Rhinology, 46, 226-230, 2008

Tissue-specific immunoglobulin E in maxillary sinus mucosa of allergic fungal sinusitis*

Yu-Tuan Chang[1] and Sheen-Yie Fang[2]

[1] Department of Otolaryngology, Tainan Municipal Hospital, Tainan, Taiwan
[2] Department of Otolaryngology, National Cheng Kung University Hospital, Tainan, Taiwan

SUMMARY

Objective: *The objectives of this prospective study were to analyze the specific immunoglobulin E (sIgE) in maxillary sinus mucosa and to determine the importance of local tissue sIgE in the patients with allergic fungal sinusitis (AFS).*

Methods: *We investigated tissue-specific IgE in the maxillary sinus mucosa. Thirty-four patients with rhinosinusitis and nasal polyposis were included in the study. The patients were divided into three groups – AFS, fungal sinusitis and chronic rhinosinusitis(CRS). The sIgE profile of the maxillary sinus mucosa was studied by the CAP method. Other parameters, such as allergic symptoms, presence of fungi hyphae and eosinophilic mucin in the sinus cavities as well as computed tomography (CT) scanning findings were also evaluated in all groups.*

Results: *All patients in the AFS group had allergic symptoms, and the serum IgE test was positive to mites or house dust, but none had a positive serum IgE response to Aspergillus. However, 85.7% of this group had tissue sIgE to Aspergillus.*

Conclusions: *The local tissue sIgE profile is more specific than the systemic sIgE profile in determining the allergic status of AFS patients. Tissue sIgE for fungi may be considered as a part of AFS diagnostic criteria.*

Key words: allergic fungal sinusitis, immunoglobulin E, CAP, pathophysiology.

INTRODUCTION

Allergic fungal sinusitis (AFS) is a noninvasive fungal rhinosinusitis that represents an allergic and immunological response to the presence of fungal hyphae in the sinus cavities [(1)]. It is a distinct form of chronic rhinosinusitis (CRS), first reported in 1983 by Katzenstein et al. [(2)]. Immunoglobulin (Ig) E mediated type I hypersensitivity to fungi has been postulated to be a pathogenic mechanism in AFS (3-7). The incidence of AFS has been estimated to be about 5~10 % of all chronic sinusitis cases going to surgery [(1,2)]. The prevalence of the disease is extremely variable depending on the different countries: particularly high in warm, humid climates such as the southern United States and India, and low in Europe [(4,6,8-11)]. Since the precise relationship between allergy and chronic sinusitis is still debatable, many investigational methods have been used for study. These include the radioallergosorbent test (RAST) [(12, 13)] and the CAP system (Pharmacia Diagnostics, Uppsala, Sweden) [(14)] to detect specific immunoglobulin E (sIgE) in the patient's serum and local secretions. However, the CAP system is both more sensitive and more rapid than RAST, without loss of specificity [(15, 16)].

The treatment of AFS involves initial sinus surgery and postoperative medications because AFS is a highly recurrent disease. Antibiotics only help if there is an acute bacterial exacerbation in the chronic disease progress. In recent decades, many postoperative treatments have been devised, such as antihistamines, oral steroids, intra-nasal corticosteroids, aeroallergen immunotherapy and other anti-allergy therapies [(8,17,18)]. It is critical to correctly diagnose AFS and to estimate the patient's immunological status. Unfortunately, we encountered some limitations in applying current AFS diagnostic criteria to patients in our practice. For example, a serum IgE test alone for the diagnosis of AFS is not an accurate indicator of local reactions involving the diseased sinus. However, as per the definition of AFS, the immunological reaction involves the diseased sinus mucosa. There is as yet no published data regarding the local immunological status of patients with AFS. Thus, in the present study, we undertook to measure, by the CAP system, tissue specific IgE in the maxillary sinus mucosa, and to compare our results with serum-specific IgE tests and the clinical manifestations of AFS patients.

MATERIALS AND METHODS

Patients

Our prospective study included 34 patients with chronic rhinosinusitis and nasal polyposis. These cases were divided into three groups – AFS, fungal sinusitis, and CRS (control group).

*Received for publication: September 20, 2007; accepted: May 9, 2008

In the first group, 14 AFS patients were included, employing the acknowledged AFS diagnostic criteria described by Bent and Kuhn [1] in 1994, which were: [1] nasal polyposis, [2] IgE mediated type I hypersensitivity, [3] eosinophilic mucus without fungal invasion into sinus tissue, [4] positive fungal stain of sinus contents, [5] typical computed tomography (CT) scan with scattered hyperdensity within the obstructed paranasal sinuses. Actually, these five characteristic criteria are not always seen in every AFS patient [9,19,20]. In our study, all participants in AFS group had allergic symptoms, nasal polyposis, eosinophilic mucus, positive fungal staining of sinus contents, and evidence of type I hypersensitivity. Thus, the diagnosis of AFS in our study was absolutely certain. Half of our AFS patients had the classic preoperative findings of AFS on CT scanning. Incidentally, Serrano and coauthors have recently pointed out that the lack of a characteristic CT scan does not necessarily rule out the diagnosis of AFS [21].

The second group included 10 fungal sinusitis patients, in which the diagnoses were confirmed by fungal hyphae identification and final pathological reports. The third group is composed by 10 CRS patients, which was defined as the control group, with inclusion criteria of [1] endoscopy confirmed middle meatus polyps, [2] CT showing bilateral mucosal disease, and [3] pathological evidence [22].

The mean (± SD) age of all patient groups was 47.8 ± 2.3 years, and 19 of 34 patients were males. The mean age of the AFS patient group was 28.4 ± 3.7 years, and 8 of 14 patients were males.

Methods

The allergic symptoms, CT scan, and serum sIgE tests were obtained for all patients before surgical intervention. Then all patients underwent endoscopic sinus surgery, and the maxillary sinus mucosa was taken for histopathological examination, mucin smear (GSA stain for fungi hyphae), fungi culture and tissue sIgE tests. Serum and tissue sIgE levels were investigated, using the CAP system (Pharmacia Diagnostics), to five common aeroallergens in Taiwan – *Dermatophagoides pteronyssinus, Dermatophagoides farinae, Candida, Aspergillus,* and *Penicillium.*

For investigation with the CAP system, maxillary sinus mucosa tissue was frozen at -70°C immediately after removal. Samples selected for study were thawed, ground, and homogenized with lysis buffer (120 mmol/L sodium chloride, 10 mmol/L Tris-hydrochloric acid, Nonidet P40, 1% deoxycholate, 0.1% sodium dodecyl sulfate [pH 6.8]) and were stored at 4°C for about 30 minutes. These samples were then centrifuged at 14,000 rpm for 20 minutes. The upper liquid layer was removed and used to run the CAP system. Serum and sinus tissue sIgE levels corresponding to the five kinds of allergens were determined. A positive result was defined as a sIgE level of 0.35 kU/L or greater.

Table 1. Three groups in our study: allergic fungal sinusitis, fungal sinusitis and chronic rhinosinusitis.

Group	Case number	Including criteria
Allergic Fungal Sinusitis	14	a. Nasal polyposis b. Type I hypersensitivity (IgE mediated) c. Eosinophilic mucus without fungal invasion into sinus tissue d. Positive fungal smear. e. Characteristic CT scan (not always necessary)
Fungal Sinusitis	10	a. Pathological proof and fungal hyphae identification b. CT scan finding
Chronic rhinosinusitis (control group)	10	a. Endoscopy confirmed middle meatus polyps b. CT showed bilateral mucosal disease c. Pathological proof

RESULTS

Allergic symptoms, results of the serum and tissue CAP tests, mucin smears, and CT findings of the three groups are shown in Table 2. All 14 patients with AFS had allergic symptoms (sneezing, itching nose, etc), and positive serum IgE responses to house dust mite and house dust. However, none had a positive serum IgE response to *Aspergillus*. In contrast, 85.7% (12/14) showed a positive tissue sIgE response to *Aspergillus*, 100% had eosinophilic mucin, and 50% had typical CT findings. All specimens with AFS showed scanty numbers of fungal hyphae on a mucous smear, however, none was noted with positive fungal culture.

Of the 10 patients with fungal sinusitis in this study, only 20% had allergic symptoms, 10% exhibited a positive serum CAP test to mites and house dust, and none demonstrated positive

Table 2. Findings in patients with allergic fungal sinusitis, fungal sinusitis and chronic rhinosinusitis.

	Allergic Fungal Sinusitis	Fungal Sinusitis	Chronic rhinosinusitis
Number	14	10	10
Allergic symptoms	14	2	3
Serum IgE to mites, dust (+)	14	1	4
Serum IgE to *Aspergillus* (+)	0	0	0
Tissue sIgE to *Aspergillus* (+)	12	0	0
Eosinophilic mucin	14	8	6
Fungal hyphae	14	10	0
Characteristic CT findings	7	0	0

tissue sIgE responses to *Aspergillus*, nor did they have typical CT findings of AFS. All specimens in this group showed many fungal hyphae on the mucous smear. In the control group (CRS), 30% had allergic symptoms, 40% exhibited positive serum CAP tests to mites and house dust, and none demonstrated positive tissue sIgE responses to *Aspergillus*, nor did they have typical findings of AFS on CT scanning.

DISCUSSION

First, it is notable that a positive fungal culture does not confirm the diagnosis of AFS, nor does a negative culture rule it out. Fungi may proliferate as saprophytic growth in diseased sinuses. This concept had proven by many studies. In 1999, Ponikau et al. [6] collected 210 consecutive CRS patients, and fungal cultures of nasal secretions were positive in 202 (96%) of them. In 2003, similar results were reported from Europe, where investigators found fungi by histology in 75.5% (28/37) of consecutive surgical chronic rhinosinusitis patients [23]. Of course, not all of these patients belonged to AFS group. Hence, allergic mucin or tissue remains the most reliable indicator of AFS, instead of fungal culture. Therefore we needed to focus on specific IgE to fungi, not fungal culture.

In our study, the AFS group showed a high proportion of positive serum IgE responses to mites and house dust (14/14), but none had a positive serum IgE response to *Aspergillus* (0/14). In the literature review, Manning and Holman reported that 66.6% patients in the AFS group tested positive for Bipolaris specific IgE by RAST and 88.9% tested positive for IgG by ELISA [10]. These results are quite different from ours. In contrast, Schubert MS reported 67 cases with AFS, with an elevation in total serum IgE at diagnosis but with no elevation of serum fungal-specific IgE [8]. The latter study results are similar to our data. There may be several possible explanations for this finding. Firstly, all AFS patients are atopic. Fungi have long been recognized as inhalant allergens, playing a role as noninvasive stimulants of mucosal inflammation. Even if an immunological response to *Aspergillus* cannot be detected in the serum, its local effect in the sinuses still can cause allergic symptoms and induce rhinosinusitis changes. Secondly, the concentration of specific IgE to fungi may be too low to be detected in serum; but high in localized inflammatory tissue [24]. That's why we can get positive tissue sIgE CAP results in most (85.7%) of AFS patients. Thirdly, we chose to detect only five kinds of sIgE in our study; it may be possible that we failed to detect other kinds of sIgE in the study participants, especially sIgE to other species of fungi.

We have noticed that serum IgE tests do not always show a good correlation between AFS and allergic reactions. In previous studies, type I allergy was defined as either a positive skin test or the detection of specific IgE in the serum [8-10]. However, Shatkin and coauthors showed that this definition omitted cases in which IgE is produced locally in the nasal or sinus mucosa, thus remaining undetectable in serum or by skin tests [24]. To find a better and more specific method for confirming the diagnosis and prognosis of AFS, our study was designed to detect, using the CAP system, tissue specific IgE to *Aspergillus* in the maxillary sinus. The sIgE profile of the maxillary sinus mucosa was studied by the CAP method instead of RAST. The CAP system has usually been compared with RAST for the detection of serum sIgE. Both of them have been used widely in clinical practice for the investigation of allergy in Taiwan. The superiority of CAP has been demonstrated in different studies [15, 16]. Additionally, the CAP system allows for quick provision of test results (i.e., within 6 hours) as compared to the modified RAST (around 3 days). In short, the CAP technique is both faster and more convenient to perform in clinical practice. The CAP technique is more sensitive than RAST without loss of specificity when 0.35 kU/L is used as the cutoff value for the detection of serum sIgE [12,13]. We used the CAP technique to detect sIgE in serum and maxillary sinus tissue with the selected cutoff value set at 0.35 kU/L in this study.

By using CAP for measuring sinus tissue sIgE, 85.7% cases had positive results to *Aspergillus* in our AFS group. Compared to the serum sIgE, tissue sIgE tests are more sensitive and accurate, and they may also provide evidence for the pathogenic mechanisms involving locally produced fungal-specific IgE. Thus, a tissue CAP test should be routinely done for patients who exhibit positive allergic symptoms and are suspected to have AFS.

Besides, we should mention the reasons why we chose sinus mucosal tissue instead of sinus mucin for local sIgE measuring. Collins and coauthors recently evaluated the sinus mucin of AFS patients by detecting fungal-specific IgE and fungal culture [20]. They proved that patients with AFS were more likely to have fungal-specific IgE in sinus mucin (71%). Total serum IgE elevation had no evident association with systemic fungal allergy. These results were similar to ours. The difference was that we used sinus mucosal tissue instead of sinus mucin. There are two reasons for chosing sinus mucosa. First, there is no published data describing the sIgE on mucosal tissue in AFS patients. Second, it is well known that the sinus mucin is composed of secretion from goblet cells, submucosal glands, and exudates from vessels. Therefore the allergic status in which sinus mucin reflects is the sum of local and systemic immunologic responses. In contrast, the mucosal tissue demonstrates mainly local immunologic status, thus it can provide more direct evidence that locally produced fungal-specific IgE plays an important role in AFS. Indeed, we reported 85.7% positive rate for sIgE to *Aspergillus* by measuring mucosal tissue in our AFS group.

Regarding the causative fungi in AFS, there are obvious differences between continents. *Aspergillus* is not the sole causative

agent but also *Bipolaris, Dreschleria, Urvularia, Curvularia*, etc. In the literature review performed by Manning and Holman [10], 263 cases of AFS in America were identified, of which 168 cases yielded positive cultures, 87% were from the dematiaceous genera, and only 13% yielded *Aspergillus*. However, in Asian, Rupa et al. [11] from India reported that *Aspergillus* species were the most common fungi isolated (95.8%) in a series of 24 patients with AFS. Fadl et al. [25] from Saudi Arabia reported 4 cases of AFS, and all grew *Aspergillus*. Goh *et al.* [26] from Malaysia also reported that *Aspergillus* species were the most common fungi (54.5%) cultured in their group. Even though the reports from Asia and the Middle East were small as compared with the Western literature review, the main fungi causing AFS was indeed influenced by geographic location. Therefore we chose *Aspergillus* as our main target of tissue sIgE CAP tests, due to our subjects were all live in Asia.

In the second group of fungal sinusitis, allergic symptoms were uncommon (2/10) in the fungal sinusitis group. Additionally, only one case had positive findings for serum IgE to mites and house dust, and none of them had positive results for serum IgE and tissue sIgE to *Aspergillus*. In all cases (10/10) hyphae were found in the smear, but none of them had positive sinus tissue sIgE to *Aspergillus*. These results confirm that patients of fungal sinusitis have fungal hyphae in their sinuses, but it does not induce a local immunological response. It also indicates that fungal sinusitis is an infective process, not an immunologically mediated disease. Its pathophysiology is quite different from AFS.

In the CRS group, some cases had allergic symptoms (30%), and some exhibited a positive serum CAP test to mites and house dust (40%). None demonstrated a positive test to *Aspergillus* in the serum CAP test or the tissue sIgE CAP test. Eosinophilic mucin was found in 60% CRS patients. It is reasonable to assume that both immunological and infectious process play an important role in CRS. As expected, fungal hyphae were not found in the smears of any of these patients.

There are many treatments for AFS, and a definite diagnosis of AFS is an important requirement for planning any treatment strategy. Post-operative medications include oral steroids, intra-nasal corticosteroids, immunotherapy and other anti-allergy therapies. [8, 17, 18] Once the tissue CAP test yields a positive result, allergy control is critical.

CONCLUSIONS

In conclusion, our understanding of AFS sinusitis has steadily evolved over the past three decades, with increasing evidence of specific hypersensitivity to the causative fungi. Our study illustrates the closer relationship between AFS and local mucosal tissue specific IgE to fungi (85.7% positive), as compared to serum sIgE. This also confirms the hypothesis that locally-produced fungal-specific IgE plays a role in the pathogenesis of AFS. According to this concept, post-operative anti-allergic treatment should not only focus on traditional systemic formulations; topical agents are also essential. Also, a positive tissue sIgE response to fungi may play a part in the diagnosis criteria of AFS.

ACKNOWLEDGEMENT

This study was granted by The National Science Council, Taiwan. (NSC95-2314-B006-017)

REFERENCES

1. Bent III JP, Kuhn FA. Diagnosis of allergic fungal sinusitis. Otolaryngol Head Neck Surg 1994; 111: 580-588.
2. Katzenstein AL, Sale SR, Greenberger PA. Allergic Aspergillus sinusitis: a newly recognized form of sinusitis. J Allergy Clin Immunol 1983; 72: 89-93.
3. Manning SC, Mabry RL, Schaefer SD, Close LG. Evidence of IgE-mediated hypersensitivity in allergic fungal sinusitis. Laryngoscope 1993; 103: 717–721.
4. Schubert MS. Allergic fungal sinusitis. Otolaryngol Clin North Am 2004; 37: 301–326.
5. Corey JP, Delsuphene KG, Ferguson BJ. Allergic fungal sinusitis: allergic, infectious, or both? Otolaryngol Head Neck Surg1995; 113: 110-119.
6. Ponikau JU, Sherris DA, Kern EB, et al. The diagnosis and incidence of allergic fungal sinusitis. Mayo Clin Proc 1999; 74: 877–884.
7. deShazo RD, Chapin K, Swain RE. Fungal sinusitis. N Engl J Med 1997; 337: 254-259.
8. Schubert MS, Goetz DW. Evaluation and treatment of allergic fungal sinusitis. I: demographics and diagnosis. J Allergy Clin Immunol 1998; 102: 387-394.
9. deShazo RD, Swain RE. Diagnostic criteria for allergic fungal sinusitis. J Allergy Clin Immunol 1995; 96: 24-35.
10. Manning SC, Holman M. Further evidence for allergic pathophysiology in allergic fungal sinusitis. Laryngoscope 1998; 108: 1485-1496.
11. Rupa V, Jacob M, Mathews MS, et al. Clinicopathological and mycological spectrum of allergic fungal sinusitis in South India. Mycoses 2002; 45: 364 –367.
12. Small P, Barrett D, Frenkiel S, Rochon L, Cohen C, Black M. Local specific IgE production in nasal polyps associated with negative skin tests and serum RAST. Ann Allergy 1985; 55: 736-739.
13. 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.
14. Axen R, Drevin H, Kober A, Yman L. A new laboratory diagnostic system applied to allergy testing. In: Johansson SGO, ed. Clinical workshop: IgE antibodies and the Pharmacia CAP system in allergy diagnosis. Uppsala, Sweden: Pharmacia, 1988: 3-7.
15. Corey JP, Nelson RS, Lai V. Comparison of modified PhadezymRAST, ImmunoCAP, and serial dilution titration skin testing by receiver operating curve analysis. Otolaryngol Head Neck Surg 1995; 112: 665-669.
16. Leimgruber A, Lantin JP, Frei PC. Comparison of two in vitro assays, RAST and CAP, when applied to the diagnosis of anaphylactic reactions to honeybee or yellow jacket venoms. Correlation with history and skin tests. Allergy 1993; 48: 415-420.
17. Roth M. Should oral steroids be the primary treatment for allergic fungal sinusitis? Ear Nose Throat J 1994; 73: 928-930.
18. Mabry RL, Marple BF, Folker RJ, et al. Immunotherapy in the treatment of allergic fungal sinusitis: three years' experience. Otolaryngol Head Neck Surg 1998; 119: 648-651.
19. Pant H, Kette FE, Smith WB, Macardle PJ, Wormald PJ. Eosinophilic mucus chronic rhinosinusitis: clinical subgroups or a homogeneous pathogenic entity? Laryngoscope 2006; 116: 1241-1247.

20. Collins M, Nair S, Smith W, Kette F, Gillis D, Wormald PJ. Role of local immunoglobulin E production in the pathophysiology of noninvasive fungal sinusitis. Laryngoscope 2004; 114: 1242-1246.
21. Serrano E, Percodani J, Uro-Coste E, Yardeni E, Abbal M, Linas MD. Value of investigation in the diagnosis of allergic fungal rhinosinusitis: results of a prospective study. J Laryngol Otol 2001; 115: 184–189.
22. Meltzer EO, Hamilos DL, Hadley JA, et al. Rhinosinusitis: developing guidance for clinical trials. J Allergy Clin Immunol. 2006; 118 (5 Suppl): S17-61.
23. Braun H, Buzina W, Freudenschuss K, Beham A, Stammberger H. Eosinophilic fungal rhinosinusitis: a common disorder in Europe? Laryngoscope 2003; 113: 264–269.
24. Shatkin JS, Delsupehe KG, Thisted RA, Corey JP. Mucosal allergy in the absence of systemic allergy in nasal polyposis and rhinitis: a meta-analysis. Otolaryngol Head Neck Surg 1994; 111: 553–556.
25. Fadl FA, Hassan KM, Faizuddin M. Allergic fungal rhinosinusitis: report of 4 cases from Saudi Arabia. Saudi Med J 2000; 21: 581–584.
26. Goh BS, Gendeh BS, Rose IM, Pit S, Samad SA. Prevalence of allergic fungal sinusitis in refractory chronic rhinosinusitis in adult Malaysians. Otolaryngol Head Neck Surg 2005; 133: 27-31.

Professor Sheen-Yie Fang
Department of Otolaryngology
Faculty of Medicine
National Cheng Kung University
138 Sheng-Li Road
Tainan
70428 Taiwan(ROC)

Tel: +88-66-235 3535ext. 5311
Fax: +88-66-237 7404
E-mail: sheen@mail.ncku.edu.tw