Published in final edited form as:

J Allergy Clin Immunol. 2012 April ; 129(4): 1159–1162. doi:10.1016/j.jaci.2011.11.045.

Increased peanut-specific IgA in saliva correlates with food challenge outcomes following peanut sublingual immunotherapy

Michael Kulis, PhD¹, Katie Saba, BS¹, Edwin H. Kim, MD¹, J. Andrew Bird, MD¹, Nikolas Kamilaris, BS¹, Brian P. Vickery, MD¹, Herman Staats, PhD², and A. Wesley Burks, MD¹

¹Department of Pediatrics, Division of Allergy and Immunology

²Department of Pathology, Duke University Medical Center, Durham, NC

Abstract

Capsule Summary—Peanut-specific IgA in saliva correlates with DBPCFC outcomes following peanut SLIT, suggesting that peanut-specific salivary IgA may be a potential biomarker for SLIT used to treat peanut allergy.

Keywords

Food allergy; peanut allergy; sublingual immunotherapy; saliva; IgA; secretory IgA

To the Editor

Antigen-specific immunotherapy has been practiced for 100 years and is the only known therapy that modulates IgE-mediated allergy. Immunotherapy is commonly administered via subcutaneous (SCIT) or sublingual (SLIT) routes, with clinical benefits demonstrated for each.¹ Although immunotherapy can readily be given to patients with aeroallergen sensitivity (i.e. pollen, grass, and pet dander) and Hymenoptera venom allergy, no such treatment is offered for food allergies. The lack of a proactive therapeutic approach for food allergies has left millions of affected individuals to rely solely on avoiding the allergen triggers, which has been associated with a decreased quality of life. Both antigen-specific and non-specific approaches for food allergies are being investigated.²

Our group has recently published the first clinical trial of SLIT for peanut allergies in a pediatric population which demonstrated a clinical desensitization effect.³ Since SLIT is administered at a mucosal surface, IgA and secretory-IgA (S-IgA) may play critical roles in immune modulation.⁴ Antigen-specific IgA and S-IgA can exclude antigen uptake at mucosal surfaces via a mechanism known as immune exclusion, which may prevent inflammatory responses.⁵ Repeated mucosal allergen exposure with SLIT has the potential to induce the production of allergen-specific IgA and S-IgA that prevent allergens from accessing the systemic immune system and inducing allergic symptoms. Deficiencies in IgA and S-IgA have both been linked to atopic disease,^{5,6} including food allergies,⁷ however, the

© 2011 American Academy of Allergy, Asthma and Immunology. Published by Mosby, Inc. All rights reserved.

Correspondence: A. Wesley Burks, MD, Department of Pediatrics, Division of Allergy and Immunology, Duke University Medical Center, Box 2644, Durham, NC 27710, Telephone: 919-681-2949, Fax: 919-668-3750, wesley.burks@duke.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

role of IgA in developing tolerance to foods is poorly understood. Previous studies using SLIT for treatment of aeroallergen sensitivity have demonstrated an increased antigen-specific IgA in serum,⁸ but there have not been any previous reports regarding salivary IgA following SLIT in humans.

We used 10 peanut SLIT subjects and 7 placebo subjects from a double-blind, placebo-controlled trial³ that underwent a double-blind placebo-controlled food challenge (DBPCFC) with peanut after 12 months in the trial and had saliva and serum samples available at baseline and at time of challenge. Approximately 5 mL of saliva were collected at each time point without the use of sialogogues in subjects that had been fasted for 30 minutes. Saliva samples were kept on ice, centrifuged to remove debris, and supernatant frozen within 24 hours after collection. Peanut-specific IgA and secretory-IgA levels in saliva were measured by ELISA. Briefly, peanut proteins from a crude peanut extract⁹ were coated on Immulon 4HBX microtiter plates (Thermo Scientific) at a concentration of 20 µg/ml for one hour and then blocked with a PBS solution containing 0.05% Tween-20 and 2% BSA for one hour. Diluted saliva samples were added and left to bind peanut antigens overnight at 4°C. Detection of IgA was carried out with goat anti-human IgA-HRP antibody (Southern Biotech; diluted 1:8000) and detection of S-IgA was performed using mouse monoclonal anti-human S-IgA antibody (Calbiochem; clone HP6141; used at 10 µg/ml), followed by an HRP-conjugated goat anti-mouse IgG1 antibody (Southern Biotech; diluted 1:20,000). Both detection systems were developed with TMB substrate (KPL) for 15 minutes, acidified with a TMB stop solution (KPL), and then read on an ELISA plate reader to determine optical density (O.D.). Saliva dilutions were initially optimized, and found to be 1:10 for IgA measurements, and 1:50 for S-IgA measurements. Total IgA and S-IgA were also measured by ELISA using an unlabeled goat anti-human IgA (Southern Biotech; 2 µg/ml) as the capture antibody, with saliva diluted 1:250 for total IgA and 1:1000 for total S-IgA; detection methods were the same as those used for the peanut-specific measurements. Peanut-specific serum IgA levels were quantified using an ImmunoCAP 100 (Phadia). Clinical outcomes were defined as DBPCFC results at 12 months into the trial.

Salivary levels of peanut-IgA increased significantly for subjects receiving SLIT but not for subjects receiving placebo (Fig. 1A). Interestingly, 3 of the 10 SLIT subjects did not have an increased salivary peanut-IgA response at the time of challenge, whereas 6 of the 10 SLIT subjects had an increase in O.D. of greater than 1.0 (Fig. 1B). Peanut-specific S-IgA levels also significantly increased in SLIT subjects but not in the placebo group (Fig. 1C). As with peanut-IgA, a subset of SLIT subjects had no rise in S-IgA (Fig. 1D). Half of the SLIT subjects exhibited a greater than 1.0 O.D. rise in S-IgA whereas only 1 of 7 placebo subjects had an increase in O.D. of approximately 1.0. The total salivary IgA and S-IgA did not change significantly between baseline and 12 months in either the SLIT or placebo groups (data not shown). Additionally, the subset of SLIT subjects that did not have increased salivary peanut-IgA exhibited no obvious differences in total salivary IgA levels compared to the SLIT subjects that had increases of greater than 1.0 O.D. in peanut-IgA. Changes in salivary peanut-IgA correlated well with changes in peanut S-IgA across all 17 subjects ($R^2=0.62$; $p=0.0002$; data not shown). Peanut-specific IgA in serum was also significantly increased in peanut SLIT subjects but not in those treated with placebo (Fig. 1E). As with the salivary IgA, a subset of SLIT subjects had only marginal increases in serum IgA (Fig. 1F).

In the clinical trial,³ the group receiving peanut SLIT consumed a median cumulative dose of 1710 mg of peanut protein whereas the placebo group consumed only 85 mg at the DBPCFC. Despite these encouraging findings, there was a wide variability in the amount of peanut tolerated in the treatment group. Correlating DBPCFC outcomes with immune parameters proved difficult in our initial report, although initial peanut-IgE levels had a

moderate correlation ($R^2=0.38$; $p=0.043$).³ Here, we examined correlations of salivary and serum peanut-IgA with DBPCFC by linear regression analysis (GraphPad Prism version 5.04). Change in salivary peanut-IgA correlated strongly with DBPCFC outcomes giving an R^2 value of 0.52 and $p=0.0011$ (Fig. 2A). For the 9 subjects (3 on SLIT) that consumed 210 mg or less of peanut protein on DBPCFC, all had less than a 0.17 O.D. increase in salivary peanut-IgA, with 6 of these subjects showing slight decreases in peanut-specific IgA. For the 6 subjects with increases in salivary peanut-IgA greater than 1.0 O.D., 4 consumed the entire 2500 mg peanut protein DBPCFC, 1 consumed 1710 mg, and the last consumed only 460 mg. Salivary peanut S-IgA changes also correlated with the amount of peanut tolerated during DBPCFC, although with lower R^2 and p -values (Fig 2B). Change in serum peanut-IgA did not show any appreciable correlation to DBPCFC outcomes (Fig 2C). Baseline peanut-IgE in serum was plotted with the change in salivary peanut-IgA showing that subjects treated with SLIT with baseline peanut-IgE < 35 kU/L have increases in salivary IgA that are not observed for the placebo group with baseline IgE < 35 kU/L, nor for the SLIT subjects with initial peanut-IgE > 100 kU/L (Fig 2D). For the 6 SLIT subjects with > 1.0 O.D. change in salivary IgA (shown in blue box) the median peanut protein consumed during DBPCFC was 2500 mg, whereas the remaining 4 SLIT subjects consumed a median of 148 mg and the placebo subjects with peanut-IgE < 35 kU/L (shown in red box) consumed a median of 85 mg.

The ease of administration and limited allergic side-effects make SLIT a particularly attractive option for food allergies. Peanut SLIT can desensitize peanut allergic subjects, however, not all are desensitized to the same extent. A marker for monitoring clinical responsiveness to SLIT is highly desirable if SLIT is to become a common clinical practice for the treatment of food allergies. Initial peanut-IgE levels may be a useful marker to select suitable candidates, as lower baseline peanut-IgE was associated with a more positive clinical outcome.³ However, to monitor progress of treatment, it seems that salivary IgA anti-peanut may serve as a potential biomarker to follow throughout therapy and could be useful to determine the efficacy of therapy. Continued study of salivary antigen-specific IgA in ongoing, larger SLIT studies for peanut and other food allergies will help define the utility of these findings.

Acknowledgments

Funding source: National Institutes of Health (NCCAM) #R01-AT004435-04

References

1. Durham SR, Leung DY. One hundred years of allergen immunotherapy: time to ring the changes. *J Allergy Clin Immunol.* Jan; 2011 127(1):3–7. [PubMed: 21211638]
2. Nowak-Węgrzyn A, Sampson HA. Future therapies for food allergies. *J Allergy Clin Immunol.* Mar; 2011 127(3):558–573. quiz 574–555. [PubMed: 21277625]
3. Kim EH, Bird JA, Kulis M, et al. Sublingual immunotherapy for peanut allergy: clinical and immunologic evidence of desensitization. *J Allergy Clin Immunol.* Mar; 2011 127(3):640–646. e641. [PubMed: 21281959]
4. Macpherson AJ, McCoy KD, Johansen FE, Brandtzaeg P. The immune geography of IgA induction and function. *Mucosal Immunol.* Jan; 2008 1(1):11–22. [PubMed: 19079156]
5. Bottcher MF, Haggstrom P, Bjorksten B, Jenmalm MC. Total and allergen-specific immunoglobulin A levels in saliva in relation to the development of allergy in infants up to 2 years of age. *Clin Exp Allergy.* Sep; 2002 32(9):1293–1298. [PubMed: 12220466]
6. Ludviksson BR, Arason GJ, Thorarensen O, Ardal B, Valdimarsson H. Allergic diseases and asthma in relation to serum immunoglobulins and salivary immunoglobulin A in pre-school children: a follow-up community-based study. *Clin Exp Allergy.* Jan; 2005 35(1):64–69. [PubMed: 15649268]

7. Janzi M, Kull I, Sjoberg R, et al. Selective IgA deficiency in early life: association to infections and allergic diseases during childhood. *Clin Immunol.* Oct; 2009 133(1):78–85. [PubMed: 19541543]
8. Scadding GW, Shamji MH, Jacobson MR, et al. Sublingual grass pollen immunotherapy is associated with increases in sublingual Foxp3-expressing cells and elevated allergen-specific immunoglobulin G4, immunoglobulin A and serum inhibitory activity for immunoglobulin E-facilitated allergen binding to B cells. *Clin Exp Allergy.* Apr; 2010 40(4):598–606. [PubMed: 20184605]
9. Pons L, Ponnappan U, Hall RA, et al. Soy immunotherapy for peanut-allergic mice: modulation of the peanut-allergic response. *J Allergy Clin Immunol.* Oct; 2004 114(4):915–921. [PubMed: 15480335]

Abbreviations

SCIT	subcutaneous immunotherapy
SLIT	sublingual immunotherapy
S-IgA	secretory-IgA
DBPCFC	double-blind, placebo-controlled food challenge
HRP	horseradish peroxidase
TMB	3, 3', 5, 5'-Tetramethylbenzidine
O.D.	optical density

Figure 1.

Peanut-specific IgA in saliva and serum. **A**, **C**, and **E** show individual data with a line indicating the median for salivary peanut-IgA, salivary peanut-secretory IgA, and serum peanut-IgA, respectively. **B**, **D**, and **F** show changes for individuals from 0 mo to 12 mo for salivary peanut-IgA, salivary peanut-secretory IgA, and serum peanut-IgA, respectively. Matched pairs were compared by Wilcoxon Signed-Rank Test; SLIT and placebo were compared with the Mann-Whitney Test.

Figure 2.

Correlations of DBPCFC or baseline peanut-IgE with changes in peanut-IgA. **A:** Salivary peanut-IgA, **B:** Salivary peanut-secretory-IgA, and **C:** serum peanut-IgA plotted with mg of peanut protein consumed during DBPCFC. Linear regression analysis was used to determine if best-fit line slopes were significantly non-zero. **D:** Scatter plot of baseline peanut-IgE and change in salivary peanut-IgA. Blue circles represent SLIT subjects; Red diamonds represent placebo subjects.