

Letter to the Editor

Increased peanut-specific IgA levels in saliva correlate with food challenge outcomes after peanut sublingual immunotherapy

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 through the subcutaneous (SCIT) or sublingual (SLIT) routes, with clinical benefits demonstrated for each.¹ Although immunotherapy can readily be given to patients with aeroallergen sensitivity (ie, 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 subjects to rely solely on avoiding the allergen triggers, which has been associated with a decreased quality of life. Both antigen-specific and nonspecific 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 that demonstrated a clinical desensitization effect.³ Because SLIT is administered at a mucosal surface, IgA and secretory IgA (S-IgA) might play critical roles in immune modulation.⁴ Antigen-specific IgA and S-IgA can exclude antigen uptake at mucosal surfaces through a mechanism known as immune exclusion, which might prevent inflammatory responses.⁵ Repeated mucosal allergen exposure with SLIT has the potential to induce the production of allergen-specific IgA and S-IgA, which 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 allergies⁷; however, the role of IgA in developing tolerance to foods is poorly understood. Previous studies using SLIT for the treatment of aeroallergen sensitivity have demonstrated an increased antigen-specific IgA level in serum,⁸ but there have not been any previous reports regarding salivary IgA levels after SLIT in human subjects.

We included 10 subjects undergoing peanut SLIT and 7 placebo-treated subjects from a double-blind, placebo-controlled trial³ who 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 the time of challenge. Approximately 5 mL of saliva was collected at each time point without the use of sialogogues in subjects who had fasted for 30 minutes. Saliva samples were kept on ice and centrifuged to remove debris, and supernatants were frozen within 24 hours after collection. Peanut-specific IgA and S-IgA levels in saliva were measured by means of ELISA. Briefly, peanut proteins from a crude peanut extract⁹ were coated on Immulon 4HBX microtiter plates (Thermo Scientific, Waltham, Mass) at a concentration of 20 µg/mL for 1 hour and then blocked with a PBS solution containing 0.05% Tween-20 and 2% BSA for 1 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–horseradish peroxidase antibody (diluted 1:8000; SouthernBiotech, Birmingham, Ala), and detection of S-IgA was performed with mouse anti-human S-IgA mAb (clone HP6141 used at 10 µg/mL; Calbiochem, Nottingham, United Kingdom), followed by an horseradish peroxidase–conjugated goat anti-

mouse IgG₁ antibody (diluted 1:20,000, SouthernBiotech). Both detection systems were developed with TMB substrate (KPL, Gaithersburg, Md) for 15 minutes, acidified with a TMB stop solution (KPL), and then read on an ELISA plate reader to determine OD. 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 levels were also measured by means of ELISA with an unlabeled goat anti-human IgA (2 µg/mL, SouthernBiotech) 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 with an ImmunoCAP 100 (Phadia, Uppsala, Sweden). Clinical outcomes were defined as DBPCFC results at 12 months into the trial.

Salivary levels of peanut-specific IgA increased significantly for subjects receiving SLIT but not for subjects receiving placebo (Fig 1, A). Interestingly, 3 of the 10 subjects undergoing SLIT did not have an increased salivary peanut-specific IgA response at the time of challenge, whereas 6 of the 10 subjects undergoing SLIT had an increase in OD of greater than 1.0 (Fig 1, B). Peanut-specific S-IgA levels also significantly increased in subjects undergoing SLIT but not in the placebo-treated group (Fig 1, C). As with peanut-specific IgA, a subset of subjects undergoing SLIT had no increase in S-IgA levels (Fig 1, D). Half of the subjects undergoing SLIT exhibited a greater than 1.0 OD increase in S-IgA levels, whereas only 1 of 7 placebo-treated subjects had an increase in OD of approximately 1.0. The total salivary IgA and S-IgA levels did not change significantly between baseline and 12 months in either the SLIT or placebo groups (data not shown). Additionally, the subset of subjects undergoing SLIT who did not have increased salivary peanut-specific IgA levels exhibited no obvious differences in total salivary IgA levels compared with those seen in the subjects undergoing SLIT who had increases of greater than 1.0 OD in peanut-specific IgA. Changes in salivary peanut-specific IgA levels correlated well with changes in peanut S-IgA levels across all 17 subjects ($R^2 = 0.62$, $P = .0002$, data not shown). Peanut-specific IgA levels in serum were also significantly increased in subjects undergoing peanut SLIT but not in those treated with placebo (Fig 1, E). As with the salivary IgA levels, a subset of subjects undergoing SLIT had only marginal increases in serum IgA levels (Fig 1, F).

In the clinical trial³ 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-specific IgE levels had a moderate correlation ($R^2 = 0.38$, $P = .043$).³ Here we examined correlations of salivary and serum peanut-specific IgA levels with DBPCFC results by means of linear regression analysis (GraphPad Prism version 5.04; GraphPad Software, Inc, La Jolla, Calif). Changes in salivary peanut-specific IgA levels correlated strongly with DBPCFC outcomes, resulting in an R^2 value of 0.52 and a P value of .0011 (Fig 2, A). For the 9 subjects (3 undergoing SLIT) who consumed 210 mg or less of peanut protein on DBPCFCs, all had less than a 0.17 OD increase in salivary peanut-specific IgA levels, with 6 of these subjects showing slight decreases in

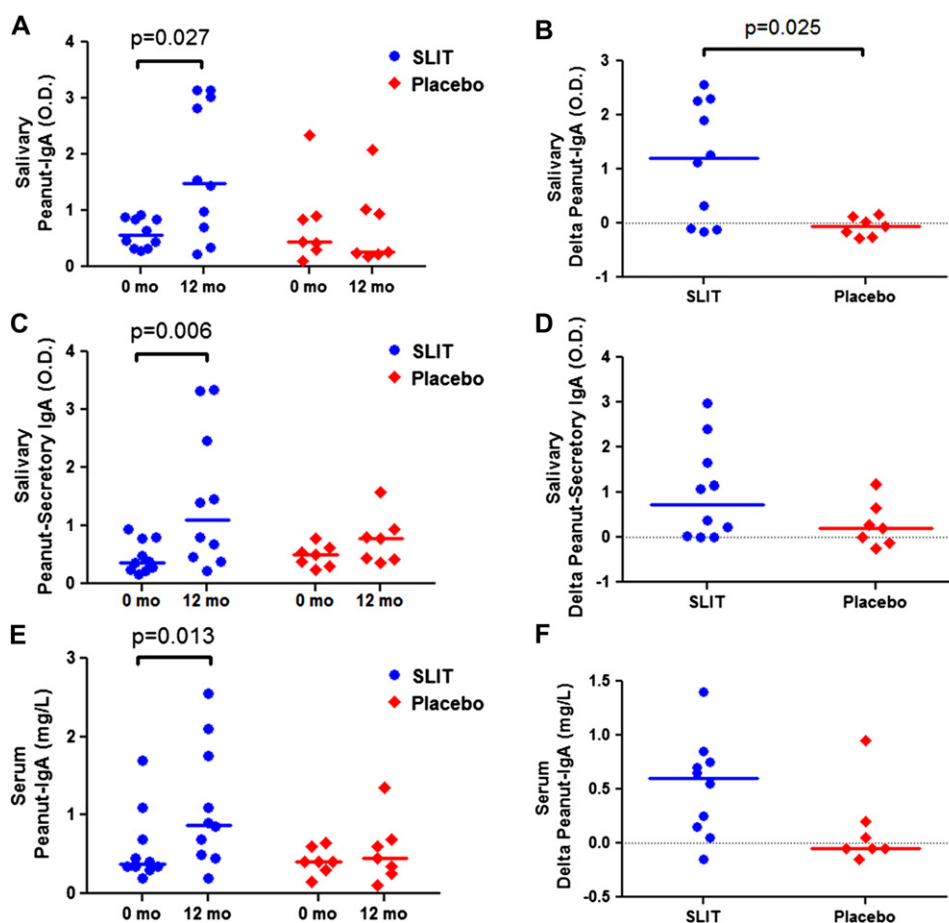


FIG 1. Peanut-specific IgA levels in saliva and serum. **A**, **C**, and **E** show individual data, with a line indicating the median for salivary peanut-specific IgA, salivary peanut-specific S-IgA, and serum peanut-specific IgA levels, respectively. **B**, **D**, and **F** show changes for subjects from 0 to 12 months for salivary peanut-specific IgA, salivary peanut-specific S-IgA, and serum peanut-specific IgA levels, respectively. Matched pairs were compared by using the Wilcoxon signed-rank test. SLIT and placebo were compared with the Mann-Whitney test.

peanut-specific IgA levels. For the 6 subjects with increases in salivary peanut-specific IgA levels of greater than 1.0 OD, 4 consumed the entire 2500-mg peanut protein DBPCFC, 1 consumed 1710 mg, and the last consumed only 460 mg. Salivary peanut-specific S-IgA changes also correlated with the amount of peanut tolerated during DBPCFCs, although with lower R^2 and P values (Fig 2, B). Changes in serum peanut-specific IgA levels did not show any appreciable correlation to DBPCFC outcomes (Fig 2, C). Baseline peanut-specific IgE levels in serum were plotted with the change in salivary peanut-specific IgA levels, showing that subjects treated with SLIT with baseline peanut-specific IgE levels of less than 35 kU/L have increases in salivary IgA levels that are not observed for the placebo group with baseline IgE levels of less than 35 kU/L nor for the subjects undergoing SLIT with initial peanut-specific IgE levels of greater than 100 kU/L (Fig 2, D). For the 6 subjects undergoing SLIT with a greater than 1.0 OD change in salivary IgA levels, the median peanut protein consumed during DBPCFCs was 2500 mg, whereas the remaining 4 subjects undergoing SLIT consumed a median of 148 mg, and the placebo-treated subjects with peanut-specific IgE levels of less than 35 kU/L consumed a median of 85 mg.

The ease of administration and limited allergic side effects make SLIT a particularly attractive option for patients with food allergies. Peanut SLIT can desensitize subjects with peanut allergy; 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-specific IgE levels might be a useful marker to select suitable candidates because lower baseline peanut-specific IgE levels were associated with a more positive clinical outcome.³ However, it seems that salivary anti-peanut IgA might serve as a potential biomarker to follow throughout therapy and could be useful in determining the efficacy of therapy to monitor the progress of treatment. Continued study of salivary antigen-specific IgA levels in ongoing, larger SLIT studies for peanut and other food allergies will help define the utility of these findings.

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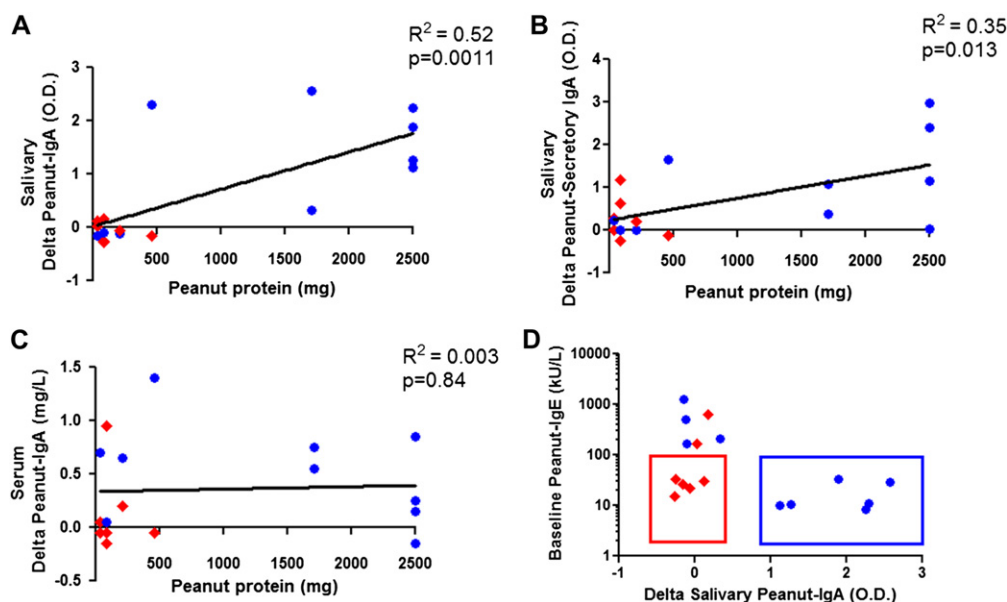


FIG 2. Correlations of DBPCFC or baseline peanut-specific IgE levels with changes in peanut-specific IgA levels. **A-C**, Salivary peanut-specific IgA (Fig 2, A), salivary peanut-specific S-IgA (Fig 2, B), and serum peanut-specific IgA (Fig 2, C) levels plotted with milligrams of peanut protein consumed during DBPCFC. Linear regression analysis was used to determine whether best-fit line slopes were significantly nonzero. **D**, Scatter plot of baseline peanut-specific IgE levels and change in salivary peanut-specific IgA levels. Blue circles represent subjects undergoing SLIT, and red diamonds represent placebo-treated subjects.

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