Histamine Induces the Generation of Monocyte-Derived Dendritic Cells that Express CD14 but not CD1a

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The local cytokine environment and the presence of stimulatory signals determine whether monocytes acquire dendritic cell or macrophage characteristics and functions. In this study, we examined the effect of histamine, a prototypic mediator of allergic inflammation, on the granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-4-driven differentiation of monocytes into monocyte-derived dendritic cells (MoDC), which typically showed CD1a+/CD14− phenotype. Monocytes from healthy adult donors were cultured with GM-CSF and IL-4 in the presence or absence of histamine, and the phenotypes and function of these cells were analyzed. Histamine induced the generation of CD1a−CD14+ cells, which exhibited cytological and phenotypical characteristics of dendritic cells (DC), showed enhanced phagocytic activity and cytokine-producing capacity, but demonstrated weak allo-stimulatory capacity compared with CD1a+CD14− MoDC. The inhibitory effects of histamine on CD1a+CD14− MoDC differentiation were antagonized by cimetidine, an H2 receptor antagonist, but not by H1 and H3 receptor blockers, and were mimicked by an H2 receptor agonist. Culture supernatant of histamine-treated monocytes also inhibited CD1a+CD14− MoDC differentiation, which was restored by the removal of IL-10. These results suggest that histamine-driven CD1a−CD14+ DC amplify their antigen-independent inflammatory reaction and may contribute to the exacerbation of allergic diseases.

Key words: cellular differentiation/dendritic cells/macrophages/monocytes

J Invest Dermatol 125:753–760, 2005

A lack of appropriate exogenous stimuli induces monocytes to undergo apoptosis, whereas under the influence of distinct signals such as proinflammatory cytokines, these cells differentiate into macrophages or dendritic cells (DC) (Katoh et al, 2000; Lanzavecchia and Zallusto, 2001). Macrophages can perform phagocytosis and produce proinflammatory or anti-inflammatory cytokines that regulate inflammatory reactions (Novak et al, 2001). In contrast, DC display the capacity to initiate primary and secondary T cell responses and the most efficient professional antigen-presenting cells (Lanzavecchia and Zallusto, 2001). DC comprise heterogeneous populations with different morphologies and molecules expressed as a function of their maturation stage (Grassi et al, 1998). Dermal DC (DDC) migrated from skin explants are divided into three subsets by phenotypic criteria: CD1a−CD14−, CD1a+CD14−, and CD1a−CD14+ (Nestle et al, 1993; Larregina et al, 2001). CD1a−CD14+ subset of DDC show decreased capacity to stimulate resting T cells when compared with the other two subsets.

These fundamental functional divergences may be of importance for the putative regulatory role of monocyte-derived DC (MoDC) or macrophages in tissues, and consequently for the outcome of local inflammatory responses (Novak et al, 1999). Thus far, several factors such as corticosteroids, interleukin (IL)-10, and signaling evoked by the engagement of FcγR1 are known to influence monocyte differentiation into DC (Buelens et al, 1997; Novak et al, 2001; Woltman et al, 2002).

Histamine is an important mediator involved in various physiological and pathological conditions including immediate hypersensitivity reaction and inflammation (Leurs et al, 1995). Recently, new immunomodulatory functions of histamine were discovered (Jutel et al, 2002) (i) histamine augments antigen-receptor-mediated T and B cell proliferation (Banu and Watanabe, 1999), (ii) enhances Th1-type responses by triggering H1 receptor (H1R), whereas Th1- and Th2-type responses are negatively regulated by H2R (Jutel et al, 2001), and (iii) alters the repertoire of cytokines and chemokines secreted by mature DC that induces the polarization of naive CD4+ T cells toward Th2 phenotypes (Caron et al, 2001; Mazzoni et al, 2001). Therefore, histamine may contribute to other mechanisms involved in the outcome of DC differentiation.

In this report, we examined the effect of histamine on the granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4-driven differentiation of monocytes into MoDC. In monocytes from non-atopic healthy individuals...
we found that (1) histamine prevents the generation of CD1a⁺CD14⁻ DC and induces CD1a⁻CD14⁺ DC, which share some phenotypic and functional features with previously described CD1a⁻CD14⁺ DDC (Nestle et al, 1993; Larregina et al, 2001); (2) the effect is mediated by H2R; and (3) it results from the histamine-induced secretion of IL-10.

Results

Histamine alters GM-CSF- and IL-4-induced differentiation of monocytes into DC At the start of culturing, the cells used to generate MoDC in a typical experiment consisted of 95% monocytes, as indicated by CD14 expression and a typical light-scattering profile. They were induced to differentiate into MoDC with IL-4 and GM-CSF over a period of 6 d. Under control conditions with GM-CSF and IL-4, the cells rapidly became non-adhered to culture plate and formed large clusters. Histamine-treated cells were also non-adhered and formed clusters. Both of them were irregularly shaped and showed extended veils (Fig 1A). The dendritic morphology with small dendrites was also observed in both of them. In contrast, macrophage colony-stimulating factor (M-CSF)-induced macrophages were adherent, roundly shaped cells containing numerous vacuoles.

Despite homogeneous cytological appearance, the expression of CD1a and CD14 by DC significantly changed when histamine was added at the start of culturing (Fig 1B). MoDC generated with GM-CSF and IL-4 expressed high levels of CD1a and low levels of CD14. In contrast, those generated in the presence of histamine expressed significantly lower levels of CD1a and higher levels of CD14. This inhibitory effect was significant at 100 nM, and highest at 100 µM, the highest concentration tested (Fig 1C). Histamine at 10 µM was used in the following experiment because it is within the concentration range of histamine detected in nasal secretions and bronchoalveolar lavages from allergic patients after allergen challenge (Wenzel et al, 1988; Liu et al, 1990; Baroody et al, 1999). The addition of histamine after 48 h, when most DC already expressed CD1a, still resulted in a significant decrease in CD1a surface expression. This downregulatory effect, however, was not observed when histamine was added on day 3 or later (data not shown). Histamine significantly inhibited the generation of CD1a⁺ mature MoDC when 100 ng per mL of lipopolysaccharide (LPS) was added on day 6 in addition to IL-4 and GM-CSF (Figs 1D and 2B).

We further examined whether histamine has any effect on the DC phenotype at day 6 (Fig 2A). Histamine induced a significant increase in MHC class II molecules, mannose receptor, CD11b, and CD68. On the other hand, the expressions of CD40 and CD80 were downregulated. No significant change was observed in CD86, CD83, CD11b, M-CSF receptor, and FXIIIa expression. LPS-matured histamine-treated DC demonstrated similar phenotypes with control-matured MoDC including CD83, except CD1a and CD14 (Fig 2B).

The viability of the cells observed when comparing the histamine-treated and untreated group using trypan blue dye exclusion was not significantly different, suggesting that the inhibitory effect of histamine on DC differentiation was not because of the induction of cell death (data not shown).

Histamine induces the generation of monocyte-derived dendritic cells (MoDC), that express CD14 but not CD1a. (A) Monocytes were cultured for 6 d in the presence or absence of 10 µM histamine in addition to interleukin (IL)-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) or macrophage colony-stimulating factor. Cytospins of control MoDC (a, d), histamine-treated cells (b, e), and control macrophages (c, f) were labeled by the May–Gru¨ nwald–Giemsa stain (a–c) or by immunostaining for HLA-DR. × 400; bar, 2 µm. (B) Monocytes were cultured for 6 d in the presence or absence of 10 µM histamine in addition to IL-4 and GM-CSF and then FACS analyzed for CD1a and CD14. (C) Cells were cultured in IL-4 and GM-CSF with different concentrations of histamine for 6 d. (D) Cells cultured in the presence of IL-4 and GM-CSF and 10 µM histamine were matured for 36 h with 100 ng per mL lipopolysaccharide at day 6. The data are representative of six independent experiments with different donors. *p < 0.05.
IL-4 than those stimulated with control DC (8.0% ± 4.2% vs 4.8% ± 3.0%, p = 0.035, n = 5). The intracellular expression of interferon-γ (IFN-γ) was not significantly different between them (histamine treated vs nil: 9.6% ± 5.4% vs 8.0% ± 3.0%, p = 0.47, n = 5). DC generated in the presence of histamine still induced a population of Th1 cells as determined by a higher number of IFN-γ-producing cells when compared with IL-4 (Th2 cells).

Next, we examined their capacity to produce cytokines and chemokines by stimulating them with 100 ng per mL of
LPS after 6 d of culturing. As reported previously (Novak et al., 2001), GM-CSF-IL-4-induced MoDC produced less proinflammatory cytokines and chemokines after stimulation with LPS compared with M-CSF-induced macrophages (Fig 4 A, B). The ability to produce proinflammatory cytokines and chemokines including IL-10, however, was significantly elevated in histamine-treated CD1a⁺/CD14⁺ cells except IL-12, which was confirmed by single-cell analysis (Fig 4 C). Endogenous production of M-CSF was not detected in control MoDC and histamine-treated DC by using ELISA (data not shown).

To investigate further as to whether histamine influences their function, the phagocytic activity of these cells was evaluated using fluorescein isothiocyanate (FITC)-labeled latex beads. Although control MoDC displayed low phagocytic activity on day 6 as reported elsewhere (Novak et al., 2001), histamine-treated DC exhibited high phagocytic activity compared with untreated MoDC (relative fluores-
H2R agonist inhibited CD1a+ DC generation from monocytes.

Role of histamine-induced cytokine production by monocyte Histamine induces the release of several cytokines from monocytes. To examine whether the inhibitory effect of histamine on DC differentiation is mediated by endogenously synthesized cytokines, MoDC were generated with IL-4 and GM-CSF in the culture supernatant of monocytes cultured for 24 h in the presence or absence of histamine. As shown in Fig 7, the supernatant of monocyte treated with histamine showed a significant inhibitory effect on the generation of CD1a+ monocyte-derived DC. This inhibitory effect was blocked by the precipitation of interleukin (IL)-10 from the supernatant before its addition to cultures used to generate DC with IL-4 and granulocyte-macrophage colony-stimulating factor, but not by precipitation of IL-1β, IL-6, IL-12, and TNF-α. The suppressive effect of the culture supernatant of histamine-treated monocytes in allogeneic and autologous MLR (1 x 10^4 of stimulator cells) was impaired by IL-10 precipitation. Representative results of four independent experiments are shown. Results are expressed as the mean ± SD of triplicate cultures.

Figure 7
The culture supernatant of histamine-treated monocytes inhibited the generation of CD1a+ dendritic cells (DC). This inhibitory effect was blocked by the precipitation of interleukin (IL)-10 from the supernatant before its addition to cultures used to generate DC with IL-4 and granulocyte-macrophage colony-stimulating factor, but not by precipitation of IL-1β, IL-6, IL-12, and TNF-α. (A) Dot-plot profiles for CD1a and CD14, and (B) the percentage of CD1a+ cells. (C) The suppressive effect of the culture supernatant of histamine-treated monocytes in allogeneic and autologous MLR (1 x 10^4 of stimulator cells) was impaired by IL-10 precipitation. Representative results of four independent experiments are shown. Results are expressed as the mean ± SD of triplicate cultures.

H2R agonist inhibited CD1a+ DC generation from monocytes.
Pretreatment of the supernatant with anti-IL-1β, -IL-6, and -IL-12 mAb did not induce any change in the phenotype and function of DC. The removal of TNF-α from the supernatant reduced CD1α expression, suggesting its enhancing role in CD1α+ DC differentiation.

**Discussion**

DC comprise heterogeneous populations with different morphologies and molecules expressed as a function of their maturation stage. In the cutaneous tissue, Langerhans cell (LC) is a unique DC that strongly expresses CD1α. DDC also express varying levels of CD1α, even though CD1α expression on DDC was consistently weaker than on LC. In addition, LC lacks the monocyte–macrophage marker CD14 and representative DDC marker FXIIIa (Larregina et al, 2001), whereas several subpopulations of DDC can be distinguished according to their differential expression of CD1α, CD14, and FXIIIa. DDC migrated from skin explants have been subdivided into three groups: CD1α+CD14−, CD1α−CD14+, and CD1α−CD14+ (Nestle et al, 1993; Larregina et al, 2001). According to their reports, CD1α−CD14+ DDC showed increased phagocytic activity and weak T cell-stimulatory potential. CD14+CD1α− DDC described by Nestle et al (1993), however, are positive for intracellular FXIIIa, whereas those reported by Larregina et al (2001) are negative for FXIIIa. This study showed that histamine prevents the IL-4/GM-CSF-driven differentiation of monocytes into CD1α+CD14+ DC, but induced their differentiation into CD1α−CD14+ FXIIIa+ cells, which exhibited increased phagocytic activity and weak allo-stimulatory capacity. It is thus suggested that the cells generated in the presence of histamine in this study are similar to DDC described by Nestle et al (1993).

In this study, histamine-induced DC were stained positively for intracellular FXIIIa and CD68. Both the molecules have been reported to be expressed in DDC, MoDC, and macrophages, but not or only weakly on LC (Caux et al, 1996; Guirouinet et al, 2001; Vakila et al, 2005). Increased expression of molecules involved in Ag capture (mannose receptor, CD11b) and decreased expression of costimulatory molecules (CD80, CD40) in histamine-treated DC may correlate with their impaired antigen-presenting function to T lymphocytes and a higher endocytic activity. These phenotypic and functional features suggest that histamine-induced DC are in a transitional state of differentiation either toward CD1α+CD14− DDC or macrophages, but not LC. Larregina et al (2001) demonstrated that CD1α−CD14+ FXIIIa− DDC differentiated into LC when cultured with TGF-β1 alone and that their allostimulatory capacity was enhanced when they were cultured with IL-4, GM-CSF, and TGF-β1. It is interesting to examine whether histamine-induced DC have the potential to differentiate into LC when cultured with TGF-β1 in addition to GM-CSF and IL-4.

The fact that dust mite inhalation aggravates eruptions in atopic dermatitis (AD) patients suggests that DDC play a role in the pathogenesis of AD (Tupker et al, 1996). As mast cells are present in the dermis and epidermis in the AD skin lesions (Imayama et al, 1995), the aggregation of FcεRI-induced by environmental allergens (e.g., dust mites and pollen) and subsequent degranulation of mast cells often occur. In addition, IgE autoantibodies are frequently detected in the circulation of severe AD patients (Valenta et al, 2000), suggesting that the degranulation of peripheral blood basophils also occurs. In AD individuals, monocytes could therefore be exposed to histamine in the skin or peripheral circulation.

In this study, histamine prevented the generation of CD1α+ DC from monocytes via H2R. In addition, this inhibitory effect was mediated by IL-10 produced endogenously by monocytes. This observation is in line with previous reports that showed that (i) the addition of IL-10 at the start of culturing with GM-CSF and IL-4 inhibits CD1α+ MoDC generation (Bue lens et al, 1997; Allavena et al, 1998), and (ii) histamine induces monocytes and MoDC to produce IL-10 (Elencov et al, 1998; Mazzoni et al, 2001; Gutzmer et al, 2002; Idzko et al, 2002). The mechanism by which IL-10 prevents CD1α+ DC generation remains unclear. IL-10 might exert a signal that causes monocytes to differentiate into macrophages because human blood monocytes cultured with IL-10 differentiate into CD16+ macrophage-like cells (Calzada-Wack et al, 1996). Alternatively, IL-10 initiates the signal that prevents IL-4-induced signal required for DC differentiation. IL-10 upregulates the gene expression of the suppressor of cytokine-signaling (SOCS)-3 (Williams et al, 2002). SOCS-3 inhibits IL-4-dependent signal transducer and activator of transcription (Stat) 6 activation of and subsequent gene induction (Haque et al, 2000). Stat-mediated signals and feedback regulation by SOCS may be involved in differentiation and maturation of DC (Jackson et al, 2004).

Recently, several groups have shown that histamine alters the repertoire of cytokines secreted by LPS-matured DC that induces the polarization of naïve CD4+ T cells toward Th2 phenotypes (Caron et al, 2001; Mazzoni et al, 2001; Gutzmer et al, 2002; Idzko et al, 2002). In this study, histamine-treated immature DC induced slightly but significantly increased intracellular expression of IL-4 in CD4+ naïve responder T cells, whereas the intracellular expression of IFN-γ was not significantly affected. Rather, DC generated in the presence of histamine still induced a population of Th1 cells as determined by a higher number of IFN-γ-producing cells when compared with IL-4 (Th2 cells). This may be explained by the much lower IL-10-producing capacity of histamine-treated immature DC when compared with LPS-matured, histamine-treated DC in this and previously reported experiments (Fig 4A–D).

In this study, we discovered a new immunomodulatory function of histamine besides its well-characterized effects in the acute inflammatory and allergic responses; it inhibits the differentiation of CD1α+ DC mostly through the action of endogenously produced IL-10. Histamine induces monocytes to differentiate into CD1α−CD14+ DC cells even in the presence of IL-4 and GM-CSF, which have an enhanced capacity to induce the production of proinflammatory cytokines and chemokines and elevated phagocytic activity in spite of a reduced antigen-presenting capacity. This suggests that histamine-driven DC amplify antigen-independent inflammatory reactions and contribute to the exacerbation of allergic disease. Alternatively, IL-10 produced by histamine-treated monocytes and DC via H2R may
induce regulatory properties of T cells because IL-10-treated DC lead to antigen-specific anergy in CD4+ T lymphocytes, which is characterized by inhibited proliferation, reduced production of IL-2, and antigen-specific suppressor activity (Jonuleit et al., 2002; Steinbrink et al., 2002). The appropriate use of HR antagonists or the use of selective agonists may be promising therapeutic tools against allergic disease.

Materials and Methods

Reagents  FITC-labeled anti-CD1a mAb (OKT6) was obtained from Ortho Diagnostics (Ritan, New Jersey). PE-, FITC-conjugated, or unconjugated anti-CD1b, CD11b, CD14, CD40, CD45RO, CD68, CD80, CD83, CD86, HLA-DR, IL-4, IL-12 (p40/p70), TNF-α, and IFN-γ were obtained from BD Pharmingen (San Diego, California). mAb against IL-10, IL-1β, IL-6, and M-CSFR were purchased from R&D Systems (Minneapolis, Minnesota). Anti-human mannose receptor (HyCult biotechnology, Uden, the Netherlands) and factor Xilla (FXilla, Lab Vision, Fremont, California) mAb were also purchased. FITC-conjugated and unconjugated F(ab)2 goat antimouse IgG Ab were acquired from Jackson Immunoresearch (West Grove, Pennsylvania). Histamine, pyrilamine (H1R antagonist), cimetidine (H2R antagonist), thioperamine (H3R antagonist), and dimaprit dihydrochloride (H2R agonist) were obtained from Sigma Chemical Co. (St Louis, Missouri). All other reagents were obtained from Sigma Chemical Co.

Cell purification and culture Whole blood was obtained from non-atopic healthy volunteers under approval of our Institutional Ethical Committee. Participants gave their written informed consent, and the study was conducted according to the Declaration of Helsinki Principles. Monocytes were isolated using Nycoprep (Nycomed, Oslo, Norway) and the Monocyte Negative Isolation Kit (Dinal Biotech, Oslo, Norway) according to the manufacturer’s instructions. The monocytes were cultured with 100 ng per mL GM-CSF and IL-4, or M-CSF (R&D Systems) in low-endotoxin RPMI 1640 (Biochrom KG, Berlin, Germany) supplemented with 10% FCS, 100 mM L-glutamine, and 1% antibiotic–antimycotic (all from Gibco BRL, Gaithersburg, Maryland) for 6 d at 37°C and 5% CO2 as described previously (Novak et al., 2001). All plastic ware and culture reagents used were tested for the presence of endotoxin with the Limulus amebocytes lysate E-Toxate multiple test. Endotoxin levels were always <10 pg/mL.

Proliferation assays Allogeneic MLR were conducted in 96-well round-bottom microtiter plates by adding different amounts of irradiated (3000 rad) DC on day 6 to 1 × 105 allogeneic and autologous naïve CD4+ T cells, which were obtained with a CD4+ isolation kit (Dynal, Oslo, Norway), and subsequent negative selection in combination with anti-CD45RO mAb plus goat anti-mouse IgG Ab-conjugated immunomagnetic beads (Dynal). After 4 d at 37°C, cell proliferation was assessed by the uptake of 3H-thymidine (1.25 μCi per well present for 16 h; Amershams, Little Chalfont, UK).

Flow cytometry Surface and intracellular molecule labeling was performed as previously reported (Novak et al., 2001). The rFI was assessed as follows: rFI = mean fluorescence intensity (MFI) (surface molecule)–MFI (control)/MFI (control). Phagocytic activity was tested by using fluorescence-labeled latex beads (Polysciences, Wallington, Pennsylvania). Briefly, cultured cells in the absence or presence of histamine on day 5 were incubated with the beads for 3 h at 37 or 0°C. After washing three times, the cells were fixed with 4% paraformaldehyde, and then the percentage of cells that performed phagocytosis was analyzed by flow cytometry.

Generation of culture supernatant from monocytes The cytokine concentration in the supernatant was harvested, and the cytokine present in it was blocked as described previously (Katoh et al., 2000). Briefly, monocytes were cultured for 24 h with or without 10 μM histamine. The supernatants were collected and centrifuged twice at 4°C, followed by immediate storage at −80°C. In blocking experiments, 500 μL of the supernatant was first treated with anti-cytokine neutralizing mAb (10 μg per mL) for 30 min at 37°C. Then, 20 μL of protein G-Sepharose (Amersham Pharmacia Biotech, Uppsala, Sweden) was added at 4°C for 2 h to precipitate bound cytokines. After centrifugation, the supernatant was collected, and then monocytes were cultured in the supernatants. Cytokine concentrations in the supernatants were examined by using commercially available ELISA kits (R&D Systems).

Statistical analysis Data are expressed as the means ± SD. Statistical differences were determined using paired and unpaired t-tests, and a p value of less than 0.05 was considered to be statistically significant.

This work is supported in part by grants from the Japanese Ministry of Education, Science, Sports and Culture, Shimizu Foundation for the Promotion of Immunology Research, and Japanese Dermatological Association for Basic Medical Research (donated by Shiseido Co. Ltd., Tokyo, Japan).

DOI: 10.1111/j.0022-202X.2005.23891.x

Manuscript received January 18, 2005; revised May 13, 2005; accepted for publication June 10, 2005

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