

Detecting local immunoglobulin E from mucosal brush biopsy of the inferior turbinates using microarray analysis

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Background: It has been previously demonstrated that local, antigen-specific immunoglobulin E (IgE) can be detected using a standard in vitro assay of lysed epithelial cells in saline, harvested via nasal mucosal brush biopsy (MBB). However, compared to surgical biopsy or serum, smaller amounts of IgE are harvested using MBB, making detection much more difficult. Microarray analysis (MA) requires less IgE for detection, making this an attractive option for MBB. The goals of this study were to compare MA to a standard IgE assay for detecting antigen-specific IgE from MBB and to test the association between the presence of multiple positive components on MA with specific IgE on standard assay and skin-prick testing (SPT) grade.

Methods: MBB samples from 18 allergic rhinitis patients, which were previously tested for antigen-specific IgE to common airborne allergens using a standard IgE assay, underwent MA for antigen-specific IgE to multiple components of airborne and food allergens. Fisher's exact probability testing was used to measure the strength of association between the 2 testing modalities for Timothy grass, ragweed, cat, *Alternaria*, and *D. farinae*.

Results: MA correlated very highly with standard assay ($p < 0.0001$) and 50% of positive antigens on MA detected multiple components to that antigen. The presence of multiple components was not associated with specific IgE levels on standard assay or SPT grade.

Conclusion: This is the first demonstration that antigen-specific IgE in saline samples can be measured using MA. The ability of MA to measure smaller amounts of IgE, with similar accuracy, may give it a potential advantage for MBB analysis in the future. © 2012 ARS-AAOA, LLC.

Key Words:

mucosal brush biopsy; microarray analysis; allergic rhinitis; inferior turbinate; local IgE

How to Cite this Article:

Reisacher WR. Detecting local immunoglobulin E from mucosal brush biopsy of the inferior turbinates using microarray analysis. *Int Forum Allergy Rhinol.* 2012;00:X-XX.

Over the past decade, there has been increasing interest in antigen-specific immunoglobulin E (IgE) present in the sinonasal tract and the role it plays in diseases such as allergic rhinitis (AR),¹ nonallergic rhinitis (NAR),²

chronic rhinosinusitis (CRS),³ allergic fungal rhinosinusitis (AFRS),^{4,5} and nasal polyposis (NP).⁶ Local IgE has also been found to be elevated in the esophageal mucosa of patients with eosinophilic esophagitis (EoE)⁷ as well as in the bronchial mucosa of both atopic and nonatopic asthma patients.⁸ Moreover, it has been demonstrated that antigen-specific IgE may be present in the sinonasal tract even in the absence of positive skin testing or in vitro testing.^{9,10}

Currently, the only methods available to harvest local IgE from epithelial cells of the sinonasal tract are mucosal brush biopsy (MBB)¹¹ and surgical biopsy of sinonasal tissue.¹² Surgical biopsy can be performed either in the operating room or in the office, but testing of children can only be done under general anesthesia. In contrast, MBB is a rapid, less invasive procedure that is performed using a cytology brush under topical anesthesia in the office. However, both methods of local IgE testing are limited by the smaller amount of IgE that can be harvested compared to serum samples. This is particularly true for MBB, which harvests

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Potential conflict of interest: W.R.R. is on the Speaker's Bureau for Thermo Fisher Scientific, who provided the microarray testing at no charge, but played no other role in this study. Presented at the American Academy of Otolaryngic Allergy Annual Meeting on September 10, 2012, Washington, DC.

Received: 27 July 2012; Revised: 18 September 2012; Accepted: 25 September 2012

DOI: 10.1002/alar.21111

View this article online at wileyonlinelibrary.com.

approximately 36% of the IgE that is harvested on surgical biopsy, measured by a standard fluorescent IgE assay.¹¹

Component-resolved diagnostic testing, using a microarray of purified or recombinant allergen components, was introduced several years ago to allow for more precise determination of food and airborne sensitizations.^{13–15} By determining the presence of cross-reactive components, microarray analysis (MA) may help predict the nature of the clinical response, particularly to food antigens, possibly avoiding the need for a double-blind placebo-controlled food challenge test.¹⁶ Studies have shown good correlation between MA and a standard fluorescent IgE assay, with high sensitivity and specificity for both techniques.¹⁷ In addition, only 400 μ L is needed for MA vs 20 mL or more for a comparable number of tests on standard assay. This ability of MA to detect smaller amounts of IgE gives it a potential advantage when used for MBB.

The central purpose of this study was to examine the utility of MA in the detection of IgE from MBB samples from the sinonasal tract, specifically compared to a standard IgE assay. MBB produces a sample of saline containing IgE at a significantly lower concentration than serum. Therefore, the first goal of the study was to determine whether or not MA can detect IgE suspended in a MBB sample. The second goal was to determine the strength of the association between MA and standard assay for testing of MBB samples, using 5 airborne study antigens. The final goal of this study was to determine whether or not MBB samples with multiple components detected from the same antigen on MA were more likely to have higher levels of specific IgE on standard assay, or higher grades on skin-prick testing (SPT).

Patients and methods

Patient sample

Twenty patients who had previously undergone MBB were selected for this study (Weill Cornell Medical College, New York City, IRB Protocol #0910010694).¹¹ All 20 patients had a clinical history of AR, age 18 years or greater, no prior history of allergy immunotherapy and positive SPT (grade 1 or higher) for at least 1 of 5 of the following antigens: Timothy grass, ragweed, cat, *Alternaria*, and *D. farinae*. As previously described, a grade 1 wheal is greater than 3 mm in diameter, but less than 5 mm; grade 2 is greater than 5 mm but less than 7 mm; grade 3 is greater than 7 mm but less than 10 mm; and grade 4 is 10 mm or greater, or any wheal associated with pseudopodia.¹⁸

Briefly, MBB had been performed on both inferior turbinates, with IgE harvested from lysed epithelial cells suspended in phosphate buffered saline (PBS) using a technique previously described.¹¹ A 1-mL sample of lysate from each patient had been sent frozen to a reference laboratory (Viracor-IBT Laboratories, Lee's Summit, MO) for a standard fluorescent IgE assay to determine total IgE levels as well as antigen-specific IgE levels for the 5 study antigens

TABLE 1. Study antigens and their components tested using microarray analysis

Antigen	Antigen component classifications (genus, species, subclass)
Timothy grass	Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 7 , Phl p 11, Phl p 12
Ragweed	Amb a 1
Cat	Fel d 1, Fel d 2 , Fel d 4
<i>Alternaria</i>	Alt a 1, Alt a 6
<i>D. farinae</i>	Der f 1, Der f 2

Antigen components in bold represent cross-reactive components.

(ImmunoCAP; Thermo Fisher Scientific, Portage, MI). Two of the patients were excluded from this study because of insufficient volume of remaining lysate for MA.

MA

Approximately 400 μ L of lysate for each patient was sent for Immuno Solid-phase Allergen Chip (ISAC) testing (Thermo Fisher Scientific, Portage, MI). Overall, the microarray contained 112 individual components; 29 species-specific food components (egg white, egg yolk, cow's milk, cod, shrimp, cashew, brazil nut, hazelnut, walnut, sesame, peanut, soy, buckwheat, wheat, and kiwi), 53 species-specific airborne components (birch, Timothy, bermuda, alder, cedar, cypress, olive, plane tree, ragweed, mugwort, goosefoot, wall pellitory, plantain, saltwort, cat, dog, horse, mouse, *Alternaria*, aspergillus, cladosporium, blomia, *D. farinae*, *D. pteronyssinus*, lepidog, cockroach, bee, paper wasp, common wasp, anisakis, and latex) and 30 components cross-reactive to tropomyosin, serum albumin, non-specific lipid transfer proteins (nsLTP), PR-10 proteins, thaumatin-like proteins (TLP), profilin, and cross-reactive carbohydrate determinants (CCD). The 5 study antigens and their respective components are listed in Table 1. Levels of specific IgE on MA are expressed in ISAC Standardized Units (ISU-E); <0.3 is undetectable, 0.3 to 0.99 is low, 1 to 14.99 is moderate/high, and ≥ 15 is very high.¹⁹ One sample of blank PBS was also sent for analysis to test for background readings.

Statistical analysis

In order to test the strength of association overall between MA and standard assay, as well as individually for each antigen, Fisher's exact probability testing was performed and 2-tailed *p* values were calculated. The mean IgE levels (kU/L) for antigens with multiple positive components vs those with only 1 positive component were compared with Student *t* test using online statistical software.²⁰ After performing an F-test to determine the significance of the difference between the variances of the 2 samples, a 2-tailed *p* value was calculated, with *p* < 0.05 considered statistically

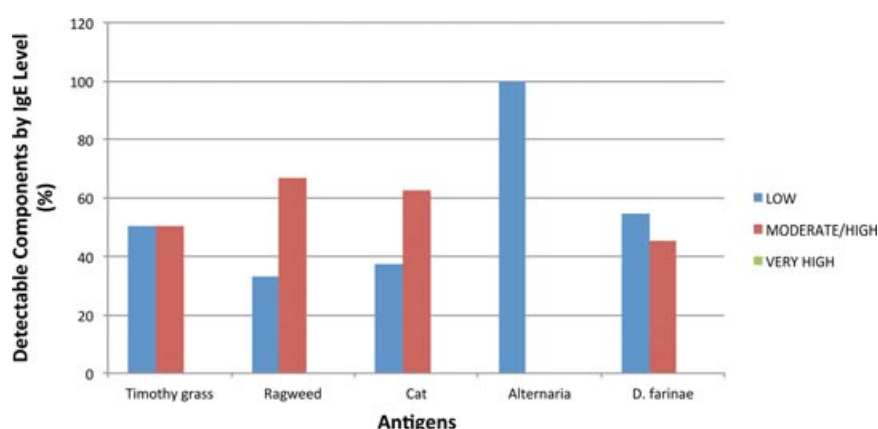


FIGURE 1. Distribution of detectable IgE levels for components of each test antigen, based on ISAC Standardized Units (ISU-E).

significant. A similar comparison was made between these 2 groups using the mean SPT grade.

Results

Results of microarray analysis

Overall, 36 of 288 (12.5%) of tests had detectable levels of IgE, which were specific to components of the study antigens. All of these positive reactions were to the species-specific components, with no positive reactions to the cross-reactive components for Timothy grass (profilin, Phl p 7, Phl p 12) or cat (serum albumin, Fel d 2). The distribution of detectable IgE categories on MA by individual antigen is shown in Figure 1. Considering all 5 study antigens together, 18 of 36 (50%) of positive reactions were in the low category, 18 of 36 (50%) were in the moderate/high category, and none of 36 (0%) were in the very high category. In addition, components for 7 different food antigens (hazelnut, shrimp, apple, peach, soybean, peanut, and celery) were detected in 3 of 18 patients, all except shrimp for the cross-reactive components of that antigen. In the blank sample, no IgE was detected for any of the species-specific or cross-reactive components on MA.

Association of MA with standard IgE assay

Fisher's exact probability testing was performed and the results are shown in Table 2. Overall, there was a strong association ($p < 0.0001$) noted between presence of specific IgE on MA and standard assay, with agreement in 88 of 90 (97.8%) of testing for the 5 study antigens. A strong association was noted for all individual antigens except ragweed, which represented the 2 cases of disagreement. In 1 of those cases, standard assay was positive, whereas MA was negative (SPT positive). In the other case, standard assay was negative, whereas MA was positive (SPT positive).

Impact of multiple positive components on IgE level and SPT

Of the 5 study antigens, multiple component testing was only available on 4 antigens in the microarray (Timothy

TABLE 2. Fisher exact probability test for association between MA and standard IgE assay for MBBT samples

Antigen (n = 18 for each)	MA positive	Standard assay positive	Two-tailed <i>p</i>
Timothy grass	6	6	<0.0001
Ragweed	3	3	0.06
Cat	7	7	<0.0001
<i>Alternaria</i>	2	2	0.007
<i>D. farinae</i>	6	6	<0.0001
Total	24	24	<0.0001

Statistically significant values are in bold.

IgE = immunoglobulin E; MA = microarray analysis; MBBT = mucosal brush biopsy testing.

grass, cat, *Alternaria*, and *D. farinae*). For these 4 antigens, 10 of 20 (50%) of positive assays detected IgE to multiple components of that antigen. The presence of multiple positive components on MA was not associated with either the level of specific IgE (kU/L) on standard assay ($p = 0.52$) or SPT grade ($p = 0.67$).

Discussion

This is the first report of antigen-specific IgE detected by MA from a sample of saline produced by MBB from the sinonasal mucosa of patients who tested positive to at least 1 of the 5 study antigens on SPT. It was also determined that the overall results of MA were strongly associated with those from standard IgE assay. MA was able to detect specific IgE with almost identical accuracy as standard IgE assay to 4 out of 5 airborne antigens. It is unknown whether the 2 discrepancies with ragweed were due to chance or some specific characteristic of the antigen. MBB from both inferior turbinates results in a 1.5 cc suspension of epithelial cells in saline. This volume of fluid is necessary in order to wash as much cellular material off of the brush as possible. It is also required for the proper functioning of the sonifier

during creation of the lysate. Moreover, at least 1 cc of lysate is recommended for ImmunoCAP testing.

The potential advantages of using MA for MBB include the much smaller sample volume needed for testing and the smaller amounts of IgE necessary for detection. MBB with ImmunoCAP analysis has been found to detect antigen-specific IgE with a frequency of approximately 25% to 50% of SPT,¹¹ but it is currently unknown whether this is due to concentrations of IgE that are too small to detect in saline, or the fact that local IgE represents a distinct subset of IgE detected on systemic testing. In the first case, concentrating the lysate down to a volume that could be used for MA may increase the yield of positive antigens to more closely approximate systemic testing. In this situation, MA would be considered superior to standard assay for local IgE testing, but if the antigen yield remained the same, MA would remain equal to standard assay and the “subset” theory would be supported. This theory may, in turn, have implications for patients who test negative for food allergies despite compelling clinical reactions, or patients who are categorized with NAR solely on the results of systemic testing.^{21,22}

The ability of MA to detect multiple components of the same antigen did not seem to add any benefit for the purposes of MBB testing for airborne allergens. In general, reactions to cross-reactive, heat-labile proteins tend to be associated with less severe reactions than species-specific, heat-stable components, but this has been found to have clinical value primarily for food antigens. The heat-labile PR-10 homologue, Ara h 8, in the peanut-sensitive individual is associated with oral cavity symptoms, whereas sensitivity to the proteolysis and heat-stable seed storage protein, Ara h 2, is associated with a greater risk of systemic reactions.²³ This information cannot be determined using SPT or in vitro testing because these methods use heterogeneous mixtures of food-specific allergens.²⁴ None of the 5 airborne allergens tested in this study were positive to any of the cross-reactive components. In addition, the presence of multiple positive components was not associated with higher levels of antigen-specific IgE on standard assay or higher SPT grades.

Although not part of the specific aims of this study, this is also the first known report of IgE specific to food antigens detected in tissue harvested from the nasal cavity. In contrast to the airborne antigens, all the detectable food-associated IgE was to cross-reactive components, except for 1 (shrimp). It should be emphasized that testing for specific IgE to food is not currently recommended in the initial evaluation of AR. However, the concept of “entropy” is

that IgE may be produced by local exposure to an antigen in the absence of systemic evidence.²⁵ While food should never be exposed to sinonasal tissue, food-specific IgE in the sinonasal mucosa may represent “spillover” from regional lymph tissue, rather than local production. Where the true boundaries lie between regional and systemic immunity is unclear at this time, but studies are currently underway to help determine the clinical significance of detectable IgE to food allergens in various locations of the respiratory and gastrointestinal tracts.

As interest in testing for local IgE grows, it is important to determine the clinical utility of currently available testing modalities. Methods to sample local IgE, such as MBB or surgical biopsy, should be standardized to allow for a more useful comparison. Part of this comparison includes the determination of sensitivity and specificity, but in order to calculate those values, the rates of true and false, positive and negative results must be known. Because testing for local IgE is different than systemic testing, these values may only be known through nasal allergen provocation testing (NAPT) or patient self-reporting, rather than SPT or standard IgE assay. Future studies are also planned to determine local, specific IgE levels to food and airborne allergens in the normal pediatric and adult populations, as well as in those patients with AR, NAR, CRS, AFRS, EoE, nasal polyps, and asthma. While this study suggests that MA and standard assay are equivalent in their abilities to detect antigen-specific IgE from a sinonasal biopsy, further studies with larger sample sizes and additional antigens will be required to verify these results and help determine the ideal specific IgE testing modality for MBB.

Conclusion

MA has essentially the identical capability of detecting antigen-specific IgE from a sample of lysed nasal epithelial cells harvested by MBB. The ability of MA to detect multiple components of an antigen does not seem to add any diagnostic advantage when considering airborne antigens, but may have important clinical implications for food antigens. If lysates from MBB are concentrated into a smaller volume, MA may ultimately prove to be a superior method of testing for local, antigen-specific IgE. ③

Acknowledgments

I gratefully acknowledge Karen Bunting, PhD, Ari Melnick, MD, Maria Bremberg, RN, and Teresa Valderrama for their valuable assistance in the completion of this study.

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