

Oral peanut immunotherapy in children with peanut anaphylaxis

Katharina Blumchen, MD,^a Helen Ulbricht,^a Ute Staden, MD,^a Kerstin Dobberstein,^a John Beschorner,^a Lucila Camargo Lopes de Oliveira, MD,^a Wayne G. Shreffler, MD, PhD,^b Hugh A. Sampson, MD,^b Bodo Niggemann, MD,^a Ulrich Wahn, MD,^a and Kirsten Beyer, MD^a Berlin, Germany, and New York, NY

Background: The only treatment option for peanut allergy is strict avoidance.

Objective: To investigate efficacy and safety of oral immunotherapy (OIT) in peanut allergy.

Methods: Twenty-three children (age, 3.2-14.3 years) with IgE-mediated peanut allergy confirmed by positive double-blind, placebo-controlled food challenge (DBPCFC) received OIT following a rush protocol with roasted peanut for 7 days. If a protective dose of at least 0.5 g peanut was not achieved, patients continued with a long-term buildup protocol using biweekly dose increases up to at least 0.5 g peanut. A maintenance phase for 8 weeks was followed by 2 weeks of peanut avoidance and a final DBPCFC. Immunologic parameters were determined.

Results: After OIT using the rush protocol, patients tolerated a median dose of only 0.15 g peanut. Twenty-two of 23 patients continued with the long-term protocol. After a median of 7 months, 14 patients reached the protective dose. At the final DBPCFC, patients tolerated a median of 1 g (range, 0.25-4 g) in comparison with 0.19 g peanut at the DBPCFC before OIT (range, 0.02-1 g). In 2.6% of 6137 total daily doses, mild to moderate side effects were observed; in 1.3%, symptoms of pulmonary obstruction were detected. OIT was discontinued in 4 of 22 patients because of adverse events. There was a significant increase in peanut-specific serum IgG₄ and a decrease in peanut-specific IL-5, IL-4, and IL-2 production by PBMCs after OIT.

Conclusion: Long-term OIT appears to be safe and of some benefit in many patients with peanut allergy. With an increase in

threshold levels and a reduction of peanut-specific T_H2 cytokine production, the induction of tolerance may be feasible in some patients. (*J Allergy Clin Immunol* 2010;■■■:■■■-■■■.)

Key words: Allergy, anaphylaxis, children, food, oral immunotherapy, peanut, specific oral tolerance induction, tolerance

Peanut allergy is a common disease with a prevalence of 1% to 2% in the pediatric population.¹⁻³ In peanut allergic individuals, ingestion of small quantities of the allergen may lead to severe and potentially life-threatening allergic reactions.⁴ Although patients try strictly to avoid peanuts in everyday life, accidental ingestions are common because of the widespread use of peanut by the food industry and problems with accurate labeling.^{5,6} Accidental exposure to peanuts is reported to be one of the most common causes of fatal and near-fatal anaphylactic reactions, especially in the pediatric population.^{7,8}

Some food allergies in early childhood, like cow's milk and hen's egg allergy, usually resolve over time.⁹ In contrast, peanut allergy tends to persist over a lifetime, and only about 20% of young children outgrow their disease.¹⁰ It has been reported that the severity of future allergic reactions to peanuts cannot be predicted from former allergic reactions in the patient's history.¹¹ Thus, most patients with peanut allergy face the fear of anaphylaxis throughout their life. This constant uncertainty has a major psychological burden on the quality of life of the children and their families.¹²

Currently, the only treatment available is strict avoidance of peanuts. In case of accidental exposure, patients need to use rescue medications such as self-injectable epinephrine.⁶ Ten years ago, the therapeutic option of subcutaneous immunotherapy with peanut extract was considered for patients with peanut allergy.^{13,14} Although patients tolerated a higher amount of peanut and showed fewer symptoms after a peanut challenge, these studies were stopped because of frequent and severe adverse reactions. Novel therapeutic approaches for peanut allergy such as treatment with anti-IgE antibodies,¹⁵ immunotherapy with mutated recombinant protein,¹⁶ or Chinese herbal medicine¹⁷ are under investigation but are not feasible yet, or the proof of efficacy in human beings is still lacking.

Therefore, the aim of our study was to investigate the efficacy and safety of oral immunotherapy (OIT) in patients with peanut allergy. We hypothesized that patients with severe peanut allergy who were at risk of anaphylaxis would be able to tolerate a sufficient amount of peanut to protect them against accidental ingestions and to increase their threshold levels after OIT. Furthermore, clinical outcomes would be paralleled by changes in immunologic parameters.

From ^athe Department of Pediatric Pneumology and Immunology, University Hospital Charité, Berlin; and ^bthe Department of Pediatrics, Mount Sinai Medical Center, New York.

Supported by the Food Allergy and Anaphylaxis Network, USA.

Disclosure of potential conflict of interest: H. A. Sampson receives research support from the Food Allergy Initiative and the NIAID, NIH; is a consultant/scientific advisor for the Food Allergy Initiative; and is 45% owner of Herbal Springs, LLC. U. Wahn receives research support from the German Research Foundation and Danone. K. Beyer receives honoraria from Dr Beckmann, Stallergenes, HAL, Nutricia, Phadia, CSL Behring, and GlaxoSmithKline and receives research support from the German Research Foundation, the European Union, the Food Allergy and Anaphylaxis Network, Phadia, and the Paul Ehrlich Institute. The rest of the authors have declared that they have no conflict of interest.

Received for publication December 2, 2009; revised April 26, 2010; accepted for publication April 27, 2010.

Reprint requests: Kirsten Beyer, MD, Department of Pediatric Pneumology and Immunology, University Hospital Charité, Augustenburger Platz 1, 13353 Berlin, Germany. E-mail: kirsten.beyer@charite.de.

0091-6749/\$36.00

© 2010 American Academy of Allergy, Asthma & Immunology
doi:10.1016/j.jaci.2010.04.030

Abbreviations used

DBPCFC: Double-blind, placebo-controlled food challenge

OIT: Oral immunotherapy

SPT: Skin prick test

METHODS**Study population**

Seventy-four peanut-sensitized children were screened either consecutively from the outpatient clinic or from a list of peanut-sensitized children followed in the Department of Pediatric Pneumology and Immunology, Charité, Berlin. Patients with unstable asthma or undergoing any kind of other immunotherapy were excluded from the study. Twenty-seven children/parents agreed to participate; inclusion criteria included being more than 3 years of age and being peanut-sensitized (peanut IgE >0.35 kU_A/L). Two refused the baseline double-blind, placebo-controlled food challenge (DBPCFC), and 2 patients were tolerant on baseline DBPCFC. Twenty-three children with IgE-mediated peanut allergy confirmed by DBPCFC were finally enrolled in the study. All patients included were on a peanut-free diet before and during the entire study. The local ethics committee approved the study, and informed consent was obtained from the subjects and their families.

Study design

During the baseline visit, patients' histories were obtained (Fig 1). A physical examination and skin prick tests (SPTs) were performed, and blood samples were collected. During a screening period of 8 weeks, patients/parents had to fill out a diary documenting asthma symptoms and allergic reactions to peanuts. After this period, children were admitted to the hospital for a baseline DBPCFC.

DBPCFCs

Patients received placebo or whole crushed roasted peanuts in boiled apple as a matrix on 2 different days in a randomized order. A maximum of 7 increasing titration steps (0.03 g, 0.06 g, 0.13 g, 0.25 g, 0.5 g, 1 g, 2 g) of whole crushed roasted peanut, corresponding to 0.0075 to 0.5 g peanut protein,¹⁸ were given at intervals of 30 minutes. If no objective reaction occurred, a single open dose of 4 g whole peanut (1 g peanut protein) was given on a third day. The procedure was stopped if objective clinical symptoms were observed (eg, urticaria, angioedema, wheezing, dyspnea, allergic rhinitis or conjunctivitis, repeated vomiting, diarrhea, tachycardia and/or drop in blood pressure) or the highest dose was reached.

Oral immunotherapy using a rush protocol

After a positive DBPCFC, OIT using an initial rush protocol was started the next day (Fig 1) on the hospital ward. Increasing doses of crushed roasted peanuts were given 2 to 4 times a day for a maximum of seven days. OIT was started with approximately 1/100 of the eliciting reaction dose during DBPCFC (Table 1). In patients who started OIT with >6 mg whole peanuts, doses were doubled each time until the maximum dose was reached. In highly reactive children who started OIT with ≤6 mg peanuts, incremental steps were initially doubled, but starting at a dose of 80 mg, doses were increased by only 20% of the previous dose because of feared side effects. The aim was to reach at least 500 mg whole crushed peanuts (= 1 small peanut kernel = 125 mg peanut protein¹⁸) in all patients. In patients who tolerated more than 500 mg whole peanut during DBPCFC, the aim during the rush phase was to achieve at least double the amount of their tolerated dose during DBPCFC. The rush protocol was stopped if the maximum dose was reached, or after 1 week.

Oral immunotherapy using a long-term buildup protocol

The general aim was to reach at least 500 mg whole peanut in all of the study subjects. Patients who tolerated 500 mg peanut or more after the rush phase went directly to the maintenance phase (Fig 1).

In case a dose of 500 mg peanut was not achieved during the rush protocol, patients were discharged and continued on their individual tolerated dose of peanut eaten on a daily basis. All individual peanut doses were weighed and supplied to the patient by the investigators. Further dose increases were performed in our outpatient department every 2 to 4 weeks following a long-term buildup protocol until the goal of at least 500 mg peanut was reached (Table 1). In children with few side effects, doses were increased up to 2 g peanut. In general, a 20% dose increase was given at each outpatient visit. If side effects occurred, increases in the dose escalation schedule were decreased to only 10%. In between dose escalation visits, the patients ingested their tolerated dose on a daily basis. When patients developed an infection, such as a viral respiratory tract infection, only 50% of the tolerated peanut dose was given for 4 to 5 days to prevent adverse reactions. Thereafter, children received 75% of the previously tolerated dose for another 4 to 5 days followed by a return to 100% of the maintenance dose. A similar approach was used during exposures to additional allergen contact, such as cat contact in a friend's house in individuals with cat allergy, or during pollen season in individuals with pollen allergy. Throughout the study, a 24-hour-per-day telephone hotline was available for immediate advice, especially for dosing instructions.

After a dose of at least 500 mg whole peanut was achieved and maintained without any side effects for 8 weeks, maintenance was considered to be stable and patients stopped OIT, thus completely avoiding exposure to peanuts for 2 weeks, followed by a final DBPCFC to determine whether higher threshold doses eliciting symptoms were attained (Fig 1). Before the start of the study and throughout the study, patients strictly avoided peanuts except for OIT dosing.

Skin prick testing

An SPT for peanut was performed as a prick-to-prick test with the natural, intact, roasted whole peanut before the therapeutic intervention (pre-OIT), after the maintenance period of OIT (post-OIT, before avoidance) and after OIT was stopped for 2 weeks, before the final DBPCFC (post-OIT, before final DBPCFC; Fig 1). Saline solution was used as a negative control, and 10 mg/mL histamine dihydrochloride (ALK-Albello, Wedel, Germany) was used as the positive control. SPTs were read after 15 minutes and the wheal size recorded.

Measurement of serum allergen-specific IgE and IgG₄

Blood samples were drawn from all patients at the time of skin testing (Fig 1) and analyzed for specific IgE to peanut, cow's milk, birch, timothy, mugwort, and house dust mite as well as specific IgG₄ to peanut by using the Phadia CAP-System FEIA (Phadia Diagnostics, Uppsala, Sweden) following the manufacturer's instructions. The lower detection limit of the Phadia CAP-System was 0.35 kU_A/L for specific IgE and 0 mg/L for peanut-specific IgG₄. In cases in which peanut-specific IgE exceeded 100 kU_A/L, further dilutions of sera were performed to obtain exact values.

In vitro stimulation of PBMCs

PBMCs of all patients were isolated at the time of skin testing (Fig 1) by density gradient separation (Lymphoprep; Fresenius Kabi AG, Bad Homburg, Germany) of heparinized venous blood. PBMCs (2×10^6 cells/500 μ L) were cultured *in vitro* for 48 hours at 37°C in 5% CO₂ with (1) crude peanut extract (50 μ g/mL, LPS-free¹⁹), (2) a casein mixture (α -s, β , and κ casein, 50 μ g/mL; Sigma-Aldrich, St Louis, Mo) as an allergen-specific negative control, and (3) medium alone (RPMI 1640 containing 10% autologous serum) as negative control in single measurements. After centrifugation, supernatants were collected and stored at -80°C for further analysis.

Measurement of cytokine production in cell culture supernatants

Supernatants were analyzed for the presence of IL-2, IL-4, IL-5, IL-10, IFN- γ , and TNF- α by a multiplex bead assay (BD cytometric bead array, human T_H1/T_H2 cytokine kit; BD Biosciences, San Diego, Calif) according to

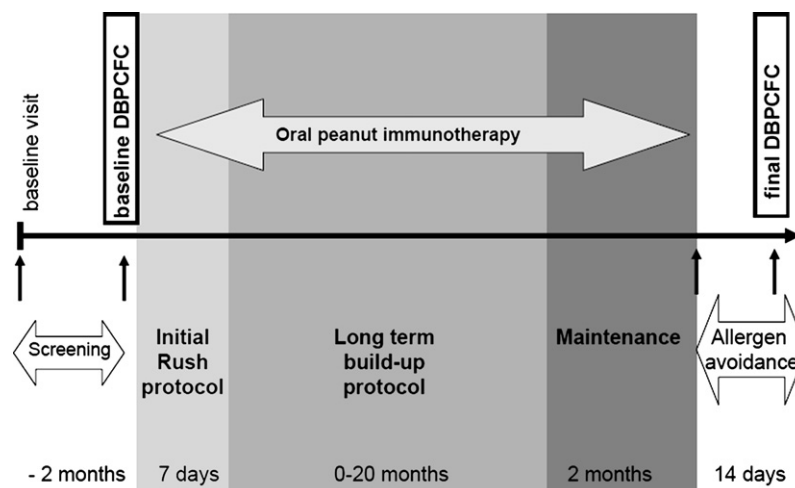


FIG 1. Study protocol. Black arrow, Visit (patient history/physical examination/blood drawing/SPT).

the manufacturer's instructions. Cytokine secretion was correlated to a standard of lyophilized human T_H1/T_H2 cytokines (10-5000 pg/mL) supplied by the manufacturer. The BD FACSCalibur was used to acquire the data, which was analyzed by BD CellQuest and BD CBA Software. Results are expressed as the amount of cytokine production detected after *in vitro* stimulation with the allergen minus the amount of cytokine detected after *in vitro* stimulation with the negative control.

Differential blood count

Differential blood cell counts were performed from EDTA-blood on an automated coulter counter (Sysmex XE-2100; Sysmex Corp, Kobe, Japan).

Statistical analysis

All statistical analyses were performed with SPSS (version 16.0 for Windows; SPSS Inc, Chicago, Ill). Continuous data are presented as medians with ranges. To compare results before and after intervention, paired data of continuous variables, such as threshold doses during DBPCFCs, IgE and IgG₄ levels, SPT results, and levels of cytokines, were used. Assuming a non-Gaussian distribution of the data, the Wilcoxon matched-pairs test was used to compare the values at baseline with those after intervention. For strength of correlation between the maximum tolerated dose following OIT using the rush protocol and peanut-specific IgE levels, the Spearman correlation coefficient was computed. To compare the safety of OIT under the rush protocol and the long-term buildup protocol, proportions of total single allergic reactions/total servings during the rush protocol or the long-term protocol for each patient were recorded as well as the number of organ-specific allergic reactions/total servings for each patient. Results of OIT under the rush protocol versus the long-term protocol were compared by Wilcoxon matched-pairs test. A 2-tailed *P* value of less than .05 was considered statistically significant.

RESULTS

Clinical responses during OIT using a rush protocol

Of the 23 children with peanut allergy between 3 and 14 years of age (median age, 5.6 years) enrolled in the study, 70% were male; 65% had asthma or increased airway reactivity. Over 80% of patients had a history of allergic reactions to peanut after accidental ingestion. The median peanut-specific IgE was 95.6 kU_A/L (range, 3-2071 kU_A/L), and 74% of patients were also sensitized to other food and/or aeroallergens.

Of the 23 patients enrolled, 22 patients finished the rush protocol. One patient dropped out during the rush protocol because of anxiety after an allergic reaction experienced during the initial DBPCFC (Fig 2). Patients received a median of

14 peanut servings (range, 9-21 servings) during the rush protocol within 1 week (range, 4-7 days). In most patients, the amount of peanut tolerated did not change during rush OIT compared with the initial food challenge (Table I). The median maximum tolerated dose at baseline DBPCFC (median, 0.13 g peanut; range, 0.015-1 g peanut) was not different from the median dose tolerated after rush OIT (median, 0.15 g peanut; range, 0.024-3 g peanut; Table I). In 17 of 22 patients, the goal to tolerate at least 500 mg whole peanut after OIT with the rush protocol was not achieved (Table I). These patients were discharged and continued on their individual tolerated dose of peanut after rush OIT. Post-rush OIT doses were taken on a daily basis and ranged from 24 mg to 400 mg peanut (Table I). Five patients reached the goal of at least 500 mg peanut at the end of the rush protocol (median, 1 g peanut; range, 0.9-3 g peanut; Table I). There was a significant difference in peanut-specific IgE levels between the group of patients who reached the goal of 500 mg or more of peanut (median peanut IgE, 9.1 kU_A/L; range, 2.97-27 kU_A/L) and those who tolerated a lesser amount after rush OIT (median peanut IgE, 212 kU_A/L; range, 14.3-2071 kU_A/L; *P* < .01). There was a significant inverse correlation between the level of specific IgE and the amount of peanut tolerated after the OIT using the rush protocol, as shown in Fig 3.

Clinical responses during OIT using the long-term buildup protocol

Of the 22 patients who underwent OIT either directly with the maintenance phase or using the long-term buildup protocol after finishing the rush protocol, 14 patients reached their maintenance dose of at least 500 mg peanut daily (Table I). One patient was a partial responder who could only consistently tolerate a smaller dose of peanut (200 mg peanut) during maintenance because of repeated subjective dyspnea, subjective lip swelling, and anxiety. Therefore, he did not qualify for the final DBPCFC. In 7 patients, OIT was discontinued during the long-term buildup phase. One patient was admitted to the hospital because of a severe infection, and the daily peanut intake had to be discontinued. The youngest patient (age, 3.1 years) stopped OIT because of dislike of the taste and spitting out OIT doses after a while on OIT. One patient did not show up in the outpatient clinic for up-dosings. Four patients were discontinued by the study physician because of adverse

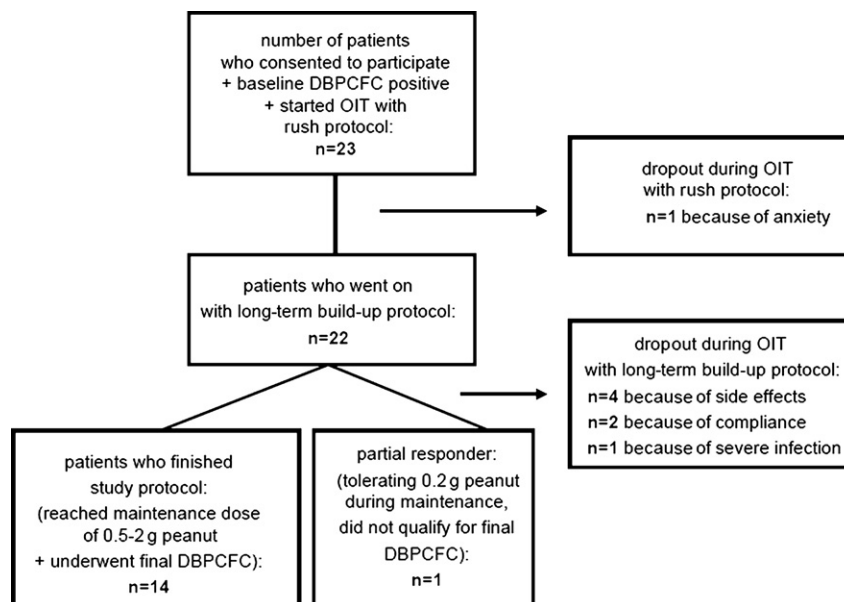
TABLE I. Comparison of the tolerated dose of peanut before OIT (at baseline DBPCFC), after the initial 7-day rush protocol, after the long-term buildup protocol, and after OIT (at final DBPCFC, after 2 weeks of peanut avoidance)

Patient no.	Maximum tolerated dose before OIT, at baseline DBPCFC (g)	Starting dose of OIT at rush phase (g)	Tolerated dose after the rush phase (g)	Daily tolerated dose after long-term buildup phase (g)*	Maximum tolerated dose after OIT, at final DBPCFC (g)†	Time of peanut exposure during long-term buildup (d)
4	0.13	0.0032	0.16	1.0	0.5	81
10	0.13	0.0032	0.08	0.5	0.25	334
7	0.5	0.012	0.16	0.5	1.0	158
12	0.25	0.006	0.9	1.0	1.0	47
14	0.06	0.0016	0.03	0.5	1.0	314
16	0.13	0.0032	0.14	0.5	1.0	251
21	1.0	0.024	3.0	2.0	2.0	88
23	0.25	0.006	0.4	1.0	4.0	230
25	0.015	0.0004	0.14	0.5	1.0	154
28	0.5	0.012	1.2	1.0	4.0	134
30	1.0	0.024	1.0	1.0	4.0	0
24	0.13	0.0032	0.05	0.5	1.0	389
22	0.25	0.006	0.4	0.5	0.5	476
18	0.06	0.0016	0.04	0.5	0.25	560
26	0.06	0.0016	0.14	0.2	No provocation	458
8	0.13	0.0032	0.16	Dropout	Dropout	Dropout
9	0.13	0.0032	0.024	Dropout‡	Dropout‡	Dropout‡
13	0.13	0.0032	0.12	Dropout	Dropout	Dropout
15	0.03	0.0008	0.04	Dropout‡	Dropout‡	Dropout‡
19	0.5	0.012	1.0	Dropout	Dropout	Dropout
20	0.13	0.0032	0.18	Dropout‡	Dropout‡	Dropout‡
29	0.25	0.006	0.12	Dropout‡	Dropout‡	Dropout‡

*Daily tolerated dose of peanut at maintenance after OIT using the long-term buildup protocol.

†Maximum tolerated dose of peanut at final DBPCFC (after OIT with the long-term buildup protocol and 2 weeks of peanut avoidance).

‡Dropout because of pulmonary adverse events.

**FIG 2.** Flow diagram of children with peanut allergy recruited into the study.

allergic reactions and were considered to be treatment failures (see section “Clinical and laboratory safety data”).

If all patients are considered in the analysis of the whole study, including all treatment failures because of side effects or being a partial responder and also including all dropouts (because of compliance, anxiety, or infection) who did not finish the protocol, 14 of 23 patients included into the study (61%) reached the goal of a final protective dose of OIT.

All 14 patients who reached their stable maintenance dose of at least 500 mg whole peanut daily completed the study protocol with a final DBPCFC (Fig 2). These 14 patients required a median of 7 months (range, 0-560 days) to reach their individual stable maintenance dose (0.5-2 g peanut) after the rush phase. The tolerated amount of peanut during maintenance was significantly greater than during the initial DBPCFC in most of the patients ($P = .002$; Table I). The median tolerated dose during baseline

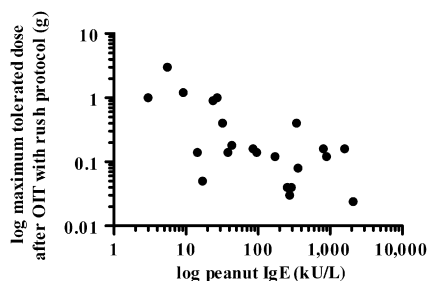


FIG 3. Correlation of peanut-specific IgE and the tolerated peanut dose after OIT following the rush protocol. Shown is the inverse correlation between level of peanut specific IgE (kU/L) at enrollment vs tolerated dose of peanut after 7 days of OIT after the rush protocol ($P = .003$; Spearman $r = -0.6$).

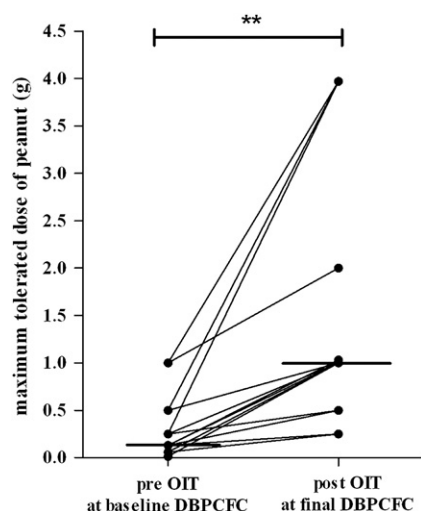


FIG 4. Clinical responses after OIT following the long-term buildup protocol. Shown are the tolerated peanut doses at baseline DBPCFC and at final DBPCFC post-OIT following the long-term buildup protocol of 14 patients who completed the whole study. The horizontal line represents the median of tolerated peanut dose. $**P < .01$.

DBPCFC (0.19 g peanut) could be increased to a median of 0.5 g peanut as a daily maintenance dose. After a median of 8 weeks (range, 7-13 weeks) on the daily stable maintenance dose, these 14 patients discontinued OIT for 2 weeks (range, 14-22 days) followed by a final DBPCFC. Even after 2 weeks off OIT, all patients showed a significant increase in threshold when baseline and final DBPCFCs were compared (Table I; Fig 4). A median 4-fold increase in the tolerated amount of peanut was seen when baseline and final DBPCFC were compared. The tolerated dose of peanut increased to a median of 1 g peanut at final DBPCFC ($P = .001$; Fig 4). Three patients (23, 28, and 30) did not react at all during DBPCFC, tolerating the highest peanut dose of 4 g (Table I).

Interestingly, despite discontinuing OIT for 2 weeks and not ingesting peanuts, 8 of these 14 patients had a higher threshold at the final DBPCFC than their maintenance peanut dose (median change, +0.5 g peanut; Table I). Only 3 patients tolerated less peanut during the final DBPCFC than their maintenance dose, indicating a breakthrough in the maintained desensitization after 2 weeks without therapy. No marked clinical or laboratory characteristic could be identified to distinguish these patients further. Three patients tolerated the same amount of peanut during maintenance and final DBPCFC (Table I).

TABLE II. Comparison of adverse allergic reactions and their treatment during the rush phase of peanut OIT and the long-term buildup with maintenance phase

Adverse reactions and treatment	OIT: rush phase	OIT: long-term buildup with maintenance***
Total single doses of OIT, n	317	6137***
Total objective symptoms,* n (%)	25 (7.9)	160 (2.6)***
Cardiovascular symptoms,* n (%)	0 (0)	0 (0)
Respiratory symptoms,* n (%)	9 (2.8)	79 (1.3)
Gastrointestinal symptoms,* n (%)	11 (3.5)	53 (0.9)
Skin symptoms,* n (%)	10 (3.2)	26 (0.4)
Upper respiratory symptoms,* n (%)	5 (1.6)	15 (0.2)
Use of epinephrine, n (%)	0 (0)	0 (0)
Use of salbutamol and cetirizine and/or prednisolone, n (%)	5 (1.6)	17 (0.3)
Use of salbutamol alone, n (%)	3 (0.9)	53 (0.9)
Use of cetirizine and/or prednisolone alone, n (%)	2 (0.6)	5 (0.08)

*Objective symptoms included cardiovascular symptoms (loss of consciousness, somnolence, hypotension), pulmonary obstruction (wheezing, dyspnea, dry cough with improvement after inhalation of β_2 -agonist), gastrointestinal symptoms (emesis, diarrhea), skin symptoms (urticaria, angioedema, flush), and upper respiratory symptoms (rhinitis, nasal congestion, conjunctivitis, hoarseness).

***%, % of treatment needed/total single doses of OIT.

***Total single doses and adverse events during long-term buildup and maintenance phase including 8 objective adverse events during 136 doses (5.8%) given at the outpatient clinic with increasing doses and 25 objective adverse events during 851 doses given with maintenance at home (2.9%).

%, % of total objective symptoms/total single doses of OIT or total events of pulmonary obstruction/total single doses of OIT.

Clinical and laboratory safety data

In 4 patients, OIT was discontinued by the study physician because of adverse allergic reactions. All 4 of these patients had stable mild to moderate asthma before the start of the study. During the long-term buildup protocol, their asthma worsened so that asthma controller therapy had to be increased. Two of these patients consistently showed a general worsening of their asthma when peanut doses were increased over a certain threshold. Three of these patients also had recurrent diarrhea and flatulence. Because of the small number of patients discontinuing OIT, no risk factors could be identified.

Clinical safety data on allergic symptoms during OIT in all patients including those who terminated OIT are presented in Table II. Only objective symptoms, not subjective symptoms such as abdominal pain, nausea, worsening of eczema, or oral pruritus, are shown. During OIT using the rush protocol, adverse reactions were frequent: of 317 total doses of OIT using the rush protocol ($n = 22$ subjects), 25 doses were associated with objective allergic symptoms. Gastrointestinal symptoms such as emesis and diarrhea followed by skin symptoms such as urticaria, angioedema, and flush were the most common. Patients experienced symptoms of pulmonary obstruction such as wheezing, dyspnea, and dry cough in nine cases of 317 total doses given during the rush phase. Eight had to be treated with an inhaled, short-acting β_2 -agonist. The 5 patients who tolerated 500 mg peanut or more after the rush protocol were able to increase their dose during the rush period with almost no or only mild subjective adverse reactions such as abdominal pain. However, all except 1 of these patients had to decrease their dose again because of either strong dislike of the taste of peanut ($n = 2$) or mild respiratory reactions after the ingestion of the OIT dose on the third day of home dosing ($n = 2$).

Reported adverse allergic symptoms were no less common in patients using the long-term buildup protocol compared with the rush protocol (Table II). In total, 6137 doses of OIT during the long-term buildup protocol (including home doses during buildup, outpatient doses when doses were increased, and home doses applied during maintenance) were administered with 1.3% resulting in pulmonary symptoms, which constituted the most common adverse reactions. All of these pulmonary episodes were treated with a short-acting β_2 -agonist, which led to a rapid improvement of symptoms. It is also noteworthy that the majority of these reactions occurred in the 4 patients who did not complete the study because of adverse reactions. Cardiovascular symptoms did not occur. No patient received self-injectable epinephrine. However, 1 patient with moderate pulmonary obstruction and hives who self-administered inhaled salbutamol, oral cetirizine, and prednisolone was admitted to the hospital for monitoring. Most of the adverse allergic reactions occurred during home dosing. On 8 occasions, mild adverse reactions with no pulmonary symptoms were observed during the incremental dosing of OIT while the patient was monitored in the outpatient clinic. Twenty-five objective adverse events occurred during 851 doses given during maintenance therapy (2.9%), and of these, 22 were reported in 1 patient during a vacation and were not initially reported to the research team. Excluding this patient, one can conclude that once patients reached the maintenance phase, adverse events were rare (2/790; 0.3%).

Oral immunotherapy did not lead to increased eosinophilia (median eosinophil count before OIT, 6.9%; range, 2.2% to 21%; vs post-OIT, 6.0%; range, 2.0% to 11.2%). OIT also did not alter sensitization to other allergens such as cow's milk, birch, timothy, mugwort, or house dust mite (data not shown).

Immunologic changes during peanut OIT

In the 14 patients who completed the study protocol, cytokine production of peanut-stimulated PBMCs *in vitro* was compared at baseline (pre-OIT) and at the end of OIT treatment (post-OIT, before avoidance) at a median of 9 months (range, 2–22 months; Figs 1 and 5). There was a highly significant reduction in the secretion of IL-5, IL-4, and IL-2 from all patients' PBMCs post-OIT (Fig 5, A–C). The detection of other cytokines measured (IFN- γ , TNF- α , and IL-10) was just above the detection limit after *in vitro* stimulation with peanut extract. A change in production of these cytokines during OIT could not be demonstrated (data not shown). Cytokine secretion was allergen-specific because *in vitro* stimulation of PBMCs with a different allergen (cow milk caseins) showed almost no detectable production of IL-4 or IL-5 but detectable and stable IL-10, IFN- γ , and TNF- α production (see this article's Table E1 in the Online Repository at www.jacionline.org). After discontinuing OIT for 2 weeks (post-OIT, after avoidance, before final DBPCFC; Fig 1), the reduction of peanut-induced IL-5, IL-4, and IL-2 secretion was stable in most of the patients (Fig 5, A–C). However, in some patients, this reduction was not stable during the 2 weeks off OIT, and cytokine levels increased after the allergen-avoidance phase; for example, 4 patients showed an increase in IL-2 production after allergen avoidance of 2 weeks, almost returning to their baseline values (Fig 5, patient # 7, 25, 28, 24). However, in most of the cases, the increases did not match the course of other cytokine levels, peanut-specific IgE, or SPT within the same patients. These changes did not correlate with the clinical outcome of the final DBPCFC (Table I) or with the length of time necessary to

achieve the maintenance OIT dose. In most cases, patients who tolerated less peanut during the final DBPCFC compared with maintenance dosing did not show an increase in peanut-specific cytokine production.

There was a small but significant reduction of peanut SPT wheal diameter after OIT (Fig 5, D). After 2 weeks of peanut avoidance, this reduction was no longer evident. Peanut-specific IgE levels as well as total IgE levels did not change after the intervention. However, after 2 weeks of peanut avoidance, peanut-specific IgE (Fig 5, E) and total IgE (data not shown) decreased significantly. As expected, peanut-specific IgG₄ levels increased significantly after OIT in all patients (Fig 5, F). After 2 weeks off OIT, there was a small but significant drop in peanut-specific IgG₄ levels for all patients (Fig 5, F).

DISCUSSION

In the current study, it has been shown that OIT using a long-term buildup phase may be a safe and effective way to treat many patients with peanut allergy to prevent severe reactions caused by accidental ingestion. Our study population belongs to the group of patients at high risk for anaphylactic reactions because they were highly sensitized and two thirds had asthma.¹¹

Clinical efficacy and safety of OIT using a rush protocol

In contrast with the recently published study on OIT in children with cow's milk allergy using a rush protocol,²⁰ efficacy of a rush protocol in peanut OIT seems to be poor in most children with peanut allergy. We found that only children with low levels of peanut-specific IgE reached a maintenance protective dose after OIT by using the rush protocol. Adverse reactions were also quite common during the rush protocol. Furthermore, most patients who achieved a protective dose during the rush phase subsequently had their dose decreased because of adverse events or refusal to take the dose because of the taste. In combination, the rush protocol does not seem like a feasible therapeutic strategy in most patients with peanut allergy.

Clinical efficacy of OIT using a long-term buildup protocol

However, a slow, long-term buildup protocol of OIT appears to be effective, as demonstrated by 2 clinical outcome parameters of our study:

1. Fourteen of 23 patients (61%) starting the study reached a maintenance stable dose of at least 500 mg whole peanut (125 mg peanut protein = 1 small peanut kernel). It is generally believed that accidental exposure to peanut is unlikely to exceed this threshold.^{15,21,22} Therefore, a well tolerated daily intake of 500 mg peanut or more is likely to provide significant protection.
2. We could also demonstrate a significant increase in the threshold dose after OIT comparing pre and post DBPCFCs. Furthermore, 8 of 14 patients tolerated even more peanuts at the final DBPCFC despite 2 weeks off OIT than during their maintenance phase. Three patients, in fact, even tolerated the highest dose given without any symptoms.

These results are similar to other published findings reported in small case reports of patients with peanut allergy in the last few years.^{23–25} Recently, Jones et al²⁶ also demonstrated that 29

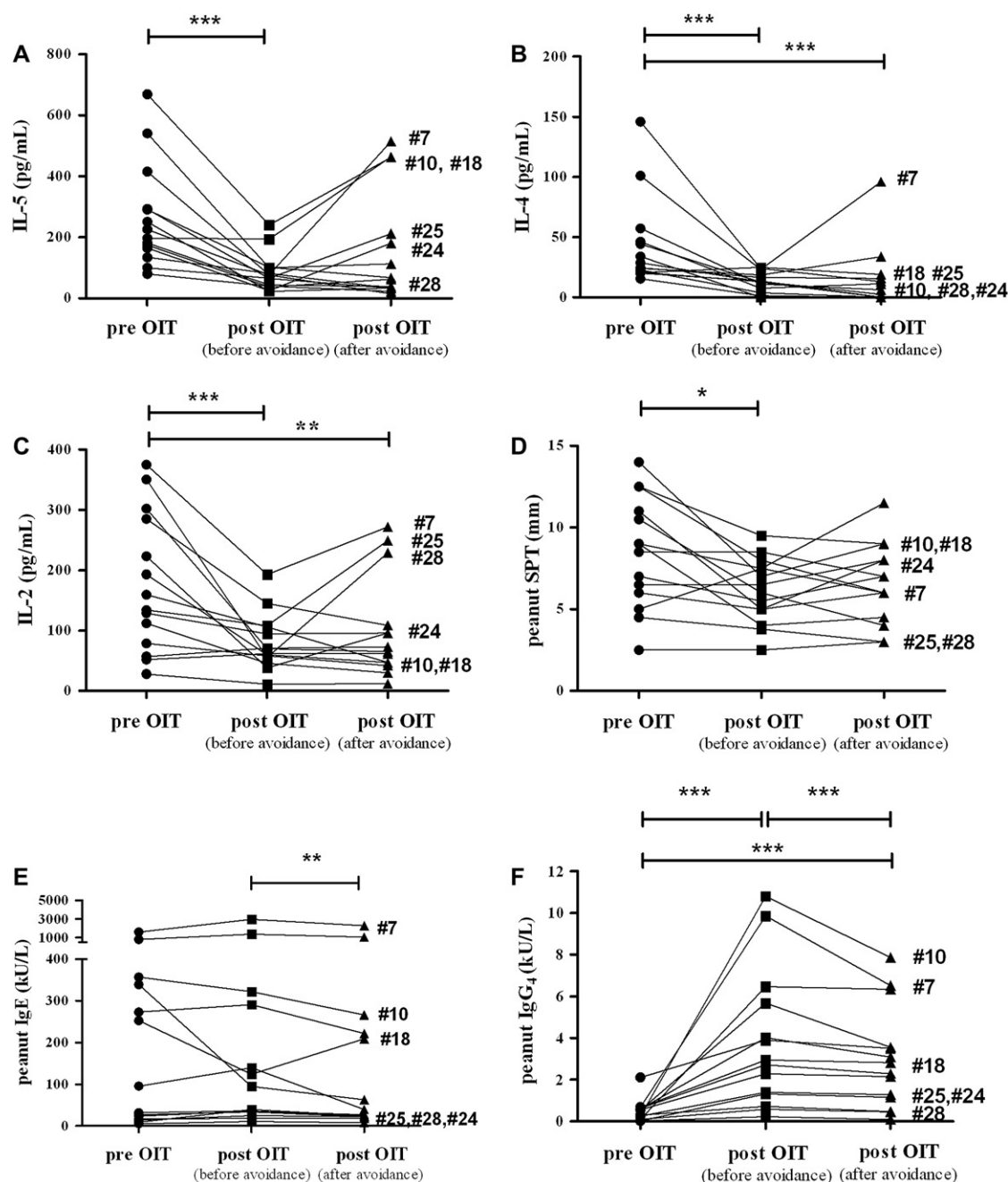


FIG 5. Comparison of laboratory and SPT results pre-OIT at baseline DBPCFC, directly post-OIT, and 2 weeks off OIT before final DBPCFC. Shown are IL-5 (A), IL-4 (B), IL-2 (C), wheal size diameter (D), peanut-specific IgE (E), and IgG₄ (F) for 14 patients who completed the study (* $P < .05$; ** $P < .01$; *** $P < .001$). Selected patients are marked (#) to follow the individual course.

patients with peanut allergy of 39 recruited (74%) could tolerate a daily dose of approximately 300 mg peanut protein during a first maintenance phase on OIT using a long-term protocol. Threshold levels also increased. However, in contrast with our study, peanut allergy was not verified by an initial food challenge, allowing for the possibility that some peanut-tolerant patients were enrolled, and making a true comparison of threshold reactivity before and after OIT difficult.

In contrast with our study, all other studies on peanut OIT to date have adopted a study design in which the increase of

threshold dose was demonstrated directly after or during ongoing OIT. Because we chose a different study design, we could show—for the first time—that even after 2 weeks of discontinuation of daily peanut exposure, ingestion of 500 mg whole peanut was safe in all but 2 of the patients who underwent OIT.

The 35% (8/23) dropout rate in the current study is quite high compared with other studies.²⁶⁻²⁸ In 4 of these patients (17%), the study was terminated early because of adverse reactions. Jones et al²⁶ reported that only 4 of 39 (10%) patients were discontinued because of repeated adverse side effects. In 2 of our patients,

compliance was lacking, maybe because they expected to reach a protective dose after 7 days following an inpatient rush protocol.

Clinical safety of OIT using a long-term buildup protocol

Using the long-term OIT buildup protocol, objective adverse reactions were quite common but not more frequent than in other OIT studies.²⁷⁻²⁹ Although our results cannot be directly compared with those of other OIT studies because we reported only objective adverse reactions, the rates seem to be quite similar to other studies when comparing the frequency of medical treatment required by the patients during OIT. For example, salbutamol inhalation was given in 0.9% of the total doses taken during our long-term buildup protocol, which is similar to the studies of Skripak et al²⁷ and Hofmann et al,²⁹ who reported β -agonist use in approximately 0.9%.

During OIT, some of our patients experienced chest symptoms that would require the use of epinephrine as recommended by the international guidelines.^{30,31} These guidelines were developed for patients' self-management in emergency situations after the accidental ingestion of an unknown amount of allergen. However, in our study, allergic reactions during OIT occurred in a controlled, monitored setting, with a known dose of peanut that had been well tolerated on a daily basis beforehand, and with various augmenting factors (eg infections, exercise). Therefore, patients on OIT and their caretakers were instructed to use self-injectable epinephrine in life-threatening events after the intake of OIT such as sudden severe wheezing or shortness of breath because of laryngeal edema, moderate pulmonary obstruction with no relief after the use of a short-acting β_2 -agonists, somnolence, and/or unconsciousness. Using these criteria for self-treatment of adverse events, none of our patients required treatment with epinephrine. Overall, we conclude that the frequency of adverse events can be considered acceptable as a therapeutic side effect. However, the risk-benefit ratio has to be evaluated further in a placebo-controlled study.

Immunologic changes during peanut OIT

T cells from children with peanut allergy show allergen-specific T_H2 cytokine production after *in vitro* stimulation with peanut.³² We hypothesized that cytokine production of children with peanut allergy would be skewed toward T_H1 cytokine production after OIT similar to results from children with peanut allergy who had spontaneously outgrown their disease.³² Interestingly, we could demonstrate peanut-specific downregulation of T_H2 cytokine production (IL-4 and IL-5) but not a shift toward T_H1 . There was a downregulation of IL-2 secretion, which is more consistent with the hypothesis of clonal anergy or clonal deletion.^{33,34} Although it has been postulated that regulatory T cells secreting IL-10 are involved in the induction of oral tolerance,³⁵ we could not demonstrate an upregulation of IL-10, which might be because that we evaluated cytokine production at a median of 9 months after OIT and may have missed the time point when peanut-specific regulatory T cells were induced. Consequently, the old concept of active suppression followed by clonal anergy/clonal deletion in oral tolerance induction is still open for discussion.^{33,34}

Our data on peanut-specific cytokine production before and after OIT differ from recently published data.²⁶ Jones et al²⁶ showed a significant increase of IL-5 and TNF- α production by PBMCs stimulated *in vitro* with peanut extract after OIT.

However, Jones et al²⁶ reported on baseline levels of cytokines that were very low in general: for example, baseline median peanut-specific IL-5 secretion was reported to be approximately 2 pg/mL, whereas our baseline IL-5 levels were much higher (median, 212 pg/mL). Furthermore, they could not detect any IL-4, IL-2, IL-10, or IFN- γ secretion after allergen stimulation.

The maintenance phase in our study was only 8 weeks. Therefore, it is not surprising that some patients still reacted after provocation and immunologic changes seemed unstable within our study group. No risk factor could be ascertained from the data to identify patients who would be either clinically or immunologically unstable after 2 weeks off OIT. Long-term tolerance was not yet established in most of our patients. However, tolerance induction with robust immunologic changes seems feasible with a prolonged time on a stable maintenance dose of OIT, as has been shown for subcutaneous immunotherapy.

Recently, studies on sublingual immunotherapy with inhalant allergens have demonstrated good clinical efficacy and safety^{36,37} when children received a stable maintenance dose for approximately 7 months. Obviously, the oral application followed by swallowing of crushed peanut in our study cannot be directly compared to sublingual immunotherapy with inhalant allergens. However, more and more promising data are published showing that the mucosal route of the gastrointestinal tract might be a good target for immunotherapy.

In conclusion, peanut OIT using a long-term buildup protocol appears to be a safe and effective treatment to protect many high-risk patients with peanut allergy against accidental reactions. Moreover, an increase in threshold levels above the daily maintenance dose and changes of immunologic parameters suggest that this form of therapy may be useful in achieving long-term tolerance induction. These promising results reinforce the need for further well controlled studies, including long-term trials with more patients within a placebo-controlled randomized study design. Until the risk-benefit ratio has been further evaluated, OIT for patients with peanut allergy cannot be considered as a therapeutic strategy for routine clinical practice.

We thank Petra Ellensohn, Margeret Oberreit, Alexander Rohrbach, and Gabriele Schulz for technical assistance in the laboratory.

Key messages

- Rush oral immunotherapy is not successful in most children with peanut allergy.
- In contrast, a long-term buildup protocol for peanut OIT appears to be safe and effective in reaching clinically relevant protective doses for high-risk patients with peanut allergy.
- With an increase in peanut threshold levels and a reduction of peanut-specific T_H2 cytokine production, the induction of long-term tolerance may be feasible following OIT in some children with peanut allergy.

REFERENCES

1. Sicherer SH, Munoz-Furlong A, Sampson HA. Prevalence of peanut and tree nut allergy in the United States determined by means of a random digit dial telephone survey: a 5-year follow-up study. *J Allergy Clin Immunol* 2003;112:1203-7.
2. Ben-Shoshan M, Kagan RS, Alizadehfard R, Joseph L, Turnbull E, St Pierre Y, et al. Is the prevalence of peanut allergy increasing? a 5-year follow-up study in children in Montreal. *J Allergy Clin Immunol* 2009;123:783-8.

3. Du Toit G, Katz Y, Sasieni P, Mesher D, Maleki SJ, Fisher HR, et al. Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. *J Allergy Clin Immunol* 2008;122:984-91.
4. Wood RA. The natural history of food allergy. *Pediatrics* 2003;111:1631-7.
5. Yu JW, Kagan R, Verreault N, Nicolas N, Joseph L, St Pierre Y, et al. Accidental ingestions in children with peanut allergy. *J Allergy Clin Immunol* 2006;118:466-72.
6. Ewan PW, Clark AT. Efficacy of a management plan based on severity assessment in longitudinal and case-controlled studies of 747 children with nut allergy: proposal for good practice. *Clin Exp Allergy* 2005;35:751-6.
7. Pumphrey RS. Lessons for management of anaphylaxis from a study of fatal reactions. *Clin Exp Allergy* 2000;30:1144-50.
8. Mehl A, Wahn U, Niggemann B. Anaphylactic reactions in children—a questionnaire-based survey in Germany. *Allergy* 2005;60:1440-5.
9. Host A, Halken S. A prospective study of cow milk allergy in Danish infants during the first 3 years of life: clinical course in relation to clinical and immunological type of hypersensitivity reaction. *Allergy* 1990;45:587-96.
10. Skolnick HS, Conover-Walker MK, Koerner CB, Sampson HA, Burks W, Wood RA. The natural history of peanut allergy. *J Allergy Clin Immunol* 2001;107:367-74.
11. Pumphrey RS, Gowland MH. Further fatal allergic reactions to food in the United Kingdom, 1999-2006. *J Allergy Clin Immunol* 2007;119:1018-9.
12. Primeau MN, Kagan R, Joseph L, Lim H, Dufresne C, Duffy C, et al. The psychological burden of peanut allergy as perceived by adults with peanut allergy and the parents of peanut-allergic children. *Clin Exp Allergy* 2000;30:1135-43.
13. Oppenheimer JJ, Nelson HS, Bock SA, Christensen F, Leung DY. Treatment of peanut allergy with rush immunotherapy. *J Allergy Clin Immunol* 1992;90:256-62.
14. Nelson HS, Lahr J, Rule R, Bock A, Leung D. Treatment of anaphylactic sensitivity to peanuts by immunotherapy with injections of aqueous peanut extract. *J Allergy Clin Immunol* 1997;99:744-51.
15. Leung DY, Sampson HA, Yunginger JW, Burks AW Jr, Schneider LC, Wortel CH, et al. Effect of anti-IgE therapy in patients with peanut allergy. *N Engl J Med* 2003;348:986-93.
16. Li XM, Srivastava K, Grishin A, Huang CK, Schofield B, Burks W, et al. Persistent protective effect of heat-killed *Escherichia coli* producing "engineered," recombinant peanut proteins in a murine model of peanut allergy. *J Allergy Clin Immunol* 2003;112:159-67.
17. Srivastava KD, Kattan JD, Zou ZM, Li JH, Zhang L, Wallenstein S, et al. The Chinese herbal medicine formula FAHF-2 completely blocks anaphylactic reactions in a murine model of peanut allergy. *J Allergy Clin Immunol* 2005;115:171-8.
18. Souci S, Fachmann W, Kraut H. Food composition and nutrition tables. 7th ed. Stuttgart: Medpharm Scientific Publishers; 2008.
19. Burks AW, Williams LW, Connaughton C, Cockrell G, O'Brien TJ, Helm RM. Identification and characterization of a second major peanut allergen, Ara h II, with use of the sera of patients with atopic dermatitis and positive peanut challenge. *J Allergy Clin Immunol* 1992;90:962-9.
20. Staden U, Blumchen K, Blankenstein N, Dannenberg N, Ulbricht H, Dobberstein K, et al. Rush oral immunotherapy in children with persistent cow's milk allergy. *J Allergy Clin Immunol* 2008;122:418-9.
21. Vadas P, Perelman B. Presence of undeclared peanut protein in chocolate bars imported from Europe. *J Food Prot* 2003;66:1932-4.
22. Schappi GF, Konrad V, Imhof D, Etter R, Wuthrich B. Hidden peanut allergens detected in various foods: findings and legal measures. *Allergy* 2001;56:1216-20.
23. Patriarca G, Nucera E, Pollastrini E, Roncallo C, De Pasquale T, Lombardo C, et al. Oral specific desensitization in food-allergic children. *Dig Dis Sci* 2007;52:1662-72.
24. Clark AT, Islam S, King Y, Deighton J, Anagnostou K, Ewan PW. Successful oral tolerance induction in severe peanut allergy. *Allergy* 2009;64:1218-20.
25. Mansfield L. Successful oral desensitization for systemic peanut allergy. *Ann Allergy Asthma Immunol* 2006;97:266-7.
26. Jones SM, Pons L, Roberts JL, Scurlock AM, Perry TT, Kulis M, et al. Clinical efficacy and immune regulation with peanut oral immunotherapy. *J Allergy Clin Immunol* 2009;124:292-300, e1-97.
27. Skripak JM, Nash SD, Rowley H, Brereton NH, Oh S, Hamilton RG, et al. A randomized, double-blind, placebo-controlled study of milk oral immunotherapy for cow's milk allergy. *J Allergy Clin Immunol* 2008;122:1154-60.
28. Longo G, Barbi E, Berti I, Meneghetti R, Pittalis A, Ronfani L, et al. Specific oral tolerance induction in children with very severe cow's milk-induced reactions. *J Allergy Clin Immunol* 2008;121:343-7.
29. Hofmann AM, Scurlock AM, Jones SM, Palmer KP, Lokhnygina Y, Steele PH, et al. Safety of a peanut oral immunotherapy protocol in children with peanut allergy. *J Allergy Clin Immunol* 2009;124:286-91, 91 e1-6.
30. Sampson HA, Munoz-Furlong A, Campbell RL, Adkinson NF Jr, Bock SA, Branum A, et al. Second symposium on the definition and management of anaphylaxis: summary report—Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. *J Allergy Clin Immunol* 2006;117:391-7.
31. Muraro A, Roberts G, Clark A, Eigenmann PA, Halken S, Lack G, et al. The management of anaphylaxis in childhood: position paper of the European Academy of Allergy and Clinical Immunology. *Allergy* 2007;62:857-71.
32. Turcanu V, Maleki SJ, Lack G. Characterization of lymphocyte responses to peanuts in normal children, peanut-allergic children, and allergic children who acquired tolerance to peanuts. *J Clin Invest* 2003;111:1065-72.
33. Weiner HL. Oral tolerance, an active immunologic process mediated by multiple mechanisms. *J Clin Invest* 2000;106:935-7.
34. Hoyne GF, Tan K, Corsin-Jimenez M, Wahl K, Stewart M, Howie SE, et al. Immunological tolerance to inhaled antigen. *Am J Respir Crit Care Med* 2000;162:S169-74.
35. Karlsson MR, Rugtveit J, Brandtzaeg P. Allergen-responsive CD4+CD25+ regulatory T cells in children who have outgrown cow's milk allergy. *J Exp Med* 2004;199:1679-88.
36. Wahn U, Tabar A, Kuna P, Halken S, Montagut A, de Beaumont O, et al. Efficacy and safety of 5-grass-pollen sublingual immunotherapy tablets in pediatric allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 2009;123:160-6, e3.
37. Bufe A, Eberle P, Franke-Beckmann E, Funck J, Kimmig M, Klimek L, et al. Safety and efficacy in children of an SQ-standardized grass allergen tablet for sublingual immunotherapy. *J Allergy Clin Immunol* 2009;123:167-73, e7.

TABLE E1. Comparison of cytokine production after *in vitro* stimulation with milk proteins of PBMCs of 14 children with peanut allergy pre-OIT and directly after peanut OIT and after 2 weeks off peanut OIT (before final DBPCFC)

Cytokines	Pre-OIT median (range), pg/mL	Post-OIT before avoidance median (range), pg/mL	Post-OIT after avoidance median (range), pg/mL
IL-4	1 (0-8)	0 (0-4)	0 (0-8)
IL-5	1 (0-24)	0 (0-7)	1 (0-9)
IL-10	220 (35-755)	149 (42-513)	135 (51-597)
IFN- γ	37 (0-237)	11 (0-584)	24 (0-190)
TNF- α	120 (0-863)	114 (21-7227)	189 (0-2168)