Letter to the Editor

Prophylactic use of sublingual allergen immunotherapy in high-risk children: A pilot study

To the Editor:

A wide body of epidemiologic evidence indicates that in children at high genetic risk of allergic diseases, programming of T_H2-polarized immunologic memory associated with progressively increasing IgE antibody production is most commonly initiated during the preschool years (reviewed in Holt et al[1] and Holt and Thomas[2]), and there is growing interest in "early intervention" aimed at arresting this process before it becomes persistent. The basis for one emerging therapeutic strategy is the consistent finding in experimental models that development of resistance to inhalant allergy is actively driven by environmental allergen exposure via the nasopharyngeal mucosa, resulting in the induction of a form of immunologic tolerance mediated by regulatory T cells, which target allergen-specific T_H2 memory cells (reviewed in Strickland et al[3]). Moreover, the successful induction of protective tolerance requires considerably higher levels of mucosal allergen exposure in animal strains expressing the atopic-equivalent "high-IgE-producer" phenotype, relative to their low–IgE-producing counterparts (reviewed in Strickland et al[3]), hinting at the operation of underlying genetic defect(s) in mucosal immune surveillance mechanisms. There is strong supporting evidence for the operation of a similar mucosal tolerance mechanism in humans, notably epidemiologic data demonstrating a positive correlation between resistance to clinically relevant sensitization and the levels of exposure to specific aeroallergens,[4-6] consistent with the immunologically cognate nature of this process.

These findings collectively provided the basis for an investigator-initiated double-blind placebo-controlled trial funded by the National Institutes of Health Immune Tolerance Network (NCT00346398) to test the hypothesis that enhancing the levels of mucosal exposure of children at high risk of inhalant allergy/asthma prior to the onset of sensitization would reduce the likelihood of subsequent sensitization and/or development of asthma. The protocol developed to test this hypothesis involved sublingual administration of a mixture of soluble allergens by using a modified cleft palate spoon; the mixture comprised 3×200 uL aliquot extracts, respectively, of house dust mite (containing 3.75 ug Der p1 plus 3.75 ug Der f1), cat (containing 11.3 ug Fel d1), and timothy grass (containing 15.0 ug Phl p5), given daily for 12 months. The individual allergen dosages were determined on the basis of solubility and represented the maximum amounts deliverable in the fluid volume used. The primary efficacy end point (proportion of participants sensitized to ≥1 allergen) was to be assessed 3 years posttreatment. It was rationalized that this process would mirror sublingual immunotherapy (SLIT), which is associated with the generation of regulatory T cells directed against the mucosally delivered allergens.[7]

The inclusion criteria in the trial initially were age 18 to 30 months, positive atopic family history coupled with personal history of atopic dermatitis and sensitization to 1 or more food allergen, and levels of serum IgE against treatment allergens of less than 0.35 kU/L; the lower age limit was subsequently reduced to 12 months early during recruitment because of the high frequency of sensitization encountered among positive atopic family history children 18 months or older at the Perth, Melbourne, and New York trial sites; on the basis of power calculations, the study plan called for 100 subjects in each arm. An important issue identified during protocol development was that in contrast to conventional SLIT in which treated adults "hold" the sublingual allergen drops under the tongue for 2 to 3 minutes to maximize mucosal absorption, infants cannot be trained in this regard and would tend to clear the mucosally deposited liquid much more rapidly, which could, in turn, limit the penetration of allergen through the mucosa to levels under the threshold required for triggering immunologic processes. Accordingly, initial recruitment was limited to 25 subjects in each arm, with serum sampling to be performed at 3 months and 6 months, for blinded interim analyses of treatment-allergen specific IgE/IgG antibodies and associated T_H-cell responses, overseen by an independent monitoring group. The emergence of differences in antibody titers between active/placebo groups that achieved acceptable statistical significance ($P < .05$ after Bonferroni correction) was to be taken as confirmation that the treatment was delivering sufficient allergen transmucosally to trigger immunologic recognition by the infant immune system, and if so, recruitment of the remaining 150 subjects would proceed. No such differences were detected by the 6-month sampling point, and accordingly recruitment was terminated and the trial reduced to pilot study status; monitoring of the original subjects continued including sample collection for humoral and cellular immunologic assessments at 12 and 24 months, and the outcome sample at 48 months. No significant differences in immunologic parameters between groups were detected up to 24 months, and no serious adverse events were reported throughout the study. A total of 18 of 25 and 19 of 25 in the active and placebo groups, respectively, completed assessments at 48 months, and a summary of relevant outcome data is shown in Table I. There were no differences between groups with respect to asthma prevalence at outcome age. There were trends toward small increases in the rates of sensitization to mite and timothy grass in the treatment group, but these are not significant after correction for multiple testing. A comparable (but again not significant) trend was also evident following reanalysis of the data after correction for site variation (see Table E1 in this article's Online Repository at www.jacionline.org). However, a limitation of the study, which makes interpretation of these data and those from the earlier interim analyses problematic, is the small sample size. Thus, the direction of these small potential effects may indicate that levels of transmucosal allergen exposure remained below the threshold required to drive tolerance induction and instead served as a weak booster (see Holt and Thomas[2]), or alternatively may simply mean that exposure did not achieve immunoactive levels.

Under these circumstances, it is not possible to draw firm conclusions concerning the potential efficacy of this overall approach for allergy prophylaxis, but it does reinforce scepticism concerning the use of soluble allergen drops in age groups that cannot be trained to "hold" sublingual allergen as per adults in whom the treatment is demonstrably immunomodulatory.[7] Better control of transmucosal allergen delivery in the infant age group, to the extent that mucosal exposure times equivalent to those in adults can be routinely achieved during repeated treatment, would appear to be the most important prerequisite for future infant

TABLE I. Univariate logistic regression analysis of 48-month outcome results

	Active	Placebo	Odds ratios (95% CI)*	Uncorrected *P* value	Corrected *P* value†
Allergic sensitization‡ (yes)					
ITT§	88.0% (22/25)	76.0% (19/25)	2.32 (0.51-10.5)	.28	1.00
PP||	88.9% (16/18)	73.7% (14/19)	2.86 (0.48-17.1)	.25	.75
Current asthma (yes)¶					
ITT§	16.0% (4/25)	16.0% (4/25)	1.00 (0.16-6.1)	.85	1.00
PP||	5.6% (1/18)	5.3% (1/19)	1.06 (0.01-87.7)	.74	.97
Allergic sensitization# to house dust mite, *Der P* (yes)					
ITT§	76.0% (19/25)	60.0% (15/25)	2.11 (0.63-7.1)	.23	1.00
PP||	77.8% (14/18)	52.6% (10/19)	3.15 (0.75-13.2)	.12	.58
Allergic sensitization# to house dust mite, *Der F* (yes)					
ITT§	72.0% (18/25)	56.0% (14/25)	2.02 (0.62-6.6)	.24	1.00
PP||	77.8% (14/18)	47.4% (9/19)	3.89 (0.93-16.3)	.06	.38
Allergic sensitization# to timothy grass (yes)					
ITT§	80.0% (20/25)	60.0% (15/25)	2.67 (0.75-9.5)	.13	.90
PP||	83.3% (15/18)	52.6% (10/19)	4.50 (0.97-20.8)	.05	.38
Allergic sensitization# to cat (yes)					
ITT§	60.0% (15/25)	60.0% (15/25)	1.00 (0.32-3.1)	1.00	1.00
PP||	66.7% (12/18)	52.6% (10/19)	1.80 (0.48-6.8)	.39	.77
Allergic sensitization to aeroallergen not** in the treatment mix (yes)					
ITT§	68.0% (17/25)	68.0% (17/25)	1.00 (0.31-3.3)	1.00	1.00
PP||	83.3% (15/18)	63.2% (12/19)	2.92 (0.62-13.8)	.18	.70

A total of 191 subjects were screened at the 3 sites—Perth (n = 78), Melbourne (n = 92), and New York (n = 18)—and 50 enrolled for the initial phase of the study (outcomes above). The major reasons for screen failure included no evidence of food sensitization (n = 66) and sensitization to 1 or more treatment allergen (n = 53).

*Odds ratios are calculated by using an unadjusted logistic regression with a Wald χ^2 test.

†Bonferroni procedure of Hochberg was used for multiple test correction of the primary end points for the ITT and PP independently.

‡Defined as having serum levels of specific IgE against any of the treatment allergens at or above 0.35 kU/L.

§Intention-to-treat (ITT) sample. All randomized participants. Participants who drop out (have missing efficacy end points) are considered treatment failures in the ITT sample.

||Per-protocol (PP) sample. Treated participants must have been on treatment throughout the study and cannot have missed more than 25% of their doses in any between-visit observation period. Participants with missing end points are considered treatment failures in the PP sample.

¶Odds ratios are calculated by using unadjusted exact logistic regression with mid *P* value to adjust for the discreteness of the distribution.

#Defined as having serum levels of specific IgE against any of the specified treatment allergen at or above 0.35 kU/L.

**Egg white, cow milk, peanut, and soy.

studies to further test this hypothesis. It is relevant to note in this context our recent demonstration that the rate-limiting step in experimental mucosal tolerance induction is the efficiency of *in situ* allergen "loading" of mucosal dendritic cells.[3] These tolerogenic dendritic cells acquire allergen via dendrites, which penetrate between mucosal tight junctions, and hence their sampling efficiency will be related directly to the concentration of allergen achieved in their immediate microenvironment (ie, on the adjacent mucosal surface), coupled with the length of exposure time.

In this context, it is noteworthy that SLIT treatment of sensitized adults and children with allergen drops is progressively being supplanted by the use of allergen-containing sublingual tablets, which solubilize directly onto the mucosal surface. One goal being sought in the development of the latter SLIT modality was to prolong the period following dosing during which the concentration of treatment allergen remains high at the point of application onto the mucosal surface, with the aim of improving allergen-driven therapeutic outcomes. In this regard, it is pertinent to note the recent demonstration for the first time in a large-scale placebo-controlled trial of sustained disease-modifying effects in presensitized atopics that persist at least 2 years posttreatment with SLIT tablets,[8] an outcome not previously achieved in comparable trials using allergen drops. These findings suggest that this modified version of SLIT should be considered for retesting of mucosal-based immunotherapy for atopic asthma prevention. Moreover, it would appear desirable to focus on children with preexisting specific IgE against treatment allergens who were deliberately excluded from the present study, in light of recent[1] and earlier evidence (reviewed in Hoyne et al[9]) suggesting that the induction of antigen-specific tolerance is typically preceded by an obligatory step involving transient activation of primary immunity to the treatment antigen.

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TABLE E1. Multivariate (to adjust for site) logistic regression analysis results

	Odds ratio (95% CI)*	Uncorrected *P* value	Corrected *P* value†
Allergic sensitization (yes)			
ITT‡	2.40 (0.51-11.3)	.27	1.00
PP§	2.95 (0.47-18.5)	.25	.75
Current asthma (yes)||			
ITT‡	1.00 (0.17-5.9)	.85	1.00
PP§	1.07 (0.01-84.1)	.75	.96
Allergic sensitization to house dust mite, *Der P* (yes)			
ITT‡	2.47 (0.63-9.6)	.19	1.00
PP§	4.66 (0.82-26.6)	.08	.42
Allergic sensitization to house dust mite, *Der F* (yes)			
ITT‡	2.28 (0.63-8.3)	.21	1.00
PP§	5.86 (1.03-33.4)	.05	.30
Allergic sensitization to timothy grass (yes)			
ITT‡	2.87 (0.77-10.7)	.12	.82
PP§	4.98 (1.00-24.8)	.05	.30
Allergic sensitization to cat (yes)			
ITT‡	1.00 (0.32-3.1)	1.00	1.00
PP§	1.82 (0.48-6.9)	.38	.77
Allergic sensitization to aeroallergen not¶ in the treatment mix (yes)			
ITT‡	1.00 (0.29-3.5)	1.00	1.00
PP§	3.23 (0.63-16.5)	.16	.64

*Odds ratios are calculated by using an unadjusted logistic regression with a Wald χ^2 test.
†Bonferroni procedure of Hochberg was used for multiple test correction of the primary end points for the ITT and PP independently.
‡Intention-to-treat (ITT) sample. All randomized participants. Participants who drop out (have missing efficacy end points) are considered treatment failures in the ITT sample.
§Per-protocol (PP) sample. Treated participants must have been on treatment throughout the study and cannot have missed more than 25% of their doses in any between-visit observation period. Participants with missing end points are considered treatment failures in the PP sample.
||Odds ratios are calculated by using an unadjusted exact logistic regression with mid *P* value to adjust for the discreteness of the distribution.
¶Egg white, cow milk, peanut, and soy.