

Kinetics of basophil hyporesponsiveness during short-course peanut oral immunotherapy



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Background: Oral immunotherapy (OIT) leads to suppression of mast cell and basophil degranulation along with changes in the adaptive immune response.

Objectives: This study aimed to determine how rapidly these effects occur during OIT and more broadly, the kinetics of basophil and mast cell suppression throughout the course of therapy.

Methods: Twenty participants, age 4 to 12 years, were enrolled in a peanut OIT trial and assessed for desensitization and sustained unresponsiveness after 9 months of therapy. Blood was collected 5 times in the first month and then intermittently throughout to quantify immunoglobulins and assess basophil activation by CD63, CD203c, and phosphorylated SYK (pSYK). **Results:** Twelve of 16 participants that completed the trial were desensitized after OIT, with 9 achieving sustained unresponsiveness after discontinuing OIT for 4 weeks. Basophil hyporesponsiveness, defined by lower CD63 expression, was detected as early as day 90. pSYK was correlated with CD63 expression, and there was a significant decrease in pSYK by day 250. CD203c expression remained unchanged throughout therapy. Interestingly, although basophil activation was decreased across the cohort during OIT, basophil activation did not correlate with individual clinical outcomes. Serum peanut-specific IgG₄ and IgA increased throughout therapy, whereas IgE remained unchanged.

Conclusions: Suppression of basophil activation occurs within the first 90 days of peanut OIT, ultimately leading to suppression of signaling through pSYK. (*J Allergy Clin Immunol* 2022;150:1144-53.)

Key words: Peanut allergy, food allergy, oral immunotherapy, basophil activation, pSYK

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Oral immunotherapy (OIT) has been studied as a potential treatment for food allergy over the past 15 years.¹⁻³ Palforzia, a characterized peanut flour for use in OIT, was recently approved by the US Food and Drug Administration to treat peanut allergy after a successful phase 3 trial.⁴ Peanut OIT studies include a buildup phase that lasts several weeks to months and a maintenance phase that lasts several months to years. Based on these protocols, the majority of participants who complete OIT become desensitized, and a smaller subset develop sustained unresponsiveness (SU), which is defined as lack of allergic response to a specified allergen dose after discontinuation of therapy.⁵⁻⁷ During OIT, peanut-specific IgE tends to increase initially then decreases after years of OIT; peanut-specific IgG₄ increases and plateaus; T_H2 cytokines including IL-4, IL-5, and IL-13 decrease; and basophil and mast cell activation are suppressed.³ There are many variables that likely impact clinical outcomes, including the age of participants, OIT dose, and the length of the treatment period.

Mechanistically, the underlying causes of mast cell and basophil suppression are not fully elucidated. It has been well established that IgE signals through the FcεRI on mast cells and basophils, which activates a signaling cascade, including the phosphorylation of SYK (pSYK), ultimately leading to

Abbreviations used

AUC: Area under the curve
DBPCFC: Double-blind, placebo-controlled food challenge
OFC: Oral food challenge
OIT: Oral immunotherapy
pSYK: Phosphorylated/phosphorylation of SYK
SPT: Skin prick test
SU: Sustained unresponsiveness

degranulation.⁸ *In vitro* studies have demonstrated actin rearrangement, alterations in calcium flux, and endocytosis of IgE following repeated antigen exposure of mast cells leads to desensitization.^{9,10} Another suppression mechanism occurs through peanut-specific IgG₄. IgG₄ may intercept peanut allergens in circulation from binding to cell surface-bound IgE, or it may disrupt the cross-linking of adjacent IgE through binding to FcγRIIB on effector cells.^{11,12} Interestingly, mast cells in the skin do not express FcγRIIB, which may account for the delay in suppression relative to basophils that express the receptor.¹³

Previous studies have demonstrated suppression of basophil and mast cell activation following OIT, measured by basophil activation tests and skin prick tests (SPTs), respectively.^{1,14} In general, prior work has demonstrated that participants who are desensitized and reach SU have suppressed basophil and mast cell activation even after weeks to months of discontinuing OIT.^{5,7,15} However, previous studies focus primarily on baseline and long-term time points to measure effector cell suppression, without investigating effector cell responses within the first few weeks.^{5,7,15} Therefore, it remains unclear how rapidly these effects occur during OIT (ie, days or weeks) and more broadly, the kinetics of basophil and mast cell suppression throughout the course of therapy. To address this gap in knowledge, we conducted a 9-month peanut OIT study in children focused on effector cell changes within days to months of initiating OIT. This study was intentionally designed with frequent peripheral blood sampling early in treatment to assess basophil activation throughout OIT. The main goal of this study was to determine the kinetics of basophil and mast cell suppression throughout short-course peanut OIT.

METHODS

Study design

Twenty participants ages 4 to 12 years of any gender, race, and ethnicity were enrolled at a single center (University of North Carolina) (Table I). Inclusion criteria included a history of allergic reaction to peanuts, peanut-specific IgE ≥ 0.35 kU/L, peanut SPT wheal ≥ 3 mm compared to the negative control, and a positive double-blind, placebo-controlled food challenge (DBPCFC). This phase 2, open-label study was conducted under Institutional Review Board approval and was registered as clinical trial NCT01814241 (Mechanisms of Desensitization During Peanut Oral Immunotherapy [PnOIT4]). Additionally, this study was carried out under Investigational New Drug (IND) 13665, allowing University of North Carolina to produce the investigational drug product in compliance with Good Manufacturing Practice (GMP) guidelines.¹⁶

A 1000-mg peanut DBPCFC (oral food challenge [OFC] 1) was performed to determine the highest tolerated cumulative dose at day −60. Participants continued to avoid peanut, serving as their own untreated control during the period between days −60 and 0. On day 0, participants underwent an initial dose escalation up to a target dose of 6 mg peanut protein (see Table E1 in this

TABLE I. Demographics of participants enrolled in the peanut OIT trial

Age, y	8.56 ± 1.50
Sex, male	11 (55)
Race	
Caucasian	17 (85)
African American	1 (5)
Native American	1 (5)
Indian	1 (5)
History	
Allergic rhinitis	9 (45)
Atopic dermatitis	11 (55)
Asthma	16 (80)
Other food allergies	9 (45)

Values are mean ± SD or n (%).

article's Online Repository at www.jacionline.org). Next, participants began the biweekly buildup phase of peanut OIT up to a target maintenance dose of 1450 mg peanut protein. After 2 weeks on the maintenance dose, a 5000-mg peanut DBPCFC (OFC 2) was performed to assess desensitization. Peanut OIT was discontinued and a DBPCFC (OFC 3) was performed after 1 or 2 weeks of peanut avoidance (denoted as day 264) depending on randomization. Participants whose DBPCFC 3 was at least 50% of DBPCFC 2 continued peanut avoidance and completed the final DBPCFC 4 (OFC 4) after 3 or 4 weeks of peanut avoidance (denoted as day 278) (Fig 1, A).

Peripheral blood was collected and SPTs were performed throughout the trial, on days −60 (OFC 1), 0, 2, 10, 14, 30, 90, 250 (OFC 2), 264 (OFC 3), and 278 (OFC 4) for immunologic assessments. On days when OFCs were performed, blood was collected prior to the DBPCFC.

DBPCFCs

The baseline DBPCFCs (OFC 1) were performed by administering the following doses: 1, 5, 15, 50, 75, 100, 250, and 500 mg peanut protein for a cumulative dose of 996 mg peanut protein. Equivalent amounts of oat flour by weight were used for the placebo portion of the DBPCFC. DBPCFCs conducted on days 250, 264, and 278 were performed by administering the following doses 10 to 15 minutes apart: 250, 500, 1000, 1000, 1000, 1250 mg peanut protein for a cumulative dose of 5000 mg protein. DBPCFC results are reported as highest tolerated cumulative dose, defined as cumulative peanut protein ingested up to but not including the dose causing discontinuation of the DBPCFC. Desensitization was defined as a highest tolerated cumulative dose of 5000 mg at OFC 2, with failure defined as a highest tolerated cumulative dose <5000 mg at OFC 2; SU was defined in desensitized participants as a highest tolerated cumulative dose ≥3750 mg at OFC 4.

SPTs

Peanut SPTs were performed at days −60, 0, 2, 10, 14, 30, 90, 250, 264, and 278 with a GREER Pick (Greer, Lenoir, NC) using a standard 1:20 dilution of peanut extract and saline and histamine controls. Wheal size was calculated as the average of the largest diameter and the perpendicular midpoint diameter. Results were reported as the peanut wheal size minus the wheal size of the saline control.

Immunoglobulin quantification

Plasma was collected at days −60, 0, 2, 10, 14, 30, 90, 250, 264, and 278 and peanut-specific IgE, IgG₄, and IgA were measured using an ImmunoCAP 100 (Thermo Fisher Scientific, Waltham, Mass), as previously described.^{7,17} Peanut-specific IgE values were converted from kU/L to ug/L using the conversion factor 2.42 for IgG₄ to IgE ratio calculations.⁶

Salivary IgA quantification

Saliva was collected at the same time points as plasma, either by suction or spitting directly into a cryovial. Saliva samples were then frozen and stored

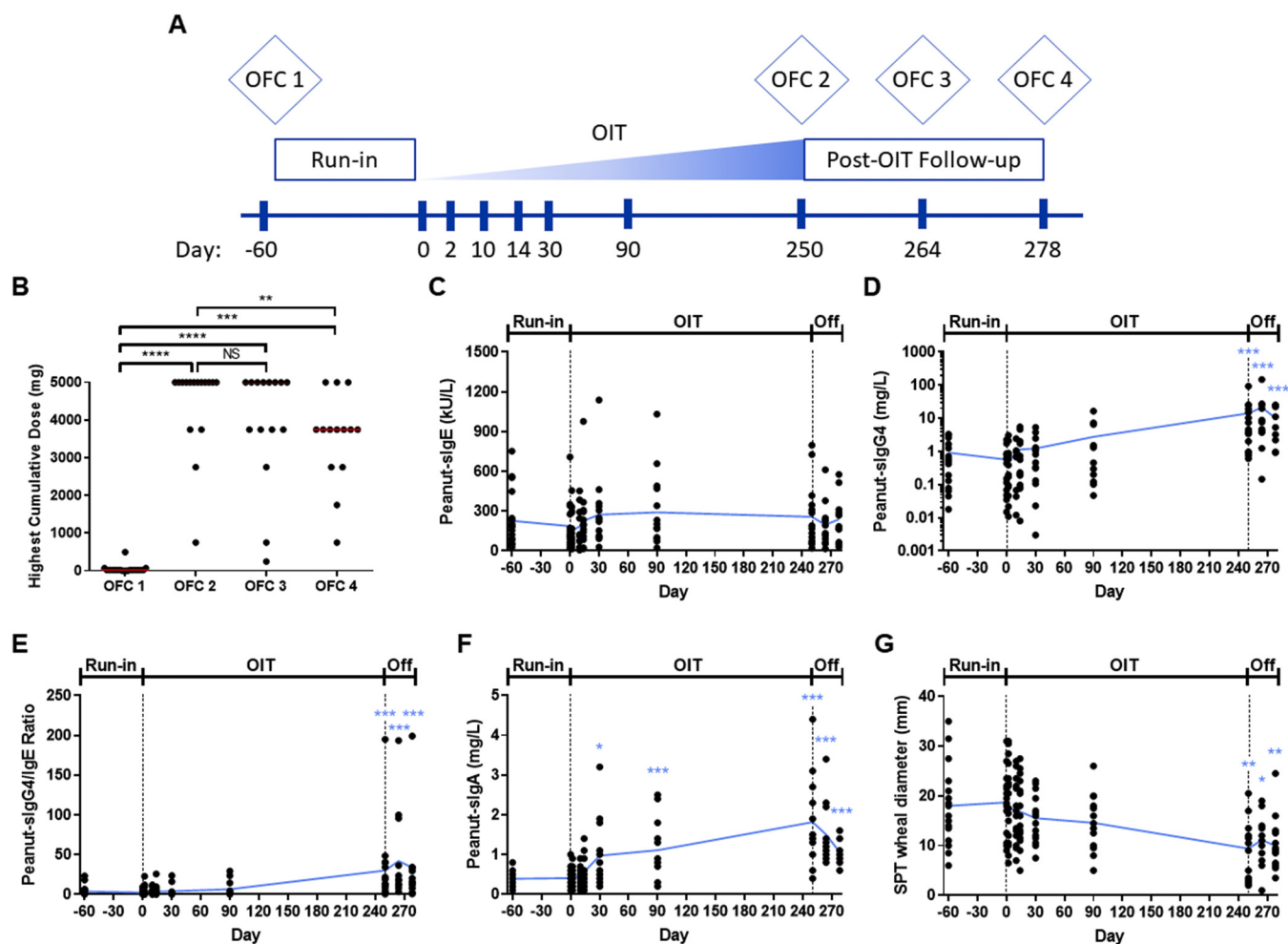


FIG 1. Clinical and serological outcomes after short-course OIT. **(A)** Overview of OIT protocol. **(B)** DBPCFC outcomes. Serum peanut-specific **(C)** IgE, **(D)** IgG₄, **(E)** IgG₄/IgE, and **(F)** IgA. **(G)** Peanut SPT outcomes. **P* < .05, ***P* < .01, ****P* < .001, *****P* < .0001, Wilcoxon matched-pairs signed rank test **(B)** or Kruskal-Wallis comparing each time point to day -60 **(C-G)**, individual data are shown with a line representing the median **(B)** or mean **(C-G)**.

long term at -80°C until analysis. For peanut-specific IgA ELISAs, 96-well plates were coated with $2\ \mu\text{g/mL}$ of anti-human IgA₁/IgA₂ (clone G18-1, BD Biosciences, San Jose, Calif) for standard curves or $20\ \mu\text{g/mL}$ peanut protein for saliva samples. Wells were blocked with 2% BSA in PBS with 0.05% Tween 20. Saliva samples were diluted 1:50. Standard curves were generated with purified human IgA (Bethyl Laboratories, Montgomery, Tex) ranging from 0.6 to 60 ng/mL. Samples were detected with a 1:1000 dilution of anti-human IgA-HRP (Southern Biotech, Birmingham, Ala). Plates were developed using tetramethylbenzidine (SeraCare, Milford, Mass); 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).

Basophil activation assays

Peripheral blood was collected in sodium heparin tubes at days -60, 0, 2, 10, 14, 30, 90, 250, 264, and 278. Basophils in whole blood were stimulated with peanut extract (extracted as previously described¹⁸) (1, 10, 100, or 1000 ng/mL), media or polyclonal anti-IgE (Bethyl Laboratories) in basophil media (RPMI with human IL-3 [2 ng/mL], Miltenyi Biotec, Bergisch Gladbach,

Germany). Samples were stained for 30 minutes with CD63 (fluorescein isothiocyanate, clone H5C6, BD Biosciences), CD203c (phycoerythrin, clone 97A6, Beckman Coulter, Brea, Calif), and CCR3 (AF647, clone 5E8, BD Biosciences). Samples were acquired on a CyAn Flow Cytometer (Beckman Coulter), and data was analyzed using FlowJo (Ashland, Ore); basophils were identified as CCR3⁺ cells in the lymphocyte gate, and CD63 and CD203c were used as markers of activation. Peanut-induced activation is presented as %CD63⁺ basophils corrected by subtracting background activation with media alone, and CD203c is presented as a stimulation index generated by dividing peanut stimulation by media alone.

To measure pSYK, blood was collected at the same time points and basophils were stimulated with peanut (1000 or 100 ng/mL) or media for 5, 10, 20, or 30 minutes. Basophils were stained for CCR3 (AF647, clone 5E8, BD Biosciences) and CD63 (fluorescein isothiocyanate, clone H5C6, BD Biosciences) for 30 minutes. Cells were permeabilized with Perm Buffer III (BD Biosciences) for 30 minutes, washed, and stained with $25\ \mu\text{L}$ of anti-pSYK at 4°C in the dark overnight (phycoerythrin, clone I120-722, BD Biosciences). Basophils were identified from the CCR3⁺ lymphocyte gate, and pSYK was quantified based on mean fluorescence intensity. CD63 was used as a

confirmation of activation. pSYK quantities are reported as a ratio of peanut activation to basophil media activation.

Statistical analysis

We compared baseline measurements of serum peanut-specific IgE, IgG₄, ratio of IgG₄/IgE, IgA, SPT, %CD63, CD203c, pSYK, areas under the curve (AUCs), and salivary peanut-specific IgA versus the corresponding measurements at each visit using the Kruskal-Wallis test (executed in R version 4.0.3, R Foundation, Vienna, Austria). Wilcoxon matched-pairs signed rank tests were performed on DBPCFC outcomes (GraphPad Prism 9.1.2, GraphPad Software, San Diego, Calif). Spearman correlations were performed on CD63 versus pSYK, and salivary versus serum IgA (GraphPad Prism 9.1.2). Longitudinal generalized estimating equations were generated to estimate changes over time in biomarkers (STATA version 17, StataCorp, College Station, Tex). *P* values <.05 were considered statistically significant.

The AUCs were calculated by using trapezoidal approximation as previously described;^{19,20} the methodology is available at <https://github.com/FACatMGH/AutoBAT>. AUC is the integral of %CD63⁺ or CD203c mean fluorescence intensity with respect to stimulation concentration or pSYK mean fluorescence intensity with respect to *in vitro* stimulation time point using trapezoid rule integration as implemented by the *trapz* package in the caTools library (version 1.18.2) executed in R (version 4.1.2).

RESULTS

Desensitization and sustained unresponsiveness occur after 9 months of peanut OIT

Sixteen of the 20 participants completed the protocol. Based on DBPCFCs performed following 9 months of peanut OIT, 12 participants (of 16 who completed the protocol, 75%) were desensitized, with 9 of those achieving SU after discontinuation of therapy (56%). At OFC 2, the median highest tolerated cumulative dose consumed increased to 5000 mg (range 750–5000 mg) peanut protein from 21 mg (range 1–496 mg) at baseline (Fig 1, B, and see Table E2 in this article's Online Repository at www.jacionline.org). After discontinuing therapy for 2 weeks, during OFC 3, the median remained at 5000 mg (range 250–5000 mg) peanut protein, with only 3 participants tolerating <5000 mg after passing OFC 2. Thirteen participants who consumed ≥2750 mg of peanut protein during OFC 3 continued to avoid peanut and were challenged again 2 weeks later. The median highest tolerated cumulative dose consumed during OFC 4 decreased to 3750 mg (range 750–5000 mg) peanut protein, with 8 of 13 subjects tolerating lower amounts of peanut protein compared to OFC 3. Despite the short course of peanut OIT, desensitization up to 5000 mg peanut protein was achieved in 75% of participants that completed the protocol and discontinuation of therapy for up to 4 weeks still led to a reaction threshold significantly higher than baseline (*P* < .001).

Peanut-specific immunoglobulins and skin prick tests are modulated throughout OIT

Serum peanut-specific IgE was not statistically different throughout the course of OIT (median: day –60: 169 kU/L, day 250: 170 kU/L) (Fig 1, C). There were marked increases in peanut-specific IgG₄ and the ratio of peanut-specific IgG₄ to IgE from baseline to the desensitization OFC at day 250 (*P* < .001) (Fig 1, D and E). Serum peanut-specific IgA was also significantly increased throughout OIT, with a sharp increase occurring as early as day 30 (*P* < .05), suggesting distinct kinetics compared to IgG₄ (Fig 1, F). SPT outcomes, presented as wheal diameter,

decreased by day 250 (*P* < .01) (Fig 1, G). Overall, these results demonstrate that decreases in peanut-specific IgE are not required for mast cell suppression throughout OIT.

Basophil activation is suppressed by short course peanut OIT

Basophil activation tests were performed throughout the course of peanut OIT to determine how quickly suppression occurred. Basophils were identified as CCR3⁺ cells in the lymphocyte gate, and activation was assessed by CD63 and CD203c (Fig 2, A). Four concentrations of peanut were used for basophil stimulation: 1000, 100, 10, and 1 ng/mL (Fig 2, B–E, and see Fig E1, A in this article's Online Repository at www.jacionline.org). In longitudinal models through day 250, suppression of basophil activation was found for all 4 doses. Basophil activation tests performed with lower concentrations (1 and 10 ng/mL peanut protein) demonstrated significant suppression of %CD63⁺ basophils by day 90 (*P* < .001) using the Kruskal-Wallis test. By the completion of OIT at day 250, suppression of basophil activation after 100-ng/mL peanut stimulation was also significant. AUC analyses were performed using the dose response across the 4 concentrations for CD63 activation (Fig 2, F). Based on AUC, CD63 activation was suppressed at the end of OIT and during the peanut avoidance phase (days 250–278) compared to baseline. However, there is a trend toward increased basophil activation off therapy.

CD203c is constitutively expressed on basophils and is upregulated during activation. During the course of OIT, the CD203c stimulation index remained nearly constant at all peanut concentrations (Fig 3, A–D, Fig E1, B). Likewise, AUC for CD203c was not significantly different at any time point or longitudinally (Fig 3, E). Because CD203c is unaltered during OIT, this does not coincide with the significant increases in highest tolerated cumulative peanut dose seen in OFCs 2, 3, and 4.

pSYK expression is suppressed in basophils during OIT and is correlated with CD63 activation

Basophil assays were performed with the addition of a pSYK antibody to better understand the signaling effects of OIT-induced basophil suppression. Basophils were again identified as CCR3⁺ cells in the lymphocyte gate; 2 doses of peanut (1000 and 100 ng/mL) and 4 stimulation times (5, 10, 20, and 30 minutes) were used to capture pSYK expression (Fig 4, A). When comparing pSYK expression at day 0 with that at day 250, all dose/time point combinations were significantly decreased, except for the 1000 ng/mL after 20-minute stimulation (Fig 4, B and C). There was a significant decrease based on the AUC analysis of pSYK over time at both peanut concentrations (*P* < .01) (Fig 4, D). A stimulation time of 30 minutes led to high activation of pSYK; there is a clear and consistent decrease by day 250 at both peanut concentrations (compared to day 0, *P* < .01), which remains suppressed at day 264 and then trends upward by day 278 (Fig 4, E). These data indicate that short course peanut OIT is effective at suppressing pSYK while participants are on OIT, although once therapy is discontinued, pSYK may recur.

pSYK was correlated with CD63 after 30 minutes at both peanut concentrations. Based on basophil activation data prior to the initiation of therapy (days –60 and 0), CD63 basophil activation was highly correlated with pSYK (Spearman *R* > 0.72 and *P* < .0001, both concentrations) (Fig 5, A and B).

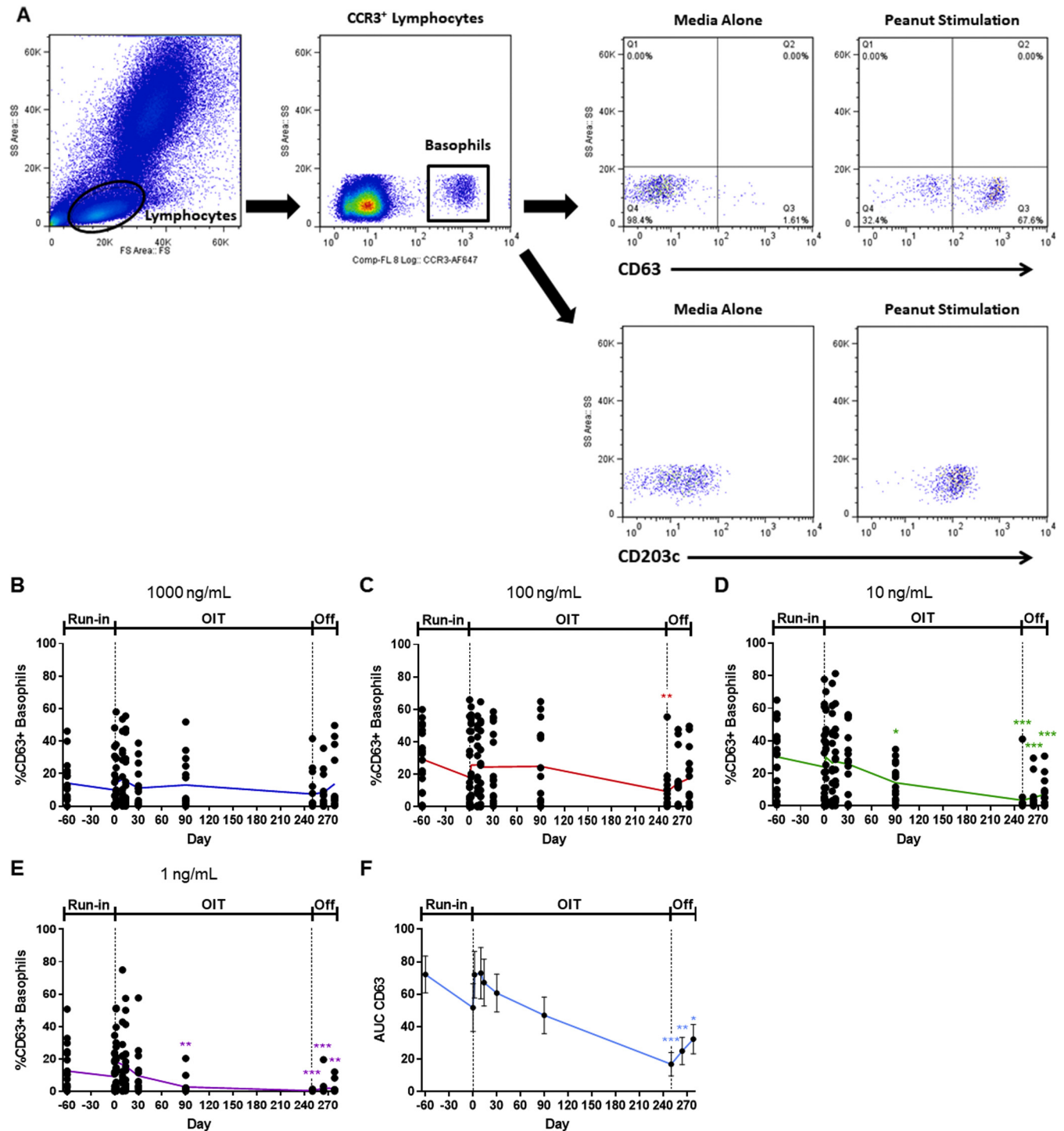


FIG 2. Whole blood basophil activation assay in response to 4 concentrations of peanut stimulation. **(A)** Flow cytometry gating strategy to identify activated basophils. Basophil activation based on CD63 expression after stimulation with **(B)** 1000 ng/mL, **(C)** 100 ng/mL, **(D)** 10 ng/mL, and **(E)** 1 ng/mL of peanut protein. AUC based on **(F)** CD63 expression of 4 peanut concentrations. * $P < .05$, ** $P < .01$, *** $P < .001$, **** $P < .0001$, Kruskal-Wallis comparing each time point to day -60. Scatter plots show individual data with a line representing the mean.

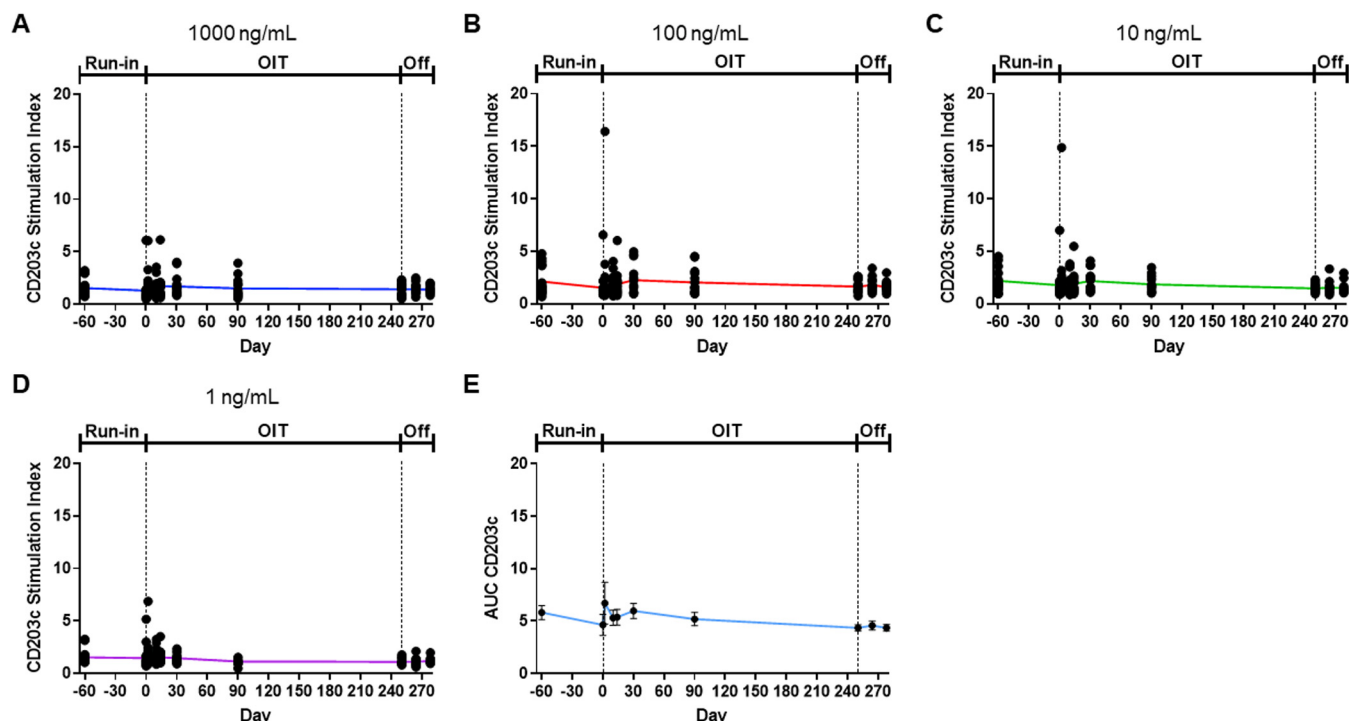


FIG 3. Whole blood basophil activation assay in response to 4 concentrations of peanut stimulation. Basophil activation based on CD203c after stimulation with (A) 1000 ng/mL, (B) 100 ng/mL, (C) 10 ng/mL, and (D) 1 ng/mL of peanut protein. AUC based on (E) CD203c of 4 peanut concentrations. Scatter plots show individual data with a line representing the mean.

When including data from all time points, CD63 was moderately correlated with pSYK (Spearman $R > 0.5$ and $P < .0001$, both concentrations) (Fig 5, C and D). Overall, these data demonstrate that pSYK is correlated with basophil activation, and both are suppressed throughout the course of peanut OIT.

Suppression of basophil activation is not associated with individual OFC outcomes

CD63 basophil activation at all 4 peanut concentrations was plotted for each of the 4 OFCs and color-coded for clinical outcomes (Fig 6). Sustained unresponsiveness was assigned to participants that consumed 5000 mg peanut protein at OFC 2 and ≥ 3750 mg at OFC 4 (black); desensitization was assigned to participants that consumed 5000 mg at OFC 2 and < 3750 mg at OFC 4 (blue); failure was assigned to participants that consumed < 5000 mg at OFC 2 (purple). At all 4 peanut concentrations, there is clear suppression of basophil activation by OFC 2 ($P < .001$ at all concentrations) (Fig 6, A-D); with 1 to 100 ng/mL peanut stimulation, basophil activation remains suppressed through OFCs 3 and 4, after therapy is discontinued (Fig 6, B-D). Interestingly, there was no grouping of participants based on clinical outcome at any OFC/peanut dose combination or over time in our longitudinal models.

Salivary peanut-specific IgA is increased during OIT

Many studies have quantified immunoglobulins in serum throughout OIT; however, little is known about mucosal immunoglobulins. We aimed to assess the local IgA response in saliva throughout OIT. Salivary peanut-specific IgA was increased by

day 90 and continued to rise through day 250 (Fig 7, A). On discontinuing OIT, mean levels of salivary peanut-specific IgA remained significantly increased on day 264 relative to baseline, but were no longer different than baseline by day 278. There was no clear clustering of salivary peanut-specific IgA based on clinical outcome at each OFC (Fig 7, B). Importantly, there was no correlation of salivary and serum peanut-specific IgA at baseline or after 9 months of OIT (Fig 7, C and D), indicating that there is a difference in local and systemic responses to OIT.

DISCUSSION

Short-course peanut OIT led to desensitization in a majority of participants completing the protocol (12 of 16), with 9 participants achieving sustained unresponsiveness 4 weeks after therapy was discontinued. Suppression of basophil activation, based on CD63 expression, occurred by day 90, which was later than hypothesized. With rush immunotherapy for venom and drug allergies, patients can be desensitized and tolerate relatively high doses of antigen or drug within hours to a few days.^{21,22} There is some evidence with rush immunotherapy for cat and birch pollen allergens that basophil activation is inhibited within 1 to 3 days of initiating desensitization.²³ These results led us to hypothesize that a similar length of time on OIT would lead to an immediate reduction in basophil activation. However, statistically meaningful suppression did not occur within the first few weeks of OIT and instead did not occur until day 90 in this study. Of note, this could be an effect of the low dose of peanut protein administered during the escalation phase during those first weeks or the short length of exposure at that point. Future studies should aim to separate these variables, where higher and lower starting doses are directly

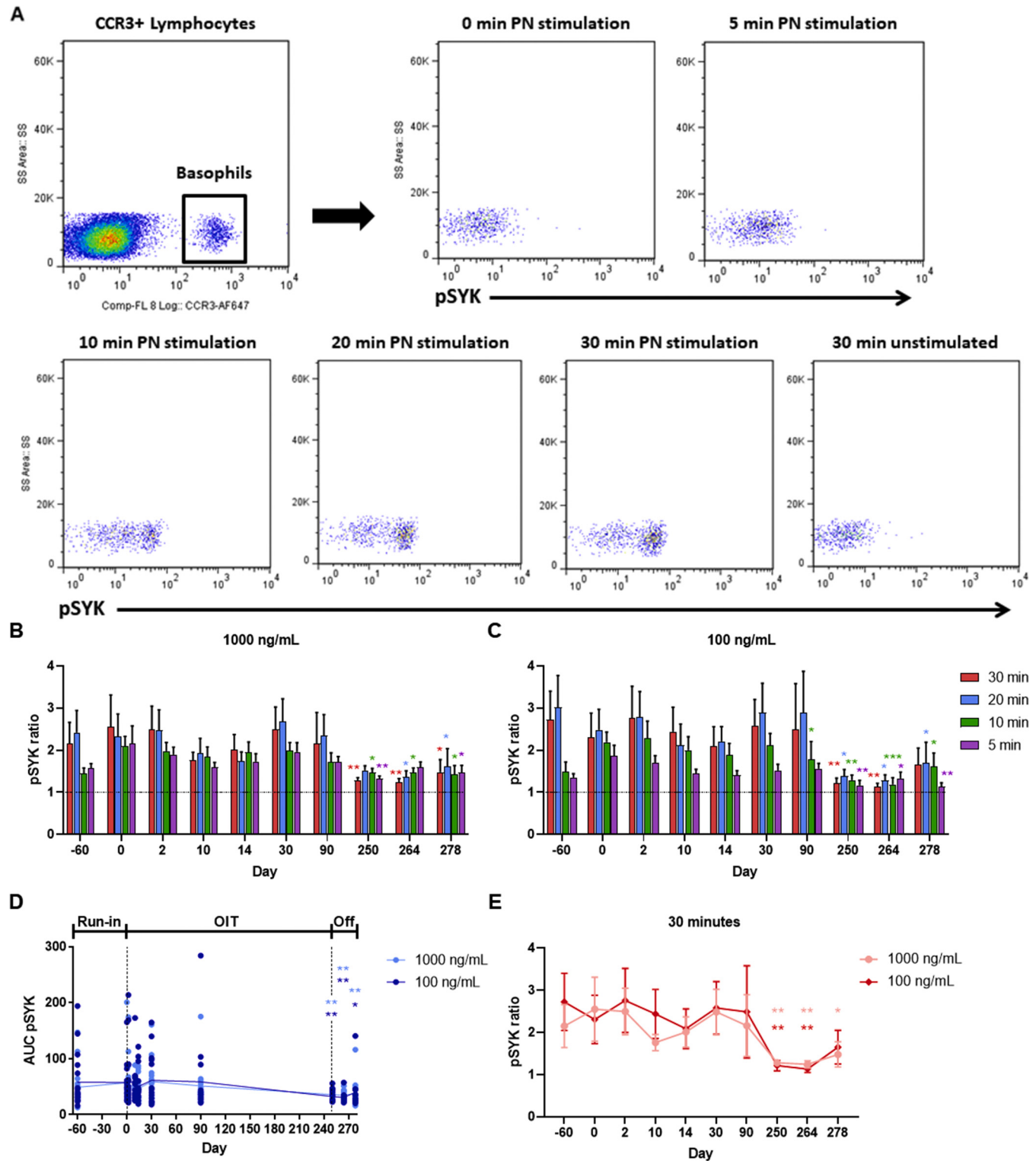


FIG 4. Quantification of pSYK within basophils. **(A)** Flow cytometry gating strategy to identify pSYK. Longitudinal pSYK response with 5-, 10-, 20-, and 30-minute stimulation with **(B)** 1000 ng/mL or **(C)** 100 ng/mL peanut (PN). **(D)** AUC pSYK of stimulation time course. **(E)** pSYK with 30-minute peanut stimulation. * $P < .05$, ** $P < .01$, Kruskal-Wallis comparing each time point to day 0. Histograms represent means; scatter plot shows individual data with a line representing the mean; line plot shows mean $\pm$ SEM.

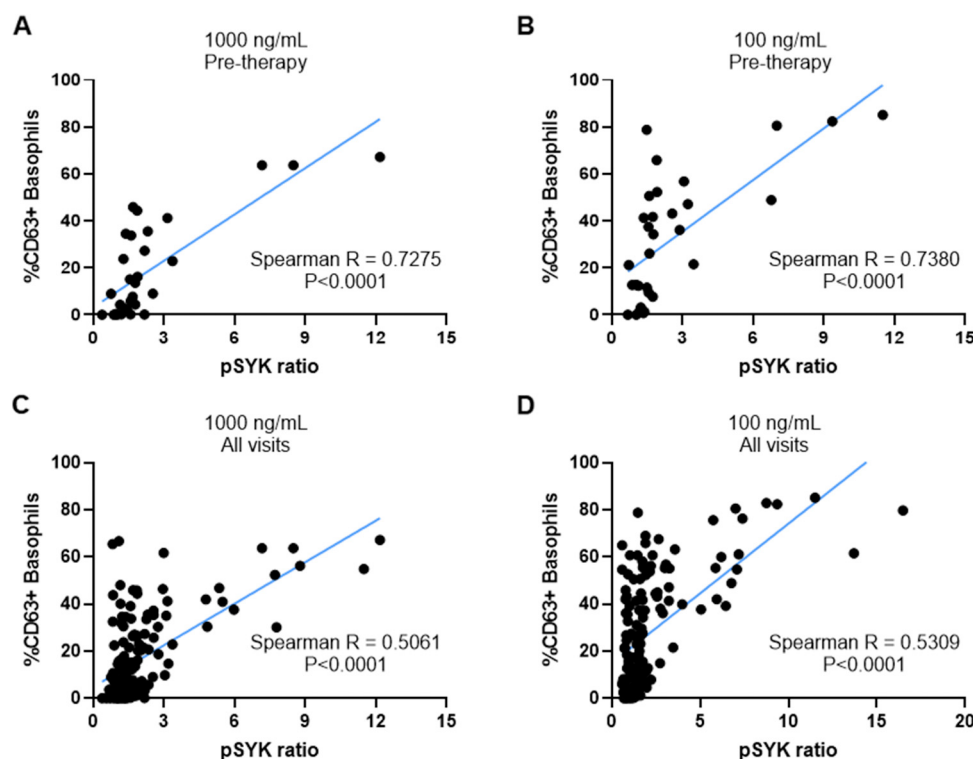


FIG 5. Correlation between CD63 expression and pSYK. Basophil activation markers on days –60 and 0 after (A) 1000 ng/mL or (B) 100 ng/mL peanut stimulation. Basophil activation markers from all time points after (C) 1000 ng/mL or (D) 100 ng/mL peanut stimulation. Spearman correlation; individual data are shown with linear regression line.

compared to determine the early suppressive effects. These results compared to the more immediate increases in IgA highlight the different kinetics of action between basophil activation suppression and the production of protective antibodies in circulation.

CD63 expression was significantly decreased from baseline by the end of therapy; CD203c expression, however, remained unchanged throughout the course of therapy, as previously shown for peanut OIT.²⁴ Interestingly, a 2-year peanut OIT study eventually demonstrated suppression of CD203c, but not within the first year of treatment.²⁵ CD63 is distinctly associated with anaphylactic degranulation of basophils, whereas CD203c is constitutively expressed on the cell surface and can be upregulated with IL-3 or specific allergens.^{26,27} IL-3 is used in basophil activation assays to prime the basophils for anaphylactic degranulation. However, priming with IL-3 can lead to nonspecific increases in CD203c, which may lead to discrepancies in CD63 and CD203c suppression following OIT.²⁸ These reasons, along with the short duration of therapy, may explain why we found a suppression of CD63 by the end of therapy in this study, whereas CD203c remained unchanged.

pSYK followed similar trends to CD63 expression, and they were highly correlated. Overall, pSYK may not be more effective than CD63 as a biomarker, but pSYK could be used as a drug target to reduce CD63 expression and degranulation. A previous study demonstrated that inhibition of pSYK with entospletinib led to >90% inhibition of CD63 expression.²⁹ A murine study showed that treatment of mast cells with omeprazole decreased pSYK, along with reduced degranulation and release of mediators after

allergen exposure.³⁰ Selectively inhibiting SYK signaling in basophils may therefore be a promising therapeutic approach.

Despite the majority of participants becoming desensitized, peanut-specific IgE was not significantly reduced throughout OIT, which is likely reflective of the short duration of OIT in this study. These data further demonstrate that change in IgE over time is not predictive or indicative of clinical outcome, and furthermore that its quantity alone is not critical to achieve desensitization. Serum peanut-specific IgG₄ and IgA, however, both significantly increased throughout OIT, with sharp increases in IgA by day 30 and by day 250 in IgG₄. These antibodies have been shown to be protective at blocking basophil and mast cell degranulation,^{11,31–34} and here their increase appears to be more crucial to desensitization than decreases in IgE. Although, the question of antibody quantity versus function remains an area of future study.³⁵

While overall basophil activation decreased in participants by OFC 4, an unexpected finding was that some participants that had the highest basophil activation reached either desensitization or SU (blue and black dots in Fig 6). There was no clear distinction of participants based on clinical outcome and basophil activation. Because their basophils are activated with peanut *ex vivo*, this may indicate that peanut is not being absorbed into circulation during the OFC. One potential explanation, which would require further studies, is that local responses in the gastrointestinal tract, including IgA production and microbiome changes, lead to low intestinal permeability and therefore low uptake of peanut during OFCs, ultimately resulting in a high tolerance to consumed peanut during the OFC. Indeed, salivary peanut-specific IgA was significantly increased throughout OIT in this study and may limit

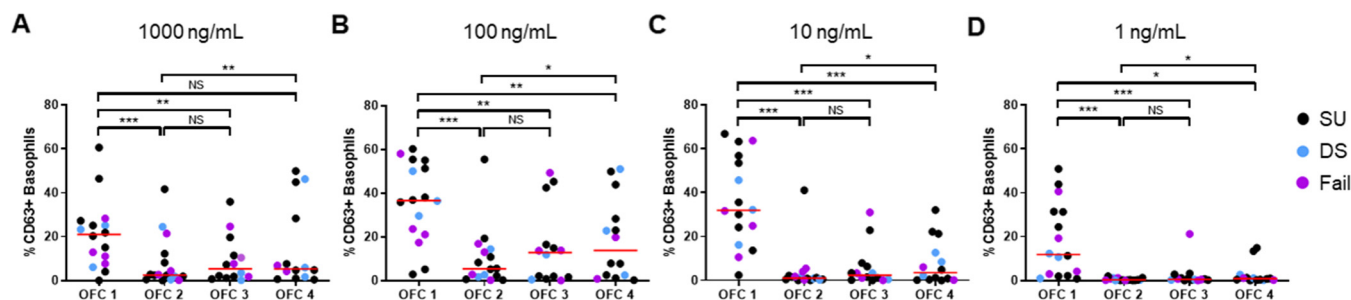


FIG 6. Basophil activation at each DBPCFC based on clinical outcome. CD63 expression after stimulation with (A) 1000 ng/mL, (B) 100 ng/mL, (C) 10 ng/mL, and (D) 1 ng/mL peanut. * $P < .05$, ** $P < .01$, *** $P < .001$, Wilcoxon matched-pairs signed rank test; individual data are shown with a line representing the median. Black dots represent participants that achieved SU; blue dots represent participants that achieved desensitization (DS); purple dots represent participants that failed. NS, Not significant.

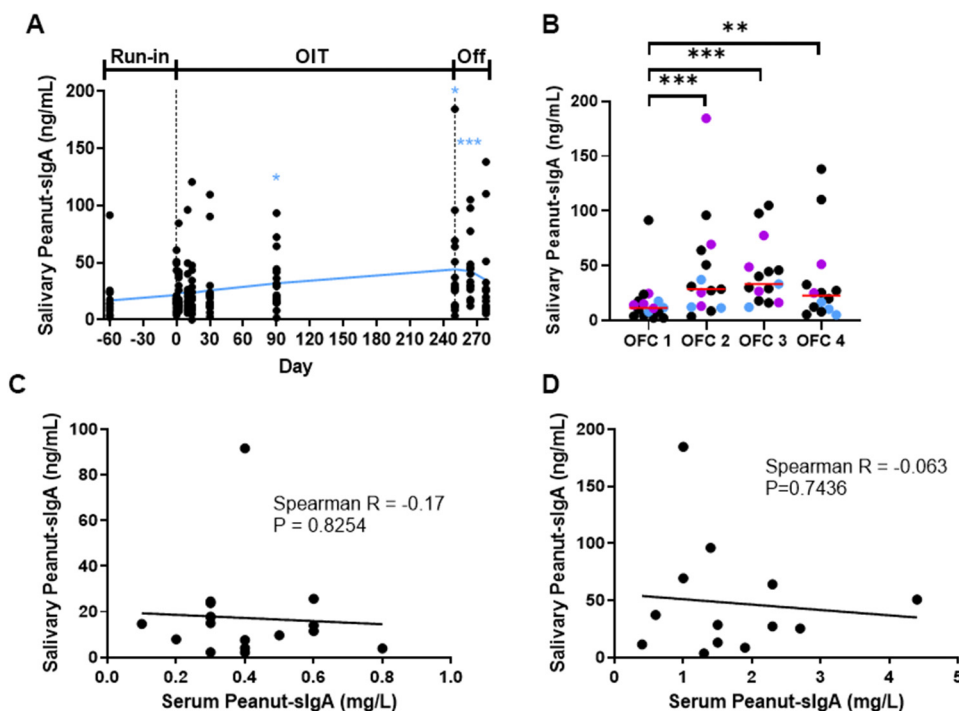


FIG 7. Salivary peanut-specific IgA throughout OIT. (A) Longitudinal quantification of peanut-specific IgA in saliva. (B) Peanut-specific IgA at each OFC. Correlation between salivary and serum peanut-specific IgA at day -60 (C) and day 250 (D). ** $P < .01$, *** $P < .001$, Kruskal-Wallis comparing each time point to day -60 (A), Wilcoxon matched-pairs signed rank test (B), or Spearman correlation (C-D); individual data are shown with a line representing the mean (A), median (B), or linear regression (C-D).

systemic absorption of peanut. Additionally, previous studies in murine models have demonstrated changes in mucosal IgA and microbiome may be critical to challenge outcomes,^{36,37} and similar studies are needed for participants on OIT.

Here, we present the first study investigating the kinetics of basophil suppression during OIT using the conventional CD63 and CD203c readouts, as well as cell signaling through pSYK. Suppression of basophil activation based on CD63 does not occur within the first few weeks of peanut OIT, and instead occurs by day 90. pSYK was similarly suppressed by the end of therapy and was correlated with CD63 activation. Overall, our study demonstrates the time course of basophil suppression and adaptive

immune responses in the first days, weeks, and months of initiating OIT, providing insight into the immunologic mechanisms of OIT.

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Key messages

- Basophil activation, based on CD63 expression, was suppressed within 90 days of peanut OIT, ultimately leading to suppression of pSYK signaling by day 250.

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