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Histamine Induces CD86 Expression and Chemokine Production by Human Immature Dendritic Cells

Gersende Caron,* Yves Delineste,* Edith Roelandts, † Catherine Duez, ‡ Nathalie Herbufft,* Giovanni Magistrelli, † Jean-Yves Bonnefoy,* Joel Pestel, ‡ and Pascale Jeannin1* 

Mast cells and immature dendritic cells (DC) are in close contact in peripheral tissues. Upon activation, mast cells release histamine, a mediator involved in the immediate hypersensitivity reaction. We therefore tested whether histamine could affect human DC activation and maturation. Histamine induces CD86 expression on immature DC in a dose-dependent (significant at 10−7 M) and transient manner (maximal after 24-h stimulation). Histamine also transiently up-regulates the expression of the costimulatory and accessory molecules, CD40, CD49d, CD54, CD80, and MHC class II. As a consequence, immature DC exposed for 24 h to histamine stimulate memory T cells more efficiently than untreated DC. In addition, histamine induces a potent production of IL-6, IL-8, monocyte chemoattractant protein 1, and macrophage-inflammatory protein 1α by immature DC and also up-regulates IL-1β, RANTES, and macrophage-inflammatory protein 1β but not TNF-α and IL-12 mRNA expression. Histamine activates immature DC through both the H1 and H2 receptors. However, histamine-treated DC do not have a phenotype of fully mature cells, as they do neither show significant changes in the expression of the chemokine receptors, CCR5, CCR7 and CXC chemokine receptor 4, nor expression of CD83 de novo. These data demonstrate that histamine activates immature DC and induces chemokine production, thereby suggesting that histamine, via stimulation of resident DC, may participate locally in T cell stimulation and in the late inflammatory reaction associated with allergic disorders. The Journal of Immunology, 2001, 166: 6000–6006.

D endritic cells (DC)2 are the most potent APCs. Located in peripheral nonlymphoid tissues, immature DC are “sentinels” that capture foreign Ags very efficiently (1, 2). After Ag challenge or stimulation with inflammatory stimuli (such as IL-1 and TNF-α), DC migrate to the secondary lymphoid organs (1–3). During their migration, they undergo modulations of phenotype and function, referred to as DC maturation. They express increased levels of surface Ags involved in T cell activation such as costimulatory molecules (e.g., CD54 and CD86) and MHC class I and II molecules (1, 2). They produce numerous cytokines (e.g., IL-1β and IL-6) and chemokines (e.g., monocyte chemoattractant protein (MCP) 1, macrophage-inflammatory protein (MIP) 1α, MIP-1β, IL-8, and RANTES) that favor lymphocyte recruitment and activation (4, 5). Moreover, they also down-regulate the expression of receptors for inflammatory chemokines (e.g., CCR1 and CCR5) and up-regulate those for constitutive chemokines (i.e., CCR7 and CXC chemokine receptor 4 (CXCR4)), therefore allowing maturing DC to migrate from the periphery to the lymph nodes (6). At the same time, they lose their capacity to process Ag and neoxpress some molecules (such as CD83 on human cells). In the T cell-dependent areas of the lymphoid organs, myeloid migratory DC have acquired potent immunostimulatory properties and sensitize recirculating naive Ag-specific T cells (1, 2).

Mast cells are present in tissues in proximity to surfaces that interface the external environment. In allergic individuals, a contact with the sensitizing allergen results in the activation of mast cells through allergen-specific IgE bound to high-affinity IgE receptors (FcεRI). Activated mast cells release preformed (e.g., histamine) and newly synthesized mediators (such as prostaglandins, leukotrienes, cytokines, and chemokines) (7, 8). Among these mediators, histamine elicits almost all of the pathologic processes involved in immediate allergic reaction (such as vasodilation, smooth muscle contraction, mucus hypersecretion, and edema formation) (9–12). Histamine also presents immunoregulatory properties. It induces E-selectin, ICAM-1, and LFA-1 expression (10, 13), IL-6 and IL-8 production by endothelial cells (14, 15), and IL-1, IL-6, IL-18, and IFN-γ production by PBMC (16–18). In parallel to these proinflammatory effects, histamine has immunosuppressive properties. It induces IL-10 and prevents LPS-induced TNF-α and IL-12 production by monocytes/macrophages (19–22). Histamine exerts its effects through three receptors, H1, H2, and H3 (10, 12). Although H1 and H2 receptors are expressed on numerous cell types, including lymphoid cells, monocytes, and endothelial cells, H3 is mainly expressed in the brain where histamine functions as a neurotransmitter. Signaling through H1 receptor involves the activation of the phospholipase C and controls most of the effects of histamine in allergic disorders (smooth muscle contraction and increased vasopermeability). Signaling through H2 receptors involves cAMP generation and controls gastric acid secretion and vasodilation (10, 12).

Because mast cells and immature DC are in close proximity throughout connective tissues (such as those from the respiratory tract and the skin), we have evaluated whether histamine released by activated mast cells may affect DC maturation.

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2Abbreviations used in this paper: DC, dendritic cell; MCP, monocyte chemoattractant protein; MIP, macrophage-inflammatory protein; MFI, mean fluorescence intensity; PI, proliferation index; LC, Langerhans cell.

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Materials and Methods

Human DC generation

PBMC were isolated from healthy volunteers by standard density gradient centrifugation on Ficoll-Paque (Amersham Pharmacia Biotech, Uppsala, Sweden). Monocytes were purified from PBMC by positive selection using a MACS (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer’s instructions. Purity assessed by FACS analysis using a FITC-labeled anti-CD14 mAb (Cymbys, Hants, U.K.) was >98%. Monocytes were cultured in complete medium (CM) consisting of RPMI 1640 medium supplemented with 10% FCS, 2 mM L-glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin, 10 mM HEPES, and 0.1 mM nonessential amino acids (all from Life Technologies, Cergy Pontoise, France) at 37°C for 24 h. After 8% and/or 30 h of culture with 10−5, 10−4 or 10−3 M histamine, total RNA was extracted using TRIzol reagent (Life Technologies, Rockville, MD) and the single-strand cDNA was synthesized using 2 μg of total RNA by RT using a oligo(dT) primer (Amersham Pharmacia Biotech). PCR were performed with cDNA corresponding to 50 ng of total RNA and primers designed to amplify the coding sequence of the cytokines and chemokines (4, 5, 23). PCR was as follows: 94°C for 30 s, 5 M histamine, total RNA was extracted using TRIzol reagent (Life Technologies, Rockville, MD) and the single-strand cDNA was synthesized using 2 μg of total RNA by RT using an oligo(dT) primer (Amersham Pharmacia Biotech). PCR were performed with cDNA corresponding to 50 ng of total RNA and primers designed to amplify the coding sequence of the cytokines and chemokines (4, 5, 23). PCR was as follows: 94°C for 3 min, 30 cycles (25 cycles for IL-8 and MIP-1α) 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min followed by a final extension at 72°C for 5 min. RNA integrity and cDNA synthesis were verified by amplifying GAPDH cDNA (5′-TTCAACACCTGTGTGTGA-3′ and 5′-ACCA CAGTCCCATGCACATAC-3′). The amplified fragments were size-separated on a 1% agarose gel and visualized by ethidium bromide.

Cytokine and chemokine quantification

Day 7 DC were stimulated as described above and the concentration of IL-6, IL-8, IL-10, IL-12p35, IL-12p40, IL-18, TNF-α, MCP-1, MIP-1α, RANTES, CCR5, CCR7, and CXCR4 was determined by RT-PCR. After 8 and/or 30 h of culture with 10−5 M histamine, total RNA was extracted using TRIzol reagent (Life Technologies, Rockville, MD) and the single-strand cDNA was synthesized using 2 μg of total RNA by RT using an oligo(dT) primer (Amersham Pharmacia Biotech). PCR were performed with cDNA corresponding to 50 ng of total RNA and primers designed to amplify the coding sequence of the cytokines and chemokines (4, 5, 23). PCR was as follows: 94°C for 3 min, 30 cycles (25 cycles for IL-8 and MIP-1α) 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min followed by a final extension at 72°C for 5 min. RNA integrity and cDNA synthesis were verified by amplifying GAPDH cDNA (5′-TTCAACACCTGTGTGTGA-3′ and 5′-ACCA CAGTCCCATGCACATAC-3′). The amplified fragments were size-separated on a 1% agarose gel and visualized by ethidium bromide.

Cytokine and chemokine quantification

Day 7 DC were stimulated as described above and the concentration of IL-6, IL-8, IL-10, IL-12, MCP-1, MIP-1α, and TNF-α was determined in the 24- or 72-h cell-free culture supernatants by ELISA (R&D Systems). Statistical analysis

Statistical analyses were performed using Student’s t test. Values of p < 0.05 were considered to be statistically significant.

Results

Histamine induces a potent and transient expression of CD86 on human immature DC

To evaluate the effect of histamine on DC, human immature DC were exposed to 10−8–10−4 M histamine and CD86 expression was analyzed by flow cytometry. CD86 expression is undetectable on immature DC (<6% positive cells in day 7 monocyte-derived DC) and is induced by histamine on a percentage of cells that increases dose dependently (Fig. 1, A and B). One day after exposure, CD86 expression is significant with 10−7 M histamine (11% ± 4% of CD86-positive cells, mean% ± SD, n = 5) and maximal with 5 × 10−5 M histamine (75% ± 10%; Fig. 1B).

In contrast to LPS (data not shown) or TNF-α (1, 2), histamine-induced CD86 expression is transient (Fig. 1C). The expression of CD86 induced by 10−5 M histamine is detectable after 6 h, maximal at 24 h (65% ± 10%, mean% ± SD, n = 5), and returns to basal level at later time points. Addition of histamine at 24 h and/or 48 h failed to sustain this effect (data not shown).

As histamine and preformed TNF-α are released concomitantly by mast cells (7, 8), we then tested the addition of these mediators together on immature DC. An additive effect of histamine and TNF-α on CD86 expression on DC is observed at any concentration used (Fig. 1D).

Finally, the observation that polymyxin B down-regulates LPS but not histamine-induced CD86 expression allows us to exclude contaminating endotoxin in histamine preparation (Fig. 1E).

In conclusion, these data show that histamine activates human immature DC as assessed by the induction of CD86 expression.

Histamine transiently up-regulates costimulatory molecule expression on human immature DC

When stimulated by proinflammatory cytokines (e.g., TNF-α) or bacterial products (e.g., LPS), DC undergo a maturation process characterized by an up-regulation of molecules related to Ag presentation, a change in chemokine receptor expression, and the neoexpression of the maturation marker CD83 (1, 2, 6). Exposure of immature DC to histamine results in a marked up-regulation of CD40, CD49d, CD54, CD80, and HLA-DR expression (Table I). As observed for CD86, histamine transiently up-regulates these molecule expressions; the effect of histamine is maximal after 1 day and undetectable after 4 days (Table I).

However, histamine-treated DC do not acquire CD83 expression (Table I) nor present veils (data not shown). At any time point analyzed, histamine does not significantly modulate CCR5 expression as assessed at the transcriptional (Fig. 2A) and traductional (Fig. 2B) levels nor induce CCR7 and CXCR4 mRNA expression (Fig. 2A). In contrast, LPS decreases CCR5 expression (Fig. 2B) and induces CCR7 and CXCR4 mRNA expression on immature DC (Fig. 2A) (24).
Histamine up-regulates costimulatory molecule expression on DC

Histamine up-regulates costimulatory molecule expression on DC in a dose dependent manner, significant at 4 × 10⁻⁷ M and maximal at 10⁻⁵ M (Fig. 3A). Histamine also induces IL-6, MCP-1, and MIP-1α production (Fig. 3B). Histamine up-regulates IL-8, IL-6, MCP-1, and MIP-1α mRNA expression and also IL-1β, MIP-1β, and RANTES mRNA expression (Fig. 3C).

In contrast, histamine does not induce the production of detectable levels of IL-10, p70 IL-12, and TNF-α by DC after 24 or 72 h (Fig. 3B) nor up-regulate IL-10, p35, and p40 IL-12, IL-18, and TNF-α mRNA expression after 8 h (Fig. 3C) or 16 h (data not shown). IL-10 is a cytokine that inhibits DC maturation (25). In agreement with the absence of detectable levels of IL-10 in the supernatant of histamine-treated DC, neutralizing anti-IL-10 Ab does not enhance the histamine effect on DC (data not shown).

Table I. Histamine up-regulates costimulatory molecule expression on DC*  

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 4</th>
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<tbody>
<tr>
<td></td>
<td>None</td>
<td>Histamine</td>
</tr>
<tr>
<td>CD40</td>
<td>157 ± 16</td>
<td>221 ± 23</td>
</tr>
<tr>
<td>CD49d</td>
<td>11 ± 2</td>
<td>20 ± 4</td>
</tr>
<tr>
<td>CD54</td>
<td>127 ± 21</td>
<td>216 ± 32</td>
</tr>
<tr>
<td>CD80</td>
<td>10 ± 1</td>
<td>19 ± 2</td>
</tr>
<tr>
<td>CD83b</td>
<td>9 ± 2</td>
<td>10 ± 3</td>
</tr>
<tr>
<td></td>
<td>(23% ± 1)</td>
<td>(3% ± 1)</td>
</tr>
<tr>
<td>CD86b</td>
<td>12 ± 4</td>
<td>177 ± 21</td>
</tr>
<tr>
<td></td>
<td>(5% ± 3)</td>
<td>(65% ± 8)</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>334 ± 21</td>
<td>555 ± 25</td>
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</table>

* Immature DC were either untreated or exposed to 10⁻⁵ M histamine or 20 ng/ml TNF-α. CD40, CD49d, CD54, CD80, CD83, CD86, and HLA-DR expression was analyzed by FACS after 1 and 4 days. Results are expressed in MFI values as mean ± SD of three separate experiments.

b In parentheses, the percentage of positive cells is shown as mean ± SD (n = 3).
As expected, LPS and TNF-α induce the production of these cytokines (except TNF-α that did not induce IL-12 production by DC) (Fig. 3, B and C). Although immature DC constitutively express IL-18 mRNA (4, 26), we failed in detecting IL-18 production, in the absence of T cells (27), after stimulation with histamine, LPS, or TNF-α for 24 or 72 h (data not shown). Thus, histamine induces the expression of the proinflammatory cytokines IL-1β and IL-6 and of the chemokines IL-8, MCP-1, MIP-1α, MIP-1β, and RANTES by human immature DC.

**Histamine enhances DC accessory capacity**

Based on the observation that histamine activates immature DC, we therefore tested in T cell proliferation assays whether histamine may enhance DC accessory capacities.

In anti-CD3 mAb assays, naive and memory CD4+ T cells were stimulated with a suboptimal concentration of anti-CD3 mAb and cocultured with DC either untreated or treated with histamine or TNF-α (Fig. 4, A and B). Histamine-treated DC stimulate the proliferation of memory T cells more efficiently than naive T cells (PI = 3.9 ± 0.5 and 1.9 ± 0.2, respectively, mean ± SD, n = 3, at 10^4 DC/well). As expected, TNF-α-treated mature DC stimulate efficiently both naive and memory T cell proliferation (PI = 4.5 ± 0.8 and 4.8 ± 0.7; Fig. 4, A and B).

CD86 is required for an efficient generation of primary MLR directed against alloantigen (28). In primary MLR assays, we therefore compared the ability of DC either untreated or treated with histamine or TNF-α (positive control) to stimulate the proliferation of T cells from different donors. Histamine-treated DC stimulate allogenic T cell proliferation although to a lower extent than TNF-α-treated DC (PI = 2.8 ± 0.4 and 5.1 ± 0.8, respectively, mean ± SD, n = 3, at 10^3 DC/well; Fig. 4C).

Thus, histamine-treated DC present enhanced costimulatory properties as assessed by an efficient stimulation of memory T cell proliferation.

**Histamine activates DC through H1 and H2 receptors**

Histamine exerts its effects through three receptors, H1, H2, and H3 (10, 12). Using specific receptor antagonists, we then analyzed which receptor is involved in histamine-induced DC activation. DC were exposed to 10^{-5} M of each of the H1, H2, or H3 receptor antagonists. In anti-CD3 mAb assays, naive and memory CD4+ T cells were stimulated with a suboptimal concentration of anti-CD3 mAb and cocultured with DC either untreated or treated with histamine or TNF-α (Fig. 4, A and B). Histamine-treated DC stimulate the proliferation of memory T cells more efficiently than naive T cells (PI = 3.9 ± 0.5 and 1.9 ± 0.2, respectively, mean ± SD, n = 3, at 10^4 DC/well). As expected, TNF-α-treated mature DC stimulate efficiently both naive and memory T cell proliferation (PI = 4.5 ± 0.8 and 4.8 ± 0.7; Fig. 4, A and B).

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Thus, histamine-treated DC present enhanced costimulatory properties as assessed by an efficient stimulation of memory T cell proliferation.
Taken together, these data show that histamine activates human amide on histamine-induced DC activation is observed (Fig. 5). After 24 h, DC were irradiated and cultured with freshly isolated CD45RO−CD4+ naive T cells (A), or with freshly isolated allogenic T cells (C), in the absence (C) or presence (A and B) of a suboptimal concentration of an anti-CD3 mAb. T cell proliferation was measured at day 3 (A and B) or day 5 (C). Results are expressed in cpm as mean of quintuplicate values and results are representative of one of three experiments.

antagonists (mepyramine, cimetidine, or thioperamide, respectively) 1 h before addition of $10^{-5}$ M histamine. None of these antagonists has an effect by itself on CD86 expression or IL-8 production by DC (Fig. 5). Results show that mepyramine and cimetidine significantly prevent histamine-induced CD86 expression (decrease of 58% ± 6 and 72% ± 5, respectively; mean ± SD, n = 4) and IL-8 production by DC (decrease of 86% ± 5 and 92% ± 4, respectively; Fig. 5). In contrast, no effect of thioperamide on histamine-induced DC activation is observed (Fig. 5). Taken together, these data show that histamine activates human DC by acting through both H1 and H2 receptors.

Discussion

We demonstrate that histamine activates human immature DC as assessed by an up-regulation of costimulatory molecule expression and by a production of proinflammatory cytokines and chemokines.

We demonstrate for the first time that histamine up-regulates numerous chemokine production. It induces IL-8, MCP-1, and MIP-1α production and up-regulates MIP-1β and RANTES mRNA expression by immature DC. All of these chemokines are involved in the recruitment of numerous cell types including T cells, monocytes, neutrophils, and immature DC (29, 30) and in the development of the cellular afflux associated with the late phase reaction (31). Furthermore, IL-8, MIP-1α, RANTES, and MCP-1 recruit mast cells/basophils and induce histamine release (29), suggesting a positive feedback that may contribute to sustain the inflammatory reaction.

In addition, histamine acts directly on immature DC to control proinflammatory cytokine production. In agreement with data reporting that histamine induces IL-1 and IL-6 production by PBMC (16, 17) and IL-6 production by endothelial cells (15), we show that histamine up-regulates the expression of the two proinflammatory cytokines by DC. Moreover, in accordance with data obtained using monocytes/macrophages (18–21), histamine does not induce TNF-α nor IL-12 production by DC. Histamine has been shown to induce IL-10 production by alveolar macrophages (22). In contrast, others reported that histamine alone did not induce IL-10 production by PBMC (18, 21). In agreement with this study, we report no detectable effect of histamine on IL-10 production by DC. Finally, although histamine induces IL-18 production by PBMC (18), we failed in detecting an effect of histamine on IL-18 production by DC in the absence of T cells (26, 27). These data point out a tight control of proinflammatory cytokine production by histamine which may differ with the nature of the target cells.

Several hours after an allergen challenge, some allergic patients develop a late inflammatory reaction (also named late phase reaction) characterized by a local infiltrate of eosinophils, memory T cells, and neutrophils that contribute to the tissue damage. The recruitment and activation of these inflammatory cells involves a series of events, including transendothelial migration of leukocytes and subsequent chemotactic movements, that are regulated by proinflammatory cytokines and chemokines (24). Based on the observations that histamine induces IL-8 production by endothelial cells (14) and that histamine challenge increases neutrophil, mast cell, and leukocyte numbers in the bronchoalveolar lavage from healthy subjects (32), it is now suggested that, in addition to its effects in the immediate reaction associated to allergic diseases, histamine also participates in the late inflammatory reaction. The present data show that histamine, in addition to inducing the production of proinflammatory cytokines IL-1 and IL-6, up-regulates chemokine production by DC and thereby evidence a direct mechanism by which histamine may participate in the initiation of the inflammatory reaction.

Although histamine-treated DC have not all of the characteristics of fully mature cells (compared with stimuli that induce DC maturation and migration such as LPS and TNF-α), they transiently express high levels of CD86 and other costimulatory molecules. In agreement with this observation, results from proliferation assays show that histamine enhances the costimulatory properties of DC. Interestingly, although histamine-treated DC are as effective as fully mature DC in stimulating memory T cell proliferation, they only slightly enhance naive T cell proliferation. Both of these observations are in agreement with the fact that memory T cells require less stringent and sustained cosignals than
naive cells to be efficiently activated (33). We also report that histamine only poorly affects the expression of the chemokine receptors CXCR5, CCR7, and CXCR4, therefore suggesting that these DC could be unable to migrate from the periphery to the lymph nodes (6). In contrast to naïve T cells that migrate to the lymph nodes, memory T cells are preferentially located in peripheral tissue (34, 35). Thus, we could speculate that histamine by activating DC may participate, at the inflammatory site, in memory T cell restimulation (36). This is reinforced by the observation that histamine-treated DC produce chemokines involved in memory T cell recruitment (such as RANTES, MCP-1, and MIP-1α) (29). Taken together, our in vitro data suggest that histamine by acting on immature DC may favor in situ-specific T cell stimulation.

In allergic patients, allergen-specific T cells and mast cells produce IL-4, a Th2 lymphokine that plays a central role in IgE synthesis and in the induction and maintenance of allergic responses (37, 38). MCP-1 stimulates IL-4 production by T cells, attracts memory effector T cells, and is required for the development of a Th2 response (39, 40). In contrast, IL-12 induces IFN-γ production and prevents the development of a Th2 response. The findings that histamine induces MCP-1 production and does not affect IL-12 production suggest that histamine may contribute to maintain a Th2 polarization in allergic diseases. However, since histamine also induces IL-18 and IFN-γ production by PBMC (18), whether histamine may modulate Th1/Th2 orientation remains to be evaluated. Nevertheless, the observation that histamine activates DC, added to data from others showing that histamine acts on B and T cells to increase Ag receptor-mediated responses (41), suggest a direct role for histamine in the development of a specific immune response. Thus, histamine may participate in the immune and inflammatory responses associated with allergic disorders.

We report that histamine activates immature DC with an effect significant at concentrations ranging from $10^{-7}$ to $10^{-4}$ M. Although it remains difficult to determine precisely the concentration of histamine in the target organ, concentrations from $10^{-8}$ to $10^{-4}$ M were reported to be comparable to those measured in tissues after mast cell degranulation (16, 19, 42). For example, after allergen challenge, a concentration of $10^{-7}$ M was detected in nasal lavages of patients with allergic rhinitis, and this concentration was diluted 10- to 100-fold by the lavage procedure (43). Moreover, upon contact with the sensitizing allergen, mast cells from allergic patients concomitantly release different mediators (such as PGE2, histamine, and preformed TNF-α) that may act in concert to stimulate DC. TNF-α synergizes with PGE2 in inducing DC maturation in vitro (44). We report here an additive effect between TNF-α and histamine in inducing DC activation. According to these different points, the in vitro effects of histamine on DC described here probably reflect its in vivo activity at the inflammatory sites. Previous studies reported that epidermal Langerhans cells (LC) are not ac-

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