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 FcεRI 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 naïve 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

Abbreviations: AD, atopic dermatitis; DC, dendritic cells; DDC, dermal dendritic cells; FITC, fluorescein isothiocyanate; GM-CSF, granulocyte-macrophage colony-stimulating factor; HR, histamine receptor; IFN-γ, interferon-γ; IL, interleukin; LC, Langerhans cells; LPS, lipopolysaccharide; mAb, monoclonal antibody; M-CSF, macrophage colony-stimulating factor; MFI, mean fluorescence intensity; MoDC, monocyte-derived dendritic cells; rFI, relative fluorescence intensity

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 1*A*). 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 1*B*). 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 1*C*). Histamine at 10 μM was used in the following experiment because it is within the concentration range of histamine detected in nasal secretions and brochoalveolar 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 1*D* and 2*B*).

We further examined whether histamine has any effect on the DC phenotype at day 6 (Fig 2*A*). 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, CD1b, 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 2*B*).

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).

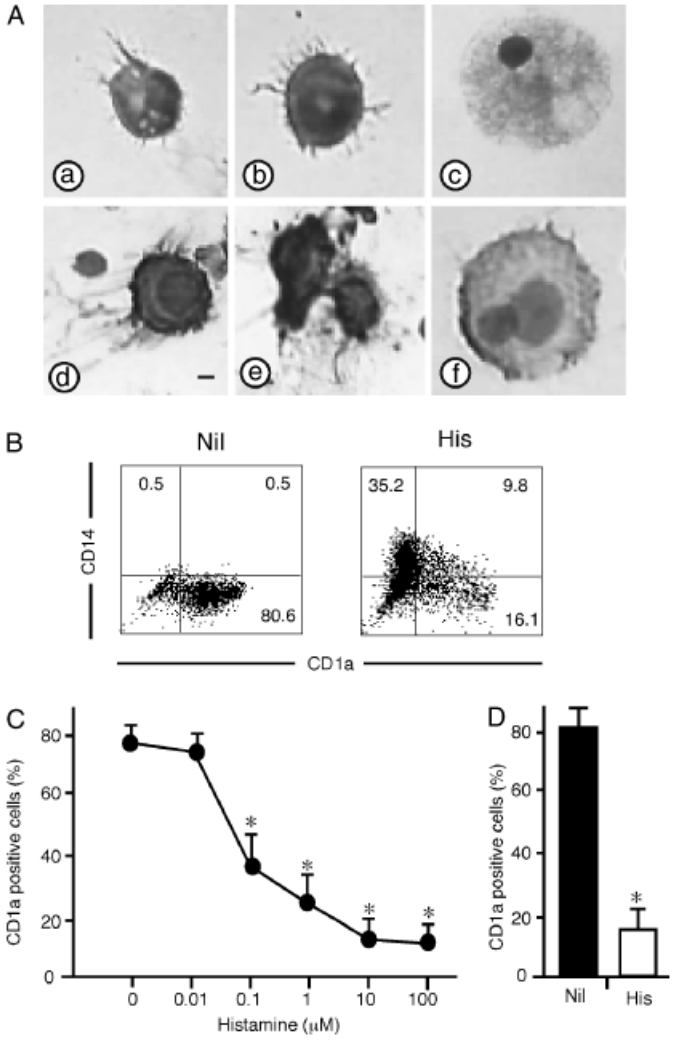

Figure 1
Histamine induces the generation of monocyte-derived dendritic cells (MoDC), that express CD14 but not CD1a. (*A*) Monocytes were cultured for 6 d in the presence or absence of 10 μM histamine in addition to interleukin (IL)-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) or 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–Grünwald–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. $^{*}p<0.05$.

Histamine alters DC function A characteristic feature of DC is their capacity to stimulate naïve T cells, as seen in the model of allogeneic mixed lymphocyte reaction (MLR). To determine the functional characteristics of cells generated in the presence of histamine, we examined whether histamine affects their T cell-stimulating capacity in MLR. As shown in Fig 3, compared with MoDC generated under control conditions, histamine-treated DC exhibited significantly reduced stimulatory activity toward allogeneic T cells. As shown in Fig 3*C*, CD4+ naïve T lymphocytes after incubation with allogeneic histamine-treated DC exhibited slightly but significantly higher intracellular expression of

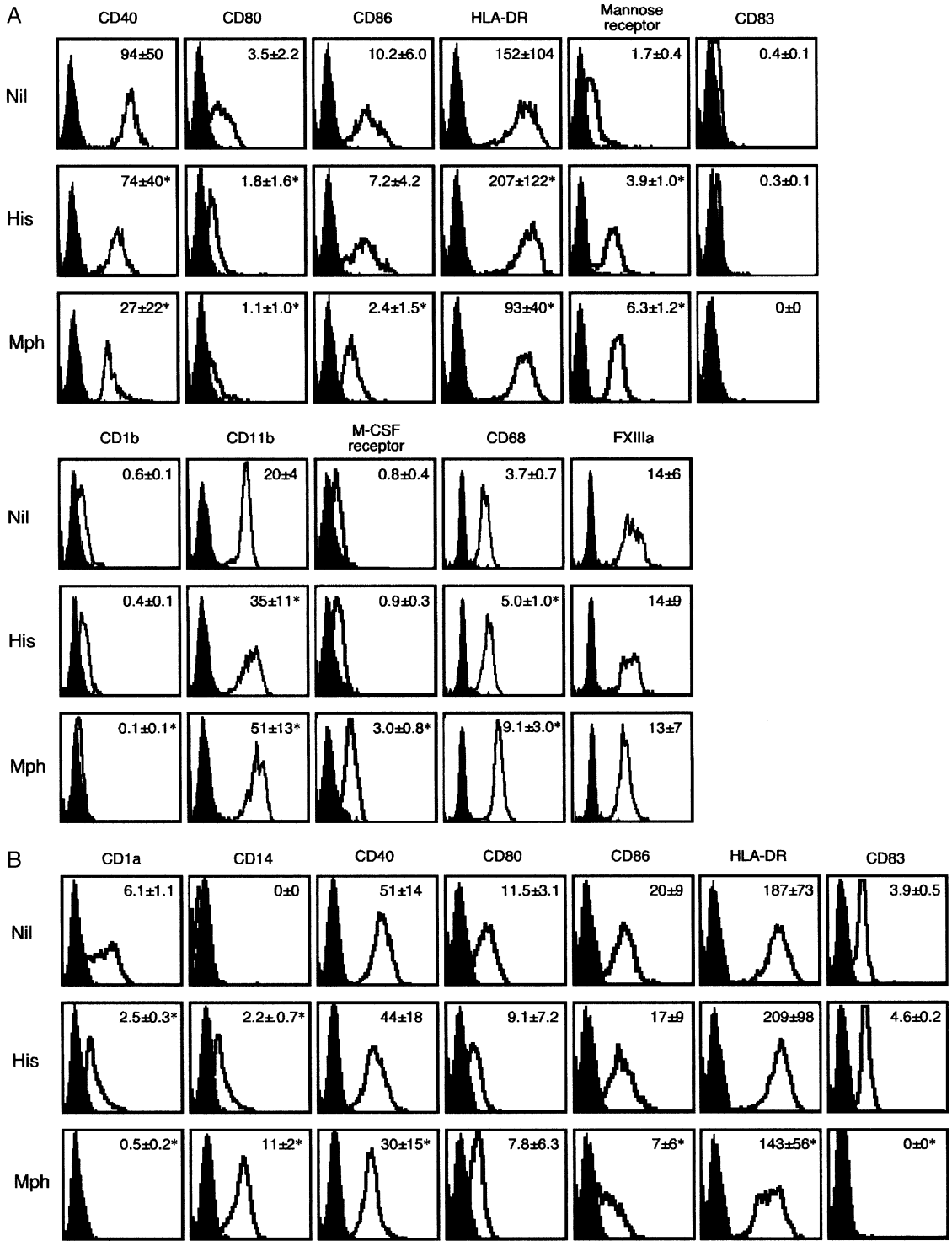

Figure 2
Effect of histamine on the phenotypes of *in vitro*-generated dendritic cells (DC). (*A*) The expression of the surface marker is shown for control DC (nil) and control macrophage colony-stimulating factor (M-CSF)-induced macrophage (Mph) without histamine in comparison with the cells cultured with histamine (His) for 6 d. (*B*) These cells were matured with 100 ng per mL lipopolysaccharide for 36 h and FACS analyzed. The overlay histograms represent the data from eight independent experiments with different donors. The intracytoplasmic staining for CD68 and FXIIIa was performed by permeabilization with saponin. The values of mean rFI ± SD from eight independent experiments are also demonstarated. Asterisk indicates statistical significance between control monocyte-derived dendritic cells and histamine- or M-CSF-treated cells.

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

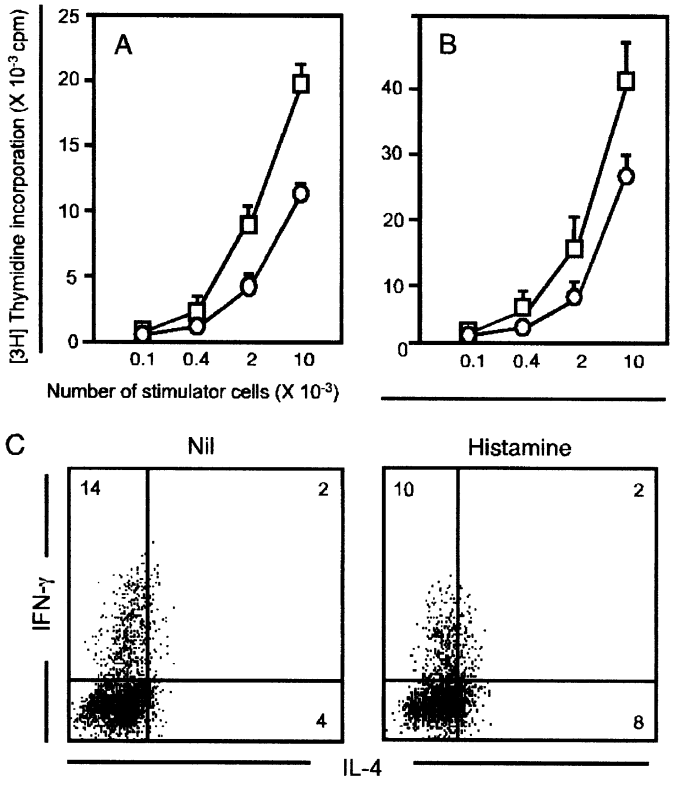

Figure 3
Suppression of the stimulatory activity of allogeneic T cells by dendritic cells (DC) generated in the presence of histamine. (*A*) Monocytes were cultured with (*open circle*) or without histamine (*open square*) in addition to interleukin (IL)-4 and granulocyte-macrophage colony-stimulating factor for 6 d. These cells were washed three times and then cocultured with purified responder allogeneic naïve CD4+ T cells for 4 d. (*B*) In day 6 the cells were matured with 100 ng per mL lipopolysaccharide for 36 h and then cocultured with naïve CD4+ T cells as described above. The data are representative of five independent experiments with different donors. Results are expressed as the mean ± SD for triplicate cultures. (*C*) Naïve CD4+ T cells were cocultured with control monocyte-derived dendritic cells or hitamine-treated DC for 96 h. T cells were expanded with 50 U per mL IL-2 for 7 d, and stimulated with 5 ng per mL PMA and 500 ng per mL ionomycin for 6 h. Brefeldin A was added during the last 2 h of culture. After fixation and permeabilization, intracellular cytokine staining for IL-4 and interferon-γ (IFN-γ) was performed. Representative dot plots of five independent experiments with different donors are shown.

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-

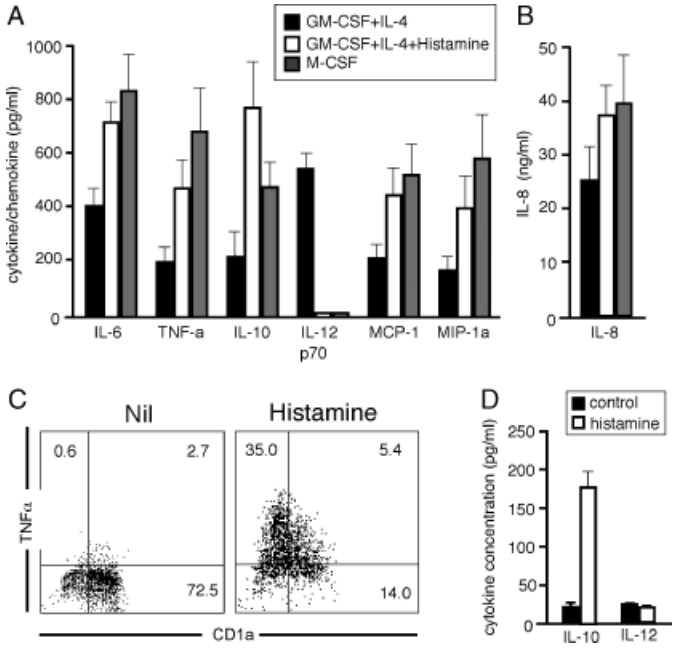

Figure 4
Capacity for producing proinflammatory cytokines and chemokines. (*A*, *B*) Monocytes were cultured with (*open square*) or without histamine (*closed square*) in addition to interleukin (IL)-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) for 6 d. Alternatively, monocytes were cultured in the presence of macrophage colony-stimulating factor (M-CSF) alone for 6 days (*hatched square*). After washing, the cells were stimulated with 100 ng per mL lipopolysaccharide for 24 h. The cytokine concentrations of the culture supernatants were estimated by ELISA. The data are mean ± SEM of four independent experiments with different donors. (*C*) Alternatively, these cells were stained with anti-CD1a fluorescein isothiocyanate and anti-TNF-α PE after fixation and permeablization. Representative dot blots of four independent experiments are shown. (*D*) Monocytes were cultured with or without histamine for 24 h, and concentrations of IL-10 and IL-12 p70 were estimated by ELISA. The data are mean percentage positive ± SEM of four independent experiments with different donors.

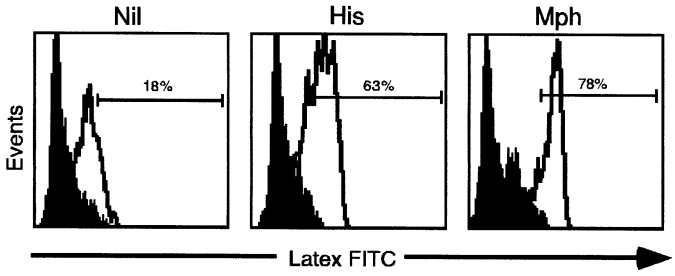

Figure 5
Effect of histamine on the phagocytic activity of dendritic cells. Monocytes cultured with (His) and without histamine (nil) in addition to interleukin-4 and granulocyte-macrophage colony-stimulating factor or macrophage colony-stimulating factor (Mph) for 6 d were tested for phagocytic capacity at 0°C (*black histogram*) and 37°C (*white histogram*) using fluorescein isothiocyanate-labeled latex beads and flow cytometry. Representative histograms (*A*) from six independent experiments with different donors are shown.

cence intensity (rFI): 20.5 ± 3.8 and 4.5 ± 1.0, respectively, p = 0.0139, n = 6) (Fig 5).

Histamine inhibits DC differentiation through H2R To elucidate the involvement of histamine receptor subtypes in the inhibition of DC differentiation, experiments using receptor subtype-selective antagonists were performed. Histamine-induced inhibition of CD1a expression and antigen-presenting capacity were completely blocked by preincubation of the monocytes with cimetidine but not with the H1R or H3R antagonists (Fig 6). In addition, the

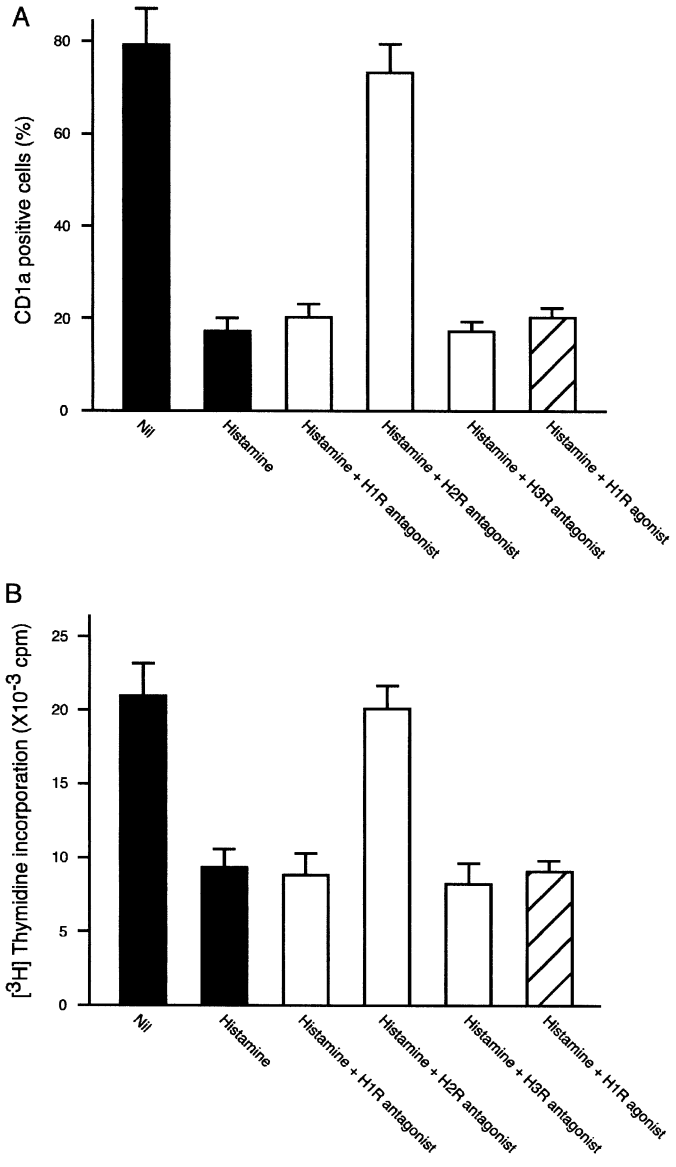

Figure 6
Histamine inhibits the generation of CD1a+ dendritic cells via H2 receptors. Monocytes were incubated with histamine receptor antagonists (H1R antagonist; 10 μM pyrilamine, H2R antagonist; 10 μM cimetidine, and H3R antagonist; 100 μM thioperamine), and after 1 h, the cells were washed three times and histamine, interleukin (IL)-4, and granulocyte-macrophage colony-stimulating factor (GM-CSF) were added. Alternatively, H2R agonist (10 μM dimaprit dihydrochloride) was added with IL-4 and GM-CSF. The cells were cultured for 6 d, their phenotypes were analyzed by flow cytometry (*A*), and their stimulatory capacity for allogeneic T cells was measured by MLR (stimulator:responder = 1:10) (*B*). The data are representative of four independent experiments with different donors.

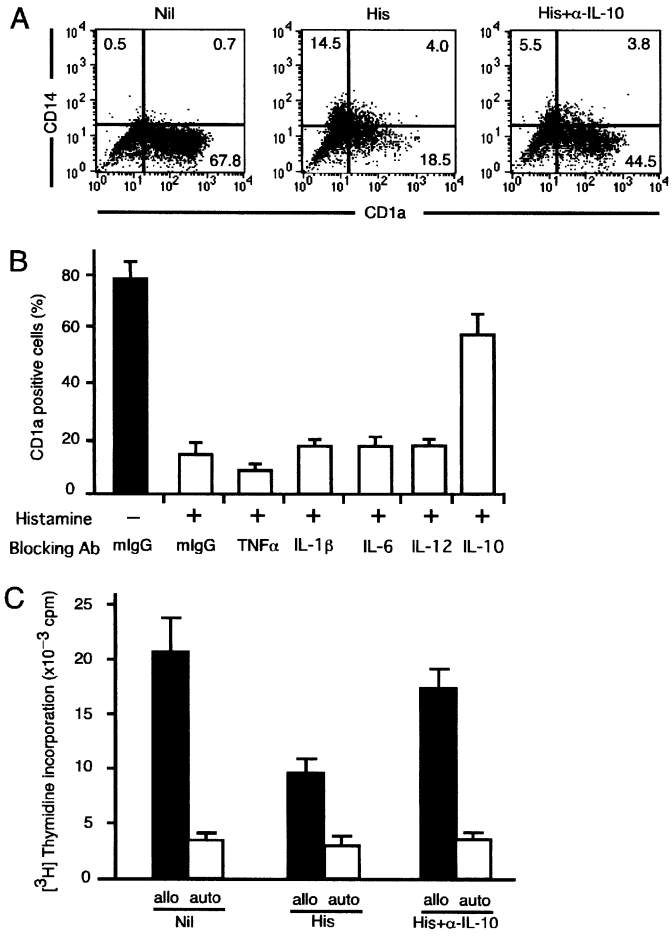

Figure 7
The culture supernatant of histamine-treated monocytes inhibited the generation of CD1a+ dendritic cells (DC). This inhibitory effect was blocked by the precipitation of interleukin (IL)-10 from the supernatant before its addition to cultures used to generate DC with IL-4 and granulocyte-macrophage 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 autologus MLR (1×10^4 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.

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 CD1a+CD14− DC generation. The removal of IL-10 with neutralizing monoclonal antibody (mAb) resulted in the restoration of CD1a+ DC differentiation inhibited by histamine as seen in the phenotype and allogeneic MLR. Endogenous production of IL-10 was 16 ± 6 pg per mL in day 5 MoDC and 184 ± 32 pg per mL in histamine-treated DC (Fig 4*D*).

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 CD1a expression, suggesting its enhancing role in CD1a+ 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 CD1a. DDC also express varying levels of CD1a, even though CD1a 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 CD1a, CD14, and FXIIIa. DDC migrated from skin explants have been subdivided into three groups: CD1a+CD14−, CD1a−CD14−, and CD1a−CD14+ (Nestle *et al*, 1993; Larregina *et al*, 2001). According to their reports, CD1a−CD14+ DDC showed increased phagocytic activity and weak T cell-stimulatory potential. CD14+CD1a− 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 CD1a+CD14− DC, but induced their differentiation into CD1a−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; Guironnet *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 CD1a+CD14− DDC or macrophages, but not LC. Larregina *et al* (2001) demonstrated that CD1a−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 CD1a+ 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 CD1a+ MoDC generation (Buelens *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 CD1a+ 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 4*A–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 CD1a+ DC mostly through the action of endogenously produced IL-10. Histamine induces monocytes to differentiate into CD1a−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 (Raritan, New Jersey). PE-, FITC-conjugated, or uncojugated 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, Minnnesota). Anti-human mannose receptor (HyCult biotechnology, Uden, the Netherlands) and factor XIIIa (FXIIIa, Lab Vision, Fremont, California) mAb were also purchased. FITC-conjugated and unconjugated F(ab)2 goat anti-mouse 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% CO_2 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 per 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 1×10^5 allogeneic and autologus 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 [^{3}H]thymidine (1.25 μCi per well present for 16 h; Amersham, 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 culture supernatant of monocytes 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 $\pm$ 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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