



Effect of allergic rhinitis on the expression of human β -defensin 2 in tonsils

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ABSTRACT

Background: Human β -defensins (HBDs) are a newly identified family of antimicrobial peptides that are expressed by epithelia on mucosal surfaces. Exposure of airway epithelial cells to T_H2 -type cytokines results in a significant decrease in the antimicrobial activity of the cells.

Objective: To investigate the effect of allergic rhinitis on the expression of HBD-2 in tonsils and adenoids. **Methods:** Palatine tonsils and adenoids were obtained from 30 patients with no history of recurrent tonsillitis. The patients were divided into 2 groups: allergic rhinitis and nonallergic rhinitis groups. Real-time polymerase chain reaction analysis was used to measure messenger RNA (mRNA) levels of HBD-2 mRNA in tonsil and adenoid tissue samples from the 2 patient groups. Immunofluorescent staining and enzyme-linked immunosorbent assay (ELISA) were used to evaluate the expression of HBD-2 protein in tonsil and adenoid tissues. The concentration of the cytokines interleukin (IL) 4, IL-5, and interferon γ (IFN- γ) in tissue homogenates was measured by ELISA.

Results: Immunofluorescent staining data demonstrated the expression of HBD-2 protein in the surface epithelia of tonsils, and a marked difference in the staining intensity was observed between 2 groups. HBD-2 mRNA and protein levels in the tonsils were significantly lower in the allergic rhinitis group than that in the nonallergic rhinitis group ($P = .03$ and $P = .04$, respectively). IL-5 and IFN- γ were not detected, and no significant difference was found in IL-4 concentrations in tonsil homogenates between the 2 groups.

Conclusion: Allergic rhinitis suppresses HBD-2, an epithelial antimicrobial peptide, in the tonsils.

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Introduction

Defensins are a newly delineated family of effector molecules with a considerable potential contribution to host defense, inflammation, and cytotoxicity in humans. Defensins have a wide range of antimicrobial activities, encompassing gram-positive and gram-negative bacteria, fungi, and viruses.^{1–4} Human β -defensin 2 (HBD-2) is the first human defensin reported to be produced after stimulation of epithelial cells by microorganisms, including gram-positive and gram-negative bacteria and fungi such as *Candida albicans*, or by cytokines, such as tumor necrosis factor α (TNF- α) and interleukin (IL) 1 β .⁵ HBD-2 is locally expressed at the messenger RNA (mRNA) and protein levels in inflammatory skin lesions, such as psoriasis.^{5,6} HBD-2 has been also found in infected lung epithelia of patients with cystic fibrosis.⁷

Adaptive immunity is known to function through specialized structures, such as tonsils and adenoids, with a characteristic mucosal-lymphoid association (mucosa-associated lymphoid tissue).⁸ HBD-2 is strongly expressed in tonsil tissue, compared with nasal and paranasal sinus mucosa and adenoids.⁹ Exposure of airway epithelial cells to T_H2 cytokines reduced levels of HBD-2 mRNA and significantly decreased the antimicrobial activity of cells.¹⁰ Furthermore, mice with allergic airway inflammation had significantly more viable bacteria in their lungs after infection.¹⁰ However, although tonsils and adenoids are important for protection against microorganisms and for induction of allergic rhinitis in children, the influence of allergic reactions on the expression of HBD-2 in tonsils and adenoids is poorly understood. The aim of the present study was to investigate the effect of allergic rhinitis on the expression of the antimicrobial peptide (AMP) HBD-2 in tonsils and adenoids.

Methods

Patients

We reviewed medical records of 30 patients who were diagnosed as having tonsil and adenoid hypertrophy and underwent tonsillectomy with/without adenoidectomy between January 2008

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and May 2010 at the Seoul National University Hospital. Indications for tonsillectomy with/without adenoidectomy were enlarged tonsils and adenoids and occurrence of sleep-disordered breathing, such as mouth breathing, snoring, and apnea during sleep.¹¹ Tonsil and adenoid size was readily identified using a tonsil and adenoid grading scale.¹² Patients with a history of recurrent tonsillitis or asthma were excluded to avoid local effects of inflammation on the tonsils and adenoids at the time of the enrollment. The study population consisted of 25 men (83%) and 5 women (17%). The median age at the time of surgery was 6.5 years (range, 3–14 years). The patients were divided into 2 groups: allergic rhinitis and nonallergic rhinitis. The allergic rhinitis group was defined as the group of patients who presented with clinical symptoms, including sneezing, itching, watery rhinorrhea, and nasal obstruction, and more than 3 positive signs on the Multiple Allergen Simultaneous Test. This study was approved by the institutional review board at Seoul National University Hospital (H-1006034320), and written informed consent was provided by the parents of patients, all of whom were minors.

Tissue Collection and Preparation

Tonsils and adenoids were obtained when patients underwent tonsillectomy and adenoidectomy, respectively. Tissue samples were cut into 3 portions. One portion was immediately flash frozen in liquid nitrogen and stored at -70°C for subsequent RNA studies. The second portion was fixed with 4% paraformaldehyde in 0.1M phosphate-buffered saline (PBS; pH 7.2) overnight at 4°C , followed by immersion in 30% sucrose solution, and then embedded in Tissue-Tek OCT compound media (Sakura Finetek USA Inc, Torrance, California) for preparing frozen sections. The third portion was prepared as a homogenate. Tissue was first put in a 2-mL tube. Before grinding with homogenizer, 1 mL of PBS was added for real-time polymerase chain reaction (PCR) or 1 mL of protein lysis buffer was added for Western blotting. The supernatant is used for study after the sample is centrifuged at $12,600 \times g$ 20 minutes at 4°C .

Real-Time Reverse Transcription–PCR for HBD-2 in Tonsil and Adenoid Tissues

Total RNA was prepared from the tonsil and adenoid tissues by using TriZol reagent (Invitrogen, Carlsbad, California). Complementary DNA (cDNA) was synthesized using Superscript reverse transcription (RT) (Invitrogen) and oligo(dT) primers (Fermentas, Burlington, Ontario, Canada). For analysis of HBD-2 (Hs00175474_ml) and *GAPDH* (Hs99999905_ml) expression, predeveloped assay reagent kits of primers and probes were purchased from Applied Biosystems (Foster City, California). Sequences of primers and probes used for PCR amplification of HBD-2 and *GAPDH* cDNA were as described in a previous report.⁹ Amplification of HBD-2 and *GAPDH* cDNA was performed in MicroAmp optical 96-well reaction plates (Applied Biosystems). Quantitative real-time PCR (Taqman) analysis was performed with ABI PRISM 7500 Sequence Detection System (Applied Biosystems). We subjected the plate (master mix of the reaction mix + 15 μL of the PCR mix + 5 μL of cDNA on a 96-well plate by Taqman protocol) to the following cycling: 50°C , 2 minutes; 95°C , 10 minutes; and 40 cycles of 95°C , 15 seconds; and 60°C , 1 minute. The mean transcript levels of genes were then normalized to the mean *GAPDH* levels.

Immunohistochemistry Staining for HBD-2

Tissue sections (4 μm) were cut from pretreated archival tissue blocks. Immunohistochemical studies for HBD-2 (1:500 [ab63982]; Abcam, Cambridge, United Kingdom) were performed on formalin-fixed, paraffin-embedded, 4- μm -thick tissue sections. Deparaffinization of all sections was performed through a series of xylene baths and rehydration through a series of graded alcohol solutions.

Immunostaining was performed with a labeled avidin-biotin technique using the rabbit polyclonal to β_2 -defensin antibody (Abcam) as the primary antibody and the Histostain-Plus Bulk Kit (Invitrogen, Camarillo, California). An unmasking reagent (Vector Laboratories Inc, Burlingame, California) was used in the pre-stain. After blocking endogenous peroxidase activity with 3% hydrogen peroxidase in methyl alcohol for 10 minutes, the tissue was blocked with serum blocking solution for 30 minutes, and then primary antibody for HBD-2 was incubated for 1 hour at room temperature. The biotinylated secondary antibody and horseradish peroxidase–streptavidin–peroxidase were used for detection of HBD-2. The 3,3'-diaminobenzidine substrate kit for peroxidase (Vector Laboratories) was used for visualizing the antibody, and a chromogen and Mayer hematoxylin (Dako, Glostrup, Denmark) counterstain was applied.

Immunofluorescent Staining for HBD-2

The tissues in Tissue-Tek OCT compound media were cryosectioned to 10- μm thickness with a cryostat (-23°C chamber) and mounted on glass slides. The tissue sections were warmed for 15 minutes at 37°C and then rehydrated with PBS for 10 minutes. The sections were then permeabilized, and nonspecific antigenic sites were blocked using a blocking solution (0.3% Triton X-100 and 10% normal donkey serum in PBS). The sections were incubated overnight at 4°C with the primary antibody, a rabbit anti-HBD-2 antibody (Abcam), diluted at 1:200. The specificity of the immunofluorescent stain was controlled by preincubation of the anti-serum with the peptide antigen (the ratio of concentration of peptide to antibody was 1:1) and omitting the primary antibody. The sections were extensively washed with PBS and then incubated in the dark for 1 hour at room temperature with the secondary antibody (anti-rabbit Alexa Fluor 488; Invitrogen) diluted in 0.01% Triton X-100 and 1% normal donkey serum in PBS to a final dilution of 1:1000. Finally, the sections were extensively washed with PBS and overlaid with 20 μL of Gel/Mount and a cover glass. The sections were examined under a confocal microscope (LSM510 META; Carl Zeiss, Jena, Germany). The immunofluorescence data were evaluated qualitatively and not quantitatively.

Enzyme-Linked Immunosorbent Assay for HBD-2 in Tissue Homogenates

Tonsil and adenoid homogenates were centrifuged at $14,700 \times g$ for 10 minutes at 4°C . The supernatant was withdrawn and diluted to a final concentration of 20 $\mu\text{g}/\text{mL}$ in PBS and then added to a polyvinyl chloride microtiter plate coated with 50 μL of the antigen solution. Nonspecific antigen-antibody reactions were blocked with 200 μL of blocking buffer and 5% nonfat dry milk and PBS in each well. After washing the plate twice with PBS, 100 μL of the antibody solution (anti-HBD2-biotin [ab83509]; Abcam), diluted at 1:400 in 1% BSA, was added and the plates were incubated for 2 hours at room temperature. After washing with PBS, a streptavidin–horseradish peroxidase solution (1:2,000) was added and allowed to react with the substrate for 15 minutes. The enzyme-substrate reaction was terminated by adding 100 μL of stop solution. We used TMB microwell peroxidase (KPL Inc, Gaithersburg, Maryland) as substrate buffer and 1M hydrochloride as stop solution. We used β_2 -defensin protein (Active [ab1257489]; Abcam) as a source of standard HBD. The absorbance at 450 nm of each well was measured using a plate reader.

Enzyme-Linked Immunosorbent Assay for IL-4, IL-5, and Interferon γ in Tissue Homogenates

The levels of IL-4, IL-5, and interferon γ (IFN- γ) were assayed in the tissue homogenates by using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis,

Minnesota) according to the manufacturer's instructions. After measuring the optical density at 450 nm, the concentrations of IL-4, IL-5, and IFN- γ were determined by interpolation from a standard curve.

Statistical Analysis

Statistical analysis was performed using SPSS statistical software, version 13.0 (SPSS Inc, Chicago, Illinois). The Mann-Whitney test was used for comparing the allergic rhinitis and nonallergic rhinitis groups (unpaired). $P < .05$ was considered statistically significant.

Results

Patient Characteristics

The clinical data of patients in the allergic rhinitis and nonallergic rhinitis groups are given in Table 1. Both groups consisted of 15 patients: the allergic rhinitis group included 13 men and 2 women (mean age, 6.0 years), and the nonallergic rhinitis group included 12 men and 3 women (mean age, 7.1 years). Statistically significant differences were observed between the 2 groups with regard to the mean (SD) serum total IgE levels (205.5 [38.3] vs 26.0 [5.0] kIU/L; $P < .001$) and peripheral blood eosinophil counts (300.1 [29.7] vs 181 [30.2]; $P = .01$). However, the size of the tonsils and adenoids did not significantly differ between the groups.

Assessment of HBD-2 mRNA Levels in Tonsil and Adenoid Tissues

Real-time RT-PCR analysis revealed that HBD-2 mRNA was expressed in both tonsil and adenoid tissues. The level of HBD-2 transcripts was significantly lower in the tonsils of the patients in the allergic rhinitis group than in those of the patients in the nonallergic rhinitis group ($P = .03$, Fig 1A). HBD-2 mRNA expression in adenoid tissue was also lower in the patients in the allergic rhinitis group compared with those in the nonallergic rhinitis group; however, this finding was not statistically significant ($P = .56$, Fig 1B).

Immunocytochemistry Staining for HBD-2

Microscopic analysis revealed positive staining for HBD-2 peptide in the surface epithelia and submucosal area of the tonsils and adenoids. Although the staining intensity was not quantitatively analyzed, qualitatively immunoreactive cells were scattered in the surface epithelia and submucosal area of the tonsils and adenoids in the nonallergic rhinitis group (Figs 2A, B, and D) compared with the allergic rhinitis group (Figs 2C and E).

Table 1

Clinical characteristics of the study patients

Characteristic	AR (n = 15)	NAR (n = 15)	P value
M:F	12:3	13:2	
Mean age, y	6.0	7.1	.19
Tonsil size	3.1	3.1	>.99
Adenoid size	2.2	2.3	.72
Serum total IgE, mean (SD), kIU/L	205.5 (38.3)	26.0 (5.0)	<.001
Peripheral blood eosinophils, mean (SD), $\times 1000/\mu\text{L}$	300.1 (29.7)	181 (30.2)	.009

Abbreviations: AR, allergic rhinitis; NAR, nonallergic rhinitis.

Immunofluorescent Staining for HBD-2

Confocal microscopic analysis revealed positive staining for HBD-2 peptide in the surface epithelia of tonsils. Although the staining intensity was not quantitatively analyzed, a qualitatively marked decrease was detected in the allergic rhinitis group (Fig 3A) compared with the nonallergic rhinitis group (Fig 3B).

ELISA for HBD-2 in Tissue Homogenate

ELISA was performed to measure the amounts of HBD-2 protein in the tonsil and adenoid tissues of patients. In patients with allergic rhinitis, the mean (SD) HBD-2 level in the tonsils was significantly lower than that in the tonsils of patients with nonallergic rhinitis (1,318 [268.4] vs 3,779 [1,078]; $P = .04$; Fig 4A); this finding demonstrated the inhibitory effect of allergic rhinitis on the expression of HBD-2 in tonsils. However, no significant difference was found in HBD-2 levels in adenoid tissue between the 2 groups ($P = .12$, Fig 4B).

Levels of IL-4, IL-5, and IFN- γ in Tissue Homogenates

IL-5 and IFN- γ in tonsils and IFN- γ in adenoids were not detected by ELISA in either group. The IL-4 level in tonsils, measured by ELISA, was low in both groups, and the difference was not significantly significant ($P = .95$, Fig 5A). In addition, the IL-4 and IL-5 levels in adenoids did not reveal any significant differences between the 2 groups ($P = .87$, Fig 5B, and $P = .99$, Fig 5C, for IL-4 and IL-5, respectively).

Discussion

Human palatine tonsils are composed of lymphoid tissues covered by a stratified, nonkeratinizing epithelium. With regard to innate host defenses, tonsil epithelia not only provide a physical barrier to invading microorganisms but also act through the synthesis of an array of specific peptides.⁸ These peptides are collectively termed AMPs. In general, AMPs are small (1–10 kDa)

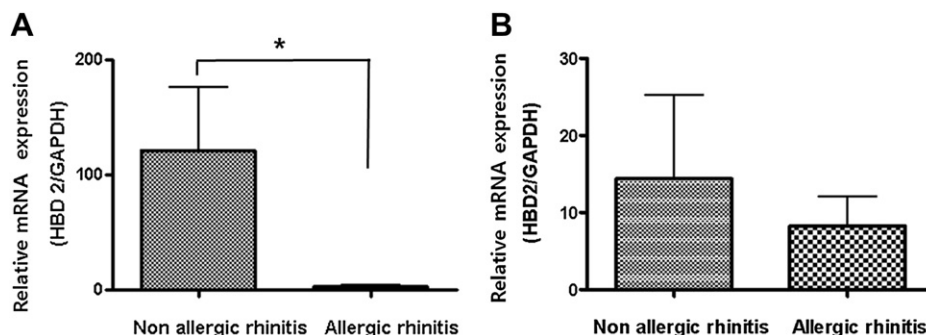


Figure 1. Human β -defensin 2 (HBD-2) messenger RNA (mRNA) expression in tonsil and adenoid tissues. The expression of HBD-2 mRNA was measured by real-time reverse transcription–polymerase chain reaction in tonsil (A) and adenoid (B). The allergic rhinitis group had a significantly lower level of HBD-2 mRNA than the nonallergic rhinitis group in tonsil tissues ($P = .03$). On the other hand, no significant difference was found between the HBD-2 mRNA levels in the adenoid tissues of the allergic rhinitis and nonallergic rhinitis groups ($P = .56$). * $P < .05$.

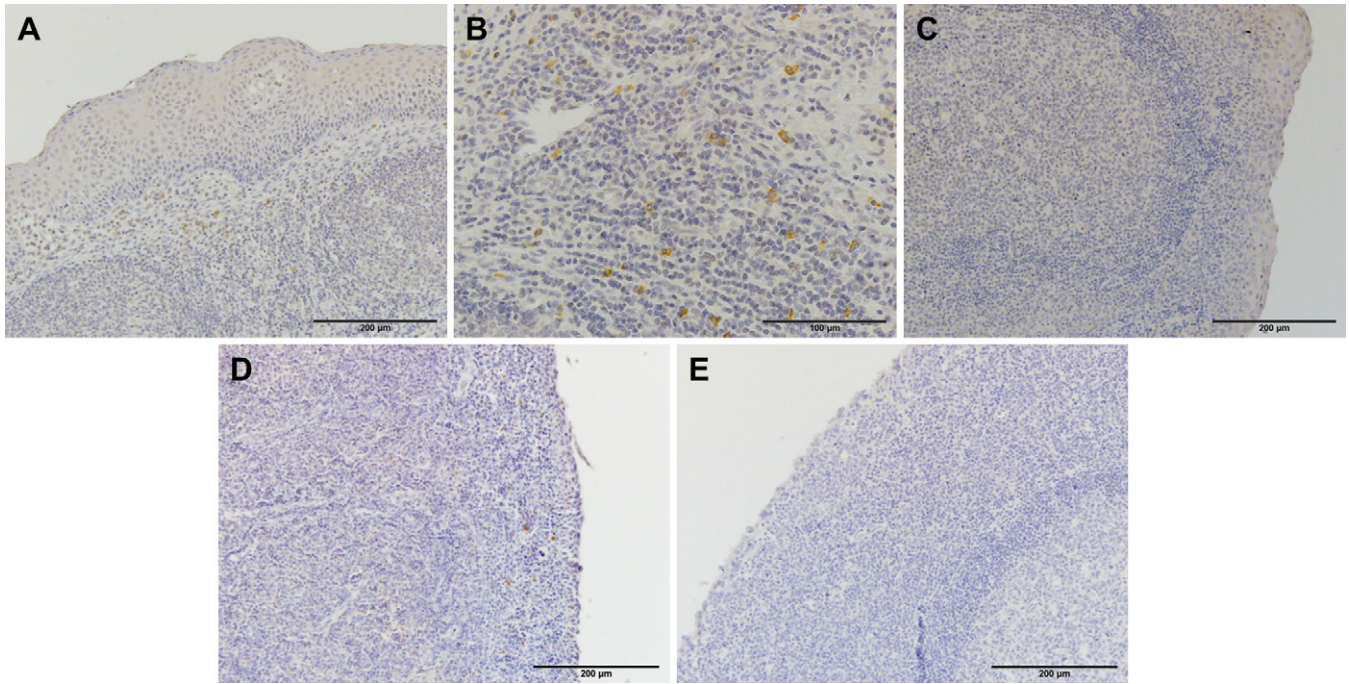


Figure 2. Immunohistochemistry staining of human β -defensin 2 (HBD-2) in tonsil (A and B) and adenoid (D) from the nonallergic rhinitis group and tonsil (C) and adenoid (E) from the allergic rhinitis group. HBD-2 protein expression in tonsil and adenoid was markedly lower in the allergic rhinitis group than that in the nonallergic rhinitis group.

and structurally diverse molecules that display broad-spectrum antimicrobial activity against a range of bacteria, fungi, and enveloped viruses.¹ Recently, the expression of many AMPs, such as HBD, cathelicidin LL-37, and liver-expressed AMP, has been demonstrated in tonsils.¹ Among them, HBD-2 is known to be an important AMP because its expression is related to allergic inflammation.⁷

The concept that the respiratory tract mucosa acts as an immune system organ is now widely accepted.⁹ It was proposed that proinflammatory stimuli stimulate the expression of HBD-2 in airway epithelia, resulting in an increase in HBD-2 mRNA and peptide abundance.^{13,14} Furthermore, HBD-2 is known to be present in higher levels in bronchoalveolar lavage or plasma specimens from patients with inflammatory lung diseases, such as cystic fibrosis,¹⁴ pneumonia,¹⁵ and atypical mycobacterial infections,¹⁶ compared with that in specimens from healthy persons. The expression of HBD-2 in human palatine tonsils has been reported previously.¹⁷

One study found that an airway allergic inflammation inhibits the antibacterial host defense.¹⁰ The antimicrobial activity of airway epithelia and the induction of antimicrobial factors are inhibited by T_H2 cytokines in vitro.¹⁰ T_H2 -based inflammation also suppresses the host defense and inhibits expression of AMPs in skin.^{18,19} The exact mechanism by which T_H2 cytokines inhibit the antimicrobial host defense is not clear yet; however, it has been speculated that T_H2 cytokines inhibit the TNF- α /nuclear factor (NF)- κ B system through activation of STAT6, which acts as a transcriptional inhibitor of NF- κ B-dependent gene expression.²⁰ Many reports have demonstrated that upstream and downstream HBD-2 is regulated by the NF- κ B and mitogen-activated protein kinase (MAPK)/AP-1 pathways. Wehkamp and colleagues²¹ reported that the parallel activation of NF- κ B and AP-1 is needed for full transcriptional activation of the HBD-2 promoter after IL-1 β stimulation and treatment of keratinocytes with supernatants of *Pseudomonas aeruginosa*. On the other hand, another study demonstrated induction of HBD-2 expression through *Fusobacterium nucleatum* in

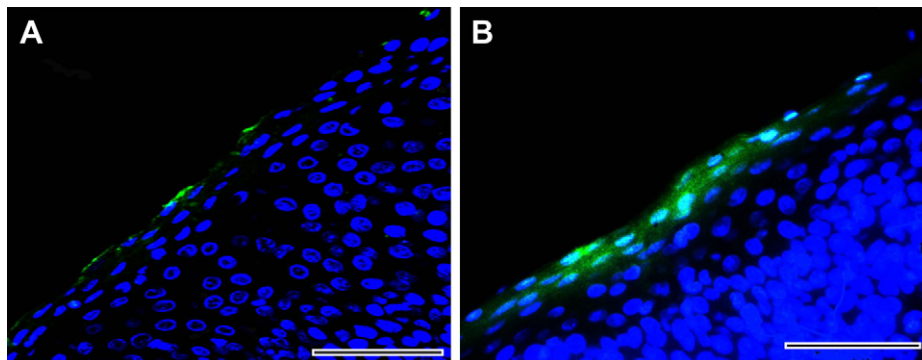


Figure 3. Immunofluorescent staining of human β -defensin 2 (HBD-2) in tonsillar epithelium in the allergic rhinitis group (A) and the nonallergic rhinitis group (B). HBD-2 protein expression in tonsils was markedly lower in the allergic rhinitis group than that in the nonallergic rhinitis group. The tissue samples were treated with an HBD-2-specific antibody and visualized with Alexa Fluor 488 (green). The nuclei were visualized by staining with DAPI blue. Scale bar = 100 μ m.

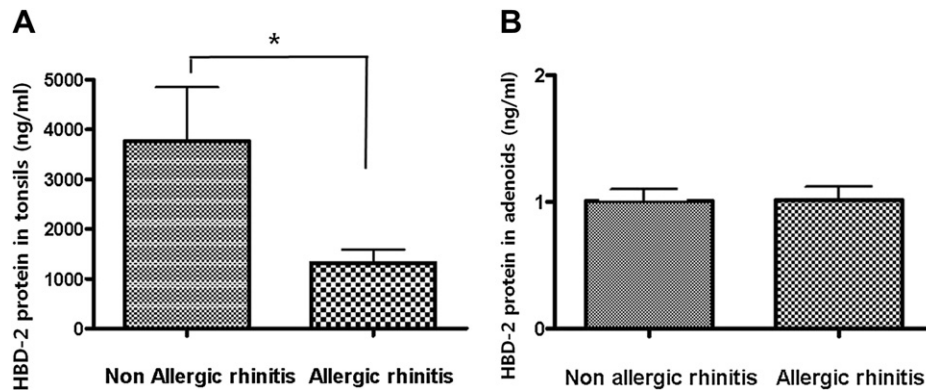


Figure 4. Assessment of human β -defensin 2 (HBD-2) protein in tonsil and adenoid tissue homogenates. HBD-2 protein levels in tissue homogenates in tonsils (A) and adenoids (B) were assessed by enzyme-linked immunosorbent assay. A significant decrease was seen in tonsillar HBD-2 protein levels in the allergic rhinitis group compared with the nonallergic rhinitis group ($P = .04$). However, no significant difference was seen in the HBD-2 protein levels of adenoids between the 2 groups ($P = .12$). * $P < .05$.

human gingival epithelial cells independent of NF- κ B activation.²² Steubesand et al²³ demonstrated that both NF- κ B and AP-1 activation are required for full upregulation of the HBD-2 mRNA after treatment with either supernatants of *C albicans* or the coculture of *C albicans* with polymorphonuclear cells. Inhibition of the NF- κ B or MAPK/AP-1 pathway significantly reduced the induction of HBD-2 expression, confirming the central role of both transcription factors.²³ This mechanism could also account for the inhibitory effect of IL-4 on the lipopolysaccharide (LPS)-induced activation of neutrophils.²⁴ A complete understanding of how the T_H2 cytokines suppress antimicrobial activity of airway epithelial cells requires additional studies.

Our study was the first one measuring local cytokine levels in tonsil and adenoid tissues of allergic and nonallergic patients. Contrary to our expectation, we could not find any differences in the levels of T_H2 cytokines, IL-4, and IL-5 in tonsil and adenoid tissues between the 2 groups. One of the reasons may be the small amount of cytokine production in tonsil and adenoid tissues (IL-4 in tonsils, 23.9 pg/mL in the allergic rhinitis group and 24.8 pg/mL in the nonallergic rhinitis group; $P = .95$; IL-4 in adenoids, 6.1 pg/mL in the allergic rhinitis group and 6.4 pg/mL in the nonallergic rhinitis group; $P = .87$; IL-5 in adenoids, 16.2 pg/mL in the allergic rhinitis group and 16.3 pg/mL in the nonallergic rhinitis group; $P = .99$). In addition, some reports have demonstrated that there are no significant differences in local T_H2 cytokine levels, regardless of allergic condition. A study performed by Lee et al,²⁵ including 20 patients (10 patients with perennial allergic rhinitis and 10 controls), found that the IL-5 level in the nasal mucosa did not differ significantly between allergic and nonallergic rhinitis

patients. Ohashi et al²⁶ compared the productions of IgE, IL-5, and IFN- γ by allergen-stimulated peripheral blood mononuclear cells between atopic and nonatopic patients. They did not find any significant differences in the levels of IgE, IL-5, and IFN- γ between the 2 groups. Silva et al²⁷ demonstrated that IL-5, IL-8, and IFN- γ cytokine levels by RT-PCR analysis in the nasal mucosa were not significantly different between atopic and nonatopic patients. A recent study reported that HBDs expression in tonsil tissues was reduced in allergic rhinitis; however, this study also failed to show local cytokine levels in tonsil tissues.²⁸

Tonsils and adenoids are specialized structures of nasal-associated lymphoid tissue, and they play important roles in the protection against microorganisms. Because HBD-2 is highly expressed in tonsils and adenoids, it is considered an important factor in defense mechanisms. Lehrer et al²⁹ reported that β -defensins act as selective chemoattractants for monocytes and that specialized antigen-presenting cells, primarily consisting of macrophages along with dendritic cells, are present in the germinal center in palatine tonsils. These findings suggest that β -defensins play an important role in the innate immunity of palatine tonsils.

Allergic rhinitis is a common disease, and its incidence is increasing in children. We tried to evaluate whether the expression of HBD-2 in tonsils and adenoids is altered in patients with allergic rhinitis, and we found that there was a significant decrease of HBD-2 mRNA expression in the tonsils of allergic rhinitis patients compared with patients with nonallergic rhinitis; however, such differences were not detected in adenoids. Allergic rhinitis patients had a significantly lower level of HBD-2 expression in tonsils than the nonallergic rhinitis patients but not in adenoid tissues. Possible

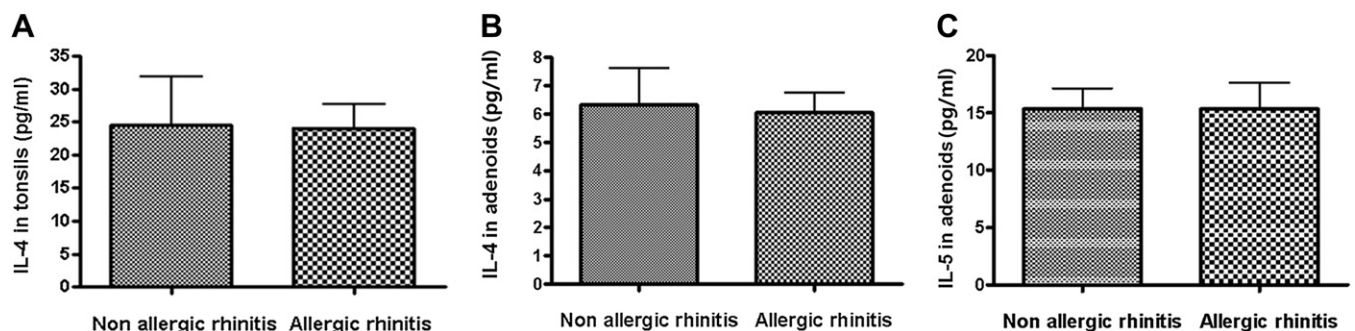


Figure 5. Cytokine levels in tonsil and adenoid tissue homogenates. The levels of interleukin (IL) 4, IL-5, and interferon γ (IFN- γ) in tonsil and adenoid tissue homogenates were measured by enzyme-linked immunosorbent assay (ELISA). (A) IL-4 in tonsils; (B) IL-4 in adenoids; and (C) IL-5 in adenoids. Cytokine levels tended to be lower in the allergic rhinitis group than those in the nonallergic rhinitis group; however, the differences between the 2 groups were not statistically significant (IL-4 in tonsils, $P = .95$; IL-4 in adenoids, $P = .87$; IL-5 in adenoids, $P = .99$). IL-5 and IFN- γ in tonsils and IFN- γ in adenoids were not detected by ELISA.

reasons for this discrepancy in HBD-2 expressions of tonsil and adenoid tissues are that HBD-2 is more abundantly expressed in the epithelia of tonsils, skin, and airway, rather than in adenoids, and that tonsil tissue was obtained with an intact epithelium, whereas adenoid epithelia were easily damaged during surgery owing to a lack of capsules.

Induction of HBD-2 by LPS has already been demonstrated in vitro.²⁴ Therefore, it is highly likely that there is a difference in the expression levels of HBD-2 in tissues according to the status of bacteria-induced inflammation. Presumably, a more intensive and prolonged exposure to microorganisms or allergic reaction is needed for the modulation of defensins in adenoids. In future studies, we will address the expression of HBD-2 in pure adenoidal epithelial cells and the difference in HBD-2 expression in inflamed tissues stimulated by bacteria or LPS between allergic and nonallergic groups.

In this study, we detected HBD-2 protein expression in palatine tonsils by immunofluorescent staining and ELISA. In addition, we demonstrated significant decreases in the expressions of HBD-2 mRNA and protein in the tonsils of allergic rhinitis patients compared with those of patients with nonallergic rhinitis. These findings suggest that the adaptive immune response can modulate the innate immune response and that the downregulation of tonsillar HBD-2 expression by allergic rhinitis might provoke an increased susceptibility to tonsillar infection in allergic rhinitis patients.

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