

Histamine and prostaglandin E₂ up-regulate the production of Th2-attracting chemokines (CCL17 and CCL22) and down-regulate IFN- γ -induced CXCL10 production by immature human dendritic cells

Anne McIlroy,¹ Gersende Caron,²
Simon Blanchard,^{1,2} Isabelle
Frémaux,² Dorothée Duluc,²
Yves Delneste,² Alain Chevailler¹
and Pascale Jeannin^{1,2}

¹Immunology and Allergy Laboratory, and

²INSERM U564, Equipe Avenir, University
Hospital of Angers, University of Angers,
Angers, France

Summary

Effector memory T helper 2 (Th2) cells that accumulate in target organs (i.e. skin or bronchial mucosa) have a central role in the pathogenesis of allergic disorders. To date, the factors that selectively trigger local production of Th2-attracting chemokines remain poorly understood. In mucosa, at the sites of allergen entry, immature dendritic cells (DC) are in close contact with mast cells. Histamine and prostaglandin E₂ (PGE₂) are two mediators released by allergen-activated mast cells that favour the polarization of maturing DC into Th2-polarizing cells. We analysed here the effects of histamine and PGE₂ on the prototypic, Th2-(CCL17, CCL22) versus Th1-(CXCL10) chemokine production by human DC. We report that histamine and PGE₂ dose-dependently up-regulate CCL17 and CCL22 by monocyte-derived immature DC. These effects were potentiated by tumour necrosis factor- α , still observed in the presence of the Th1-cytokine interferon- γ (IFN- γ) and abolished by the immunomodulatory cytokine interleukin-10. In addition, histamine and PGE₂ down-regulated IFN- γ -induced CXCL10 production by monocyte-derived DC. These properties of histamine and PGE₂ were observed at the transcriptional level and were mediated mainly through H₂ receptors for histamine and through EP2 and EP4 receptors for PGE₂. Finally, histamine and PGE₂ also up-regulated CCL17 and CCL22 and decreased IFN- γ -induced CXCL10 production by purified human myeloid DC. In conclusion, these data show that, in addition to polarizing DC into mature cells that promote naïve T-cell differentiation into Th2 cells, histamine and PGE₂ may act on immature DC to trigger local Th2 cell recruitment through a selective control of Th1/Th2-attracting chemokine production, thereby contributing to maintain a microenvironment favourable to persistent immunoglobulin E synthesis.

Keywords: allergy; chemokines; dendritic cells; histamine; PGE₂

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Correspondence: Dr P. Jeannin, Immunology
and Allergy Laboratory, University
Hospital of Angers, F-49933 Angers, France.
Email: pascale.jeannin@univ-angers.fr
Senior author: Pascale Jeannin

The first two authors contributed equally to
this work.

Introduction

Allergic disorders, such as allergic asthma and atopic dermatitis, are characterized by an immediate hypersensitivity reaction followed by a late inflammatory reaction (late-phase response).^{1,2} The immediate reaction, observed early upon contact with the sensitizing allergen, is consecutive to the IgE-dependent activation of mast cells that subsequently release stored mediators preformed in granules, such as histamine or tumour necrosis factor- α (TNF- α), or newly synthesized such as the arachidonic acid metabolite prostaglandin (PG)E₂.

Histamine and PGE₂ act on numerous cell types through G-protein-coupled receptors termed H₁–4 and E prostanoid receptors (EP), EP₁–4, respectively.^{3,4} Histamine is responsible for most of the clinical manifestations associated with the immediate reaction (i.e. vasodilatation, smooth muscle cell contraction and mucus hyper-secretion).³ In contrast to histamine, PGE₂ is also produced by antigen-presenting cells, epithelial cells and endothelial cells. Although commonly considered as a proinflammatory mediator involved in the pathogenesis of several diseases, PGE₂ seems to prevent allergen-induced bronchoconstriction.⁴

The late inflammatory reaction, that occurs several hours after contact with the allergen, is characterized by tissue infiltration by eosinophils and memory T lymphocytes (mainly CD4⁺ T helper 2 (Th2) cells) that contribute to tissue damage.^{1,2} This infiltration is tightly controlled by chemokines such as CCL17 (TARC or thymus and activation-regulated chemokine) and CCL22 (MDC or macrophage-derived chemokine) that selectively attract Th2 memory T cells.¹ Both these chemokines have a crucial role in the pathophysiology of allergic asthma^{5,6} and atopic dermatitis.^{7–9} Interferon- γ (IFN- γ) is also often present at sites of allergic inflammation and is thought to contribute to the disease. IFN- γ appears to be involved in the induction of CXCL10, a chemoattractant for Th1 cells that participates in airway hyper-reactivity and inflammation, in the activation of inflammatory cells such as eosinophils and in keratinocyte and bronchial epithelial cell apoptosis.^{10–13}

In addition to their roles in the immediate hypersensitivity reaction, histamine and PGE₂ modulate the production of pro- and anti-inflammatory cytokines involved in the pathophysiology of the late phase reaction. Histamine up-regulates interleukin (IL)-6, IL-8, monocyte chemoattractant protein-1 and IL-10 production by myeloid cells, IL-8 and IL-6 production by endothelial cells and IFN- γ synthesis by T cells.^{3,14–16} In contrast, PGE₂ exhibits some immunosuppressive properties: it decreases chemokine production by numerous cell types^{17,18} down-regulates proliferation and IFN- γ production by T cells¹⁹ and inhibits late-phase bronchoconstriction and eosinophil infiltration.⁴

Dendritic cells (DC) play a central role in the pathogenesis of allergic disorders through their ability to initiate and amplify Th2 immune responses.^{20,21} Some factors released early by activated mast cells at the time of allergen exposure, such as thymus stromal lymphopoietin (TSLP), histamine and PGE₂, promote the generation of mature DC (also called DC2) that produce low levels of IL-12 and favour the differentiation of naive T cells into Th2-effector cells. TSLP acts on human monocyte-derived DC to induce the generation of mature DC2.²² We and others previously reported that histamine is a DC2-polarizing but not a DC-maturation factor. Histamine, by itself, transiently activates human immature DC.^{16,23} However, it decreases IL-12p70 production by lipopolysaccharide (LPS)- or polyI:C-maturing DC and favours DC2 generation.^{23,24} Among the prostaglandins, PGE₂ is the most potent modulator of DC functions.²⁵ PGE₂ decreases LPS-induced IL-12p70 production by immature DC, favours DC2 generation^{21,26–28} and also selectively induces IL-12p40 production (an IL-12 antagonist) by TNF- α -maturing DC.^{29,30} Contradictory data were obtained when PGE₂ was added to monocyte-differentiating cells³¹ or to maturing DC in complemented serum-free

medium.²⁵ Finally, although one study reported that PGE₂ up-regulated the expression of the maturation marker CD83 on DC cultured in serum-free medium²⁵ it is not a DC maturation by itself. PGE₂ does not switch immature DC into CD83-positive DC with migratory properties^{28,29,32,33} but acts together with different DC maturation factors to switch on DC migratory properties.^{32,34}

Immature DC present in tissues of allergic individuals may also contribute to the maintenance of a Th2-polarized microenvironment required for local IgE synthesis by favouring Th2 memory cell recruitment and activation.³⁵ DC in mucosa of allergic individuals may take up and present allergens to memory T cells more efficiently (through an up-regulation of immunoglobulin E (IgE) receptors).³⁶ Moreover, immature DC are an important source of Th1- and Th2-attracting chemokines (such as CCL17, CCL22 and CXCL10).^{27,37,38} To identify factors that may act on immature DC to induce Th2 chemokine production is crucial to prevent the allergic immune cascade.

The aim of this study was to evaluate whether histamine and PGE₂, two mast cell mediators produced upon contact with allergen may affect the production of Th1-/Th2-attracting chemokines by human DC. We show that histamine and PGE₂ up-regulate Th2-attracting chemokine production by immature human DC and prevent the IFN- γ -induced CXCL10 production.

Materials and methods

Generation of monocyte-derived dendritic cells (mo-DC)

Peripheral blood mononuclear cells (PBMC) were isolated from non-atopic healthy blood donors by standard density gradient centrifugation on Ficoll-Paque (Amersham Biosciences, Uppsala, Sweden). Monocytes were purified from PBMC by positive selection using a magnetic cell separator (MACS technology; Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. Purity, assessed by fluorescence-activated cell sorting (FACS) analysis using a fluorescein isothiocyanate (FITC)-labelled anti-CD14 monoclonal antibody (mAb; BD Biosciences, San Diego, CA) was 98%. Dendritic cells were differentiated from monocytes by culture in complete medium (CM) consisting of RPMI-1640 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 50 U/ml penicillin, 50 μ g/ml streptomycin, 10 mM HEPES, and 0.1 mM non-essential amino acids (all from Biowhittaker, Verriers, Belgium) at 10⁶ cells/ml in six-well culture plates (Costar, Cambridge, MA) in the presence of 20 ng/ml IL-4 and 20 ng/ml granulocyte-macrophage colony-stimulating factor (kindly provided by Dr F. Brière, Schering Plough, France).

Purification of peripheral blood myeloid dendritic cells

After depletion of CD19⁺ B cells, myeloid DC were isolated from PBMC by positive selection with anti-BDCA1 Ab-coated microbeads (MACS technology); purity, assessed by FACS analysis using a FITC-labelled anti-BDCA1 mAb (Miltenyi Biotec), was more than 95%.

Cell stimulation

Dendritic cells at 0.7×10^6 cells/ml in 96-well flat-bottom plates (Costar) were exposed to different concentrations of histamine or PGE₂ (both from Sigma, St. Louis, MO) in the absence or presence of 2 or 25 ng/ml TNF- α , 20 ng/ml IL-10 or 20 ng/ml IFN- γ (all from R & D Systems, Abingdon, UK). In some experiments, DC were exposed to 1 μ M of the H1, H2 or H3/H4 receptor antagonists (mepyramine, cimetidine and thioperamide, respectively; all from Sigma) 1 hr before addition of 1 μ M histamine. In others experiments, immature DC were treated with 1 μ M of the following EP receptor agonists (all from Cayman, Ann-Arbor, MI): Sulprostone (selective for EP1/EP3, EP3 > EP1), Butaprost (EP2), 17-phenyl-w-trinor (17-Ph) (EP1/EP3, EP1 > EP3), and PGE₁ alcohol (PGE₁ alc) (EP3/EP4). To evaluate the signalling pathways involved in the effects of histamine and PGE₂, immature mo-DC were exposed to 100 nM calphostin C (a protein kinase C inhibitor) or H89 (a protein kinase A (PKA) inhibitor) (both from Calbiochem, San Diego, CA) just before addition of 1 μ M histamine or PGE₂.

Chemokine quantification

CCL17, CCL22 and CXCL10 levels were determined in the culture supernatants by enzyme-linked immunosorbent assay (ELISA; R & D Systems; sensitivity = 10 pg/ml); results are expressed in ng/ml as mean $\pm$ SD. Using histamine receptor antagonists, results are expressed in percent of inhibition (CCL17 and CCL22) or increase (CXCL10) as follows: $[1 - (C - A)/(B - A)] \times 100$ or $(A - C)/(A - B) \times 100$, respectively, where A is the concentration of chemokines in the absence of stimulus, B is the concentration of chemokines in the presence of 1 μ M histamine alone, and C is the concentration of chemokines in the presence of histamine plus the histamine receptor antagonist tested. In experiments using EP receptor agonists, results are expressed in percent of increase (CCL17 and CCL22) or inhibition (CXCL10) as follows: $(C - A)/(B - A) \times 100$ or $(A - C)/(A - B) \times 100$, respectively, where A is the concentration of chemokine in the absence of stimulus, B is the concentration of chemokines in the presence of 1 μ M PGE₂, and C is the concentration of chemokines in the presence of the EP receptor agonist tested. Using protein kinase inhibitors, results are expressed in percent of inhibition (CCL22) or increase

(CXCL10) as follows: $[1 - (C - A)/(B - A)] \times 100$ or $(A - C)/(A - B) \times 100$, respectively, where A is the concentration of chemokines in the absence of stimulus, B is the concentration of chemokines in the presence of histamine or PGE₂, and C is the concentration of chemokines in the presence of histamine or PGE₂ plus the protein kinase inhibitor tested.

Analysis of chemokine mRNA expression by reverse transcription-polymerase chain reaction (RT-PCR)

The expression of the mRNA encoding CCL17, CCL22 and CXCL10 was determined by RT-PCR. The following primers were used: CCL17: 5'-CAC TGA AGA TGC TGG CCC TGG TCA C-3' and 5'-AGA CCT CTC AAG GCT TTG CAG G-3', CCL22: 5'-ACT GCA CTC CTG GTT GTC CTC G-3' and 5'-GCC TCG GGC AGG AGT CTG AGG TCC AGT AG-3', CXCL10: 5'-TGT ACG CTG TAC CTG CAT CAG C-3' and 5'-CAT TGT AGC AAT GAT CTC AAC ACG TGG-3'. Briefly, total RNA was extracted using trizol reagent (Invitrogen, Carlsbad, CA), and single stranded cDNA was synthesized using 2 μ g total RNA. PCR reactions were performed with cDNA corresponding to 50 ng total RNA. The PCR reaction was as follows: 94° for 5 min; 30 cycles at 94° for 30 s, 60° for 30 s, and 72° for 1 min; followed by a final extension at 72° for 5 min. RNA integrity and cDNA synthesis was verified by amplifying glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA. The amplified fragments were size-separated on a 1% agarose gel and visualized by ethidium bromide. The relative levels of chemokine mRNA expression were determined by densitometry analysis of gel band intensity using the histogram function of Adobe Photoshop software (version 7.0). For each experimental condition, band intensity corresponding to chemokine mRNA expression was normalized to the signal of GAPDH mRNA expression. Modulation of CCL17, CCL22 and CXCL10 mRNA expression was calculated as a ratio of band intensity in histamine- or PGE₂-stimulated cells compared with unstimulated cells in the absence or presence of IFN- γ .

Statistical analysis

Statistical analysis was performed using the Wilcoxon paired test. All reported *P*-values are two-tailed.

Results**Histamine and PGE₂ trigger CCL17 and CCL22 production by human mo-DC**

We evaluated whether histamine and PGE₂ may affect the production of the Th2-attracting chemokines CCL17 and CCL22 by immature mo-DC. As previously reported^{27,37}

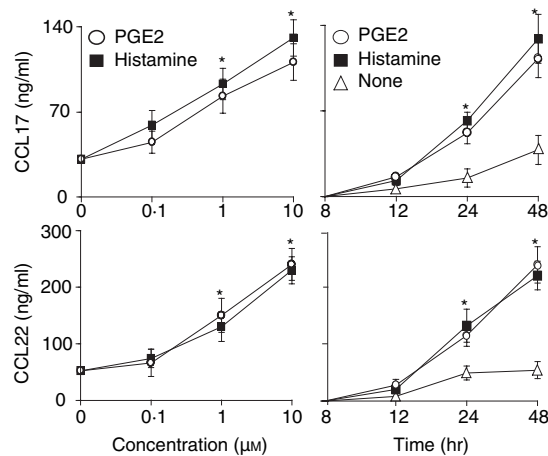


Figure 1. Histamine and PGE₂ up-regulate CCL17 and CCL22 production by immature human monocyte-derived DC. Monocyte-derived DC were not or were incubated with 0.1–10 μ M histamine (■) or PGE₂ (○) (left panels) or with 10 μ M histamine (■) or PGE₂ (○) (right panels). CCL17 (upper panels) and CCL22 (lower panels) were quantified by ELISA in the 48 hr supernatants (left panels) or at the indicated time-points (right panels). Results are expressed as mean $\pm$ SD of five separate experiments. * P < 0.05.

immature human mo-DC constitutively produce CCL17 and CCL22 (Fig. 1) and express the mRNA encoding both chemokines (Fig. 2a). Histamine and PGE₂ up-regulate CCL17 and CCL22 production by immature mo-DC (Fig. 1). This increase in CCL17 and CCL22 production is dose- (significant at 1 μ M and maximum at 10 μ M, the highest concentration tested) (Fig. 1, left panel) and time-dependent (significant at 24 hr and maximal at 48 hr, the latest time point analysed; Fig. 1, right panel). The up-regulation of CCL17 and CCL22 production is associated with an increase of the corresponding mRNA expression, as assessed by RT-PCR (Fig. 2a) and densitometry analysis (Fig. 2b).

Histamine and PGE₂ synergize with TNF- α in up-regulating CCL17 and CCL22

TNF- α , a potent DC-stimulatory factor, is preformed in mast cells and released upon IgE-dependent activation. We therefore analysed whether TNF- α may affect histamine- and PGE₂-induced up-regulation of CCL17 and CCL22 production. TNF- α induces CCL17³⁸ and CCL22²⁷ production by immature mo-DC, with an effect detectable at 2 ng/ml (Fig. 3a, b). The maximal production of CCL17 and CCL22 was induced using 25 ng/ml TNF- α (429 ± 52 and 861 ± 102 ng/ml, respectively; mean $\pm$ SD, $n = 4$) (Fig. 3a, b). Histamine and PGE₂ synergize with a suboptimal concentration of TNF- α (2 ng/ml) in up-regulating CCL17 (Fig. 3a) and CCL22 production (Fig. 3b), with a significant effect at 0.01 μ M and 0.1 μ M, respectively.

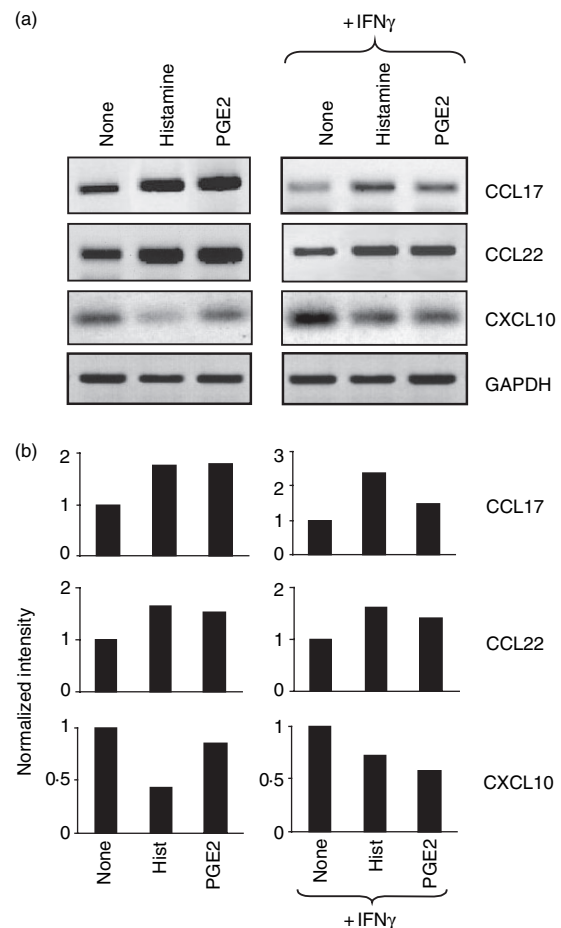


Figure 2. Histamine and PGE₂ modulate CCL17, CCL22 and CXCL10 mRNA expression. (a) Monocyte-derived DC were either untreated or exposed to 10 μ M histamine or PGE₂ in the absence (left panels) or presence of 20 ng/ml IFN- γ (right panels). After 8 hr incubation, CCL17, CCL22 and CXCL10 mRNA expression was evaluated by RT-PCR. RNA integrity and cDNA synthesis was verified by amplifying GAPDH cDNA. Results are representative of one out of five experiments. (b) Densitometry analysis. CCL17, CCL22 and CXCL10 mRNA expression after stimulation with histamine or PGE₂ is expressed as a ratio of relative band intensity in comparison with mRNA expression without stimulation (left panels) or stimulated with IFN- γ alone (right panels).

In parallel, and whatever the concentration tested (from 1 to 50 ng/ml), IL-1 β does not induce CCL17 and CCL22 production by mo-DC nor does it modulate the effect of PGE₂ and histamine (data not shown).

Finally, we evaluated whether IL-10, a late immunoregulatory cytokine present locally in chronic inflammation, may affect the up-regulation of CCL17 and CCL22 production by histamine and PGE₂. As previously observed on human monocytes³⁹ IL-10 decreases CCL22 production by human immature mo-DC (Fig. 3c). Interestingly, IL-10 also decreases the constitutive production of CCL17 by mo-DC (Fig. 3c) and prevents the up-regulation of

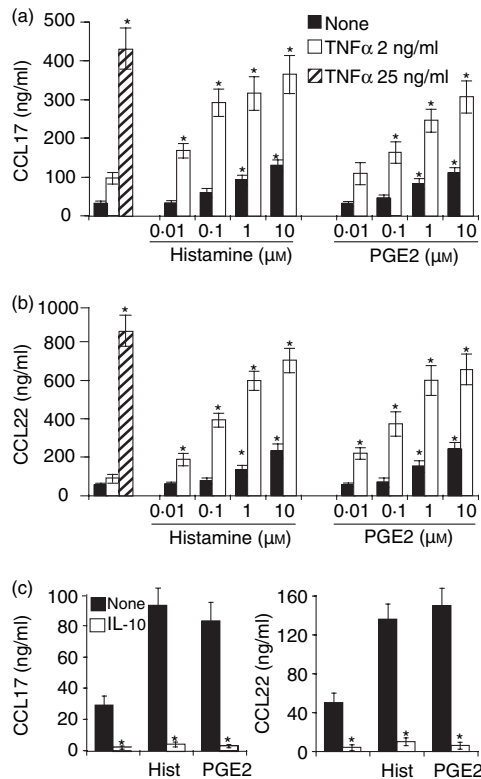


Figure 3. The effects of histamine and PGE₂ were potentiated by TNF- α and prevented by IL-10. (a & b) Monocyte-derived DC were not or were incubated with 0.01–10 μ M histamine or PGE₂ in the absence (■) or presence (□) of 2 ng/ml TNF- α . (c) DC were stimulated with 1 μ M histamine or PGE₂ in the absence (■) or presence (□) of 25 ng/ml IL-10. (a–c) CCL17 (a and c left panel) and CCL22 (b and c right panel) were quantified by ELISA in the 48 hr supernatants. As a control, the production of CCL17 (a) and CCL22 (b) in the presence of 25 ng/ml TNF- α is indicated (hatched histogram). Results are expressed as mean $\pm$ SD of four experiments. * P < 0.05.

CCL17 and CCL22 production induced by histamine and PGE₂ (Fig. 3c). As expected,^{26,40} histamine and PGE₂, used in the absence of maturation factors, do not induce

IL-10 production by human immature DC (data not shown) and the addition of a neutralizing anti-IL10 antibody does not affect the up-regulation of CCL17 and CCL22 production induced by histamine and PGE₂ (data not shown).

Histamine and PGE₂ modulate CCL17 and CCL22 production by human peripheral blood myeloid DC

We extended the observations obtained with mo-DC to freshly purified peripheral blood myeloid DC. These cells constitutively produce CCL17 and CCL22³⁷ (Fig. 4a, b) and histamine and PGE₂ up-regulate their production (Fig. 4a, b). Histamine and PGE₂ also synergize with 2 ng/ml TNF- α in up-regulating CCL17 (Fig. 4a) and CCL22 production (Fig. 4b) by purified blood myeloid DC. As for mo-DC, IL-10 decreases the constitutive production of CCL17 and CCL22 by peripheral blood myeloid DC and their up-regulation by histamine and PGE₂ (data not shown).

Histamine and PGE₂ prevent IFN- γ -induced CXCL10 production

IFN- γ , produced by activated T and natural killer cells, is a potent Th1-polarizing factor²¹ that also favours Th1 cell recruitment by inducing CXCL10 production by different cell types.⁴¹ IFN- γ is expressed together with IL-4 at the inflammatory sites in allergic diseases.¹² We show here that IFN- γ induces CXCL10 production by immature mo-DC (Fig. 5a) and decreases the constitutive production of CCL17 and CCL22⁴¹ (Fig. 5b, c). We then analysed Th1 (CXCL10)- versus Th2 (CCL17 and CCL22)-attracting chemokine production by human DC exposed to histamine and PGE₂ in the presence of IFN- γ . Surprisingly, histamine and PGE₂ prevent IFN- γ -induced CXCL10 production by human immature mo-DC (Fig. 5a) and by peripheral blood myeloid DC (Fig. 4c). This effect is dose-dependent, significant at 0.1 μ M

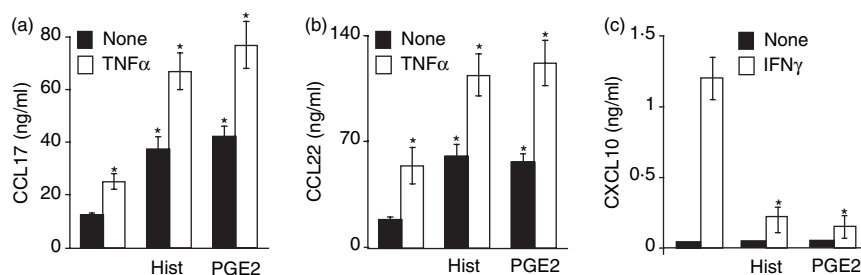


Figure 4. Histamine and PGE₂ modulate CCL17, CCL22 and CXCL10 production by peripheral blood myeloid DC. (a & b) Freshly isolated peripheral blood myeloid DC were not or were stimulated with 1 μ M histamine or PGE₂ in the absence (■) or presence (□) of 2 ng/ml TNF- α . CCL17 (a) and CCL22 (b) were quantified by ELISA in the 48 hr supernatants. (c) Freshly purified peripheral blood myeloid DC were not or were stimulated with 1 μ M histamine or PGE₂ in the absence (■) or presence (□) of 20 ng/ml IFN- γ . CXCL10 was quantified by ELISA in the 48 hr supernatants. Results are expressed as mean $\pm$ SD of four experiments. * P < 0.05.

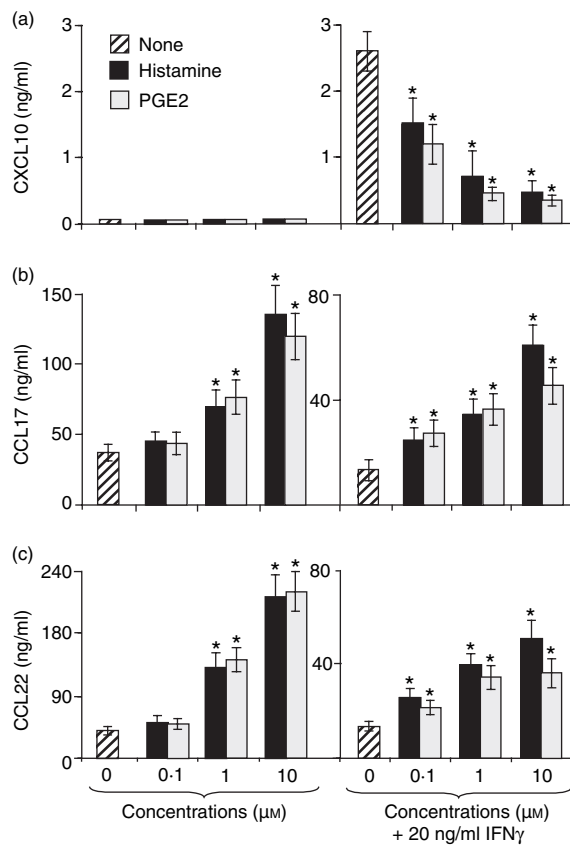


Figure 5. Histamine and PGE₂ decreased IFN γ -induced CXCL10 production. (a–c) Monocyte-derived DC were not (hatched histograms) or were incubated with 0.1–10 μ M histamine (black histograms) or PGE₂ (grey histograms) in the absence or presence of 20 ng/ml IFN- γ . CXCL10 (a), CCL17 (b) and CCL22 (c) were quantified by ELISA in the 48 hr supernatants. Results are expressed as mean $\pm$ SD of five experiments. * P < 0.05.

(Fig. 5a) and observed at the transcriptional level (Fig. 2a, b). Moreover, even in the presence of IFN- γ , histamine and PGE₂ retain the ability to up-regulate CCL17 and CCL22 production by immature DC (Fig. 5b, c).

The H2 receptor is involved in the regulation of CCL17, CCL22 and CXCL10 by histamine

DC express the four histamine receptors (H1–4).^{3,24} We tested which of these receptors is involved in the regulation of CCL17, CCL22 and CXCL10 expression in mo-DC using the specific receptor antagonists mepyramine (H1), cimetidine (H2) or thioperamide (H3 > H4). Results show that the H2 receptor antagonist cimetidine dramatically reduces the effect of histamine on CCL17, CCL22 and CXCL10 production (Fig. 6a) by mo-DC. In contrast, we failed in detecting an effect of the H1 (mepyramine) and H3 and H4 receptor antagonists (thioperamide) (Fig. 6a). In parallel, we failed in detecting an effect of clobenpropit, an H3R antagonist and H4R agonist (data

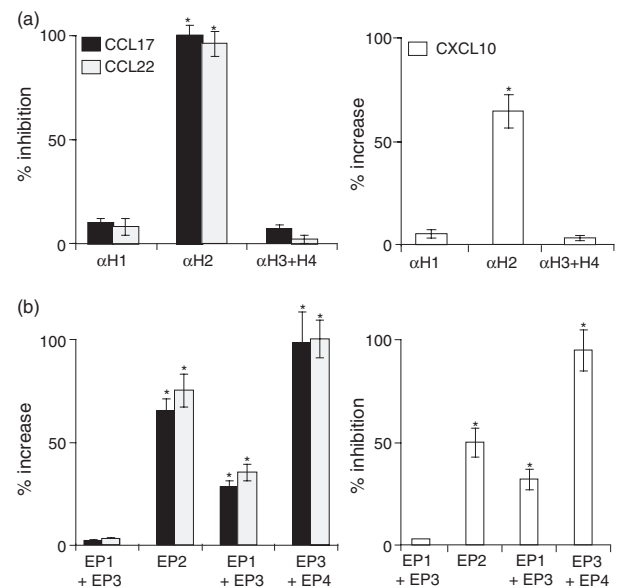


Figure 6. Histamine and PGE₂ receptors involved in the modulation of chemokine production. (a) Monocyte-derived DC were incubated with 1 μ M histamine in the absence or presence of 1 μ M of H1, H2 or H3 receptor antagonists (mepyramine, cimetidine or thioperamide, respectively). (b) Monocyte-derived DC were incubated with 1 μ M of the EP receptor agonists: Sulprostone (EP1 + EP3), Butaprost (EP2), 17-phenyl-w-trinor (EP1 + EP3), and PGE₁ alcohol (EP3/EP4). CCL17 (black bars), CCL22 (grey) and CXCL10 (white) were quantified by ELISA in the 48 hr supernatants. Results are expressed as a percentage of inhibition of CCL17 or CCL22 or as a percentage of increase of CXCL10 production, as described in Materials and methods (mean $\pm$ SD of three separate experiments). * P < 0.05.

not shown). Together, these data suggest that histamine may modulate CCL17, CCL22 and CXCL10 production by acting mainly through the H2 receptor.

EP2/EP4 receptors are involved in the regulation of CCL17, CCL22 and CXCL10 production by PGE₂

Human monocyte-derived DC express the mRNA encoding EP2, EP3 and EP4.^{28,33} We analysed which of the PGE₂ receptors was involved in the effect of PGE₂ on chemokine production by using the EP receptor agonists Sulprostone (EP1 + EP3), Butaprost (EP2), 17-Ph (EP1 + EP3) and PGE₁ alcohol (EP3 + EP4) at concentrations ranging from 0.1 to 10 μ M. Results showed that EP2 and EP4, and to a lower extent EP3 receptors, are involved in the modulation of CCL17, CCL22 and CXCL10 induced by PGE₂ (Fig. 6b). More precisely, the significant effect of PGE₁ alcohol and Butaprost suggest the involvement of EP2 and EP4. Surprisingly, the EP1 + EP3 agonist Sulprostone had no detectable effect, while, in contrast, 17-Ph (another EP1 + EP3 agonist), modulated the production of CCL17, CCL22 and CXCL10 by mo-DC. This could be explained by the

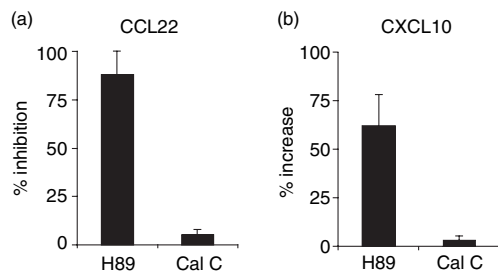


Figure 7. Involvement of the cAMP/PKA pathway in the modulation of chemokine production by histamine and PGE₂. (a & b) Monocyte-derived DC were incubated with 100 nM of a PKA (H89) or a PKC (calphostin C) inhibitor, and stimulated with histamine and PGE₂ in the absence (a) or presence (b) of 20 ng/ml IFN- γ . CCL22 (a) and CXCL10 (b) were quantified by ELISA in the 24 hr supernatants. Results are expressed as a percentage of inhibition of CCL22 or as a percentage of increase of CXCL10 production, as described in Materials and methods (mean $\pm$ SD of three separate experiments).

ability of 17-Ph to activate the EP4 receptor when used at high concentrations (1 μ M).⁴² In agreement with this hypothesis, 17-Ph had no effect on chemokine production when used at lower concentrations (0.5 μ M) (data not shown). The inability of Sulprostone to mimic the PGE₂-dependent modulation of chemokine production allowed excluding the involvement of EP1 and EP3. Together, these data show that PGE₂ modulates Th1-versus Th2-attracting chemokine synthesis by immature human DC by acting mainly through EP2 and EP4 receptors.

The effects of histamine and PGE₂ on CCL17, CCL22 and CXCL10 production by mo-DC are mediated through cAMP/PKA activation

The induction of CCL22 and the inhibition of CXCL10 production are associated to an elevation of intracellular cAMP levels and the activation of PKA.^{43,44} Binding of histamine on H2 receptors, and of PGE₂ on EP2 and EP4 receptors, results in the activation of the cAMP/PKA pathway.^{45,46} We therefore evaluated the implication of the cAMP/PKA pathway in the effects of histamine and PGE₂ on chemokine production by mo-DC. In contrast to the PKC inhibitor calphostin C, the PKA inhibitor H89 inhibits the effects of histamine and PGE₂ on the up-regulation of CCL22 (Fig. 7a) and CCL17 (data not shown) production, and on the down-regulation of IFN- γ -induced CXCL10 production (Fig. 7b). These data suggest the involvement of the cAMP/PKA pathway in the effects of histamine and PGE₂ on human immature mo-DC.

Discussion

The late-phase response associated with allergic disorders is characterized by an infiltrate of Th2 effector T cells and

eosinophils involved in the inflammatory process and tissue damage. To identify factors that trigger locally the production of Th2-attracting chemokines is therefore of importance. We report here for the first time that histamine and PGE₂ up-regulate Th2- and down-regulate Th1-attracting chemokine production by immature DC, suggesting that these mediators, released early during the allergic reaction, may participate in the selective recruitment of Th2 lymphocytes and eosinophils.

Several observations suggest an active role for the Th2-attracting chemokines CCL17 and CCL22 in the physiopathology of allergic disorders. CCL17 and CCL22 participate in airway hyper-reactivity and lung inflammation.^{1,5} Elevated levels of CCL17 and CCL22 are detected in bronchoalveolar lavage and in the sera of allergic asthmatic patients.^{6,47} In addition, serum CCL22 and CCL17 levels seem to correlate with the severity of atopic dermatitis.^{8,9} DC are a major source of chemokines and especially of CCL17 and CCL22.^{27,37} Their induction has mainly been investigated in response to maturation factors such as LPS^{27,37} and to the Th2-cytokines IL-4 and IL-13.^{38,39} We report here that histamine and PGE₂ up-regulate CCL17 and CCL22 production by immature myeloid DC. In agreement with this observation, PGE₂ has been reported to up-regulate CCL22 production by murine spleen cells.⁴³ Moreover, previous data reporting that PGE₂ does not affect CCL22 production by DC were performed in the presence of LPS.²⁷ TSLP, a mediator also released by IgE-activated mast cells, has been also recently reported to up-regulate CCL17 and CCL22 production by DC.²² In contrast to TSLP²² histamine^{16,23} and PGE₂^{28,29,32,33} are not DC maturation factors by themselves. In addition, we observed that TNF- α , a mediator preformed in mast cells granules and released concomitantly with histamine and PGE₂, synergizes with histamine and PGE₂ in up-regulating CCL17 and CCL22 production. Histamine has also been reported to synergize with TNF- α in inducing IL-8 production by human DC.¹⁶ Collectively, these data suggest that mediators released early by IgE-activated mast cells may co-operate to induce the synthesis of CCL17 and CCL22 by immature DC. It is thus tempting to speculate that, *in vivo*, these mediators may also act on other CCL17- or CCL22-producing cells such as endothelial cells (which can be also stimulated by histamine,¹⁴) or keratinocytes.^{7,9}

Although monocyte-derived immature DC and peripheral blood myeloid DC exhibited an up-regulation of CCL17 and CCL22 production in response to histamine and PGE₂, peripheral blood myeloid DC produced lower levels of these chemokines than monocyte-derived DC. This observation is in agreement with previous data showing that the ability of DC to produce CCL17 and CCL22 decreases with the maturation status.^{48,49} While monocyte-derived immature DC, used in this study, is a homogeneous CD86 negative population of immature DC,

peripheral blood myeloid DC is a heterogeneous population with differences in the levels of maturation or activation.⁵⁰

IFN- γ is often present in allergic inflammatory sites and seems to participate in the disease.^{11–13} In contrast to histamine and PGE₂, IFN- γ is a potent DC1-polarizing factor²¹ that favours Th1 recruitment through CXCL10 production. We report here that histamine and PGE₂ counteract IFN- γ -induced CXCL10 production, and similar results were observed for the other IFN- γ -inducible chemokines CXCL9 and CXCL11 (data not shown). In addition, IFN- γ decreases the constitutive production of Th2-attracting chemokines by immature DC. This effect is prevented by PGE₂ and histamine. We and others previously reported that histamine and PGE₂ partly prevented IFN- γ -induced maturing DC polarization into DC1.^{21,23} Together, these studies show that histamine and PGE₂ versus IFN- γ have dual effects on (i) the production of Th1- versus Th2-attracting chemokines by immature DC and (ii) the control of maturing DC polarization. Finally, although IFN- γ decreases Th2 chemokine production by myeloid cells, it up-regulates CCL17 and CCL22 mRNA expression and CCL22 production by keratinocytes.⁷ Taken together, these observations go some way to explain the persistence of a Th2 context in allergic disorders, even in the presence of IFN- γ .

PGE₂ and histamine have been reported to up-regulate *in vitro* LPS-induced IL-10 production by DC.^{3,26} IL-10 is an immunoregulatory cytokine that limits subsequent inflammatory responses. Interestingly, IL-10 prevents (i) the effect of histamine and PGE₂ on Th2 chemokine production and (ii) the constitutive production of Th2-chemokines by DC. To date, the role of IL-10 (and of IL-10-producing regulatory T cells) in the physiopathology of allergic disorders remains unclear.⁵¹ However, in agreement with our observation, a study reported that helminth infection decreases allergic disorders through increased IL-10 production, thereby suggesting an immunosuppressive effect of IL-10 in allergic disorders.⁵²

The activity of PGE₂ is mediated by four receptors (EP1–4).⁴ Activation of EP2, EP3 and EP4 modulates intracellular cAMP concentrations, while EP1 increases intracellular calcium. The use of EP agonists suggests that EP2 and EP4 are mainly involved in the effect of PGE₂ on chemokine production by human DC. This observation is in agreement with data showing the involvement of EP2/4 in the inhibition of IL-12 production by human DC²⁸ and in the modulation of human DC migratory capacity induced by PGE₂.^{32,33} Moreover, in contrast to murine DC, EP1 mRNA has not been detected in human DC.^{28,33}

Immature human DC express functional H1 and H2 receptors.^{3,23} Recently, the expression of H3R and H4R at the protein level has also been reported on human immature DC.²⁴ Using specific antagonists for H1, H2 and

H3/H4, we show that histamine modulates chemokine production by acting mainly through H2 receptors. In agreement with this observation, the H2 receptor has been previously reported in the modulation of IL-12 and IL-10 production by DC.^{23,40} Interestingly, we failed to detect an effect of histamine on Langerhans cells (derived from monocytes cultured with transforming growth factor- β), a result in agreement with recent data reporting that H1 and H2 receptors are not expressed on Langerhans cells.⁵³ The H1 receptor controls most of the effects of histamine in the immediate hypersensitivity reaction and H1 antagonists are currently used in the treatment of allergic disorders. Our observations, together with previous data^{16,23,40} point out the potential interest of anti-H2 molecules in allergic disorders.

The cAMP/PKA pathway, associated with H2⁴⁵, EP2 and EP4⁴⁶ receptors, is involved in the effects of histamine and PGE₂ on the up-regulation of CCL17/22 production and the down-regulation of IFN- γ -induced CXCL10 production. This result is in agreement with a previous study showing that neuropeptides that signal through the cAMP/PKA pathway have a similar effect on CCL22 and CXCL10 production by murine DC.⁴⁴

In the presence of additional DC-maturation factors, histamine and PGE₂ polarize DC into mature DC2 and enhance their migratory properties.^{21,23,26–28,32,34,54} Interestingly, we report here that PGE₂ and histamine act on immature DC to modulate Th2-attracting chemokine production. Together, these data suggest that, upon contact with allergens, IgE-activated mast cells release two mediators that may act in concert to contribute to: (i) the local recruitment of Th2 memory T cells; and (ii) the perpetuation of allergic diseases by favouring naïve T cell polarization into Th2 effector cells. The recruitment of Th2 lymphocytes may contribute to maintain a microenvironment compatible with IgE synthesis and may therefore constitute an amplification loop in allergic disorders.³⁵ These data also confer to immature DC, present locally at the site of allergic inflammation, a potent role in the physiopathology of allergic diseases through a tight control of Th1/Th2 chemokine production.

The reason for the development of a Th2-polarized response upon contact with allergen is still a debate.⁵⁵ Our study contributes to identify histamine and PGE₂ as two factors present in allergic disorders that may selectively control effector memory Th2 cell recruitment. This observation, added to the role of histamine, PGE₂ and TSLP in DC2 generation, suggests that these three mediators, released by IgE-activated mast cells, may contribute to the development and perpetuation of allergic diseases by favouring Th2-effector cell generation and recruitment. Interestingly, PGE₂ is also produced by numerous myeloid cells and, in allergic patients, these cells produce high levels of PGE₂.^{56,57} Histamine can also be synthesized by

myeloid cells.⁵⁸ Finally, TSLP is also produced by epithelial cells.²² Whether a deregulation in the production of these mediators (in the absence of an IgE- or IL-4-established environment) could be the initial factor at the origin of IgE production and allergen-sensitization should be evaluated.

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