

The influence of tonsillectomy on allergic diseases in pediatric patients

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ABSTRACT

Background: The influence of tonsillectomy on allergic airway diseases is not well known.

Objectives: In the present study, the influence of tonsillectomy on perennial allergic rhinitis (PAR) and bronchial asthma (BA) among pediatric subjects was prospectively investigated.

Methods: The tonsillectomy (surgery group) and the age-matched non-surgical subjects (control group) were examined and followed prospectively. In addition, immunological analysis was conducted.

Results: After *in vitro* allergen stimulation, the production of a small number of allergen-specific Th2 cells was induced in the tonsillar cells, even in sensitized subjects. Flow cytometry analysis detected more effector regulatory T cells (Tregs) in the tonsils than in peripheral blood. Clinically, after surgery, the PAR and BA symptoms improved in the surgery group but not in the control group. The total IgE in the surgery group was significantly lower than in the control group; after surgery, IgE levels slightly increased but remained lower. The post-operative *Dermatophagoides farinae* (Der f)-specific IgE level increased in the sensitized subjects but not in the non-sensitized subjects.

Conclusion: Tonsillectomy did not improve the underlying mechanisms of the allergy, however the decreased risk of infection and upper airway obstruction could lead to improved symptoms of allergic airway diseases.

1. Introduction

The tonsils are part of mucosa-associated lymphoid tissue and located in the respiratory tract gateway, where various foreign antigens, such as inhaled and food allergens and microbes, can enter. The various mucous membranes of the body are involved in the development of mucosal immune system. Based on their anatomical location and the development of crypt structures that involve lymphoepithelial symbiosis [1], the tonsils have been considered an organ suitable for capturing antigens. Furthermore, because tonsils have efferent but not afferent lymphatic vessels, they are expected to induce the production of memory cells [2], which spread to the peripheral blood through the mucosal immune system [3,4]. In the literature, the effector cells for polio and

nasal influenza vaccines were described as being induced in the tonsils and migrating to the effector sites. Moreover, the immune cells induced in the tonsils could cause psoriasis [5] and IgA nephropathy [6] in the effector sites. However, the function of tonsils, particularly in allergic diseases, remains unclear.

Regulatory T cells (Tregs) play critical roles in the induction of immune tolerance to self and foreign antigens [7]. The percentage of Tregs (e.g., FoxP3⁺CD4⁺ T cells) was reported higher in palatine tonsils than in peripheral blood mononuclear cells (PBMCs), and the generation of allergen-specific Tregs for oral tolerance to common allergens occurs in human tonsils [8]. Through a common mucosal immune system, the Tregs induced in the tonsils are probably continuously supplied to mucosal effector sites for protection from overwhelming immune

Abbreviations: AMC, adenoid mononuclear cells; PBMCs, peripheral blood mononuclear cells; eTregs, effector regulatory T cells; BA, bronchial asthma; Der p, *Dermatophagoides pteronyssinus*; Der f, *Dermatophagoides farinae*; HDM, house dust mite; rTregs, resting Treg cells; PAR, perennial allergic rhinitis; RT, recurrent tonsillitis; TMCs, tonsil mononuclear cells; SMS, symptom medication score.

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responses.

The nasopharyngeal tonsils (i.e., adenoids) and the palatine tonsils grow during childhood [9] and have an important role in the development of immunity in children. However, surgical removal of the adenoids and palatine tonsils is frequently performed in pediatric patients who have tonsillar hypertrophy or recurrent tonsillitis (RT) [10,11]. Removal of the tonsils might result in alteration of the immune response in effector sites due to the lack of induced memory cells or regulatory cells in the tonsils.

As mentioned above, the function of tonsils in allergic diseases remains unclear. In a recent study, FoxP3+CD4⁺ T cells were subdivided into two phenotypically and functionally distinct subpopulations based on their CD45RA expression: CD45RA-Foxp3^{high} effector Tregs (eTregs) and CD45RA + Foxp3^{low} naïve Tregs [12,13]. However, FoxP3+CD4⁺ T cells do not necessarily have a role in immune regulation.

In the present study, the influence of tonsillectomy on allergic rhinitis (AR) and bronchial asthma (BA) was prospectively examined among pediatric patients. In addition, the functional analysis of Tregs was conducted.

2. Materials and methods

2.1. Subjects

The study included subjects diagnosed with tonsillar hypertrophy or RT, defined as ≥ 4 times per year, and underwent tonsillectomy between March 2012 and November 2014 at Chiba University Hospital or Chiba Children's Hospital. Pediatric patients who visited the otolaryngology department of Chiba Children's Hospital during the same period for allergic diseases, otitis media, or otic fistula surgery and were not diagnosed with tonsillar hypertrophy or RT were enrolled as the control group. Children who had craniofacial or airway abnormalities, syndromic diagnoses, systemic diseases, or suspicious congenital or acquired immune deficiency were excluded. This study was approved by the ethics committees of Chiba University and Chiba Children's Hospital and was performed in accordance with the principles stated in the Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects. Written informed consent for study participation was obtained from the parents of all children.

2.2. Examined items

2.2.1. Diagnosis of allergic airway diseases and evaluation of symptoms

The presence of complications and changes in the symptoms of AR or BA, the frequency of fever, and the *Dermatophagoides farina* (Der f)-specific and total IgE levels were recorded before, and 1 year and 2 years after study participation or surgery. The changes in these variables were compared between the surgery and control groups as well as within each group. In subjects not diagnosed with asthma or AR at the time of study participation, the incidence of AR and BA was examined during the follow-up period. Atopic individuals were diagnosed based on a specific IgE level ≥ 2 (≥ 0.70 UA/mL) based on fluorescent enzyme immunoassay (SRL, Tokyo, Japan) of the tested allergens including house dust mite, cedar pollen, cat dander, and egg white. Non-atopic individuals were defined as those without specific IgEs to these representative four allergens tested. In Japan, house dust mite, cedar pollen, and cat dander are the most major inhaled antigens that cause allergic rhinitis [14–16]. Most of the Japanese population who are sensitized to aeroallergens is positive to at least one of these four allergens.

A diagnosis of perennial AR (PAR) was based on the following criteria: (1) representative AR symptoms of paroxysmal sneezing, rhinorrhea, and nasal congestion; (2) positive sensitization to Der f. The severity of the symptoms was evaluated on a four-point scale (0, none; 1, mild; 2, moderate; 3, severe) in accordance with the Practical Guidelines for the Treatment of Allergic Rhinitis in Japan [17] and based on

interviews with the parents. In addition, the use of anti-allergy drugs, such as antihistamines, leukotriene receptor antagonists, and nasal steroids was examined and assessed as medication scores according to guidelines [17]. The use of antihistamine or leukotriene receptor antagonist was scored as 1 and nasal steroids as 2.

The diagnosis of BA was made by pediatric specialists based on symptoms and reversible bronchoconstriction. Patients or their parents were asked to complete a daily diary of respiratory symptoms (e.g., recurrent wheezing, dyspnea, exercise-induced symptoms, and waking up at night because of symptoms); these diaries were checked by a doctor. In accordance with the Japanese Guidelines for Childhood Asthma 2014, true asthma severity was differentiated from apparent severity, and the current treatment was taken into consideration [18]. True asthma severity was evaluated on a four-point scale: 0, in remission; 1, intermittent; 2, mild persistent; 3, moderate persistent; 4, severe persistent.

2.2.2. Analysis of Der f-specific interleukin (IL)-4- and IL-5-producing cells in tonsillar mononuclear cells (TMCs), adenoid mononuclear cells (AMCs), and PBMCs

The mite-specific peripheral blood cells that produce IL-4 and IL-5 and the tonsils of 16 subjects who underwent tonsillectomy at Chiba University Hospital were examined. The tonsillar mononuclear cells (TMCs) and adenoid mononuclear cells (AMCs) were isolated immediately after surgery and their number compared. The tissues were cut into small pieces and subjected to two cycles of grinding with a medium and sieved through a 70- μ m mesh. The cells were purified using density gradient centrifugation (Lympholyte-H; Cedarlane, Hornby, Ontario, Canada). Similarly, PBMCs were collected immediately before the operation and isolated from whole peripheral blood at a maximum of 10 mL.

The number of IL-4- or IL-5-producing cells that were stimulated with *Dermatophagoides pteronyssinus* (Der p) 1 and Der p 2 was determined using enzyme-linked immunospot assay. The 96-well sterile filter plates (Millipore, Billerica, MA, USA) were coated with a monoclonal antibody to human IL-4 or IL-5 overnight at 4 °C (Mabtech AB, Nacka Strand, Sweden) followed by blocking for over 30 min using an RPMI 1640 medium supplemented with 10% fetal bovine serum (Gibco, Thermo Fisher Scientific, Carlsbad, CA, USA). After discarding the medium, the TMCs, AMCs, and PBMCs (3×10^5 cells/well) were cultured for 48 h at 37 °C in a fresh medium alone or in 2 μ g/mL of Der p 1 and 1 μ g/mL of Der p 2 (Indoor Biotechnologies, Charlottesville, VA, USA) in RPMI 1640 complete medium. The plates were incubated with a biotinylated monoclonal antibody against human IL-4 or IL-5 (Mabtech) for 2 h and with streptavidin-conjugated alkaline phosphatase (Mabtech) for 1 h at 37 °C. The plates were then incubated with BCIP/NBTPLUS (Mabtech). Specific spots were calculated by subtracting the number of spots in the control medium from the mean number of spots in the stimulated wells.

2.2.3. Analysis of the functional subgroups of Tregs

The phenotypical subpopulations of Tregs in TMCs, AMCs, and PBMCs were analyzed. The TMCs, AMCs, and PBMCs were stained with PerCP-Cy5.5-conjugated anti-human CD4 antibody, PE anti-human CD3 antibody (Biolegend, Uithoorn, The Netherlands), APC anti-45RA antibody (BD Biosciences, San Diego, CA, USA), PE anti-human CD25, or FITC anti-Foxp3 using a Foxp3/Transcription Factor Staining Buffer Set (eBioscience, San Diego, CA, USA). The cells were analyzed using a FACS Calibur instrument and data were interpreted using the Cellquest (Becton Dickinson, Mountain View, CA, USA) and Flow-Jo (Tree Star, Inc., San Carlos, CA, USA) software programs.

2.2.4. Questionnaire-based long-term follow-up of subjects

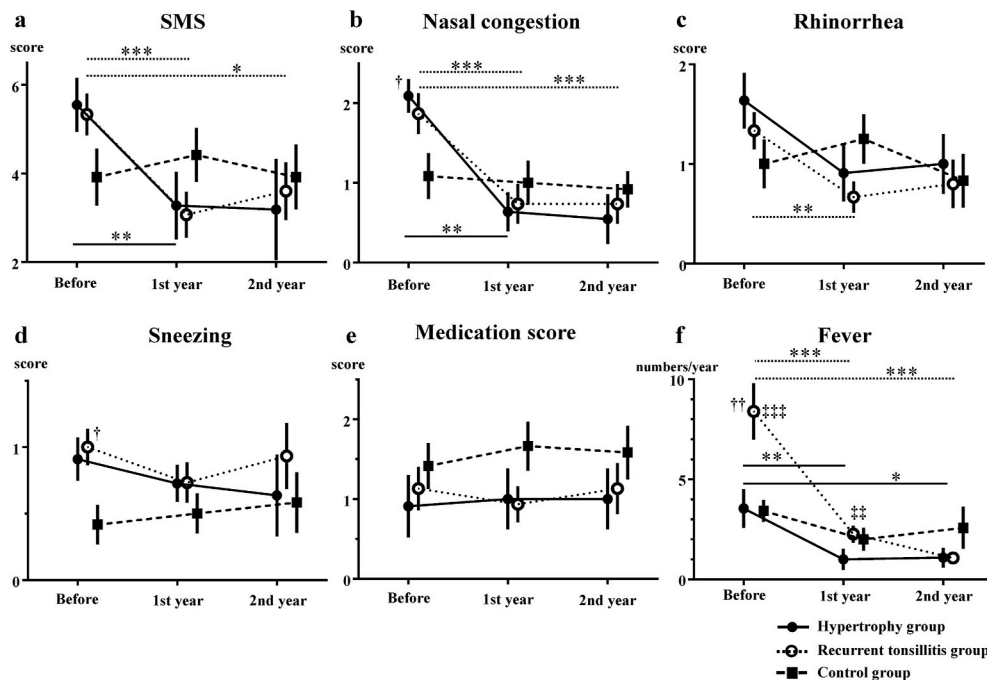
In August 2018, a questionnaire was distributed to examine the severity of nasal symptoms and asthmatic responses as well as the incidence of fever in the past year, in subjects with AR and in those

Table 1

The background data of the enrolled pediatric patients at the time of entry. (two years follow up group).

Two years follow up group		Surgery group (n = 53)		Control Group (n = 21)	P-value (vs control)	
Backgrounds		Hypertrophy (n = 26)	Recurrent Tonsillitis (n = 27)		Hypertrophy	Recurrent Tonsillitis
Age, years	Mean (SD)	4.39 (1.36)	4.92 (1.09)	4.48 (1.26)	–	–
Gender, boy	N (%)	16(62)	19(70)	10(48)	0.39	1.00
Total IgE, IU/mL	Mean (SD)	185.42 (319.46)	360.79 (445.67)	605.9 (529.2)	0.0008	0.079
Specific IgE to Der. f, UA/mL	Mean (SD)	23.9 (48.92)	41.32 (70.01)	99.28 (127.2)	0.014	0.105
Sensitization to Der. f	N (%)	12(46)	16(59)	15(71)	1.00	0.44
Atopic individuals	N (%)	14 (54)	18(67)	16(76)	0.072	0.54
Non-atopic individuals	N (%)	12 (46)	9(33)	5(24)		
SMS in PAR subjects	Score (SD)	5.55 (1.92)	5.33(1.78)	3.92 (2.14)	0.086	0.064
Comorbidities of allergic airway diseases						
PAR without BA	N (%)	9 (35)	6(22)	2(9.5)	0.081	0.44
BA without PAR	N (%)	6 (23)	2(74)	3(14)	0.71	0.64
Both PAR and BA	N (%)	2 (7.7)	9(33)	10(48)	0.0060	0.38
Neither PAR nor BA	N (%)	9(35)	10(37)	6(29)	0.76	0.76

Der. f: Dermatophagoides farina, PAR: perennial allergic rhinitis, BA: bronchial asthma, SMS: symptom medication score.

**Fig. 1.** The changes of symptoms in children with PAR for 2 years

The symptom changes in children with PAR including the hypertrophy group (n = 11), recurrent tonsillitis group (n = 15) and control group (n = 12). (a) SMS, (b) nasal congestion, (c) rhinorrhea, (d) sneezing, (e) medication score, and (f) the number of fevers per year.

The data represent the means $\pm$ SEMs.

*p < 0.05, **p < 0.01, ***p < 0.001: intragroup comparison of surgery group (vs. before surgery, –between hypertrophy group, between recurrent tonsillitis group). †p < 0.05, ††p < 0.01: comparison between groups (vs. control group).

†††p < 0.001, ††††p < 0.0001: comparison between the recurrent tonsillitis and hypertrophy group.

without AR or BA at the time of enrollment in this study.

2.3. Statistical analysis

The number and proportion of subjects in each category were presented as nominal variables for the background characteristics. The mean and standard deviation were presented as continuous variables. The Fisher's exact test and the Mann-Whitney *U* test were used for comparison of baseline characteristics. The Wilcoxon's signed rank sum test was used for comparison of each symptom or medication score, the frequency of fever, and IgE titer between surgery and control groups. The Wilcoxon's signed rank sum test was also used for comparison of the number of Der p-specific IL-4- and IL-5-producing cells and phenotypical subpopulations of Tregs. The statistical analyses were performed at a two-sided significance level of 5%. IBM SPSS statistics 23® software was used for the analyses.

3. Results

3.1. Background of subjects

A total of 53 subjects, 2–7 years of age, who underwent tonsillectomy (surgery group) and 21 age-matched subjects who did not undergo tonsillectomy (control group) were enrolled in the present study. The surgery group was divided into the non-recurrent tonsillitis patients (hypertrophy group) and the recurrent tonsillitis patients (RT group). The hypertrophy group and RT groups included 26 and 27 cases, respectively. The clinical background of the subjects is shown in Table 1. The surgery group had low total IgE and specific IgE to Der. f; however, but high SMS as compared with the control group before surgery.

3.2. Changes of symptoms in children with PAR within 2 years

AR was present in 11 subjects in the hypertrophy group, 15 subjects

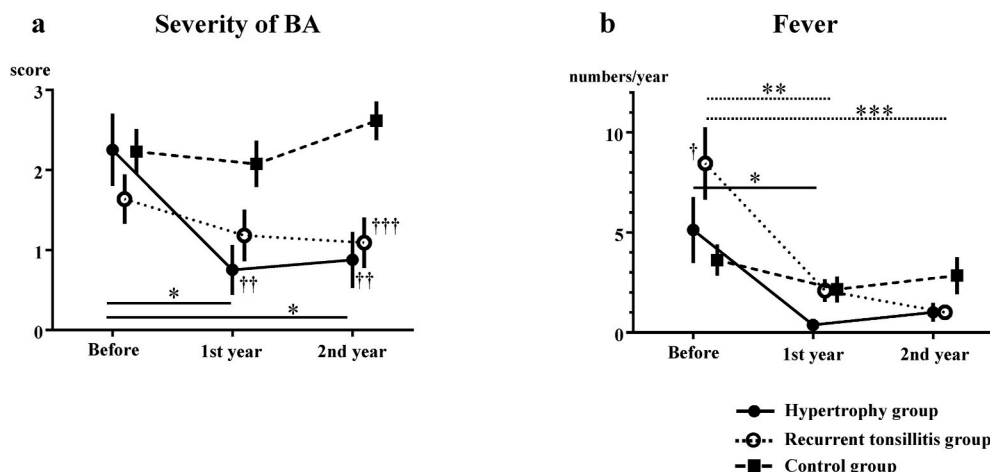


Fig. 2. The changes of symptoms in children with BA for 2 years.

The symptom changes in children with BA including the hypertrophy group ($n = 8$), recurrent tonsillitis group ($n = 11$) and control group ($n = 13$). (a) The severity of BA, and (b) the number of fevers per year. The data represent the means $\pm$ SEMs.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$: intragroup comparison of surgery group (vs. before surgery, –between hypertrophy group, between recurrent tonsillitis group). † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$: comparison between groups (vs. control group).

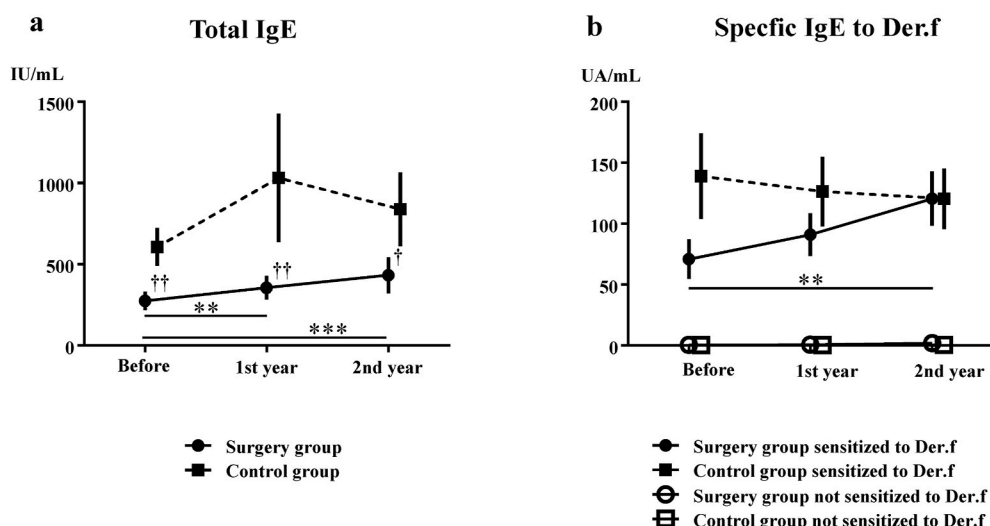


Fig. 3. Changes in the total and Der f-specific IgE within 2 years.

The changes of IgE in all children including surgery group ($n = 53$) and control group ($n = 21$). (i) The total IgE, (j) Der f-specific IgE. The data represent the means $\pm$ SEMs.

** $p < 0.01$, *** $p < 0.001$: intragroup comparison of surgery group (vs. before surgery). † $p < 0.05$, †† $p < 0.01$: comparison between groups (vs. control group).

in the RT group and 12 subjects in the control group. The changes in nasal symptoms, medications for AR, and frequency of fever before, and 1 year and 2 years after study participation or surgery were compared between the groups (Fig. 1). In both the hypertrophy group and the RT groups, the nasal congestion scores at 1 year after surgery decreased significantly compared with the preoperative scores; the frequency of

fever also improved. The rhinorrhea scores at 1 year after surgery decreased significantly in the RT group but not significantly in the hypertrophy group. Although significant improvement was not observed in the hypertrophy group, the score also decreased. Significant change was not observed in sneezing and medication scores in AR patients. The nasal congestion scores in the hypertrophy group and the sneezing scores in

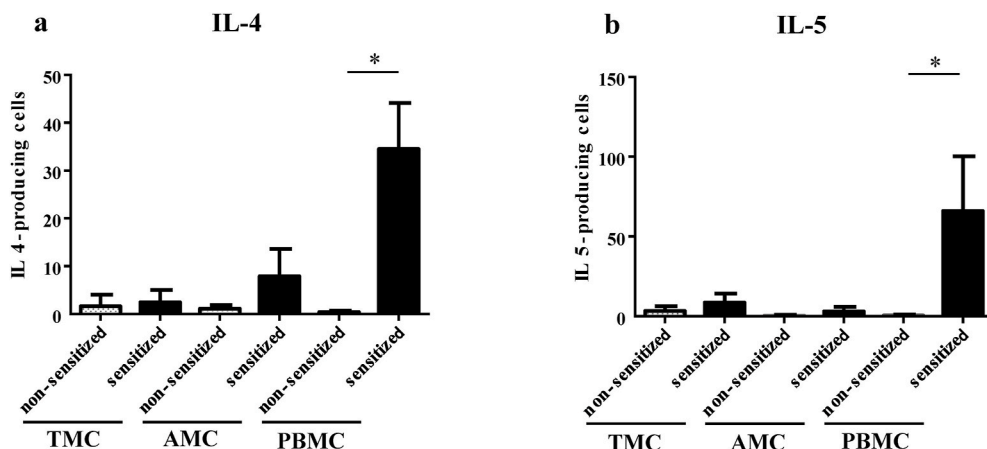


Fig. 4. The number of Der p 1- and Der p 2-specific cytokine-producing cells in the TMCs, AMCs, and PBMCs.

(a) The number of Der p 1- and Der p 2-specific IL-4-producing cells. A total of 12 TMCs, 12 AMCs, and 8 PBMCs from subjects without Der p-specific antibody ($n = 6$, $n = 7$, and $n = 4$, respectively) and subjects with Der p-specific antibody ($n = 6$, $n = 5$, and $n = 4$, respectively).

(b) The number of Der p 1- and Der p 2-specific IL-5-producing cells. A total of 12 TMCs, 12 AMCs, and 9 PBMCs from subjects without Der p-specific antibody ($n = 6$, $n = 7$, and $n = 5$, respectively) and subjects with Der p-specific antibody ($n = 6$, $n = 5$, and $n = 4$, respectively).

The data represent the means $\pm$ SEMs.

* $p < 0.05$.

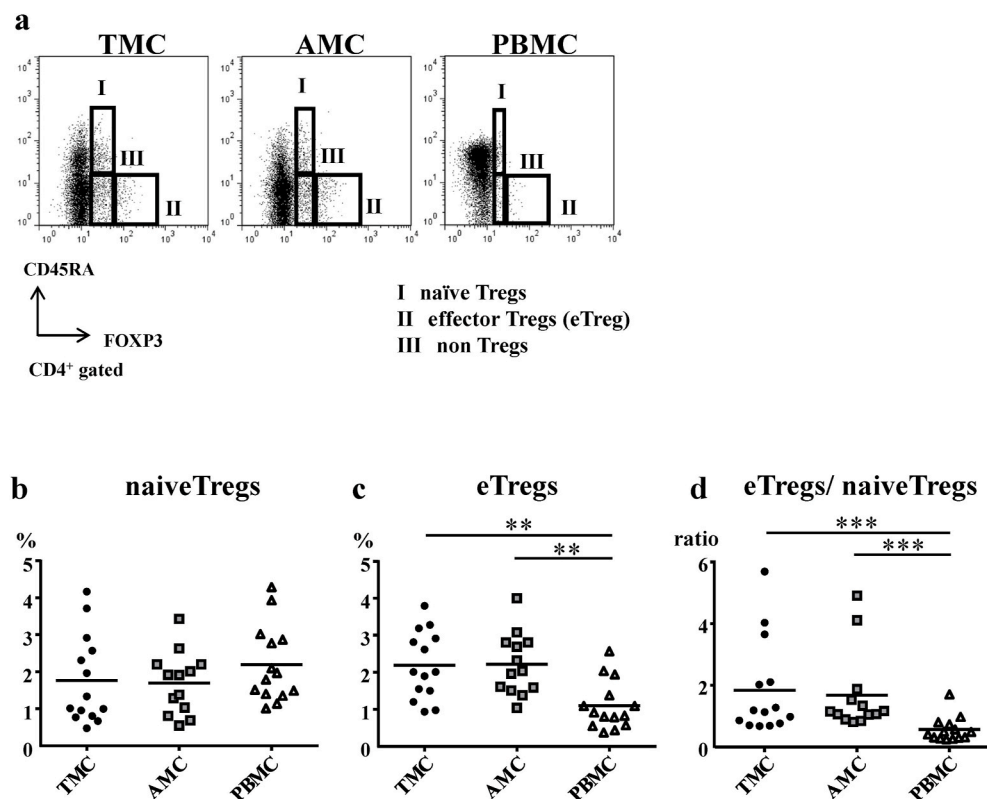


Fig. 5. Subpopulations of Tregs in the tonsils and PBMCs.

(a) Foxp3+CD4⁺ T cells can be subdivided into subpopulations based on their expression of CD45RA, (b) The percentages of rTregs, (c) eTregs, (d) eTregs/rTregs ratio. (a) A total of 16 TMCs, 15 AMCs, and 16 PBMCs, (b), (c), (d), A total of 14 TMCs, 13 AMCs, and 14 PBMCs. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

the RT group before surgery were significantly higher than in the control group. The frequency of fever was higher in the RT group than that in the hypertrophy group before surgery and at 1 year after surgery, however, significant difference was not observed at 2 years after surgery. In the control group, significant changes were not observed in the nasal congestion and sneezing scores, frequency of fever, and medication scores for 2 years.

3.3. Changes of symptoms in children with BA within 2 years

BA was present in 8 subjects in the hypertrophy group, 11 subjects in the RT group and 13 subjects in the control group. The changes in BA severity and frequency of fever before, and 1 year and 2 years after study participation or surgery are shown in Fig. 2. Obvious changes were not observed in the control group in BA severity and frequency of fever. In the hypertrophy group, the BA severity and frequency of fever were significantly improved after surgery compared with study entry. The hypertrophy and the RT groups had similar preoperative BA severity scores compared with the control group, however, significantly lower BA severity scores were observed at 2 years after surgery.

3.4. Changes in the total and Der f-specific IgE within 2 years

The levels and changes in the total and Der f-specific IgE are shown in Fig. 3. At study entry, the total IgE level was significantly lower in the surgery group than in the control group. After surgery, the total IgE level slightly increased in the surgery group. Before and 1 year after surgery, the Der f-specific IgE level in the sensitized group tended to be lower in the surgery group than in the control group. After surgery, the Der f-specific IgE level increased significantly in the sensitized group but not in the non-sensitized group.

3.5. Number of Der p 1- and Der p 2-specific cytokine-producing cells in TMCs, AMCs, and PBMCs

As shown in Fig. 4, the Der p-specific IL-4- and IL-5-producing cells were clearly detected in PBMCs but only few were observed in TMCs in the sensitized subjects and in the non-sensitized subjects; however, the number was not significantly different. There was no difference in the number of these cells between TMCs and AMCs.

3.6. Phenotypical subpopulations of Tregs in TMCs, AMCs, and PBMCs

The phenotypical subpopulations of Tregs in TMCs, AMCs, and PBMCs were analyzed (Fig. 5). Compared with PBMCs, TMCs had a similar number of naïve Tregs but a significantly higher number of eTregs. The number of eTregs and the ratio of naïve Tregs to eTregs in TMCs and PBMCs were not significantly different in atopic and non-atopic individuals (Fig. 6a). However, the ratio of naïve Tregs to eTregs in TMCs was significantly lower in subjects with RT than in those without RT (Fig. 6b).

3.7. Questionnaire-based long-term follow-up of subjects

The questionnaire survey on allergy symptoms was conducted in 46 subjects in the surgery group and 12 subjects in the control group at a mean of 67.4 months (range, 56–79 months) after study participation. The hypertrophy group included 22 cases in which AR was present in 9 cases, and the RT group included 24 cases in which AR was present in 13 cases. (Table 2). The comparison between groups regarding changes in nasal symptoms, frequency of fever, and medication score are shown in Fig. 7. The preoperative scores for nasal congestion and rhinorrhea were higher in both the hypertrophy group and the RT groups than in the control group. However, the scores decreased after surgery and tended to be lower than in the control group. In particular, the nasal congestion score improved significantly in both groups after surgery.

There were 3 cases of newly developed AR in the surgery group and 1

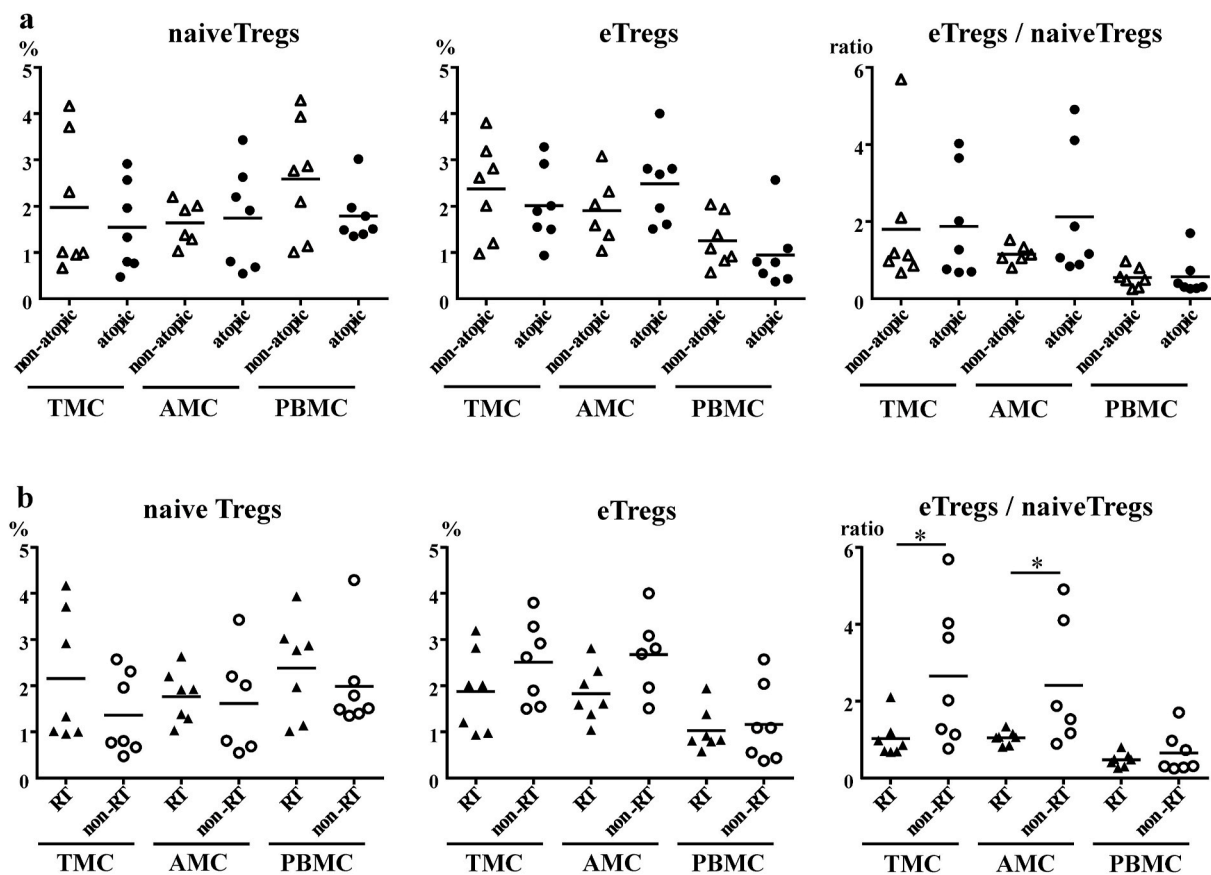


Fig. 6. Functional subpopulations of Tregs in the tonsils and PBMCs from atopic and non-atopic subjects and from patients with and without RT.

(a) Comparison of rTregs, eTregs, and the eTregs/rTregs ratio between atopic and non-atopic children. A total of 14 TMCs and PBMC from atopic ($n = 7$) and non-atopic ($n = 7$), and a total of 13 AMCs from atopic ($n = 7$) and non-atopic ($n = 6$) subjects.

(b) Comparison of rTregs, eTregs, and the eTregs/rTregs ratio between children with and without RT. A total of 14 TMCs and PBMCs with RT ($n = 7$) and without RT ($n = 7$), and a total of 13 AMCs with ($n = 7$) and without RT ($n = 6$) subjects.

Table 2

The background data of the enrolled pediatric patients at the time of entry. (long-term follow up group).

Long-term follow up group		Surgery group ($n = 46$)		Control Group ($n = 12$)	P-value (vs control)	
Backgrounds		Hypertrophy ($n = 22$)			Hypertrophy	Recurrent Tonsillitis
			Recurrent Tonsillitis ($n = 24$)			
Age, years	Mean (SD)	4.36 (1.46)	4.92 (1.11)	4.33 (1.03)	–	–
Gender, boy	N (%)	13(59)	18(75)	5(42)	0.48	0.071
Total IgE, IU/mL	Mean (SD)	205.1 (336.6)	357.0 (452.5)	691.3 (620.3)	0.0087	0.098
Specific IgE to Der. f, UA/mL	Mean (SD)	27.24 (51.4)	42.3 (74.1)	136.2 (146.8)	0.020	0.048
Sensitization to Der. f	N (%)	10(45)	14(58)	9(75)	0.15	0.47
Atopic individuals	N (%)	12(55)	16(67)	9(75)	0.73	1.00
Non-atopic individuals	N (%)	10(45)	8(33)	3(25)		
SMS in PAR subjects	Score (SD)	5.67 (2.11)	5.46(1.87)	4.50 (2.29)	0.263	0.354
Comorbidities of allergic airway diseases						
PAR without BA	N (%)	7(32)	5(21)	2(17)	0.44	1.00
BA without PAR	N (%)	5(23)	1(42)	1(8.3)	0.38	1.00
Both PAR and BA	N (%)	2(9.1)	8(33)	6(50)	0.059	0.47
Neither PAR nor BA	N (%)	8(36)	10(42)	3(25)	0.29	0.47

Der. f: Dermatophagoides farina, PAR: perennial allergic rhinitis, BA: bronchial asthma, SMS: symptom medication score.

case in the control group within 2 years after surgery. There were 2 cases of newly developed BA cases in the surgery group and 1 case in the control group within 2 years after surgery.

4. Discussion

The incidence of allergic diseases has been increasing worldwide. The influence of tonsils on the onset or development of allergic disease symptoms in the airway remains unclear. In the present study, a significant number of Th2 cytokines that produce memory cells were

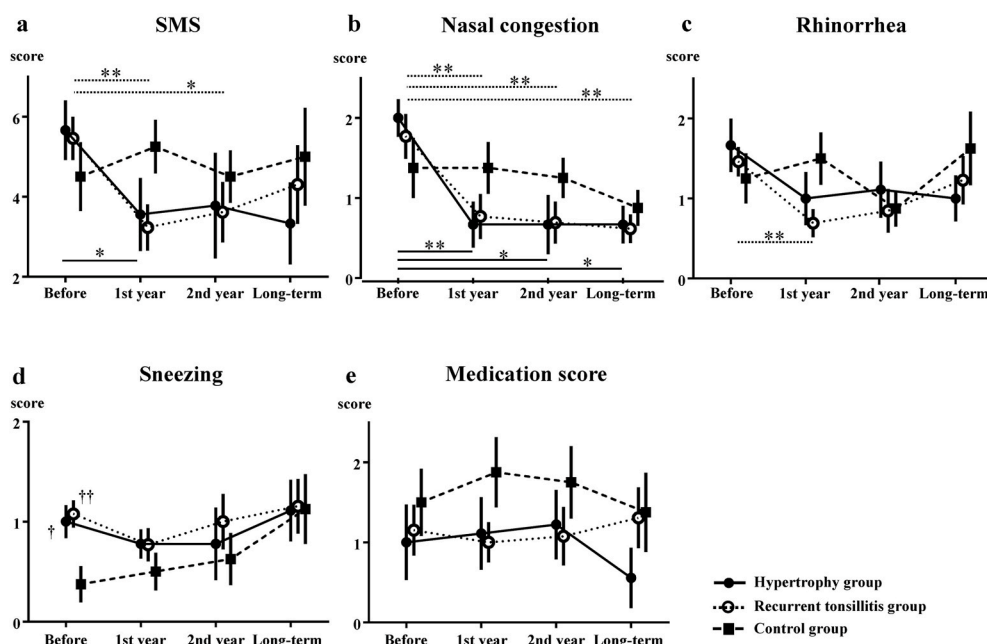


Fig. 7. The changes in children with PAR for several years.

(a) The changes in the SMS, (b) nasal congestion, (c) rhinorrhea, (d) sneezing, and (e) medication score. hypertrophy group ($n = 9$), recurrent tonsillitis group ($n = 13$) and control group ($n = 8$).

The data represent the means $\pm$ SEMs. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$: intragroup comparison of surgery group (vs. before surgery, –between hypertrophy group, between recurrent tonsillitis group).

† $p < 0.05$, †† $p < 0.01$: comparison between groups (vs. control group).

detected in PBMCs but only a few were observed in the TMCs in the sensitized group. Furthermore, the abundant eTregs in the tonsils might have had an important role in the suppression of Th2 cells. However, difference was not observed in the number of eTregs between atopic and non-atopic patients. In this study, the antigen specificity of Tregs was not examined, therefore, the exact role of these Treg cell types remains unclear. The significantly higher ratio of eTregs to naïve Tregs in the tonsils and the significantly decreased frequency of fever after tonsillectomy in subjects with recurrent infection than in subjects without recurrent infection indicate the tonsils were negatively involved in the immune defense response to infection under these conditions.

Because the tonsils contain only efferent and not afferent lymphatic ducts, memory immune cells migrate and induce immune responses in other mucosal tissues, which is involved in homing. The abundant eTregs in the tonsils might have the ability to move to other mucosal tissues and suppress the immune response. In sensitized subjects, Tregs may inhibit allergic reactions and delay the onset of allergic diseases. Therefore, tonsillectomy may increase the risk of developing or exacerbating the symptoms of AR and BA. In the present study, the increased postoperative levels of specific and total IgE indicated tonsillectomy contributes to immune mechanisms. However, based on the course of subjects who underwent tonsillectomy, how Tregs influenced the onset of allergic disease was unclear which may be due to the small number of subjects who developed allergy in both the surgery and control groups and the difficult comparison of the two groups resulting from the small number of cases.

However, among the symptoms of established allergic disease, nasal congestion and rhinorrhea markedly and continuously improved after tonsillectomy. Several possible reasons for these results include improved aeration in the upper respiratory tract and the decreased morbidity of infectious disease after tonsillectomy. In the present study population, nasal congestion and rhinorrhea continued to improve for 2 years after surgery, however, sneezing did not significantly change and the medication scores showed only mild improvement compared with the control group. Furthermore, because IgE production did not deteriorate, tonsillectomy likely did not directly improve the allergic pathology. Tonsillectomy would not be expected to modify natural history of allergy because there are other sites of lymphatic tissue in the upper airway around the Waldeyer's tonsillar ring. Instead, nasal congestion was likely improved by correction of the stenoses in the nasopharynx

and oropharynx, and the improved rhinorrhea may have been involved in the reduced incidence of infection. Complicated acute airway infection exacerbates the symptoms of BA and AR, and the most common trigger for BA exacerbation is respiratory tract infection [19–22]. In the present study, the symptoms after 4–6 years of follow-up were similar between the surgery group and the control group.

AR and BA are frequently exacerbated by recurrent infection, therefore, their association is important in examining the natural history of airway allergies. Regarding clinical symptoms, the relationship between children with and without recurrent infection were investigated, however, detailed subgroup analysis with TMCs or AMCs could not be performed due to the small sample size, which is a limitation of the study. Furthermore, the antigen specificity of Tregs was not examined, therefore, the exact role of these Treg cell types remains unclear.

5. Conclusion

Although tonsillectomy did not appear to improve the allergic pathology, the decreased risk of infection and upper airway obstruction could lead to improved symptoms of allergic diseases in the airway. The functionally activated eTregs in the tonsils may inhibit excessive local immune responses, however their actual role in allergic diseases remains unknown. Further studies are needed to clarify this important issue.

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Declaration of competing interest

The authors declare no conflicts of interest associated with this manuscript.

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References

- [1] M. Perry, A. Whyte, Immunology of the tonsils, *Immunol. Today* 19 (1998) 414–421, [https://doi.org/10.1016/S0167-5699\(98\)01307-3](https://doi.org/10.1016/S0167-5699(98)01307-3).
- [2] P. Brandtzaeg, Potential of nasopharynx-associated lymphoid tissue for vaccine responses in the airways, *Am. J. Respir. Crit. Care Med.* 183 (2011) 1595–1604, <https://doi.org/10.1164/rccm.201011-1783OC>.
- [3] P.L. Ogra, Mucosal immune response in the ear, nose and throat, *Pediatr. Infect. Dis. J.* 19 (2000), <https://doi.org/10.1097/00006454-200005001-00002>. S4-8.
- [4] M.J. van Kempen, G.T. Rijkers, P.B. Van Cauwenberge, The immune response in adenoids and tonsils, *Int. Arch. Allergy Immunol.* 122 (2000) 8–19, <https://doi.org/10.1159/000024354>.
- [5] J.E. Gudjonsson, A. Johnston, H. Sigmundsdottir, H. Valdimarsson, Immunopathogenic mechanisms in psoriasis, *Clin. Exp. Immunol.* 135 (2004) 1–8, <https://doi.org/10.1111/j.1365-2249.2004.02310.x>.
- [6] H. Meng, H. Ohtake, A. Ishida, N. Ohta, S. Kakehata, M. Yamakawa, IgA production and tonsillar focal infection in IgA nephropathy, *J. Clin. Exp. Hematop.* 52 (2012) 161–170, <https://doi.org/10.3960/jslrt.52.161>.
- [7] S. Sakaguchi, T. Yamaguchi, T. Nomura, M. Ono, Regulatory T cells and immune tolerance, *Cell* 133 (2008) 775–787, <https://doi.org/10.1016/j.cell.2008.05.009>.
- [8] O. Palomares, B. Rückert, T. Jartti, C. Kuecuksezer Umut, T. Puhakka, E. Gomez, B. Fahrner Heinz, A. Speiser, A. Jung, W. Kwok William, L. Kalogjera, M. Akdis, A. Akdis Cezmi, Induction and maintenance of allergen-specific FOXP3+ Treg cells in human tonsils as potential first-line organs of oral tolerance, *J. Allergy Clin. Immunol.* 129 (2012) 510–520, <https://doi.org/10.1016/j.jaci.2011.09.031>, 20. e1-9.
- [9] A. Akcay, C.O. Kara, E. Dagdeviren, M. Zencir, Variation in tonsil size in 4- to 17-year-old schoolchildren, *J. Otolaryngol.* 35 (2006) 270–274, <https://doi.org/10.2310/7070.2005.0118>.
- [10] C.L. Marcus, L.J. Brooks, K.A. Draper, D. Gozal, A.C. Halbower, J. Jones, M. S. Schechter, S.D. Ward, S.H. Sheldon, R.N. Shiffman, C. Lehmann, K. Spruyt, Diagnosis and management of childhood obstructive sleep apnea syndrome, *Pediatrics* 130 (2012) e714–755, <https://doi.org/10.1542/peds.2012-1672>.
- [11] M.J. Burton, P.P. Glasziou, Tonsillectomy or adeno-tonsillectomy versus non-surgical treatment for chronic/recurrent acute tonsillitis, *Cochrane Database Syst. Rev.* (2014) CD001802, <https://doi.org/10.1002/14651858.CD001802.pub3>.
- [12] M. Miyara, Y. Yoshioka, A. Kitoh, T. Shima, K. Wing, A. Niwa, C. Parizot, C. Taffin, T. Heike, D. Valeyre, A. Mathian, T. Nakahata, T. Yamaguchi, T. Nomura, M. Ono, Z. Amoura, G. Gorochoy, S. Sakaguchi, Functional delineation and differentiation dynamics of human CD4(+) T cells expressing the FoxP3 transcription factor, *Immunity* 30 (2009) 899–911, <https://doi.org/10.1016/j.immuni.2009.03.019>.
- [13] D. Sugiyama, H. Nishikawa, Y. Maeda, M. Nishioka, A. Tanemura, I. Katatama, S. Ezoe, Y. Kanakura, E. Sato, Y. Fukumori, J. Karbach, E. Jager, S. Sakaguchi, Anti-CCR4 mAb selectively depletes effector-type FoxP3+CD4+ regulatory T cells, evoking antitumor immune responses in humans, *Proc. Natl. Acad. Sci. U. S. A.* 110 (2013) 17945–17950, <https://doi.org/10.1073/pnas.1316796110>.
- [14] M. Sakashita, T. Hirota, M. Harada, R. Nakamichi, T. Tsunoda, Y. Osawa, A. Kojima, M. Okamoto, D. Suzuki, S. Kubo, Y. Imoto, Y. Nakamura, M. Tamari, S. Fujieda, Prevalence of allergic rhinitis and sensitization to common aeroallergens in a Japanese population, *Int. Arch. Allergy Immunol.* 151 (2010) 255–261, <https://doi.org/10.1159/000242363>.
- [15] Y. Osawa, D. Suzuki, Y. Ito, N. Narita, Y. Ohshima, Y. Ishihara, Y. Ishihara, S. Tsuchida, S. Fujieda, Prevalence of inhaled antigen sensitization and nasal eosinophils in Japanese children under two years old, *Int. J. Pediatr. Otorhinolaryngol.* 76 (2012) 189–193, <https://doi.org/10.1016/j.ijporl.2011.10.024>.
- [16] S. Masuda, T. Fujisawa, H. Katsumata, J. Atsuta, K. Iguchi, High prevalence and young onset of allergic rhinitis in children with bronchial asthma, *Pediatr. Allergy Immunol.* 19 (2008) 517–522, <https://doi.org/10.1111/j.1399-3038.2007.00675.x>.
- [17] K. Okubo, [Practical guideline for the management of allergic rhinitis in Japan], *Arerugi* 62 (2013) 1458–1463, <https://doi.org/10.15036/arerugi.62.1458>.
- [18] Y. Hamasaki, Y. Kohno, M. Ebisawa, N. Kondo, S. Nishima, T. Nishimuta, A. Morikawa, Japanese guideline for childhood asthma 2014, *Allergol. Int.* 63 (2014) 335–356, <https://doi.org/10.2332/allergolint.14-RAI-0767>.
- [19] S.L. Friedlander, W.W. Busse, The role of rhinovirus in asthma exacerbations, *J. Allergy Clin. Immunol.* 116 (2005) 267–273, <https://doi.org/10.1016/j.jaci.2005.06.003>.
- [20] Y. Okamoto, Z. Matsuzaki, T. Matsuoka, S. Endo, H. Yamamoto, H. Chazono, S. Horiguchi, T. Hanazawa, Influence of viral infection on the development of nasal hypersensitivity, *Clin. Exp. Allergy* 35 (5) (2005) 679–684, <https://doi.org/10.1111/j.1365-2222.2005.02216.x>.
- [21] H. Hammad, M. Chieppa, F. Perros, M.A. Willart, R.N. Germain, B.N. Lambrecht, House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells, *Nat. Med.* 15 (4) (2009) 410–416, <https://doi.org/10.1038/nm.1946>.
- [22] O.P. Alho, R. Karttunen, T.J. Karttunen, Nasal mucosa in natural colds: effects of allergic rhinitis and susceptibility to recurrent sinusitis, *Clin. Exp. Immunol.* 137 (2) (2004) 366–372, <https://doi.org/10.1111/j.1365-2249.2004.02530.x>.