeumler W: Pathogenetic and therapeutic implications of the histamine H₄ receptor in inflammatory skin diseases and pruritus. *Front Biosci (Schol Ed)* 2011;3:985–994.
- 3 Packard KA, Khan MM: Effects of histamine on Th1/Th2 cytokine balance. *Int Immunopharmacol* 2003;3:909–920.

- 4 Osna N, Elliott K, Khan MM: Regulation of interleukin-10 secretion by histamine in Th2 cells and splenocytes. *Int Immunopharmacol* 2001;1:85–96.
- 5 Gutzmer R, Diestel C, Mommert S, Kother B, Stark H, Wittmann M, Werfel T: Histamine H4 receptor stimulation suppresses IL-12p70 production and mediates chemotaxis in human monocyte-derived dendritic cells. *J Immunol* 2005;174:5224–5232.
- 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;108:1865–1873.
- 7 Banchereau J, Steinman RM: Dendritic cells and the control of immunity. *Nature* 1998;392:245–252.
- 8 van Rijt LS, van Kessel CH, Boogaard I, Lambrecht BN: Respiratory viral infections and asthma pathogenesis: a critical role for dendritic cells? *J Clin Virol* 2005;34:161–169.
- 9 Nestle FO, Turka LA, Nickoloff BJ: Characterization of dermal dendritic cells in psoriasis. Autostimulation of T lymphocytes and induction of Th1 type cytokines. *J Clin Invest* 1994;94:202–209.
- 10 Thomas R, Davis LS, Lipsky PE: Rheumatoid synovium is enriched in mature antigen-presenting dendritic cells. *J Immunol* 1994;152:2613–2623.
- 11 Cravens PD, Lipsky PE: Dendritic cells, chemokine receptors and autoimmune inflammatory diseases. *Immunol Cell Biol* 2002;80:497–505.
- 12 Tschoep K, Manning TC, Harlin H, George C, Johnson M, Gajewski TF: Disparate functions of immature and mature human myeloid dendritic cells: implications for dendritic cell-based vaccines. *J Leukoc Biol* 2003;74:69–80.
- 13 Gutzmer R, Langer K, Lisewski M, Mommert S, Rieckborn D, Kapp A, Werfel T: Expression and function of histamine receptors 1 and 2 on human monocyte-derived dendritic cells. *J Allergy Clin Immunol* 2002;109:524–531.
- 14 van Rijn RM, van Marle A, Chazot PL, Lange-meijer E, Qin Y, Shenton FC, Lim HD, Zuiderveld OP, Sansuk K, Dy M, Smit MJ, Tensen CP, Bakker RA, Leurs R: Cloning and characterization of dominant negative splice variants of the human histamine H4 receptor. *Biochem J* 2008;414:121–131.
- 15 Sander K, Kottke T, Tanrikulu Y, Proschak E, Weizel L, Schneider EH, Seifert R, Schneider G, Stark H: 2,4-Diaminopyrimidines as histamine H4 receptor ligands – scaffold optimization and pharmacological characterization. *Bioorg Med Chem* 2009;17:7186–7196.
- 16 Goordyal P, Isaacson PG: Immunocytochemical characterization of monocyte colonies of human bone marrow: a clue to the origin of Langerhans cells and interdigitating reticulum cells. *J Pathol* 1985;146:189–195.
- 17 Bosma BM, Metselaar HJ, Tra WM, Mancham S, Kuipers EJ, Tilanus HW, Kwekkeboom J: Impairment of circulating myeloid dendritic cells in immunosuppressed liver transplant recipients. *Clin Exp Immunol* 2007;149:525–534.
- 18 Idzko M, La Sala A, Ferrari D, Panther E, Herouy Y, Dichmann S, Mockenhaupt M, Di Virgilio F, Girolomoni G, Norgauer J: Expression and function of histamine receptors in human monocyte-derived dendritic cells. *J Allergy Clin Immunol* 2002;109:839–846.
- 19 Amaral MM, Davio C, Ceballos A, Salamone G, Canones C, Geffner J, Vermeulen M: Histamine improves antigen uptake and cross-presentation by dendritic cells. *J Immunol* 2007;179:3425–3433.
- 20 McIlroy A, Caron G, Blanchard S, Fremaux I, Duluc D, Delneste Y, Chevailler A, Jeannin P: Histamine and prostaglandin E up-regulate the production of Th2-attracting chemokines (CCL17 and CCL22) and down-regulate IFN-gamma-induced CXCL10 production by immature human dendritic cells. *Immunology* 2006;117:507–516.
- 21 O'Doherty U, Steinman RM, Peng M, Cameron PU, Gezelter S, Kopeloff I, Swiggard WJ, Pope M, Bhardwaj N: Dendritic cells freshly isolated from human blood express CD4 and mature into typical immunostimulatory dendritic cells after culture in monocyte-conditioned medium. *J Exp Med* 1993;178:1067–1076.
- 22 Sallusto F, Lanzavecchia A: Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J Exp Med* 1994;179:1109–1118.
- 23 Ferstl R, Akdis CA, O'Mahony L: Histamine regulation of innate and adaptive immunity. *Front Biosci* 2012;17:40–53.
- 24 Mazzoni A, Siraganian RP, Leifer CA, Segal DM: Dendritic cell modulation by mast cells controls the Th1/Th2 balance in responding T cells. *J Immunol* 2006;177:3577–3581.
- 25 Jutel M, Blaser K, Akdis CA: The role of histamine in regulation of immune responses. *Chem Immunol Allergy* 2006;91:174–187.
- 26 Risoan MC, Soumelis V, Kadowaki N, Grouard G, Briere F, de Waal Malefyt R, Liu YJ: Reciprocal control of T helper cell and dendritic cell differentiation. *Science* 1999;283:1183–1186.
- 27 Langenkamp A, Messi M, Lanzavecchia A, Sallusto F: Kinetics of dendritic cell activation: impact on priming of Th1, Th2 and nonpolarized T cells. *Nat Immunol* 2000;1:311–316.
- 28 Gschwandtnr M, Mommert S, Kother B, Werfel T, Gutzmer R: The histamine H4 receptor is highly expressed on plasmacytoid dendritic cells in psoriasis and histamine regulates their cytokine production and migration. *J Invest Dermatol* 2011;131:1668–1676.
- 29 Gschwandtnr M, Bunk H, Kother B, Thurnmond RL, Kietzmann M, Werfel T, Baumer W, Gutzmer R: Histamine down-regulates IL27 production in antigen-presenting cells. *J Leukoc Biol* 2012;92:21–29.
- 30 van der Pouw Kraan TC, Snijders A, Boeijs LC, de Groot ER, Alewijnse AE, Leurs R, Aarden LA: Histamine inhibits the production of interleukin-12 through interaction with H2 receptors. *J Clin Invest* 1998;102:1866–1873.
- 31 Elenkov IJ, Webster E, Papanicolaou DA, Fleisher TA, Chrousos GP, Wilder RL: Histamine potentially suppresses human IL-12 and stimulates IL-10 production via H2 receptors. *J Immunol* 1998;161:2586–2593.
- 32 Caron G, Delneste Y, Roelandts E, Duez C, Bonnefoy JY, Pestel J, Jeannin P: Histamine polarizes human dendritic cells into Th2 cell-promoting effector dendritic cells. *J Immunol* 2001;167:3682–3686.
- 33 Cassatella MA, Gasperini S, Calzetti F, Bertagnin A, Luster AD, McDonald PP: Regulated production of the interferon-gamma-inducible protein-10 (IP-10) chemokine by human neutrophils. *Eur J Immunol* 1997;27:111–115.
- 34 Sallusto F, Lanzavecchia A: Understanding dendritic cell and T-lymphocyte traffic through the analysis of chemokine receptor expression. *Immunol Rev* 2000;177:134–140.
- 35 Gasperini S, Marchi M, Calzetti F, Laudanna C, Vicentini L, Olsen H, Murphy M, Liao F, Farber J, Cassatella MA: Gene expression and production of the monokine induced by IFN-gamma (MIG), IFN-inducible T cell alpha chemotactant (I-Tac), and IFN-gamma-inducible protein-10 (IP-10) chemokines by human neutrophils. *J Immunol* 1999;162:4928–4937.
- 36 Flier J, Boorsma DM, Bruynzeel DP, Van Beek PJ, Stoof TJ, Scheper RJ, Willemze R, Tensen CP: The CXCR3 activating chemokines IP-10, MIG, and IP-9 are expressed in allergic but not in irritant patch test reactions. *J Invest Dermatol* 1999;113:574–578.
- 37 Medoff BD, Sauty A, Tager AM, Maclean JA, Smith RN, Mathew A, Dufour JH, Luster AD: IFN-gamma-inducible protein 10 (CXCL10) contributes to airway hyperreactivity and airway inflammation in a mouse model of asthma. *J Immunol* 2002;168:5278–5286.
- 38 Villagomez MT, Bae SJ, Ogawa I, Takenaka M, Katayama I: Tumour necrosis factor-alpha but not interferon-gamma is the main inducer of inducible protein-10 in skin fibroblasts from patients with atopic dermatitis. *Br J Dermatol* 2004;150:910–916.
- 39 Giustizieri ML, Mascia F, Frezzolini A, De Pita O, Chinni LM, Giannetti A, Girolomoni G, Pastore S: Keratinocytes from patients with atopic dermatitis and psoriasis show a distinct chemokine production profile in response to T cell-derived cytokines. *J Allergy Clin Immunol* 2001;107:871–877.
- 40 Fife BT, Kennedy KJ, Paniagua MC, Lukacs NW, Kunkel SL, Luster AD, Karpus WJ: CXCL10 (IFN-gamma-inducible protein-10) control of encephalitogenic CD4+ T cell accumulation in the central nervous system during experimental autoimmune encephalomyelitis. *J Immunol* 2001;166:7617–7624.
- 41 Singh UP, Venkataraman C, Singh R, Lillard JW Jr: CXCR3 axis: role in inflammatory bowel disease and its therapeutic implication. *Endocr Metab Immune Disord Drug Targets* 2007;7:111–123.