METHODS

OFCs

At study entry, an OFC was conducted with peanut flour to a cumulative amount of 1044 mg of peanut protein administered in doses (1, 3, 10, 30, 100, 300, and 600 mg) every 15 minutes by using a modified PRACTALL Protocol.[E1] The OFC was repeated at week 52 to a cumulative dose of 5044 mg of peanut protein administered in doses (1, 3, 10, 30, 100, 300, 600, 1000, and 3000 mg) per protocol. Lightly roasted peanut flour (Golden Peanut Company, Alpharetta, Ga) was used for peanut OFC, and placebo OFC was conducted with organic oat flour (Arrowhead Mills, Golden, Colo) in equivalent volumes. Each OFC was scored as a pass or failure by an OFC scorer who was blinded to treatment assignment through week 52. Subjects who successfully consumed the total OFC dose were scored as a pass. Inability to tolerate the total OFC challenge dose because of persistent allergic symptoms (eg, hives, wheezing, vomiting, and laryngeal edema) was scored as a failure. Persistent symptoms were defined as those that required treatment for resolution or those that worsened over time. Transient symptoms that resolved completely before the next dose (within 15 minutes) without treatment did not result in termination of the OFC.

T-cell assay methods

Blood samples. Blood samples were obtained as coded specimens in 10-mL sodium-heparin BD Vacutainer tubes (BD Biosciences, San Jose, Calif) at the 5 clinical sites. Whole blood was shipped overnight in temperature-controlled Greenbox shipping containers (ThermoSafe, Arlington Heights, Ill) assembled according to standard operating procedures. Temperature loggers were included to ensure that temperatures were maintained at between 20°C and 30°C. Samples from the Icahn School of Medicine at Mount Sinai clinical site were stored at room temperature and processed the next day to maintain consistency with the other sites.

Cell isolation and stimulation. Whole blood was spun for plasma collection, and PBMCs were isolated by using Ficoll-Paque PLUS (GE Healthcare, Piscataway, NJ), washed, and cultured in AIM V Medium (Thermo Fisher, Grand Island, NY) with 2.5% autologous plasma. Cells (4×10^6) were plated in 1 mL in 24-well culture plates in the presence or absence of 100 μg of crude peanut extract (CPE) or anti-CD3/CD28 stimulation beads (Thermo Fisher) as a positive control. Cells (8×10^6) were used for each of the media and CPE conditions, and 4×10^6 cells were used for anti-CD3/CD28. CPE had been cleaned of endotoxin by using Detoxi-Gel columns (Thermo Fisher). Brefeldin A (BD Biosciences) was added for the last 4 hours of a 6-hour culture with stimulants.

Staining and flow cytometry. Cells were harvested and stained with Live/Dead Fixable Aqua Dead Cell Stain (Thermo Fisher), followed by staining for surface markers: CD3–allophycocyanin-Cy7 (eBioscience, San Diego, Calif); CD4–Brilliant Violet 605 (BV605), CD25-BV650, and CD127-BV785 (from BioLegend, San Diego, Calif); and CCR4–peridinin-chlorophyll-protein complex/Cy5.5, CCR6–phycoerythrin-Cy7, and CXCR5–Alexa Fluor 488 (BD Biosciences). After fixation with 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, Pa) and permeabilization buffer (eBioscience), intracellular staining was performed with CD154-PE (eBioscience), IFN-γ–Alexa Fluor 700, IL-10–PE-CF594, IL-13–BD Horizon-v450 (all from BD Biosciences), and IL-4–Alexa Fluor 647 (BioLegend). Cells were acquired on a BD LSRFortessa maintained according to standard operating procedures in the Human Immune Monitoring Core at the Icahn School of Medicine at Mount Sinai. Data analysis was performed with FlowJo Software (Ashland, Ore).

REFERENCE

E1. Syed A, Garcia MA, Lyu SC, Bucayu R, Kohil A, Ishida S, et al. Peanut oral immunotherapy results in increased antigen-induced regulatory T-cell function and hypomethylation of forkhead box protein 3 (FOXP3). J Allergy Clin Immunol 2014;133:500-10.

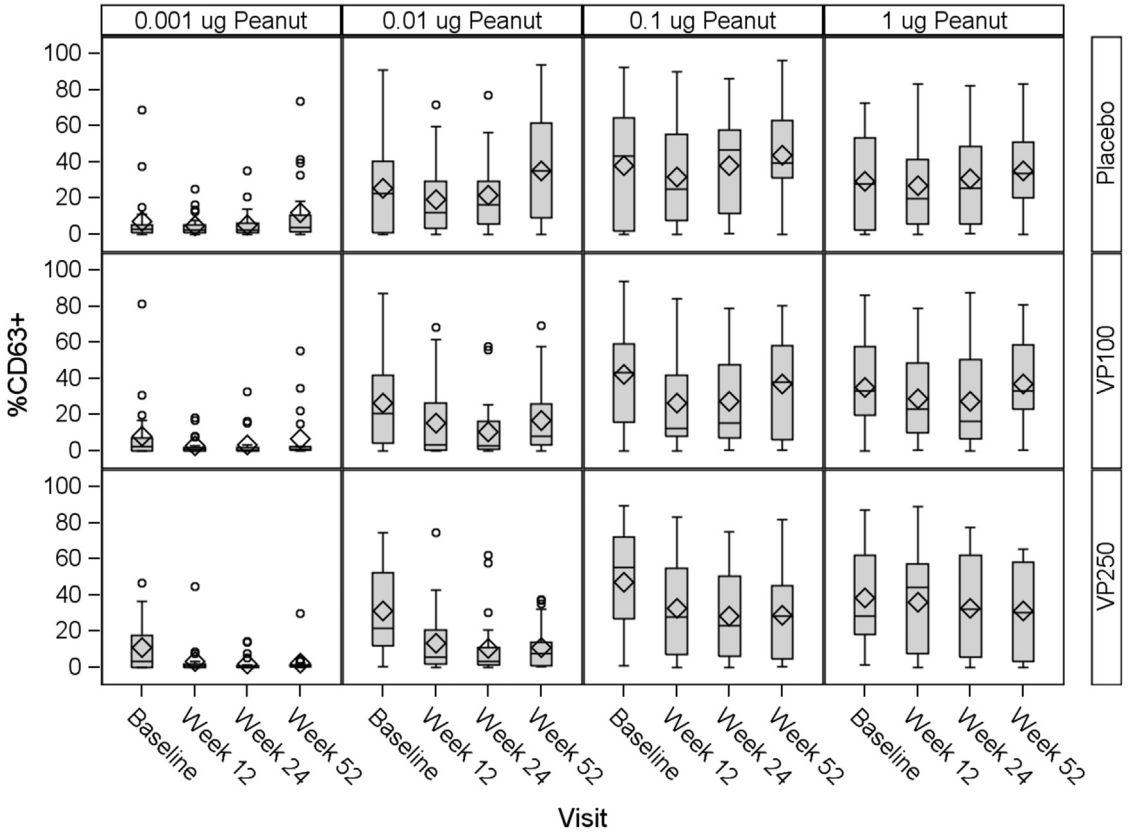

FIG E1. Effect of peanut EPIT on basophil activation (percentage of $CD63^+$ basophils) over time by treatment group. *Top row*, Placebo group; *middle row*, VP100 group; *bottom row*, VP250 group. Cells were stimulated with 0.001 μg/mL peanut extract *(column 1)*, 0.01 μg/mL peanut extract *(column 2)*, 0.1 μg/mL peanut extract *(column 3)*, or 1 μg/mL peanut extract *(column 4)*. Significant differences over time were observed only at a stimulation dose of 0.01 μg of peanut extract ($P < .0001$) when evaluating for a global treatment effect. *Diamonds* represent mean values.

TABLE E1. Inclusion and exclusion criteria

Inclusion criteria

Participants who met *all* of the following criteria were eligible for enrollment as study participants:

- Age 4-25 years, all of either sex and any race and ethnicity at enrollment
- Physician-diagnosed peanut allergy OR convincing history of peanut allergy
- Positive SPT response to peanut (wheal diameter ≥3 mm larger than that elicited by the saline control) OR detectable peanut-specific IgE (ImmunoCAP >0.35 kU_A/L)
- Positive reaction to a cumulative dose of ≤1044 mg of peanut protein in the initial qualifying OFC
- Use of an effective method of contraception by female subjects of childbearing potential to prevent pregnancy and agreement to continue to practice an acceptable method of contraception for the duration of their participation in the study
- Ability to perform spirometric maneuvers in accordance with American Thoracic Society guidelines: children aged 4-11 years who have documented inability to adequately perform spirometry can be enrolled if peak expiratory flow is greater than 80% of predicted value.
- Provision of signed informed consent forms and assent, where indicated

Exclusion criteria

Participants who met *any* of these criteria were *not* eligible for study enrollment:

- History of anaphylaxis to peanut resulting in hypotension, neurological compromise, or requirement for mechanical ventilation
- Participation in a study using an investigational new drug in the last 30 days
- Participation in any interventional study for the treatment of food allergy in the past 6 months
- Pregnancy or lactation
- Current or known allergy to the Viaskin Peanut/Viaskin Placebo patch or excipients
- Current or known allergy to the placebo allergen (oat flour) in OFCs
- Currently in a build-up phase of any allergen immunotherapy
- Severe or poorly controlled atopic dermatitis or greater than a mild flare of active disease at enrollment
- FEV_1 <80% of predicted value or any clinical features of moderate or severe persistent asthma at baseline (as defined by the 2007 National Heart, Lung, and Blood Institute guidelines) and greater than high daily doses of inhaled corticosteroids (>500 μg of fluticasone or equivalent)
- Use of steroid medications in the following manners: history of daily oral steroid dosing for >1 month during the past year, burst of steroid in the past 3 months, or >1 burst oral steroid course in the past year or any use of oral or parenteral steroids for a nonasthma indication within the past 30 days
- Asthma requiring >1 hospitalization in the past year for or >1 emergency department visit in the past 6 months for asthma
- Any previous intubation/mechanical ventilation caused by allergies or asthma
- Use of omalizumab or other nontraditional forms of allergen immunotherapy or immunomodulatory or biologic therapy in the past year
- Use of β-adrenergic blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, or calcium-channel blockers in the past 30 days
- Inability to discontinue antihistamines for skin testing and OFCs
- History of alcohol or drug abuse
- History of cardiovascular disease, uncontrolled hypertension, arrhythmias, chronic lung disease, active eosinophilic gastrointestinal disease, or other medical conditions, including immunologic disorders or HIV infection, which, in the opinion of the investigator, make the participant unsuitable for treatment or at increased risk of anaphylaxis or poor outcome

TABLE E2. Skin reaction grading system

Grade	Skin reaction (clinic assessment)	Skin reaction (participant assessment)
Grade 0	Negative	Normal skin, no reaction
Grade 1A	Only erythema	Redness only
Grade 1B	Erythema, infiltration	Redness and hard or stiff skin
Grade 2 (++)	Erythema, few papules	Redness and a few bumps
Grade 3 (+++)	Erythema, many or spreading papules	Redness with many bumps or spreading bumps
Grade 4 (++++)	Erythema, vesicles	Redness with blisters

TABLE E3. CoFAR grading system for allergic reactions

Grade 1: Mild	Grade 2: Moderate	Grade 3: Severe	Grade 4: Life-threatening	Grade 5: Death
Transient or mild discomfort (<48 h), no or minimal medical intervention/therapy required. These symptoms can include pruritus, swelling or rash, abdominal discomfort, or other transient symptoms.	Symptoms that produce mild-to-moderate limitation in activity. Some assistance might be needed, but no or minimal intervention/therapy is required. Hospitalization is possible. These symptoms can include persistent hives, wheezing without dyspnea, abdominal discomfort/increased vomiting, or other symptoms.	Marked limitation in activity. Some assistance is usually required; medical intervention/therapy required, and hospitalization is possible. Symptoms can include bronchospasm with dyspnea, severe abdominal pain, throat tightness with hoarseness, and transient hypotension among others. Parenteral medication(s) are usually indicated.	Extreme limitation in activity. Significant assistance is required; significant medical/therapy is required. Intervention is required; hospitalization is probable. Symptoms might include persistent hypotension and/or hypoxia with resultant decreased level of consciousness associated with collapse and/or incontinence or other life-threatening symptoms.	Death

TABLE E4. Week 52 OFC results by treatment group and baseline age

	Treatment group						
	Placebo		VP100		VP250		
	4-11 y	>11 y	4-11 y	>11 y	4-11 y	>11 y	Total
Week 52 SCD (mg of protein)							
Np.	17	5	16	5	18	7	68
Median	14	144	144	144	144	144	144
Minimum	1	4	44	44	0	44	0
Maximum	2044	5044	2044	2044	2044	2044	5044
Change in SCD (mg of protein)*							
No.	17	5	16	5	18	7	68
Median	0	0	71.5	0	130	100	40
Minimum	−440	−300	0	−300	0	−300	−440
Maximum	1600	4600	1900	2040	2040	1600	4600

*When comparing age groups within each treatment group: $P = .67$ for placebo, $P = .59$ for VP100, and $P = .19$ for VP250 participants.

TABLE E5. Logistic regression for baseline factors with treatment success as outcome

	P value, Wald χ^2 test		
	Model with no treatment interaction	Model with significant treatment interaction	
Variable	*P* value	*P* value for main effect	*P* value for interaction term
Age	.0469	.0880	.0057
Sex	.2910	—	—
Allergic rhinitis	.4541	—	—
Atopic dermatitis	.7209	—	—
Asthma (physician's diagnosis)	.9943	—	—
Additional food allergy (unknown = no)	.2651	—	—
AD total score	.2272	—	—
Peanut SPT score	.6301	—	—
Baseline OFC dose at first symptom	.0547	—	—
High vs low baseline OFC SCD*	.0001	—	—
Log_{10} total IgE	.1982	—	—
High vs low peanut IgE†	.6445	—	—
Log_{10} peanut IgG_4	.8053	—	—
Log_{10} peanut IgG_4/IgE ratio	.9417	—	—
Peanut IgE (%)	.2779	—	—
Log_{10} milk IgE	.7291	—	—
Log_{10} egg IgE	.7792	—	—
Peanut, 1 μg	.3156	—	—
Peanut, 0.1 μg	.3722	—	—
Peanut, 0.01 μg	.2922	—	—
Peanut, 0.001 μg	.9826	—	—

*The model using continuous baseline OFC SCD had poor fit, and therefore baseline OFC SCD was divided into high (SCD ≥ 44 mg) and low (SCD < 44 mg) values. The cut point of 44 mg was selected because it was the overall median.

†The model using continuous baseline log_{10} peanut IgE levels had poor fit, and therefore baseline log_{10} peanut IgE levels were divided into high (peanut IgE ≥ 95 kU_A/L) and low (peanut IgE < 95 kU_A/L) values. The cut point of 95 kU_A/L was selected because it was the overall median.

TABLE E6. *P* values from comparisons of percentage of doses per participant with symptoms between treatment groups*

Variable	Placebo vs VP100 vs VP250	Placebo vs VP100	Placebo vs VP250	VP100 vs VP250
Total no. of doses	.2956	.8812	.1518	.2396
Any reaction	<.0001	<.0001	<.0001	.9208
Patch-site reaction	<.0001	<.0001	<.0001	.8579
Reaction extended past patch site	<.0001	<.0001	<.0001	.1139
Non–patch-site reaction	.0771	.0708	1.0000	.0793
Skin symptoms	.2394	.2363	.6836	.1465
Respiratory symptoms	.1210	.1594	1.0000	.1594
Gastrointestinal symptoms	.3529	.3321	1.0000	.3321
Other symptoms	.9975	.9769	1.0000	.9769
Mild symptoms	.1707	.1282	1.0000	.1425
Moderate symptoms	.3529	.3321	1.0000	.3321
Severe symptoms	1.0000	1.0000	1.0000	1.0000
Duration >8 h	<.0001	<.0001	<.0001	.1964
Treated	<.0001	<.0001	<.0001	.5986
Treated with topical steroids	<.0001	.0001	<.0001	.4041
Treated with oral antihistamines	<.0001	<.0001	.0002	.6176
Treated with epinephrine	1.0000	1.0000	1.0000	1.0000
Grade 2 patch-site reaction	<.0001	<.0001	<.0001	.7130
Grade 3 patch-site reaction	.0734	.0424	.0251	.8252
Grade 4 patch-site reaction	.3529	.3321	1.0000	.3321
Grade 2 past patch reaction	<.0001	<.0001	<.0001	.4648
Grade 3 past patch reaction	.0487	1.0000	.0874	.0940
Grade 4 past patch reaction	1.0000	1.0000	1.0000	1.0000

**P* values from comparisons of all 3 treatment groups simultaneously are from the Kruskal-Wallis test. *P* values from pairwise comparisons of treatment groups are from the Wilcoxon rank sum test.

TABLE E7. Medians, lower quartiles, and upper quartiles of peanut-responsive cells per million $CD4^+$ T cells after adjustment for media control or cytokine expression as a percentage of $CD154^+$ cells

	Treatment group															
	Placebo				VP100				VP250				All			
	No.	Median	LQ	UQ	No.	Median	LQ	UQ	No.	Median	LQ	UQ	No.	Median	LQ	UQ
$CD4^+$ cells/10^6																
$CD154^+$																
Baseline	22	185	50	473	21	359	74	475	23	224	79	418	66	225	74	461
Week 24	21	158	45	380	20	126	57	260	19	203	115	289	60	186	54	294
Week 52	21	207	56	404	16	223	66	323	22	77	−16	194	59	151	22	265
$IL\text{-}4^+CD154^+$																
Baseline	22	128	38	421	21	238	40	351	23	121	44	275	66	150	43	317
Week 24	21	82	11	267	20	84	15	130	19	108	21	150	60	95	15	160
Week 52	21	73	3	188	16	92	13	204	22	46	−6	88	59	62	1	161
$IL\text{-}13^+CD154^+$																
Baseline	22	121	19	252	21	192	32	329	23	122	41	287	66	134	41	296
Week 24	21	70	29	247	20	75	16	107	19	86	53	141	60	79	24	142
Week 52	21	130	20	189	16	130	29	234	22	61	22	90	59	71	20	165
$IFN\text{-}\gamma^+CD154^+$																
Baseline	22	2	−2	8	21	5	1	15	23	2	0	5	66	3	−1	8
Week 24	21	1	−2	4	20	2	0	6	19	1	−3	6	60	1	−2	5
Week 52	21	1	−4	6	16	0	−5	2	22	−2	−11	5	59	0	−7	4
$IL\text{-}10^+CD154^+$																
Baseline	22	6	1	13	21	9	4	17	23	6	4	14	66	7	2	16
Week 24	21	4	1	11	20	8	1	12	19	15	7	22	60	9	3	15
Week 52	21	7	2	17	16	11	0	18	22	8	0	15	59	7	0	15
$CD154^+$ cells (%)																
$IL\text{-}4^+/CD154^+$																
Baseline	24	48	25	62	21	54	37	65	23	50	35	62	68	50	32	64
Week 24	21	43	29	56	20	37	20	43	19	41	29	47	60	38	22	49
Week 52	21	42	15	48	16	41	21	54	22	35	24	40	59	38	21	46
$IL\text{-}13^+/CD154^+$																
Baseline	24	39	12	57	21	47	30	59	23	42	24	56	68	42	21	57
Week 24	21	32	21	50	20	28	11	37	19	32	23	40	60	30	16	41
Week 52	21	36	11	41	16	39	16	47	22	26	16	38	59	34	13	41
$IFN\text{-}\gamma^+/CD154^+$																
Baseline	24	2	1	4	21	3	1	4	23	2	1	6	68	3	1	5
Week 24	21	2	1	3	20	3	2	5	19	2	1	4	60	2	1	4
Week 52	21	3	2	5	16	2	1	6	22	4	1	5	59	3	1	5
$IL\text{-}10^+/CD154^+$																
Baseline	24	4	2	6	21	4	3	5	23	4	3	6	68	4	3	6
Week 24	21	3	2	4	20	5	4	7	19	7	5	9	60	5	3	8
Week 52	21	6	5	8	16	8	4	15	22	9	7	13	59	7	5	12

LQ, Lower quartile; *UQ*, upper quartile.