

Total and Antigen- (Fungi, Mites and Staphylococcal Enterotoxins) Specific IgEs in Nasal Polyps Is Related to Local Eosinophilic Inflammation

Yoshinori Matsuwaki Keisuke Uno Tetsushi Okushi Nobuyoshi Otori
Hiroshi Moriyama

Department of Otorhinolaryngology, Jikei University School of Medicine, Tokyo, Japan

Key Words

Rhinosinusitis · Immunoglobulin E · Nasal polyps

Abstract

Background: Total and specific immunoglobulin E (IgE) in nasal polyps (NP) is related to local eosinophilic inflammation. However, it remains unclear which antigens cause a stronger accumulation of eosinophils in NP. We investigated whether local IgEs to specific antigens correlated with local eosinophil accumulation. **Methods:** Total IgE, antigen-specific IgEs [5 species of fungi, staphylococcal enterotoxins A and B (SEA and SEB), cedar pollen, ragweed pollen and house-dust mites] and eosinophil cationic protein (ECP) were measured in sinus mucosa homogenates and serum from 29 patients with chronic rhinosinusitis with NP (CRSwNP), 8 patients with allergic fungal rhinosinusitis (AFRS) and 8 control subjects without CRS. The level of ECP in NP was analyzed for correlations with the total and specific IgE levels in the serum and NP. **Results:** In CRSwNP, antigen-specific IgEs [*Alternaria* > mite (*Dp*) > *Aspergillus* > SEB > *Penicillium* > mite (*Df*), but not the other fungi, SEA or pollens] in the sinus mucosa correlated significantly with the local ECP. In AFRS, antigen-specific IgEs [*Penicillium* > mite (*Df*) > *Alternaria*, but not the other fungi, SEs or pollens] in the sinus mucosa correlated significantly with the local ECP.

Conclusions: Local total IgE and antigen (fungi, mites and SEs)-specific IgEs in NP are related to the local eosinophilic inflammation of AFRS and CRSwNP.

Copyright © 2013 S. Karger AG, Basel

Introduction

Tissue eosinophilia is a hallmark of nasal polyps (NP) and is thought to be a risk factor for disease recurrence [1, 2]. However, the pathogenesis of chronic rhinosinusitis (CRS) remains controversial. In Europe, patients with bilateral NP are frequently linked to asthma and aspirin sensitivity [3]. Furthermore, bilateral NP are the most predominant type, accounting for 80–90% of NP in Europe and the USA, and are usually associated with tissue eosinophilia [4]. However, in Japan, the ratio of eosinophilic CRS (ECRS) differs from that in Europe and in the USA. Our previous study found that about 40% of CRS with NP (CRSwNP) was ECRS, with a frequent association with asthma and aspirin sensitivity, while the remaining roughly 60% was noneosinophilic CRS (NECRS) which was rarely comorbid with asthma [1, 2]. Thus, when we investigate the pathogenesis of CRSwNP, we should study ECRS and NECRS as separate groups. Allergic fungal rhinosinusitis (AFRS) should be considered

at the same time, because of its eosinophilic NP and frequent disease recurrence, similar to ECRS [5, 6].

A multitude of factors has been proposed to be key agents in the development of NP, including superantigens, fungi, eosinophils and immunoglobulin E (IgE). Recent studies have investigated the roles of local IgE production against staphylococcal superantigens in the pathogenesis of CRSwNP [7]. The mean eosinophil counts in NP tended to be higher in superantigen toxin-positive patients with NP compared with patients without toxin detection [8]. Local polyclonal IgE, including IgE specific for staphylococcal enterotoxins A and B (SEA and SEB), was demonstrated in 50% of bilateral eosinophilic NP, suggesting a possible role for superantigens as disease modifiers in CRS [7]. AFRS is a representative systemic IgE/IgG-mediated disease, which is a specific allergy for fungi [6]. Local IgE-positive cells in AFRS are increased [9]. Not only antifungal, also antinonfungal IgE, was expressed in sinus tissues of AFRS, compared to the control [10]. Local total IgE correlated significantly with local IL-5, eosinophil cationic protein (ECP) and with the number of eosinophils in NP [7]. However, it remains unclear which antigens cause stronger accumulation of eosinophils in the local sinus mucosa. Thus, we hypothesized that levels of local IgE to airborne antigens (staphylococcal superantigens, fungi, house-dust mites and pollens) are much higher in ECRS and AFRS, and that local IgE probably induces local eosinophilia in CRS. We investigated whether local IgEs to specific antigens correlated with local eosinophil accumulation.

Materials and Methods

Materials

The ECP ELISA kit was from MBL (Nagoya, Japan). Total serum IgE and antigen-specific IgEs were measured by fluorometric 2-site ELISA using a Pharmacia Cap System™ (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). Dako REAL Target Retrieval Solution and Dako LSAB2 kit/HRP were from Dako (Dako Japan, Tokyo, Japan). Goat IgG antibody to human IgE (catalog No. 0751-1004) was from KPL (Gaithersburg, Md., USA). Goat IgG antibody, as a negative control, was from Acris Antibodies GmbH (Herford, Germany). Anti-mast cell chymase (CC1) antibody and mouse IgG, as a negative control, were from Santa Cruz Biotechnology. Plasma cell mouse anti-human monoclonal antibody and mouse IgG2a, as a negative control, were from LifeSpan BioScience.

Diagnosis of ECRS, NECRS and AFRS

CRS and AFRS were diagnosed in accordance with the criteria in 'Rhinosinusitis: developing guidance for clinical trials' [11]. The diagnostic criteria for ECRS were: (1) CRSwNP and (2) mucosal

eosinophilia of 120 per high-power field (HPF) or more and/or eosinophilic mucin [1]. The diagnostic criteria for NECRS were: (1) CRSwNP and (2) mucosal eosinophilia below 120/HPF with no eosinophilic mucin [1].

Patients

This study included 29 patients with CRSwNP and 8 with AFRS who had undergone endoscopic sinus surgery as well as 8 normal sinus control patients with pituitary adenoma who had undergone endoscopic transsphenoidal pituitary surgery. All surgeries were performed at the Department of Otorhinolaryngology at the Jikei University School of Medicine. The diagnoses of the CRSwNP patients were NECRS in 14 and ECRS in 15. Table 1 shows the patients' background data. Based on the presurgery data, peripheral eosinophil counts were significantly higher in the ECRS and AFRS patients than in the NECRS patients and controls. Peripheral basophil counts, total IgE and fungi-specific IgE were significantly higher in the AFRS patients than in the other groups. These results are reasonable in light of the diagnostic criteria. The study was approved by the ethics committee of the Jikei University Hospital, and all patients gave their informed written consent.

ECP and Serum IgE and Local IgE

Total and specific IgE levels in sinus tissue were measured by using a Pharmacia CAP system according to previous reports [7]. Briefly, NP and normal sinus mucosa harvested during surgery were weighed and then homogenized with no dilution. The total volume of solution extracted from each homogenate was measured. The extracted solutions and serum from the same patients were subdivided into several tubes and stored at below -40°C until assay. The levels of total IgE, antigen (*Alternaria*, *Aspergillus*, *Candida*, *Cladosporium*, *Penicillium*, SEA, SEB, house-dust mites (Dp, Df), cedar pollen and ragweed pollen)-specific IgEs and ECP were measured using the CAP system.

Immunohistochemistry

Cryostat sections of partially separated NP and normal sinus mucosa harvested during surgery were prepared (6 µm), mounted on glass slides, packed in aluminum foil and stored at -40°C until staining. For immunohistochemical staining, specimens were fixed in Carnoy's fluid (60% ethanol, 30% chloroform and 10% glacial acetic acid) and then blocked for 20 min with 0.3% hydrogen peroxide in TBS (Tris-buffered-saline) containing 0.1% sodium azide. Antihuman IgE antibody (1:500) or the negative control was applied, and the specimens were incubated for 2 h at room temperature. The signal was detected using the labeled streptavidin-biotin technique conjugated with peroxidase according to the manufacturer's instructions (Dako). Peroxidase activity was detected using AEC substrate chromogen, which results in a red-stained precipitate. Anti-mast cell chymase antibody (1:50), anti-plasma cell antibody (1:1,000) and negative control antibodies were applied, and the specimens were incubated for 15 h at 4°C. The signal was detected using the labeled streptavidin-biotin technique conjugated with peroxidase. Peroxidase activity was detected using DAB chromogen, which results in a brown-stained precipitate. Finally, the sections were counterstained with hematoxylin and mounted.

Table 1. Patient background clinical data

	Control	CRSwNP		AFRS
		NECRS	ECRS	
Number (female:male)	8 (3:5)	14 (4:10)	15 (4:11)	8 (1:7)
Age, years	53.1±14.5	43.9±17.3	48.4±13.5	53.4±12.6
Peripheral eosinophils, /ml	130±48	154±108	451±197*, †	516±419*, †
Peripheral basophils, /ml	23±13	30±20	48±28	67±47*, †
Total IgE, IU/ml	84±104	110±147	178±240	1,005±788*, †, ‡
Specific IgE, AU/ml				
Fungi	0±0	0.0±0.1	0.0±0.1	8.7±10.8*, †, ‡
SEA + SEB	0±0	0.6±2.0	0.0±0.0	0.8±1.6
Mites	3.5±7.5	0.5±1.3	1.9±5.3	1.5±2.0
Cedar pollen	2.5±4.0	3.6±6.8	12.8±27.0	32.5±39.4†
Comorbid asthma, n (%)	0/8 (0)	0/13 (0)	6/14 (42.9)	3/8 (67)
Comorbid allergic rhinitis, n (%)	3/7 (42.8)	5/13 (38.5)	9/14 (64.3)	7/8 (87.5)
CT scores (L&M)	0±0	7.9±3.3*	10.2±4.4*	12.5±7.3*
FEV1.0%	89.0±18.0	83.7±11.8	81.3±16.3	73.1±9.4

Values are mean ± SE.

* p < 0.05 vs. control; † p < 0.05 vs. NECRS; ‡ p < 0.05 vs. ECRS.

Statistics

Data were summarized and presented as the mean ± SD. When the distribution of data was regular and the dispersion of each group was equal, one-way ANOVA with repeated measures and Tukey's honestly significant difference test were used to compare more than 2 variables; when the distribution of data was not regular or the dispersion of each group was not equal, the Kruskal-Wallis and Steel-Dwass tests were used. Pearson's product moment correlation coefficient was applied to define the relationships between the peripheral eosinophil count and the local ECP level. Spearman's rank correlation coefficient was applied to define the relationships between IgE (serum and local homogenate extracts) and the local ECP level. All statistical analyses were performed using SPSS version 16.0J (Chicago, Ill., USA). Significance was established at the p < 0.05 level.

Results

Local and Peripheral Eosinophilia

Local ECP levels in NP from ECRS and AFRS were significantly increased compared with NECRS and the control subjects, as were peripheral eosinophils (fig. 1a). The peripheral eosinophil count correlated significantly with local ECP in the sinus tissues of all subjects ($r_s = 0.661$), suggesting that local eosinophilia can be estimated from the peripheral eosinophil count before surgery (fig. 1b).

Serum and Local Total IgE Levels

Serum total IgE was significantly higher in the AFRS patients than in the other groups. Both the serum and local total IgE levels were significantly increased in AFRS compared with NECRS and the controls, while only the local total IgE was significantly increased in ECRS compared with NECRS and the controls (fig. 1c, d).

Expression and Distribution of Local IgE

IgE-positive cells were detected in the subepithelium from AFRS, ECRS and NECRS, but not the controls (fig. 2). The number of IgE-positive cells was similar to the local total IgE results (fig. 1d). Double immunostainings and morphologic findings showed that IgE-positive cells were mainly involved with plasma cells and mast cells (fig. 2e, f).

Local IgE Correlated with Local Eosinophilia

The local total IgE correlated significantly with the local ECP in all subjects ($r_s = 0.655$), control ($r_s = 0.810$), CRSwNP ($r_s = 0.529$) and AFRS ($r_s = 0.833$), suggesting that the total IgE in the sinus mucosa is related to local eosinophilia, regardless of the presence of chronic sinus disease (table 2).

In all subjects, all local fungi-specific IgEs (*Aspergillus* $r_s = 0.585$ > *Alternaria* $r_s = 0.577$ > *Penicillium* $r_s = 0.549$ > *Cladosporium* $r_s = 0.521$ > *Candida* $r_s = 0.512$) corre-

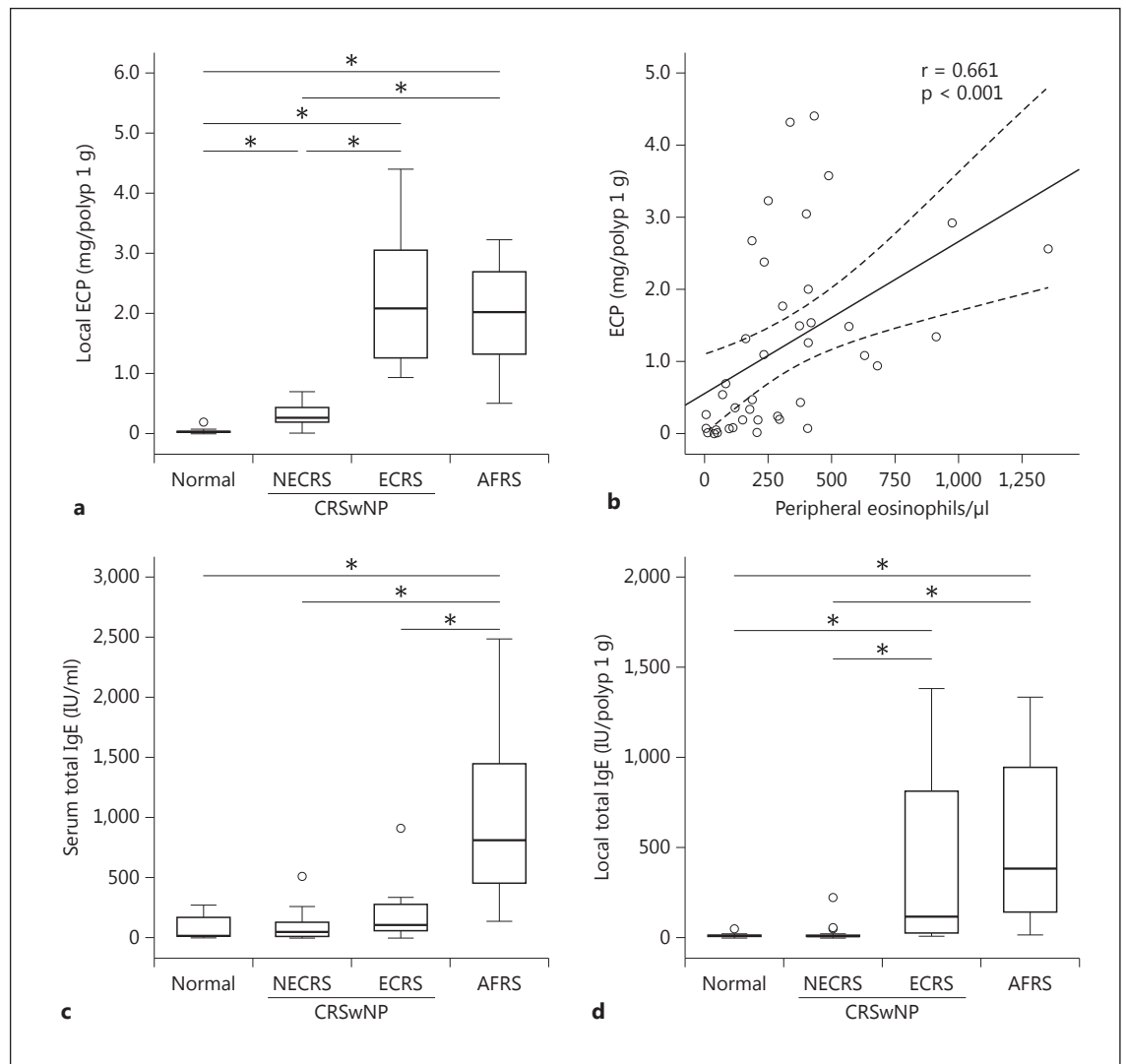


Fig. 1. Comparisons of local ECP and serum IgE and local total IgE. Local ECP (**a**), serum total IgE (**c**) and local total IgE (**d**) were compared between CRSwNP (NECRS, ECRS), AFRS and controls. One-way ANOVA with repeated measures and Tukey's honestly significant difference test were used to compare more than 2 vari-

ables. * Significance was established at the $p < 0.05$ level. **b** Pearson's product moment correlation coefficient was applied to define the relationships between the peripheral eosinophil count and the local ECP level.

lated more strongly with local ECP than the other local antigen-specific IgEs (mite (*Dp*) $r_s = 0.479 > \text{SEA } r_s = 0.471 > \text{mite (Df)} r_s = 0.465 > \text{SEB } r_s = 0.460$). Local pollen-specific IgE had no correlation with local ECP. Furthermore, not only local IgE, but also serum fungi-specific IgE (*Cladosporium* $r_s = 0.329 > \text{Alternaria } r_s = 0.311$) correlated significantly with the local ECP (table 2).

In CRSwNP patients, antigen-specific IgEs (*Alternaria* $r_s = 0.462 > \text{mite (Dp)} r_s = 0.444 > \text{Aspergillus } r_s = 0.418 > \text{SEB } r_s = 0.414 > \text{Penicillium } r_s = 0.394 > \text{mite (Df)} r_s = 0.370$, but not the other fungi, SEA or pollens) in the sinus

mucosa (but none of the serum IgEs) correlated significantly with local ECP.

In AFRS patients, antigen-specific IgEs (*Penicillium* $r_s = 0.857 > \text{mite (Df)} r_s = 0.810 > \text{Alternaria } r_s = 0.714$, but not the other fungi, SEs or pollens) in the sinus mucosa (but none of the serum IgEs) correlated significantly with local ECP.

In normal control subjects, the correlation coefficient (r_s) between antigen-specific IgE and local ECP was not calculated, as local specific IgEs in normal subjects were almost below the detection threshold.

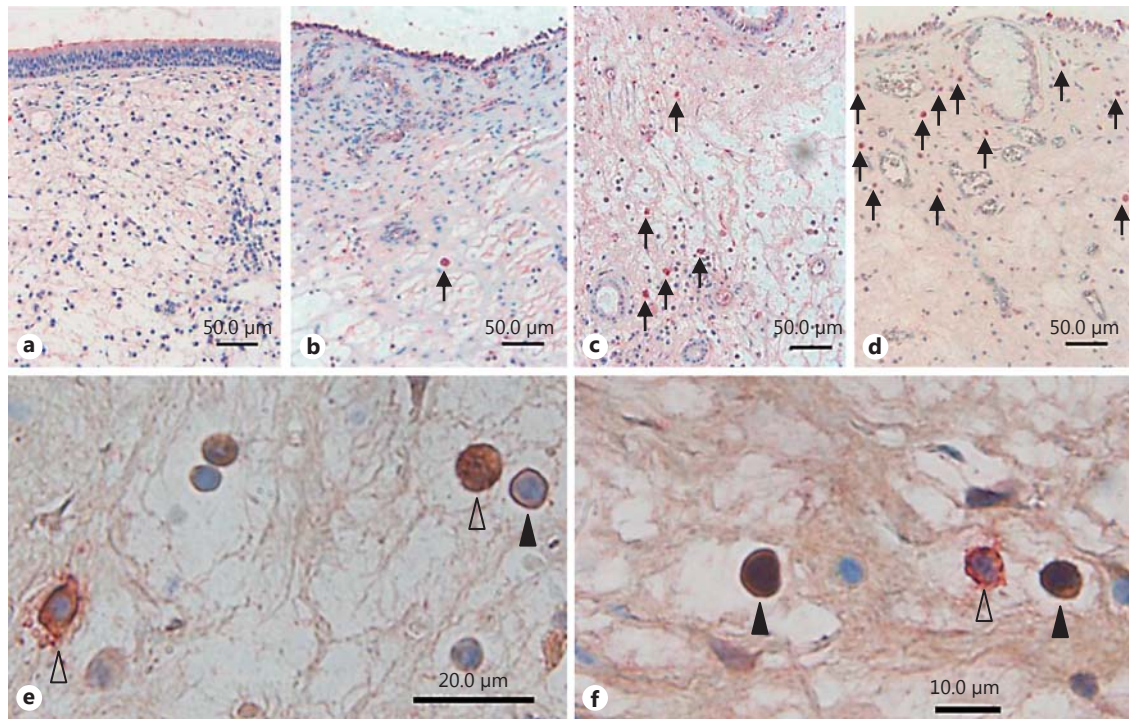


Fig. 2. The expression and distribution of local IgE in sinus mucosa and NP. IgE-positive cells (indicated by an arrow) were detected in AFRS (**d**), ECRS (**c**) and NECRS (**b**) subepithelium, but not in controls (**a**). **e** IgE (red)/mast cell chymase (brown) double immunostaining shows that both markers are positive for mast

cells with IgE (Δ), while the cell single-stained with IgE (red) looks morphologically like a plasma cell ($\blacktriangle$). **f** IgE (red)/plasma cells (brown) double immunostaining shows that both markers are positive for plasma cells with IgE ($\blacktriangle$), and the cell single-stained with IgE (red) looks morphologically like a mast cell (Δ).

Table 2. Correlation between IgE (serum, local) and the local ECP level

	Correlation coefficient (r_s)							
	Patients (total)		Controls		CRSwNP		AFRS	
	serum	local	serum	local	serum	local	serum	local
Total IgE	0.285	0.655**	0.323	0.810*	0.037	0.529**	-0.214	0.833**
Specific IgE								
<i>Alternaria</i>	0.311*	0.577**	-	-	-	0.462*	-0.119	0.714*
<i>Aspergillus</i>	0.260	0.585**	-	-	-0.077	0.418*	-0.333	0.619
<i>Candida</i>	0.155	0.512**	-	-	-	0.289	-0.335	0.619
<i>Cladosporium</i>	0.329*	0.521**	-	-	-	0.340	0.575	0.611
<i>Penicillium</i>	0.222	0.549**	-	-	0.068	0.394*	-0.091	0.857**
SEA	0.130	0.471**	-	-	-0.050	0.316	-0.109	0.464
SEB	0.207	0.460**	-	-	0.022	0.414*	0.063	0.469
House dust mite (<i>Dp</i>)	0.131	0.479**	-	-	0.042	0.444*	0.049	0.659
House dust mite (<i>Df</i>)	0.099	0.465**	-	-	0.036	0.370*	-0.493	0.810*
Cedar pollen	0.093	0.214	-	-	-0.007	0.234	-0.240	0.143
Ragweed pollen	0.242	0.214	-	-	0.192	0.246	0.023	-0.082

Spearman's rank correlation coefficient was applied to define the relationships between IgE (serum, local) and the local ECP level.

* $p < 0.05$; ** $p < 0.01$.

Discussion

We found that local total IgE and antigen (fungi, mites and SEs)-specific IgEs in nasal polyps are related to local eosinophilic inflammation of CRSwNP and AFRS. These conclusions are based on the following observations. (1) Local total IgE was significantly increased in both ECRS and AFRS compared with NECRS and the controls. (2) Serum total IgE was significantly higher in AFRS patients than in the other groups. (3) IgE-positive cells were immunohistochemically detected in AFRS, ECRS and NECRS subepithelium, but not in controls. (4) The local total IgE correlated significantly with the local ECP, regardless of the presence of chronic sinus disease. (5) In total subjects, all local fungi-specific IgEs correlated more strongly with local ECP than the other local antigen-specific IgEs. (6) In CRSwNP patients, antigen-specific IgEs (*Alternaria* > mite (*Dp*) > *Aspergillus* > SEB > *Penicillium* > mite (*Df*), but not the other fungi, SEA or pollens) in the sinus mucosa (but not serum IgEs) correlated significantly with local ECP. (7) In AFRS patients, antigen-specific IgEs (*Penicillium* > mite (*Df*) > *Alternaria*, but not other fungi, SEs or pollens) in the sinus mucosa (but not serum IgEs) correlated significantly with local ECP.

Although the role of local IgE production plays against SEs and mites in the pathogenesis of CRSwNP has been described [7–10], our findings indicate that fungi are also important allergens that cause eosinophilic inflammation, not only in AFRS but also in CRSwNP. Fungi are environmentally ubiquitous and, except when invasive, are generally not looked upon as being harmful/pathological. However, epidemiological and clinical reports recognize associations between asthma and allergic diseases and the presence of, or exposure to, airborne fungi such as *Alternaria* (in North America), *Cladosporium* and *Aspergillus* (in Europe) [12]. Allergic sensitivity to, and the airborne presence of, *Alternaria* are associated with the development and persistence of asthma [13]. In a large multi-country, cross-sectional study, sensitization to *Alternaria* or *Cladosporium*, but not to pollens or cats, was found to be a significant risk factor for severe asthma (odds ratio: 2.34) [14], while exposure to *Alternaria* was a risk factor for respiratory arrest in patients with asthma [15]. In vitro, *Alternaria* can directly induce eosinophilic activation and degranulation [16–18]. It also induces Th2 cytokine production in the human airway epithelium, the initial interface with airborne pathogens and allergens [19]. Thus, both acquired and innate immune responses to environmental fungi may increase the production of cytokines and provide the cellular activation signals that

lead to eosinophilic inflammation in both AFRS and CRSwNP patients.

We also found actual amounts of local IgE and IgE-positive cells in NP, suspecting the possibility of local IgE production. Previous reports support our data, showing that nasal mast cells [20] and basophils [21] induce IgE synthesis. Allergen-driven class switching to IgE was confirmed in the nasal mucosa [22, 23]. A recent study indicated that both Th17 cells and IL-17 promote IgE production and exert direct proallergic effects at the level of B cells [24]. IL-17 expression was associated with allergic diseases, such as asthma [25] and CRSwNP comorbid with asthma [26]. Here, we showed that both the serum and local total IgE levels were significantly increased in AFRS, while only local total IgE, but not serum IgE, was increased in ECRS. Furthermore, local total IgE and local ECP of AFRS were more strongly correlated than those of CRSwNP. These results suggest several possible pathogenetic scenarios for AFRS and ECRS: (1) AFRS is actually a systemic IgE-mediated disease, (2) local IgE production is increased in both AFRS and ECRS, but is much higher in AFRS, and (3) local IgE produced in NP overflows into a blood vessel in AFRS. AFRS is most likely a hypertrophic IgE-mediated disease at both the systemic and local levels. We still understand only the tip of the iceberg regarding the pathogenesis of these diseases, and much further investigation is needed.

Disclosure Statement

The authors declare that no financial or other conflict of interest exists in relation to the content of this article.

References

- ▶ 1 Matsuwaki Y, Ookushi T, Asaka D, Mori E, Nakajima T, Yoshida T, Kojima J, Chiba S, Ootori N, Moriyama H: Chronic rhinosinusitis: risk factors for the recurrence of chronic rhinosinusitis based on 5-year follow-up after endoscopic sinus surgery. *Int Arch Allergy Immunol* 2008;146(suppl 1):77–81.
- ▶ 2 Nakayama T, Yoshikawa M, Asaka D, Okushi T, Matsuwaki Y, Otori N, Hama T, Moriyama H: Mucosal eosinophilia and recurrence of nasal polyps – new classification of chronic rhinosinusitis. *Rhinology* 2011;49:392–396.
- ▶ 3 Stammberger H: Surgical treatment of nasal polyps: past, present, and future. *Allergy* 1999;54:7–11.
- ▶ 4 Stoop AE, van der Heijden HA, Biewenga J, van der Baan S: Eosinophils in nasal polyps and nasal mucosa: an immunohistochemical study. *J Allergy Clin Immunol* 1993;91:616–622.

- ▶5 Matsuwaki Y, Yanagi K, Nakajima T, Moriyama H: Allergic fungal sinusitis. *Nihon Jibiinkoka Gakkai Kaiho* 2002;105:1157–1165.
- ▶6 Bent JP 3rd, Kuhn FA: Antifungal activity against allergic fungal sinusitis organisms. *Laryngoscope* 1996;106:1331–1334.
- ▶7 Bachert C, Gevaert P, Holtappels G, Johansson SG, van Cauwenberge P: Total and specific IgE in nasal polyps is related to local eosinophilic inflammation. *J Allergy Clin Immunol* 2001;107:607–614.
- ▶8 Seiberling KA, Conley DB, Tripathi A, Grammer LC, Shuh L, Haines GK 3rd, Schleimer R, Kern RC: Superantigens and chronic rhinosinusitis: detection of staphylococcal exotoxins in nasal polyps. *Laryngoscope* 2005;115:1580–1585.
- ▶9 Wise SK, Ahn CN, Lathers DM, Mulligan RM, Schlosser RJ: Antigen-specific IgE in sinus mucosa of allergic fungal rhinosinusitis patients. *Am J Rhinol* 2008;22:451–456.
- ▶10 Ahn CN, Wise SK, Lathers DM, Mulligan RM, Harvey RJ, Schlosser RJ: Local production of antigen-specific IgE in different anatomic subsites of allergic fungal rhinosinusitis patients. *Otolaryngol Head Neck Surg* 2009;141:97–103.
- ▶11 Meltzer EO, Hamilos DL, Hadley JA, Lanza DC, Marple BF, Nicklas RA, Adinoff AD, Bachert C, Borish L, Chinchilli VM, Danzig MR, Ferguson BJ, Fokkens WJ, Jenkins SG, Lund VJ, Mafee MF, Naclerio RM, Pawankar R, Ponikau JU, Schubert MS, Slavin RG, Stewart MG, Togias A, Wald ER, Winther B: Rhinosinusitis: developing guidance for clinical trials. *J Allergy Clin Immunol* 2006;118:S17–S61.
- ▶12 Denning DW, O'Driscoll BR, Hogaboam CM, Bowyer P, Niven RM: The link between fungi and severe asthma: a summary of the evidence. *Eur Respir J* 2006;27:615–626.
- ▶13 Bush RK, Prochnau JJ: *Alternaria*-induced asthma. *J Allergy Clin Immunol* 2004;113:227–234.
- ▶14 Zureik M, Neukirch C, Leynaert B, Liard R, Bousquet J, Neukirch F: Sensitisation to airborne moulds and severity of asthma: cross-sectional study from European Community Respiratory Health Survey. *BMJ Clin Res Ed* 2002;325:411–414.
- ▶15 O'Hollaren MT, Yunginger JW, Offord KP, Somers MJ, O'Connell EJ, Ballard DJ, Sachs MI: Exposure to an aeroallergen as a possible precipitating factor in respiratory arrest in young patients with asthma. *N Engl J Med* 1991;324:359–363.
- ▶16 Inoue Y, Matsuwaki Y, Shin SH, Ponikau JU, Kita H: Nonpathogenic, environmental fungi induce activation and degranulation of human eosinophils. *J Immunol* 2005;175:5439–5447.
- ▶17 Matsuwaki Y, Wada K, White TA, Benson LM, Charlesworth MC, Checkel JL, Inoue Y, Hotta K, Ponikau JU, Lawrence CB, Kita H: Recognition of fungal protease activities induces cellular activation and eosinophil-derived neurotoxin release in human eosinophils. *J Immunol* 2009;183:6708–6716.
- ▶18 Matsuwaki Y, Wada K, Moriyama H, Kita H: Human eosinophil innate response to *Alternaria* fungus through protease-activated receptor-2. *Int Arch Allergy Immunol* 2011;155(suppl 1):123–128.
- ▶19 Matsuwaki Y, Wada K, White T, Moriyama H, Kita H: *Alternaria* fungus induces the production of GM-CSF, interleukin-6 and interleukin-8 and calcium signaling in human airway epithelium through protease-activated receptor 2. *Int Arch Allergy Immunol* 2012;158(suppl 1):19–29.
- ▶20 Pawankar R, Okuda M, Yssel H, Okumura K, Ra C: Nasal mast cells in perennial allergic rhinitis exhibit increased expression of the Fc epsilonRI, CD40L, IL-4, and IL-13, and can induce IgE synthesis in B cells. *J Clin Invest* 1997;99:1492–1499.
- ▶21 Yanagihara Y, Kajiwaru K, Basaki Y, Ikizawa K, Ebisawa M, Ra C, Tachimoto H, Saito H: Cultured basophils but not cultured mast cells induce human IgE synthesis in B cells after immunologic stimulation. *Clin Exp Immunol* 1998;111:136–143.
- ▶22 Coker HA, Durham SR, Gould HJ: Local somatic hypermutation and class switch recombination in the nasal mucosa of allergic rhinitis patients. *J Immunol* 2003;171:5602–5610.
- ▶23 Takhar P, Smurthwaite L, Coker HA, Fear DJ, Banfield GK, Carr VA, Durham SR, Gould HJ: Allergen drives class switching to IgE in the nasal mucosa in allergic rhinitis. *J Immunol* 2005;174:5024–5032.
- ▶24 Milovanovic M, Drozdenko G, Weise C, Babina M, Worm M: Interleukin-17a promotes IgE production in human B cells. *J Invest Dermatol* 2010;130:2621–2628.
- ▶25 Agache I, Ciobanu C, Agache C, Anghel M: Increased serum IL-17 is an independent risk factor for severe asthma. *Respir Med* 2010;104:1131–1137.
- ▶26 Saitoh T, Kusunoki T, Yao T, Kawano K, Kojima Y, Miyahara K, Onoda J, Yokoi H, Ikeda K: Role of interleukin-17a in the eosinophil accumulation and mucosal remodeling in chronic rhinosinusitis with nasal polyps associated with asthma. *Int Arch Allergy Immunol* 2010;151:8–16.