

## Original Article

# Peanut-Specific IgG4 and IgA in Saliva Are Modulated by Peanut Oral Immunotherapy

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**What is already known about this topic?** Serum peanut-specific immunoglobulin G4 (IgG4) and IgA are significantly increased after peanut oral immunotherapy, but do not correlate to an individual's likelihood of success on therapy. Immunoglobulin responses have yet to be studied at the oral mucosal surface.

**What does this article add to our knowledge?** Peanut oral immunotherapy induces substantial increases in allergen-specific IgG4 and IgA in saliva. Salivary peanut IgA is higher at baseline in subjects that do not achieve desensitization, suggesting the increase during oral immunotherapy is beneficial for desensitization.

**How does this study impact current management guidelines?** These data provide insight into oral immunotherapy –induced mucosal responses and suggest utility of these easily obtained samples for biomarker development.

**BACKGROUND:** Antigen-specific immunoglobulin responses have yet to be studied at the oral mucosal surface during peanut oral immunotherapy (PnOIT).

**OBJECTIVE:** We aimed to quantify salivary peanut-specific IgG4 (PNsIgG4) and IgA (PNsIgA) and total IgG4 and IgA in participants from the Immune Tolerance Network's IMPACT study, a phase 2 PnOIT trial.

**METHODS:** Peanut-allergic children, aged 12 months to younger than 48 months at screening, were enrolled and randomized to PnOIT or placebo oral immunotherapy (OIT) for 134 weeks. Per-protocol analysis included 69 PnOIT and 23 placebo participants. Double-blind, placebo-controlled food challenges were conducted at weeks 134 and 160 to assess desensitization and remission, respectively. Saliva samples were collected at baseline and 30, 82, 134, and 160 weeks to quantify PNsIgG4, PNsIgA, and total IgG4 and IgA.

**RESULTS:** Participants who received PnOIT experienced significant increases in PNsIgG4 in saliva, whereas participants on placebo did not ( $P < .01$  at all time points). The PNsIgA/total IgA ratio was also significantly increased in participants treated with

PnOIT when compared with those receiving placebo at 30 and 82 weeks ( $P < .05$ ). During PnOIT, desensitized participants had increased PNsIgA that plateaued, whereas the not desensitized/no remission group did not change over time. Interestingly, when the PnOIT group was evaluated by clinical outcome, PNsIgA was higher at baseline in the not desensitized/no remission group than in the desensitized/remission group ( $P < .05$ ).

**CONCLUSIONS:** PnOIT induces substantial increases in allergen-specific IgG4 and IgA in saliva. These data provide insight into OIT-induced mucosal responses and suggest the utility of these easily obtained samples for biomarker development. © 2022 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2022;■:■-■)

**Key words:** Peanut allergy; Oral immunotherapy; IgA; IgG4; Saliva

## INTRODUCTION

Peanut allergy is a growing public health concern affecting 2% of the U.S. population.<sup>1</sup> The current standard of care is

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Conflicts of interest: J. M. Smeekens has received funding from an NIH T32 training grant. S. M. Jones reports grants from NIH-NIAID, Food Allergy Research & Education (FARE), Aimmune Therapeutic, DBV Technologies, Astellas, Inc., Sanofi, Inc., Regeneron, Inc., and Genentech, Inc.; and personal fees from Food Allergy Research and Education, EMMES Corporation, and Aimmune Therapeutics. M. D. Kulis has received research support from NIH and the Department of Defense (DoD), and consulting fees from Ukko. The rest of the authors declare that they have no relevant conflicts of interest.

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**Abbreviations used***DBPCFC*- Double-blind placebo-controlled food challenge*IgA*- IgE*IgG4*- Immunoglobulin A*E*- G4*ITN*- Immune Tolerance Network*OIT*- Oral immunotherapy*PnOIT*- Peanut oral immunotherapy*PNsIgA*- Peanut-specific IgA*PNsIgE*- Peanut-specific IgE*PNsIgG4*- Peanut-specific IgG4

avoidance and ready access to epinephrine, although food-specific immunotherapies are actively being investigated. Oral immunotherapy (OIT) has been studied for the last 15 years,<sup>2,3</sup> and is an effective treatment option for peanut allergy. Indeed, 1 peanut oral immunotherapy (PnOIT) product, Palforzia, recently gained U.S. Food and Drug Administration (FDA) approval after a successful phase 3 study.<sup>4</sup> The biological mechanisms of PnOIT remain unclear, but consistent changes in systemic immunoglobulin levels have been observed.<sup>5</sup> Notably, peanut-specific immunoglobulin G4 (PNsIgG4) in plasma increases sharply within the first few months with some studies also showing increases in peanut-specific IgA (PNsIgA) throughout therapy.<sup>6,7</sup> Peanut-specific IgE (PNsIgE) increases for the first 6 months on OIT, and then decreases below baseline levels after approximately 12 months.<sup>8,9</sup> Whereas these changes are consistently observed with treatment, they have had little success when used as biomarkers to predict OIT outcomes.<sup>10</sup> However, because OIT is administered at the oral and gastrointestinal mucosal surfaces, antigen-specific immunoglobulin responses at these sites may be more informative.

Oral tolerance is a naturally occurring process in nonallergic individuals that typically results in elevated levels of serum antigen-specific IgG4 and IgA.<sup>11</sup> For example, in the Learning Early About Peanut Allergy (LEAP) trial, early introduction of peanut in infants who were protected from peanut allergy resulted in substantial increases in PNsIgG4, not seen in the infants avoiding peanut.<sup>12</sup> In a separate study, serum antigen-specific IgA2 was shown to play a role in tolerance in subjects who outgrew their egg allergy.<sup>13</sup> Collectively, these studies suggest antigen-specific IgG4 and IgA may dampen IgE responses. Food allergen-specific immunotherapies are employed to induce increased production of antigen-specific IgG4 and IgA, which have been demonstrated to block effector cell degranulation.<sup>7,14,15</sup> Whereas these effects have been demonstrated with serum antigen-specific immunoglobulins, the effects at mucosal surfaces are not well-defined.

To evaluate local immune responses, we utilized samples from the Immune Tolerance Network's (ITN's) IMPACT study, a phase 2 randomized, placebo-controlled trial of PnOIT.<sup>16</sup> Briefly, the IMPACT trial was designed to study desensitization and remission after PnOIT. Specifically, children aged 12 months to younger than 48 months with double-blind, placebo-controlled food challenge (DBPCFC)—confirmed peanut allergy were assigned to PnOIT (n = 96) or placebo OIT (n = 50) for 134 weeks. Participants were assessed for desensitization and remission by DBPCFCs at week 134 (end of treatment) and 160 (after 6 months of avoidance), respectively. The 69 participants who completed the protocol (ie, the per-protocol population)

and passed the DBPCFC at weeks 134 and 160 were categorized as desensitized/remission (n = 19), participants that passed the DBPCFC at week 134 but failed at week 160 were categorized as desensitized/no remission (n = 40), and participants that did not pass the DBPCFC at week 134 were categorized as not desensitized/no remission (n = 10) (Table 1). To assess the immunological response to PnOIT at the oral mucosal surface and its potential to predict treatment outcomes, we aimed to quantify salivary PNsIgG4 and PNsIgA and total IgG4 and IgA in the ITN IMPACT trial and compare these with their sera counterparts.

**METHODS****Clinical trial description**

The ITN IMPACT clinical trial (NCT01867671) information can be found elsewhere.<sup>16</sup>

**Saliva samples**

Saliva was collected either by suction with a syringe or by spitting directly into a cryovial after first rinsing the mouth with water or a damp washcloth. Saliva samples were then frozen and stored long term at  $-80^{\circ}\text{C}$  until analysis. Samples were deidentified and researchers measured IgA and IgG4 while blinded.

**IgG4 and IgA enzyme-linked immunosorbent assays**

For PNsIgG4 and total IgG4 enzyme-linked immunosorbent assays (ELISAs), 96-well plates were coated with 2  $\mu\text{g}/\text{mL}$  of anti-human IgG4 (clone G17-4, BD Biosciences, San Jose, CA) for standard curves or 20  $\mu\text{g}/\text{mL}$  peanut protein (extracted as described previously<sup>17</sup>) for saliva samples. Wells were blocked with 2% bovine serum albumin in phosphate-buffered saline with 0.05% Tween 20. Saliva samples were diluted 1:10 for PNsIgG4 and 1:20 for total IgG4. Standard curves were generated with native human IgG4 protein (Abcam, Cambridge, MA) ranging from 0.24 to 250 ng/mL. Samples and standards were detected with a 1:1000 dilution of antihuman IgG4 Fc-HRP (clone HP6025; Southern Biotech, Birmingham, AL). Plates were developed using tetramethyl benzidine (TMB) (SeraCare, Milford, MA); the reaction was stopped by using 1% HCl (SeraCare), and the results were read at 450 nm by using a microplate spectrophotometer (BioTek, Winooski, VT).

For PNsIgA and total IgA ELISAs, 96-well plates were coated with 2  $\mu\text{g}/\text{mL}$  of antihuman IgA1/IgA2 (clone G18-1; BD Biosciences, San Jose, CA) for standard curves or 20  $\mu\text{g}/\text{mL}$  peanut protein for saliva samples. Wells were blocked with 2% bovine serum albumin in phosphate-buffered saline with 0.05% Tween 20. Saliva samples were diluted 1:50 for PNsIgA and 1:20,000 for total IgA. Standard curves were generated with purified human IgA (Bethyl Labs, Montgomery, TX) ranging from 0.6 to 60 ng/mL. Samples were detected with a 1:1000 dilution of antihuman IgA-HRP (Southern Biotech). Plates were developed using TMB (SeraCare, Milford, MA); the reaction was stopped by using 1% HCl (SeraCare), and the results were read at 450 nm by using a microplate spectrophotometer (BioTek, Winooski, VT).

**Serum IgE, IgA, and IgG4 quantification**

Serum immunoglobulins were quantified by the ImmunoCAP 1000 System (Phadia-ThermoFisher, Waltham, MA) as previously described.<sup>16</sup>

**Statistics**

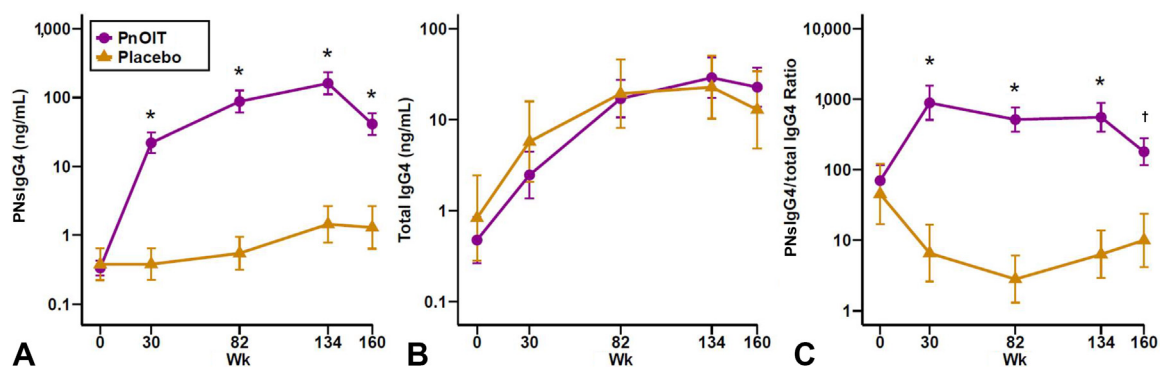
Saliva and serum antibody data were analyzed for participants in the per-protocol population who were study-compliant through the

**TABLE I.** Demographic characteristics of subjects on PnOIT\*

Demographic category	Desensitized/remission (n = 19)	Desensitized/no remission (n = 40)	Not desensitized/no remission (n = 10)
Age (mo)	30 (24–38.4)	39.6 (35.7–46.8)	40.8 (36.9–41.7)
Sex			
Female	7 (37%)	11 (28%)	3 (30%)
Male	12 (63%)	29 (72%)	7 (70%)
Race			
Asian	3 (16%)	6 (15%)	2 (20%)
Black	0 (0%)	1 (2%)	0 (0%)
Mixed	2 (11%)	9 (22%)	2 (20%)
White	14 (74%)	24 (60%)	6 (60%)
Other food allergy			
At screening	12 (63%)	21 (52%)	7 (70%)
At end of study	15 (79%)	25 (62%)	8 (80%)
PNsIgE (serum, kU/L)	16.5 (10.38–46.5)	69.5 (38–196.75)	135.4 (50.5–316.75)
Total IgE (serum, kU/L)	486 (192.5–703.75)	400.5 (192.5–617.75)	452 (247.25–772.5)

IQR, Interquartile range.

\*Data are n (%) or median (IQR).

**FIGURE 1.** PNsIgG4 and total IgG4 in saliva. (A) PNsIgG4, (B) total IgG4, and (C) PNsIgG4/total IgG4 ratio throughout the course of peanut (purple) or placebo (gold) OIT. Data are shown as mean  $\pm$  standard error of the mean. \* $P < .01$ ; † $P < .05$ .

avoidance phase and had an evaluable blinded DBPCFC at the end of the maintenance and avoidance phases (per-protocol population for the secondary end point). For comparisons between placebo and PnOIT groups, a linear mixed model was used with adjustment for baseline levels. For comparisons among PnOIT outcome groups, a linear mixed model was used without adjustment for baseline levels. The threshold for significance was  $P$  less than 0.05 (2-sided). All analyses were performed with SAS Version 9.4 (SAS Institute Inc., Cary, NC) and R version 3.2.4 (R Foundation for Statistical Computing, Vienna, Austria).

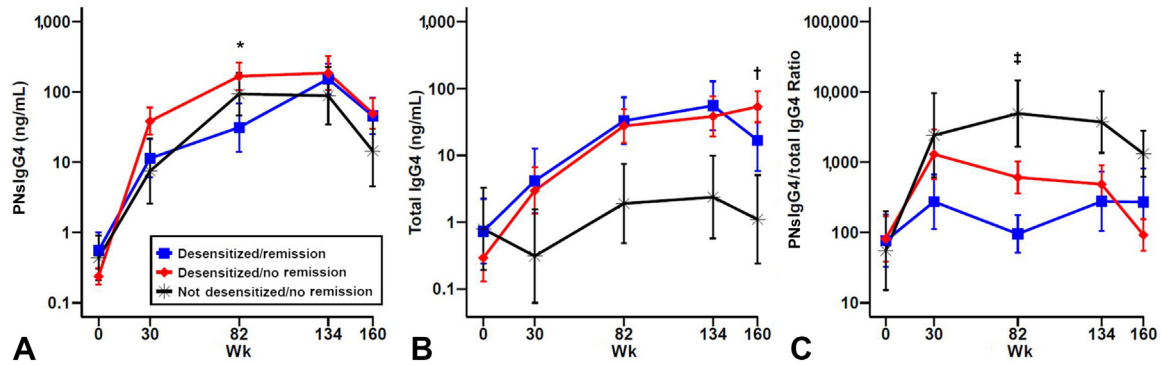
## RESULTS

### Salivary IgG4 during PnOIT treatment and avoidance

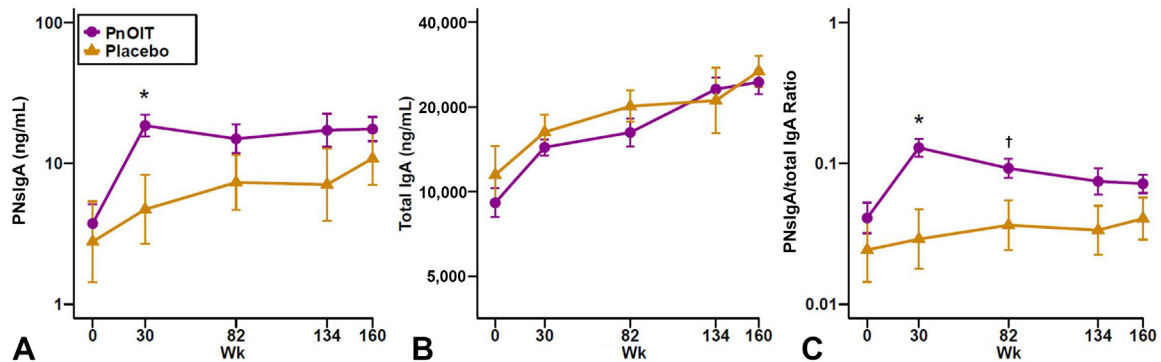
Saliva and serum samples were collected at baseline and at 30, 82, 134, and 160 weeks for quantification of salivary PNsIgG4 and total IgG4, and serum PNsIgG4. Participants receiving PnOIT demonstrated significant increases in PNsIgG4 in saliva, whereas participants receiving placebo did not (placebo vs PnOIT;  $P < .01$  at all time points; Figure 1, A). After therapy was discontinued at week 134, PNsIgG4 sharply decreased in the PnOIT group. Total IgG4 was similar between placebo and

PnOIT groups at baseline and increased in both treatment groups over time (Figure 1, B). When looking at the ratio of PNsIgG4 to total IgG4 in the PnOIT group, there was a sharp increase at week 30, which plateaued through the remaining maintenance period and decreased during avoidance (weeks 134–160) (Figure 1, C). The PNsIgG4 to total IgG4 ratio did not increase in the placebo group during the trial.

For the 69 participants who completed the protocol, when stratified by clinical outcome, a significant difference was noted in PNsIgG4 between the desensitized/remission and the desensitized/no remission groups at week 82 (Figure 2, A). For total IgG4, the not desensitized/no remission group trended toward lower levels throughout the course of treatment, and when therapy was stopped, there were statistically lower levels of total IgG4 between the not desensitized/no remission and the desensitized/no remission groups at week 160 (Figure 2, B). Taking into account the ratio of PNsIgG4 to total IgG4, the desensitized/remission group increased from baseline, and remained constant throughout the course of therapy, in comparison with the other groups in which, on average, there was an increase at week 30 and it remained elevated throughout treatment at week 134 (Figure 2, C). Interestingly, the desensitized/



**FIGURE 2.** PNslgG4 and total IgG4 in saliva by clinical outcome. (A) PNslgG4, (B) total IgG4, and (C) PNslgG4/total IgG4 ratio in desensitized/remission (blue), desensitized/no remission (red), and not desensitized/no remission (black) OIT participants. Data are shown as mean  $\pm$  standard error of the mean. \* $P < .05$  for desensitized/remission versus desensitized/no remission; † $P < .05$  for desensitized/no remission versus not desensitized/no remission; ‡ $P < .05$  for desensitized/remission versus not desensitized/no remission.



**FIGURE 3.** PNslgA and total IgA in saliva. (A) PNslgA, (B) total IgA, and (C) PNslgA/total IgA ratio throughout the course of peanut (purple) or placebo (gold) OIT. Data are shown as mean  $\pm$  standard error of the mean. \* $P < .01$ ; † $P < .05$ .

remission group on average remained constant even off therapy, whereas the other groups had a trend toward a rapid decrease off treatment. Overall, PnOIT causes large increases in salivary PNslgG4, but was not predictive of clinical outcome.

### Salivary IgA during PnOIT treatment and avoidance

The PNslgA increased in participants treated with PnOIT and reached a plateau by week 30. The PNslgA was significantly higher in the PnOIT group than in the placebo group at week 30 (Figure 3, A). Total IgA was similar between placebo and PnOIT groups with both increasing over time (Figure 3, B). When PNslgA was normalized to total IgA, the ratio of PNslgA to total IgA increased sharply in the PnOIT group by week 30 and gradually declined thereafter, whereas only small changes were observed in the placebo group (Figure 3, C).

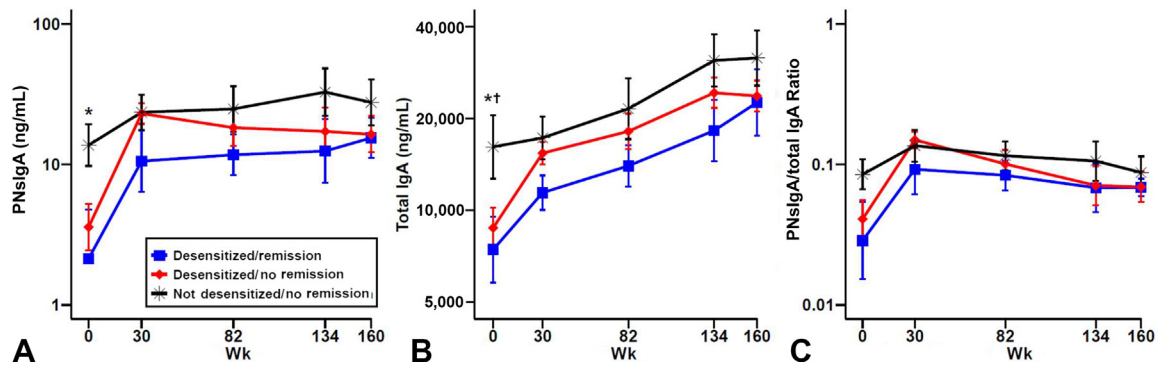
When stratified by clinical outcome within the PnOIT group, the not desensitized/no remission group had significantly higher salivary PNslgA at baseline compared with the desensitized/remission group (Figure 4, A). The same held true for total IgA (Figure 4, B). When we normalized PNslgA to total IgA, there was a similar trend as observed for PNslgA (Figure 4, C). There is also a trend for PNslgA and total IgA in the not desensitized/no remission group at weeks 134 and 160 with higher levels noted when compared with both desensitized groups.

### Correlations between salivary and serum immunoglobulin responses

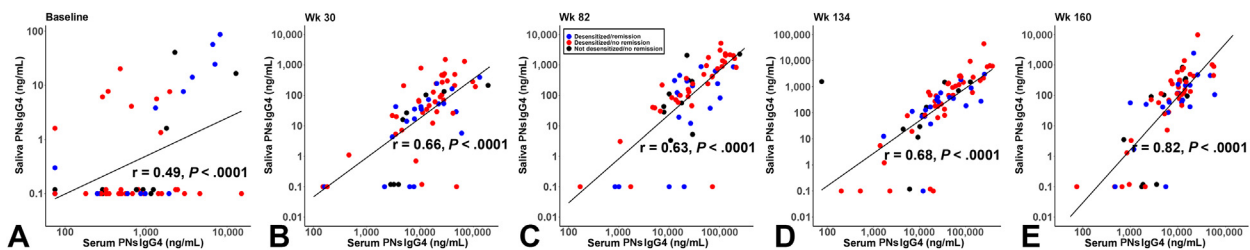
To investigate local versus systemic responses, we compared salivary PNslgG4 to serum PNslgG4. Correlation plots were produced for each time point, and statistically significant correlations were identified ( $P < .0001$  for all time points; Figure 5). These results indicate a high correlation between local and systemic PNslgG4 production. In contrast, serum PNslgA was below the limit of detection for greater than 80% of samples during treatment and avoidance and, therefore, could not be correlated with salivary PNslgA.

### DISCUSSION

To investigate the effects of PnOIT on salivary immunoglobulins, we utilized saliva samples collected from the ITN IMPACT trial. Compared with the placebo group, the PnOIT group had significantly increased PNslgG4 throughout the course of therapy, indicating a relationship between peanut exposure and production of PNslgG4. There were similar levels of total IgG4 in the placebo and PnOIT groups, which was not surprising, because PnOIT only modulates the peanut-specific immune response. However, the increase in salivary IgG4 over



**FIGURE 4.** PNslgA and total IgA in saliva by clinical outcome. (A) PNslgA, (B) total IgA, and (C) PNslgA/total IgA ratio in desensitized/remission (blue), desensitized/no remission (red), and not desensitized/no remission (black) OIT participants. Data are shown as mean  $\pm$  standard error of the mean. \* $P < .05$  for desensitized/remission versus not desensitized/no remission; † $P < .05$  for desensitized/no remission versus not desensitized/no remission.



**FIGURE 5.** Correlations between salivary and serum PNslgG4. Correlations in the PnOIT group at (A) week 0, (B) week 30, (C) week 82, (D) week 134, and (E) week 160. Lines represent linear regression lines and gray bands represent the 95% confidence intervals around the regression lines.

time was unexpected and may be due to the developing immune system in these young children.

The PnOIT group had a sharp increase in PNslgA by 30 weeks compared with the placebo group. These findings indicate a pronounced effect by PnOIT to enhance PNslgA production at the oral mucosal surface. Daily administration of peanut antigen drives PNslgA during build-up (weeks 0–30) and plateaus thereafter, indicating a maximum threshold for antigen-specific IgA production. Interestingly, serum PNslgA was not detectable in the vast majority of subjects, indicating a more robust local response to orally administered peanut antigen. One interpretation of this finding is that salivary PNslgA may play a role in the desensitization and remission mechanisms of PnOIT and may reflect the amount of PNslgA present at the gastrointestinal mucosal surface, as was shown in a mouse model with ovalbumin.<sup>18</sup> Furthermore, mucosal IgA is responsible for immune exclusion,<sup>19</sup> and in the context of PnOIT, may prevent peanut from being absorbed systemically. Further mechanistic studies are warranted to determine the role of mucosal IgA following OIT.

The ITN IMPACT trial had well-defined end points for desensitization and remission based on DBPCFC outcomes at weeks 134 and 160. By using samples from this trial, we hypothesized that there would be distinct characteristics in the remission group. Contrary to what we expected, the desensitized/remission group did not have significantly higher salivary PNslgG4 or PNslgA than the other OIT outcome groups. In

fact, the not desensitized/no remission group had significantly higher salivary PNslgA at baseline. This surprising data suggests that the sharp increase from weeks 0 to 30 is associated with desensitization and that the increase in the response rather than the quantity of the response is indicative of desensitization. Of note, a previous study examined salivary IgA after peanut sublingual immunotherapy and similarly found the increase in PNslgA was correlated with increasing amounts of peanut protein tolerated during DBPCFC.<sup>20</sup> One possible interpretation of these results is that high levels of mucosal IgA may sequester the peanut antigen, preventing uptake by tolerogenic dendritic cells that are responsible for tolerance induction. Alternatively, the significantly higher PNslgA in the not desensitized/no remission group at baseline may be an indicator of inflammation, which may limit successful outcomes on OIT.<sup>21</sup> Therefore, high levels of mucosal IgA at baseline may not be advantageous and, instead, may indicate a decreased likelihood of tolerance induction.

The clinical implications of this work are 2-fold: predicting which participants may respond positively to PnOIT and monitoring outcomes throughout the course of therapy. Currently, there are no accepted assays available to predict participant-specific responses to OIT, although thresholds for baseline PNslgE in serum may be informative.<sup>9,22</sup> Monitoring OIT is also challenging owing to lack of predictive biomarkers. Direct basophil activation testing has emerged as a potential assay to monitor OIT,<sup>22,23</sup> although these types of assays are technically difficult and require whole blood that needs to be processed

quickly. Indirect basophil activation testing has recently been reported to have high diagnostic accuracy<sup>24</sup>; however, it has not been tested in monitoring OIT outcomes. Alternatively, saliva is easily accessible and noninvasive and can be stored frozen for later analysis. Here, although the sample size of groups stratified by clinical outcome was relatively small, we demonstrated that high levels of PNslgA in saliva at baseline may indicate decreased likelihood of desensitization, which would be an impactful predictor if validated in other larger trials. Similarly, increases in PNslgA within 30 weeks of starting OIT may be utilized to monitor successful outcomes.

In conclusion, we demonstrated that PnOIT has a substantial effect on PNslgA and PNslgG4 in saliva compared with placebo treatment. These data give insight into mucosal responses induced by PnOIT and suggest a role for mucosal IgA and IgG4 in PnOIT-induced treatment outcomes. It is noteworthy that, when therapy was discontinued, PNslgG4 decreased sharply, whereas PNslgA remained elevated. Importantly, both PNslgG4 and PNslgA remain higher than baseline levels even when therapy was discontinued. Because saliva is easily obtained, biomarkers within this sample type may provide further mechanistic insights as well as prove useful for monitoring clinical outcomes.

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