Regulatory effect of histamine H1 receptor antagonist on the expression of messenger RNA encoding CC chemokines in the human nasal mucosa

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Background: Allergic rhinitis is characterized by tissue accumulation of inflammatory cells. CC chemokines, including monocyte chemotactic protein (MCP) 1, MCP-3, RANTES, and eotaxin, are thought to play an important role in inducing selective recruitment of these cells to the allergic inflammatory site. Furthermore, MCPs have been indicated as histamine-releasing factors. Histamine is an important mediator in the pathogenesis of nasal allergy. The regulation of histamine may have a role in the management of allergic inflammation.
Objective: The objectives of this study were to investigate the expression of MCP-1, MCP-3, RANTES, and eotaxin in the nasal mucosa of patients with allergic rhinitis and to clarify the effect of histamine and antihistamine on the regulation of the expression of these CC chemokines.
Methods: By using a semiquantitative reverse transcriptase PCR technique, the numbers of copies of messenger RNA encoding MCP-1, MCP-3, RANTES, and eotaxin were measured in explant cultures of human nasal mucosa. In culture medium, specific antigen or histamine was added. Furthermore, the effect of preincubation with the antihistamine carebastine was estimated.
Results: Mite antigen ($1:2 \times 10^4$ dilution) and histamine (10^{-4} to 10^{-3} mol/L) upregulated the messenger RNA expression of these CC chemokines at 3- to 10-fold increases. Carebastine (10^{-7} to 10^{-6} mol/L) inhibited this upregulation.
Conclusion: Our results suggest that histamine may induce CC chemokine production in the nasal mucosa of patients with allergic rhinitis. This indicates that there may be a prolonged inflammatory cycle in the histamine-MCP axis in allergic rhinitis. The regulation of histamine-CC chemokine interaction could lead to new therapeutic approaches in the treatment of nasal allergy. (J Allergy Clin Immunol 2001;107:123-8.)

Key words: *Nasal allergy, CC chemokine, monocyte chemotactic protein 1, monocyte chemotactic protein 3, RANTES, eotaxin, histamine, carebastine*

Abbreviations used
HRF: Histamine-releasing factor
IPR: Immediate-phase response
LPR: Late-phase response
MCP: Monocyte chemotactic protein
RT-PCR: Reverse transcriptase PCR

Allergic rhinitis is associated with the recruitment and activation of inflammatory cells, particularly eosinophils, T lymphocytes, and basophils.[1-3] Moreover, infiltration of these cells are thought to contribute to the protraction of allergic inflammation.[4] Accumulating evidence on the properties of the CC chemokines has led to suggestions that certain of these mediators may play an important role in inducing selective recruitment of inflammatory cells to the mucosa in allergic disease.[5,6] Furthermore, in the late-phase response (LPR) of nasal allergy, rather than persistent allergens, histamine release is believed to be the result of the action of histamine-releasing factors (HRFs), and most molecules described as HRFs belong to the CC chemokine class.[7-10]

Among CC chemokines, monocyte chemotactic protein (MCP) 1 has been thought to contribute to the activity of HRFs.[9,10] On the other hand, eotaxin has been thought to be selectively chemotactic for eosinophils and basophils in vitro.[11,12] MCP-3 and RANTES combine the properties of MCP-1 and eotaxin.[13,14] Although chemokines often have similar in vitro functions, their pattern of expression in an allergic inflammatory site determines which chemokines play a pathologic role. In view of these observations, we thought it important to investigate the expression and its regulation of these CC chemokines in the nasal mucosa of patients with allergic rhinitis.

The regulation of chemokine production may contribute to the treatment of nasal allergy. Studies were undertaken to determine whether (1) messenger (m)RNA encoding MCP-1, MCP-3, RANTES, and eotaxin can be detected at the site of allergic rhinitis; (2) specific antigen and histamine, a major mediator of the immediate-phase response (IPR) of nasal allergy can induce expression of mRNA encoding these chemokines; and (3) the histamine H1 receptor antagonist carebastine, which is the active metabolite of ebastine, can inhibit the expression of these chemokine mRNA.

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METHODS

Study subjects

Eight patients with allergic rhinitis (M/F ratio, 3:5; mean age ± SE, 26 ± 3 years) and 6 nonatopic subjects with chronic hypertrophic rhinitis (M/F ratio, 4:2; mean age ± SE, 41 ± 3 years) were included in this study after giving informed consent. All patients with allergic rhinitis had positive allergy responses for mite nasal allergy, such as clinical history, rhinoscopic examination, a nasal smear for eosinophilia, intradermal testing, or determination of serum-specific mite IgE antibodies (CAP system, Pharmacia Diagnostics, Sweden), as well as positive nasal provocation test responses and negative test responses for other common aeroallergens. Nonatopic subjects had negative responses on all tests. Nasal eosinophilia was not observed in these patients. Their serum IgE levels were 24 ± 4 IU/mL (mean ± SEM). All patients refrained from taking topical corticosteroids for a period of 4 weeks before biopsy. The patients who were treated with immunotherapy were not included in this study.

Preparation of explant culture of human nasal mucosa

During surgery, we took pieces of the nasal inferior turbinate mucosa (approximate weight, 2-4 g) and cut them into 2- to 3-mm cubic fragments. About 10 fragments were put into 10 mL of McCoy's 2+ tissue culture medium (GIBCO, Grand Island, NY) containing 100 μg/mL penicillin, 100 μg/mL streptomycin sulfate, and 50 μg/mL gentamicin sulfate and were incubated with 5% carbon dioxide at 37°C for 24 hours. The nasal mucosal tissue was collected and homogenized in ISOGEN (Nippon Gene, Tokyo, Japan) and stored at –70°C.

Semiquantitative reverse transcriptase (PCR)

Total RNA was collected by using a single-step method with the guanidium thiocyanate-phenol-chloroform extraction method.[15] Reverse transcriptase (RT) PCR reactions were performed according to the manufacturer's protocol (Takara RNA PCR Kit, Takara Biochemicals, Tokyo, Japan). Briefly, reverse transcription with oligo dT primers was performed for 30 minutes at 55°C with 1 μg of total RNA. PCR was carried out, and each cycle consisted of 1 minute for denaturation at 94°C, 1 minute for annealing at 55°C, and 1 minute for elongation at 74°C; a total of 18 cycles were performed. For each parameter, specific primers were set as shown in Table I. All products were confirmed as the specific amplification products by sequencing (data not shown). The products of each reaction were subjected to electrophoresis on 1.5% agarose gel. The gel was stained with ethidium bromide. Ethidium bromide intensity was measured and standardized by using the β-actin mRNA level.

Effect of antigen, histamine, and carebastine on CC chemokine expression

Mite antigen *(Dermatophagoides pteronyssinus)* extract, a gift of Torii Pharmaceutical Co (Tokyo, Japan), was applied to the culture medium at a dilution of $1:2 \times 10^4$ (0.22 μg of protein nitrogen/mL). This concentration was determined according to our previous study.[16] Histamine dihydrochloride (ICN Biomedicals, Inc, Aurora, Ohio) was added to the culture medium at a concentration of 10^{-5} to 10^{-3} mol/L. This concentration was based on the study that demonstrated histamine-induced upregulation of IL-8.[17] To investigate the effect of the histamine H1 receptor antagonist carebastine on CC chemokine expression, nasal mucosal tissue was preincubated in the medium containing 10^{-7} or 10^{-6} mol/L carebastine, a gift from Dainippon Pharmaceutical Co, Ltd (Osaka, Japan), for 30 minutes. After preincubation, mite antigen or histamine was added and incubated for 24 hours. Then total RNA was extracted by the method mentioned above.

Statistical analysis

The data presented are expressed as the mean ± SEM. Statistical significance for intergroup comparison was determined by using 1-way ANOVA and subsequently by using the Dunnett method. The differences were considered significant only when the P value was less than .05.

RESULTS

Basal expression and mite antigen and histamine stimulation

As shown in Fig 1, the sample of patients with nasal allergy expressed a single band corresponding to MCP-1, MCP-3, RANTES, eotaxin, and histamine receptor type 1 mRNAs. Both house dust mite antigen and histamine upregulated the mRNA expression of chemokines. House dust mite antigen upregulated the mRNA for chemokines (induction compared with control: MCP-1, 6.3 ± 2.1-fold; MCP-3, 3.1 ± 3.0-fold; RANTES, 5.4 ± 1.2-fold; eotaxin, 1.5 ± 1.1-fold; $P < .05$). Moreover, histamine (10^{-4} mol/L and 10^{-3} mol/L) upregulated the chemokines dose dependently, except for MCP-1, which was upregulated to the maximum extent by 10^{-4} mol/L histamine. A histamine level of 10^{-5} mol/L did not affect chemokine expression (Fig 2). Four of 6 samples from nonatopic subjects expressed eotaxin mRNAs, and 2 of them expressed MCP-3 mRNAs. MCP-1 and RANTES were not observed in the samples from nonatopic subjects. Histamine neither induced nor upregulated chemokine expression in these samples (data not shown).

Effect of carebastine on chemokine expression

Overnight culture with carebastine did not affect the chemokine expression (data not shown). In the specimen stimulated by mite antigen (n = 6), 10^{-6} mol/L carebastine tended to decrease the enhanced expression of chemokines (Table II). In contrast, in the specimen stimulated by 10^{-3} mol/L histamine (n = 8), carebastine (10^{-7} and 10^{-6} mol/L) significantly decreased the enhanced expression of MCP-1 (60% ± 2% and 10% ± 2% compared with control, $P < .01$), MCP-3 (62% ± 2% and 6% ± 1% compared with control, $P < .01$), and RANTES (58% ± 2% and 5% ± 2% compared with control, $P < .01$) in a dose-dependent fashion. The enhanced expression of eotaxin mRNA was suppressed only by 10^{-6} mol/L carebastine (10% ± 3% compared with control, $P < .01$; Fig 3, *A* and *B*).

DISCUSSION

The production of MCP-1, MCP-3, RANTES, and eotaxin in the human nasal mucosa has been well documented; however, a study comparing the mRNA expression of these 4 chemokines has not been carried out.[18-21] We stimulated the explant culture of nasal mucosa with antigen and demonstrated the expression of these 4 chemokines at the same time. However, it was not certain how the reactions after binding of antigen to specific IgE

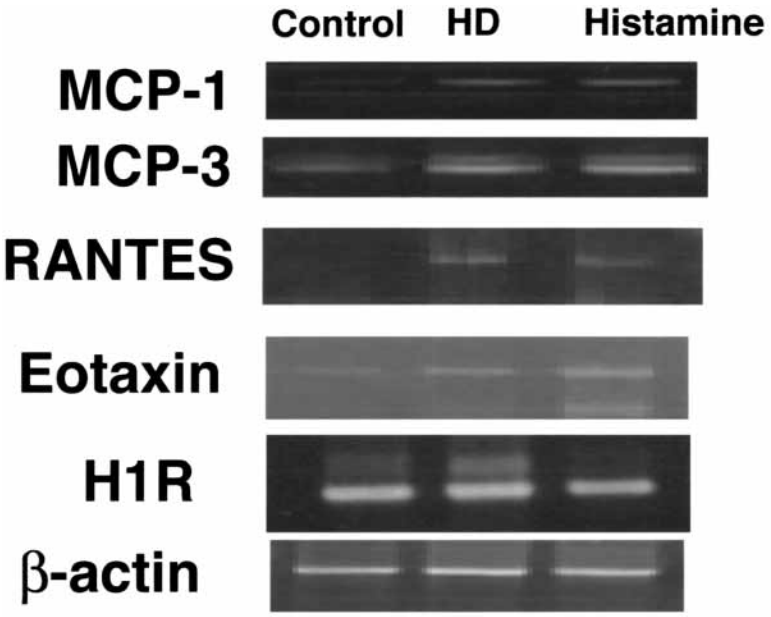

FIG 1. Effects of house dust mite antigen (HD) and histamine on mRNA expression of chemokines. Single bands corresponding to each chemokine and histamine receptor type 1 (H1R) are shown. House dust mite antigen and 10^{-3} mol/L histamine upregulated chemokine mRNA.

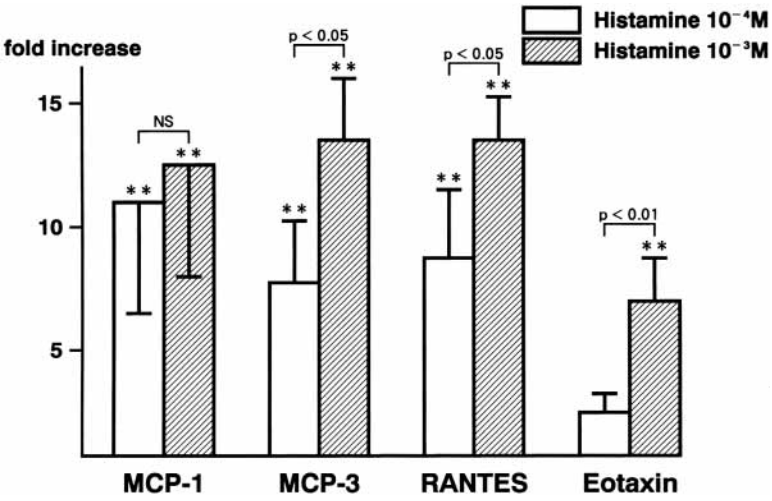

FIG 2. Effect of histamine on mRNA expression of chemokines. Each chemokine's mRNA calibrated with β-actin mRNA, and their increases by 10^{-4} mol/L and 10^{-3} mol/L histamine were calculated. *Columns* indicate fold induction compared with control induction. In addition to MCP-1, each chemokine's mRNA was upregulated in a dose-dependent fashion. $^{**}P < .01$ versus control. *NS,* Not significant.

led to the expression of CC chemokines. Previously, we have shown that mite antigen induces a remarkable amount of histamine release from nasal scrapings at the dilution of $1{:}2 \times 10^4$ (the same concentration of antigen used in our study).[16] Therefore we thought that histamine might also be released from the explant culture of nasal mucosa in our study, and it would mediate chemokine production from the nasal mucosa. Our hypothesis for the effects of histamine was in agreement with the findings of other studies demonstrating histamine-induced production of IL-8 from endothelial and epithelial cells.[17,22] Furthermore, carebastine, the active metabolite of the antihistamine ebastine, inhibited chemokine upregulation induced by antigen or histamine. This result also suggests that histamine is one of the major stimuli that induce CC chemokine expression in the nasal mucosa of patients with allergic rhinitis.

In the nasal mucosa major cell sources of CC chemokines are macrophages, epithelial cells, endothelial cells, and fibroblasts.[23] Among these cells in the nasal

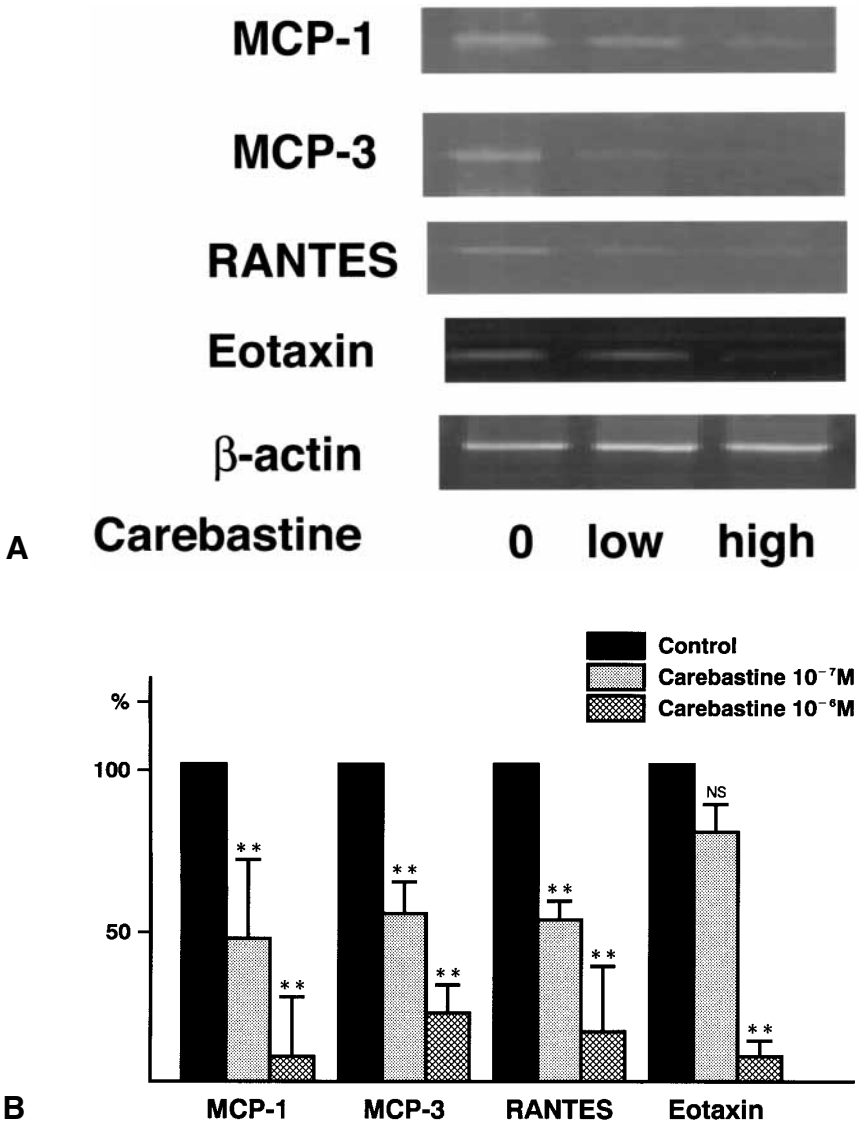

FIG 3. Effect of carebastine on mRNA expression of chemokines. Effect of carebastine was studied on histamine-induced chemokines. **A,** A typical RT-PCR product of each chemokine. All products were decreased by carebastine in a low (10^{-7} mol/L) and high (10^{-6} mol/L) dose. **B,** RT-PCR products were calibrated with β-actin, and percentage of baseline value was quantified. Carebastine 10^{-7} mol/L and 10^{-6} mol/L dose dependently reduced mRNA of each chemokine. $P < .01$ versus control. *NS,* Not significant.

mucosa, endothelial cells and epithelial cells have been reported to express histamine H1 receptor.[24,25] Cultured human skin fibroblasts have been suggested to express histamine H1 receptor.[26] These findings also prompted us to hypothesize that histamine released during the IPR might activate H1 receptor–expressing cells and induce CC chemokine production. Intracellular mechanisms of histamine action have been reported to include intracellular calcium change.[27] The exact process and the possibility of chemokine production through the H2 and H3 receptors should be elucidated by future investigations.

In the LPR of nasal allergy, histamine release from basophils is thought to be stimulated by HRFs. MCPs act as HRFs, and MCP is upregulated by histamine. Through forming this prolonged inflammatory cycle, the histamine-CC chemokine interaction may contribute to the protraction of nasal allergy. Among CC chemokines, MCPs, as accelerators of this cycle, are considered to be important factors for the pathogenesis of allergic inflammation. Our result showed that antihistamines successfully inhibited MCP expression. This strongly suggests that blockade of histamine H1 receptor is a good tactic to terminate the prolonged inflammatory cycle.

It has been thought that antihistamines are an effective treatment for the sneezing and watery rhinorrhea associated with the IPR of allergic rhinitis but have a negligible

TABLE I. Specific primers for chemokines

MCP-1 5′	5′-AGCATGAAAGTCTCTGCCGCC-3′
MCP-1 3′	5′-TCAAGTCTTCGCAGTTTGGG-3′
MCP-3 5′	5′-AACATGAAAGCCTCTGCAGCAC-3′
MCP-3 3′	5′-TGTTCAAAGCTTTGGAGTTTG-3′
RANTES 5′	5′-ACCACACCCTGCTGCTTTGCCTACATTGCC-3′
RANTES 3′	5′-CTCCCGAACCCATTTCTTCTCTTGGGTTGGC-3′
Eotaxin 5′	5′-CCCAACCACCTGCTGCTTTAACCTG-3′
Eotaxin 3′	5′-TGGCTTTGGAGTTGGAGATTTTTGG-3′
H1R 5′	5′-AGCACTATCTGCTTGGTCAC-3′
H1R 3′	5′-TGGCCACATAGTCCATGGAAAGC-3′
β-actin 5′	5′-GTGGGGCGCCCCAGGCACCA-3′
β-actin 3′	5′-CTCCTTAATGTCACGCACGATTTC-3′

H1R, Histamine type 1 receptor.

TABLE II. Inhibitory effect of carebastine on house dust mite antigen–induced chemokines

			MCP-1		MCP-3		RANTES		Eotaxin	
Patient No.	**Sex**	**Age (y)**	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**
1	F	22	90	ND	ND	ND	89	25	98	47
2	M	14	79	19	97	50	61	8	78	15
3	F	20	86	ND	94	68	96	12	96	24
4	F	18	94	36	60	12	81	15	82	10
5	M	35	Nd	21	Nd	ND	Nd	22	Nd	39
6	F	33	Nd	ND	Nd	ND	Nd	3	Nd	5

mRNA expression was standardized with β-actin mRNA, and percentage of baseline value was calculated.
Low, Carebastine 10^{-7} mol/L; *High,* carebastine 10^{-6} mol/L; *ND,* not detectable; *Nd,* not done.

effect on nasal congestion, which is the main symptom of the LPR. Recently, several studies have confirmed the anti-inflammatory effect of antihistamines.[28,29] It has been reported that antihistamines used for seasonal allergic rhinitis with decongestants improved coexisting mild seasonal asthma symptoms and pulmonary functions.[30] Antihistamines may contribute to the treatment of chronic nasal congestion through reducing the basal level of allergic inflammation. In our study the possibility of an anti-inflammatory effect of carebastine was also demonstrated. The concentration of carebastine used in our study was 0.1 to 1.0 μmol/L. According to the study that investigated the human plasma level of carebastine, it was not far from the dose of clinical use.[31] Therefore our hypothesis could also be elucidated by future in vivo investigations.

In patient samples with nasal allergy, the inhibitory effect of carebastine for eotaxin mRNA expression was not remarkable, as compared with other chemokines. Constitutive expression of eotaxin has been observed in nasal tissue and other organs.[11,19] Among CC chemokines, eotaxin seems to be regulated in a different fashion under the influence of allergic reactions.

Besides antigen, C3a, C5a, and other stimuli can induce histamine release from the mast cell. In nonallergic inflammation histamine may also act as an important mediator. Furthermore, viral infections have been known to upregulate chemokine production.[32] The interaction between histamine and CC chemokines is not specific in allergic inflammation. However, the population and activity of effector cells may be different in allergic and nonallergic inflammation.[2,33] Therefore the effect of this interaction is thought to be more important in allergic inflammation.

In conclusion, histamine release is induced by either IgE-mediated reactions or CC chemokines and, on the other hand, histamine induces CC chemokine production. Antihistamines could terminate this prolonged inflammatory cycle of the allergic inflammation axis. The regulation of the histamine-CC chemokine interaction could be a new therapeutic approach in nasal allergy.

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