

ORIGINAL ARTICLE

Preseasonal prophylactic treatment with antihistamines suppresses IL–5 but not IL-33 mRNA expression in the nasal mucosa of patients with seasonal allergic rhinitis caused by Japanese cedar pollen

YOSHIAKI KITAMURA¹, HIROYUKI MIZUGUCHI², HIROTAKA OGISHI²,
WAKANA KURODA³, MASASHI HATTORI², HIROYUKI FUKUI² & NORIAKI TAKEDA¹

¹Department of Otolaryngology and, ²Department of Molecular Pharmacology, Institute of Health Biosciences, University of Tokushima Graduate School, Tokushima and ³Department of Otolaryngology, Yashima General Hospital, Takamatsu, Japan

Abstract

Conclusions: These findings suggest that the down-regulation of interleukin (IL)–5 gene expression in collaboration with the suppression of histamine H₁ receptor (H1R) gene expression in the nasal mucosa provides the basis for better therapeutic effects of preseasonal prophylactic treatment with antihistamines in patients with seasonal allergic rhinitis caused by Japanese cedar pollen. **Objectives:** The effects of prophylactic administration of antihistamines on the expression of IL–5 and IL–33 mRNA in the nasal mucosa of the patients with pollinosis were investigated. **Methods:** Eight patients had already visited the hospital before the peak pollen period and started preseasonal prophylactic treatment with antihistamines. Seventeen patients who first visited the hospital during the peak pollen period were designated as the no treatment group. After local anesthesia, nasal mucosa was obtained by scraping the inferior concha with a small spatula during the peak pollen period. **Results:** During the peak pollen period, the expression of IL–5 mRNA, but not that of IL–33 mRNA, in the nasal mucosa of patients receiving preseasonal prophylactic treatment with antihistamines was significantly lower in comparison with that of patients without treatment. Moreover, there was a significant correlation between the expression of IL–5 mRNA and the nasal symptoms or the expression of H1R mRNA.

Keywords: histamine-cytokine network, histamine H1 receptor, prophylaxis

Introduction

Recently, preseasonal prophylactic treatment with antihistamines was reported to increase their efficacy in patients with seasonal allergic rhinitis caused by Japanese cedar pollen, suggesting that antihistamines have greater inhibitory effects on allergic rhinitis symptoms when administered before the pollen season [1,2]. Accordingly, their administration 2–4 weeks before the onset of the pollen season is recommended in patients with pollinosis in Japan [3]. In a previous study, we suggested that the suppression of histamine H₁ receptor (H1R) gene expression in the nasal mucosa contributes to the slow-onset effects of

preseasonal treatment with antihistamines [4]. During the peak pollen period, the expression of H1R mRNA in the nasal mucosa and the scores of sneezing and watery rhinorrhea in the patients receiving preseasonal prophylactic treatment with antihistamines were significantly suppressed in comparison with those of patients without treatment. Moreover, there was a significant correlation between the nasal symptoms and the expression of H1R mRNA in both groups of patients with or without preseasonal prophylactic treatment.

Recently, several lines of evidence have suggested that in addition to their histamine-blocking effect at H1R, antihistamines possess anti-inflammatory

Correspondence: Noriaki Takeda MD PhD, Department of Otolaryngology, Institute of Health Biosciences, University of Tokushima Graduate School, 3-18-15 Kuramoto, Tokushima 770-8505, Japan. Tel: +81 88 633 7169. Fax: +81 88 633 7170. E-mail: takeda@clin.med.tokushima-u.ac.jp

(Received 16 September 2011; accepted 13 November 2011)

ISSN 0001-6489 print/ISSN 1651-2251 online © 2012 Informa Healthcare
DOI: 10.3109/00016489.2011.644804

activity through a histamine-cytokine network [5-7]. In the present study, an attempt was made to investigate the effects of preseasonal treatment with antihistamines on the expression of Th2 cytokines such as interleukin (IL)-5 and IL-33 in the nasal mucosa of patients with seasonal allergic rhinitis caused by Japanese cedar pollen. This is because in our preliminary study, we measured Th2 cytokines including IL-4, IL-5, IL-9, and IL-13 in the nasal mucosa scraping and only detected IL-5 in this group. At that time, we also detected IL-33, a cytokine that induces eosinophilic infiltration like IL-5.

Material and methods

Patients

We used the same 25 patients with seasonal allergic rhinitis caused by Japanese cedar pollen included in our previous study that was carried out during the 2009 cedar pollen season in Japan [4]. Among the patients, eight (three males, five females; mean age 47.9 years) had visited the Department of Otolaryngology, Yashima General Hospital, before the peak pollen period (from February 20 to March 26, 2009) and started preseasonal prophylactic treatment with antihistamines (ebastine 10 mg/day p.o. in five patients, fexofenadine 120 mg/day p.o. in three patients). The treatment lasted for 17.9 ± 11.0 days (mean $\pm$ SD) before the follow-up visit during the peak pollen period, when the nasal symptoms were evaluated and the scraping of the nasal mucosa was then obtained in patients by an otolaryngologist, as described elsewhere [4]. Sneezing, watery rhinorrhea, and nasal obstruction were separately scored on a 0-5 point scale before nasal sampling. Under local anesthesia with 4% lidocaine, the surface of each patient's inferior nasal concha was scratched with a small spatula and a scraping of the nasal mucosa was obtained.

Another 17 patients with seasonal allergic rhinitis caused by Japanese cedar pollen (8 males, 9 females; mean age 46.3 years) who first visited the hospital during the peak pollen period, but did not take antihistamines or any other treatment, were designated as the no treatment group. This study was approved by the Ethical Committees of Tokushima University Hospital and Yashima General Hospital, and written informed consent was obtained from each patient before inclusion in the study.

Real-time quantitative RT-PCR

In the present study, we remeasured levels of IL-5 and IL-33 mRNA in the remaining scraping of nasal

mucosa samples of the same patients, in which we previously measured H1R mRNA levels [4]. The scrapings of the nasal mucosa were frozen in RNAlater[®] (Applied Biosystems, Foster City, CA, USA) and stored in a tube at -80°C until use. Total RNA was isolated using the RNeasy-RNAqueous-Micro Kit (Applied Biosystems) in accordance with the manufacturer's instructions. RNA samples were reverse-transcribed to cDNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). TaqMan primers and probe were designed using Primer Express primer design software (Applied Biosystems). The sequences of the IL-5 primers were as follows: sense primer, 5'-cactgaagaaatctttcagggaat-3'; antisense primer, 5'-cag-taccccccttgacacagtt-3'. The sequences of the IL-33 primers were as follows: sense primer, 5'-aggcctt-cactgaaacagg-3'; antisense primer, 5'-taccaaagg-caaagcactcc-3'. Universal Probe Library #47 and #27 were used for the IL-5 and IL-33 probes, respectively (Roche). The PUM1 primer and probe kit (Hs 00206469-m1, Applied Biosystems) was used as an internal standard. The transcripts were used for a 40 cycle, 3 step polymerase chain reaction (PCR) with the GeneAmp 7300 Sequence Detection System (Applied Biosystems).

Statistical analysis

Statistical analysis was performed using the unpaired *t* test and the Spearman rank correlation method.

Results

During the peak pollen period, the level of IL-5 mRNA in the nasal mucosa of patients with seasonal allergic rhinitis caused by Japanese cedar pollen receiving preseasonal prophylactic treatment with antihistamines was significantly lower, compared with that of patients without treatment (Figure 1a) ($p < 0.05$). However, there was no difference in the level of IL-33 mRNA in the nasal mucosa of patients between the prophylactic treatment group and the no treatment group (Figure 1b).

Spearman rank correlation analysis showed a significant positive correlation between the expression of IL-5 mRNA in the nasal mucosa and the sum of sneezing and watery rhinorrhea scores in both groups of patients with or without preseasonal prophylactic treatment (Figure 1c) ($r = 0.51$, $p < 0.01$).

Furthermore, there was a significant positive correlation between nasal expression of IL-5 mRNA and that of H1RmRNA in both groups of patients (Figure 1d) ($r = 0.79$, $p < 0.01$).

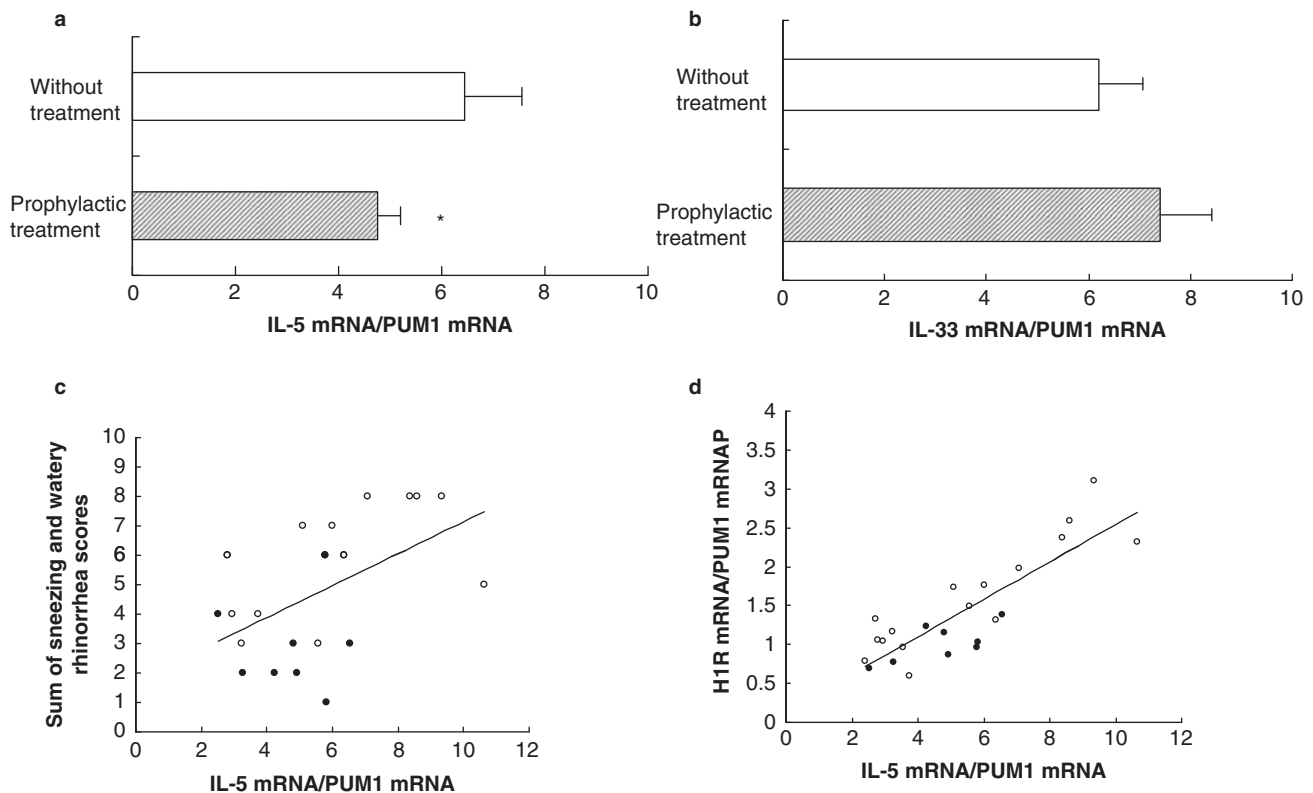


Figure 1. Comparison of the level of IL-5 (a) and IL-33 (b) mRNA in the nasal mucosa in patients with or without preseasonal prophylactic treatment with antihistamines. Data are presented as mean $\pm$ SD. * $p < 0.05$ vs no treatment group. (c) Relationship between the expression of IL-5 mRNA in the nasal mucosa measured in the present study and the sum of sneezing and watery rhinorrhea scores that had been reported in our previous study [4] of the same patients with (filled circles) or without (open circles) preseasonal prophylactic treatment with antihistamines ($r = 0.51$, $p < 0.01$). (d) Relationship between the expression of IL-5 mRNA measured in the present study and that of H1RmRNA that had been reported in our previous study [4] in the nasal mucosa of the same patients with (filled circles) or without (open circles) preseasonal prophylactic treatment with antihistamines ($r = 0.79$, $p < 0.01$).

Discussion

In the present study, the effects of preseasonal treatment with antihistamines on the expression of Th2 cytokines such as IL-5 and IL-33 in the nasal mucosa of patients with seasonal allergic rhinitis caused by Japanese cedar pollen were investigated. This is because in our preliminary study, we measured Th2 cytokines including IL-4, IL-5, IL-9, and IL-13 in the nasal mucosa scraping and only detected IL-5 in this group. At that time, we also detected IL-33, a cytokine that induces eosinophilic infiltration like IL-5. During the peak pollen period, the expression of IL-5 mRNA, but not IL-33 mRNA, in the nasal mucosa of patients with pollinosis receiving preseasonal prophylactic treatment with antihistamine was significantly lower than in those without treatment (Figure 1 a,b). These findings suggested that the suppression of IL-5 gene expression in the nasal mucosa contributes to the slow-onset effects of preseasonal treatment with antihistamines. IL-5 is a Th2 cytokine that plays a key role in eosinophil

activation [8]. Evidence of a pathogenic role of eosinophils in allergic rhinitis has been well established [9,10]. Recently, it has been reported that terfenadine, an antihistamine, inhibited the secretion of IL-5 in human peripheral blood mononuclear cells [11] and that cetirizine, another antihistamine, was reported to inhibit eosinophil chemotaxis in vitro [12]. Therefore, it is suggested that antihistamines possess anti-inflammatory activity to inhibit eosinophil chemotaxis in the nasal mucosa by inhibiting the gene expression of IL-5, in addition to their histamine-blocking effect at H1R.

Subsequently, there was a significant positive correlation between the expression of IL-5 mRNA in the nasal mucosa and the sum of sneezing and watery rhinorrhea scores (Figure 1c). Therefore, it is suggested that down-regulation of the gene expression of IL-5 in the nasal mucosa by preseasonal prophylactic treatment with antihistamines has better therapeutic effects in patients with pollinosis, probably because of their inhibitory effect on eosinophil chemotaxis in the nasal mucosa.

Moreover, there was a significant positive correlation between the expression of IL-5 mRNA in the nasal mucosa and that of H1R mRNA (Figure 1d). Thus, antihistamines seem to down-regulate the gene expression of IL-5 in the nasal mucosa through suppression of H1R signaling. We have shown that histamine up-regulates H1R mRNA in HeLa cells and that histamine-induced up-regulation of H1R gene expression was suppressed by antihistamines [13]. We have also shown that repeated pretreatment with antihistamines suppressed provocation-induced up-regulation of both H1R and IL-4 gene expressions in nasal allergy model rats [14]. On the other hand, GATA-3 is a master regulator of Th2 differentiation and regulates trans-activation of Th2 cytokines including IL-4, IL-5, and IL-13 [15,16]. It was also reported that the expression of GATA-3 is induced by IL-4 through STAT6 activation [17,18]. Therefore, down-regulation of IL-4 gene expression by treatment with antihistamines could cause suppression of IL-5 gene up-regulation through GATA-3 down-regulation. However, the mechanisms underlying the inhibition of IL-5 gene expression by antihistamines remain to be investigated.

IL-33 is a cytokine belonging to the IL-1 superfamily and is known to induce helper T cells, mast cells, eosinophils, and basophils to produce Th2 cytokines [19]. Recently, Sakashita et al. reported that serum levels of IL-33 were significantly higher in patients with Japanese cedar pollinosis than in controls, suggesting a role of IL-33 in the pathogenesis of allergic rhinitis [20]. But, because no suppression of IL-33 mRNA in the nasal mucosa was observed in the present study, it is not likely that IL-33 is involved in the therapeutic effects of preseasonal treatment with antihistamine for pollinosis.

In conclusion, the expression of IL-5 mRNA, but not that of IL-33 mRNA, in the nasal mucosa of patients with seasonal allergic rhinitis caused by Japanese cedar pollen receiving preseasonal prophylactic treatment with antihistamines was significantly lower in comparison with that of patients without treatment during the peak pollen period. Moreover, there was a significant correlation between the expression of IL-5 mRNA and the nasal symptoms or the expression of H1R mRNA. These findings suggest that the down-regulation of IL-5 gene expression in collaboration with the suppression of H1R gene expression in the nasal mucosa provides the basis for better therapeutic effects of preseasonal prophylactic treatment with antihistamines in patients with pollinosis.

Acknowledgment

This work was financially supported by Grant-in-Aid from the Ministry of Health and Welfare of Japan

(Suppression of histamine H1 receptor gene expression using natural sources: a new therapeutic strategy for allergic disease).

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

- [1] Stern MA, Darnel R, Tudor D. Can an antihistamine delay appearance of hay fever symptoms when given prior to pollen season? *Allergy* 1997;52:440–4.
- [2] Kurowski M, Kuna O, Gorski P. Montelukast plus cetirizine in the prophylactic treatment of seasonal allergic rhinitis: influence on clinical symptoms and nasal allergic inflammation. *Allergy* 2004;59:280–8.
- [3] Okubo K, Kurono Y, Fujieda S, Ogino S, Uchio E, Odajima H, et al. Japanese guideline for allergic rhinitis. *Allergol Int* 2011;60:171–89.
- [4] Mizuguchi H, Kitamura Y, Kondo Y, Kuroda W, Yoshida H, Miyamoto Y, et al. Pre-seasonal prophylactic treatment with antihistamines suppresses histamine H₁ receptor mRNA expression in the nasal mucosa of patients with pollinosis. *Methods Find Exp Clin Pharmacol* 2010;32:745–8.
- [5] Igaz P, Novák I, Lázaár E, Horváth B, Héninger E, Falus A. Bidirectional communication between histamine and cytokines. *Inflamm Res* 2001;50:123–8.
- [6] Marone G, Granata F, Spadaro G, Genovese A, Triggiani M. The histamine-cytokine network in allergic inflammation. *J Allergy Clin Immunol* 2003;112(Suppl 4):S83–8.
- [7] Packard KA, Khan MM. Effects of histamine on Th1/Th2 cytokine balance. *Int Immunopharmacol* 2003;3:909–20.
- [8] Kouro T, Takatsu K. IL-5- and eosinophil-mediated inflammation: from discovery to therapy. *Int Immunol* 2009;21:1303–9.
- [9] Pelikan Z, Pelikan-Filipek M. Cytologic changes in the nasal secretions during the late nasal response. *J Allergy Clin Immunol* 1989;83:1068–79.
- [10] Pipkorn U, Karlsson G, Enerback L. The cellular response of the human allergic mucosa to natural allergen exposure. *J Allergy Clin Immunol* 1988;82:1046–54.
- [11] Munakata Y, Umezawa Y, Iwata S, Dong RP, Yoshida S, Ishii T, et al. Specific inhibition of TH2-type cytokine production from human peripheral T cells by terfenadine in vitro. *Clin Exp Allergy* 1999;29:1281–6.
- [12] De Vos C, Joseph M, Leprevost C, Vorng H, Tomassini M, Capron M, et al. Inhibition of human eosinophil chemotaxis and of the IgE-dependent stimulation of human blood platelets by cetirizine. *Int Arch Allergy Appl Immunol* 1989;88:212–15.
- [13] Das AK, Yoshimura S, Mishima R, Fujimoto K, Mizuguchi H, Dev S, et al. Stimulation of histamine H₁ receptor up-regulates histamine H₁ receptor itself through activation of receptor gene transcription. *J Pharmacol Sci* 2007;103:374–82.
- [14] Mizuguchi H, Hatano M, Matsushita C, Umehara H, Kuroda W, Kitamura et al. Repeated pre-treatment with antihistamines suppresses transcriptional up-regulations of histamine H₁ receptor and interleukin-4 genes in toluene-2,4-diisocyanate-sensitized rats. *J Pharmacol Sci* 2008;108:480–6.

- [15] Zheng W, Flavell RA. The transcription factor GATA-3 is necessary and sufficient for TH2 cytokine gene expression in CD4 T cells. *Cell* 1997;89:587–96.
- [16] Barnes PJ. Role of GATA-3 in allergic diseases. *Curr Mol Med* 2008;8:330–4.
- [17] Murphy KM, Reiner SL. The lineage decisions of helper T cells. *Nat Rev Immunol* 2002;2:933–44.
- [18] Murphy KM, Ouyang J, Farrar D, Yang J, Ranganath S, Asnagli H, et al. Signaling and transcription in T helper development. *Annu Rev Immunol* 2000;18:451–94.
- [19] Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 2005;23:479–90.
- [20] Sakashita M, Yoshimoto T, Hirota T, Harada M, Okubo K, Osawa Y, et al. Association of serum interleukin-33 level and the interleukin-33 genetic variant with Japanese cedar pollinosis. *Clin Exp Allergy* 2008;38:1875–81.