Randomized controlled trial of primary prevention of atopy using house dust mite allergen oral immunotherapy in early childhood

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Background: Children born to atopic parents are at increased risk of sensitization to environmental allergens.

Objective: We sought to demonstrate proof of concept for oral immunotherapy to high-dose house dust mite (HDM) allergen in infancy in the prevention of allergen sensitization and allergic diseases.

Methods: This was a prospective, randomized, double-blind, placebo-controlled, proof-of-concept study involving 111 infants less than 1 year of age at high risk of atopy (≥2 first-degree relatives with allergic disease) but with negative skin prick test responses to common allergens at randomization. HDM extract (active) and appropriate placebo solution were administered orally twice daily for 12 months, and children were assessed every 3 months. Coprimary outcomes were cumulative sensitization to HDM and sensitization to any common allergen during treatment, whereas development of eczema, wheeze, and food allergy were secondary outcomes. All adverse events were recorded.

Results: There was a significant ($P = .03$) reduction in sensitization to any common allergen (16.0%; 95% CI, 1.7% to 30.4%) in the active (5 [9.4%]) compared with placebo (13 [25.5%]) treatment groups. There was no treatment effect on the coprimary outcome of HDM sensitization and the secondary outcomes of eczema, wheeze, and food allergy. The intervention was well tolerated, with no differences between active and placebo treatments in numbers or nature of adverse events.

Conclusion: Prophylactic HDM oral immunotherapy is well tolerated in children at high heredity risk. The results met the trial's prespecified criteria for proof of concept in reducing sensitization to any allergen; however, no significant preventive effect was observed on HDM sensitization or allergy-related symptoms. (J Allergy Clin Immunol 2015;136:1541-7.)

Key words: *Atopy, house dust mite, oral immunotherapy, primary prevention, randomized controlled trial, allergen, infant, early childhood*

The emergence of atopic diseases, such as asthma, as a generational epidemic over the last 30 years has had a major health and socioeconomic effect. Atopy is defined as the genetic propensity to produce IgE antibodies in response to exposure to allergens and is assessed as a positive skin prick test (SPT) response or the presence of specific IgE to 1 or more common allergens. The United Kingdom has one of the highest prevalences of atopic disease, with 1 in 4 persons being affected and 5.4 million persons receiving treatment for asthma, which is associated with more than £1 billion in annual health care costs.[1] In the United States more than 25 million persons are affected with asthma alone.[2] Similarly, in other countries atopic diseases present a major problem.

The global burden of allergic diseases demands effective disease prevention strategies. Given the early-life origins of atopy and asthma, primary prevention efforts have to commence soon after birth to be most effective.[3] We have shown previously that extensive lifestyle adjustments, including allergen avoidance, can significantly reduce sensitization[4-6]; however, such measures are complex and expensive and therefore impractical. Instead, it is preferable to target the immune system before sensitization has developed. There is evidence of delayed maturity at birth in both T_H2 (allergy favoring) and T_H1 (counterbalancing) responses,[7] with regulatory T (Treg) cells maintaining a balance between the T_H1 and T_H2 effector T helper subsets.[8] In children with atopic heredity, this balance might never be achieved, and a T_H2 predominance persists, leading to sensitization and, eventually, development of clinically evident allergic diseases, such as eczema, rhinitis, and asthma. A strong and adequate immune stimulation at a very young age is required to overcome maturational deficiencies in the developing immune system and counter allergen-specific T_H2 bias. One such stimulus is the administration of a potent and prevalent allergen, such as house

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Abbreviations used
HDM: House dust mite
IMP: Investigational medical product
SPT: Skin prick test
Treg: Regulatory T

dust mite (HDM). The gut is the primary site of Treg cell stimulation to exogenous antigens during the first 18 months of life, and the majority of naive Treg cells in infancy express the gut-homing receptor $\alpha4\beta7$.[9] Thus an oral route of exposure in this period of life is likely to be most effective in inducing tolerance to common allergens by stimulating Treg cell induction and function.

Here we report the findings of the first ever primary prevention study in infancy using allergen immunotherapy to prevent the development of allergic sensitization. The aim was to provide proof of concept for further studies to confirm that this treatment reduces the risk of sensitization and, subsequently, development of allergic diseases, including asthma. In this double-blind placebo-controlled trial we sought to demonstrate proof of concept that oral administration of HDM extract can prevent development of atopy by reducing sensitization to HDM and other common allergens. We hypothesized that exposure to a ubiquitous allergen, such as HDM, would have both allergen-specific (HDM) and nonspecific (other common allergens) preventive effects. For that reason, sensitization to HDM and sensitization to any of the tested allergens were set as coprimary outcomes.

METHODS

Study design

This was an investigator-initiated, prospective, randomized, double-blind, placebo-controlled, phase IIa study conducted between January 2011 and October 2014 (see Fig E1 in this article's Online Repository at www.jacionline.org) involving 2 study sites: the National Institute of Health Research Southampton Respiratory Biomedical Research Unit based at the University Hospital Southampton and the David Hide Asthma and Allergy Research Centre, St Mary's Hospital, Isle of Wight, United Kingdom. Approval was obtained from the Medicines and Healthcare products Regulatory Agency (Eudract no 2009-015679-28), ethics approval was given by the South Central Ethics Committee (09/H0504/124), and participants' parents provided written informed consent.

Participants

Suitable participants aged 5 to 9 months were recruited by using flyers and through screening of potential families face to face at primary care child health clinics. Inclusion criteria were high risk of atopy based on heredity (≥2 first-degree family members with allergic diseases [asthma, allergic rhinoconjunctivitis, eczema, or food allergy]) but negative SPT responses to HDM, grass pollen, cat, peanut, milk, and egg (ALK-Abelló, Hørsholm, Denmark). Infants who were born premature (<37 weeks' gestation) or had major health problems were excluded.

Intervention

The intervention was glycerinated allergen extract of HDM (SIW08), consisting of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* in equal parts (ALK-Abelló), and normal saline served as placebo, with both products being formulated as drops for oral administration. Participants in the active group received 2000 standard treatment units per day, which is equivalent to 11 μg of major allergens (Der p 1, Der f 1, and Der 2), a dose previously shown in older children and adults with clinical allergy to be well tolerated and inducing a significant immunologic response.[10]

Randomization

Computer-generated block randomization was performed by ALK-Abelló; investigational medical product (IMP) arrived in both centers prerandomized and was allocated sequentially to participants. Group allocation remained blind to participants and the whole study team.

Procedures

SPTs with common food allergens and aeroallergens were performed by research nurses at baseline and every 3 months for the duration of the study by using standardized reagents from ALK-Abelló. The wheal size diameters were measured and recorded by nurses. The mean wheal diameter (longest diameter plus diameter perpendicular to it divided by 2) was calculated, which determined the final wheal size. An SPT response was regarded as positive when the mean wheal diameter was at least 3 mm larger than that elicited by the negative control. At the initial visit, SPTs were carried out to HDM, grass pollen, cat, peanut, milk, and egg, and only those infants with negative responses to all allergens were recruited. Participants then underwent assessments by using the validated International Study of Asthma and Allergies in Childhood and study-specific questionnaires and SCORAD forms to assess the presence of wheeze, eczema, food allergy, and severity of eczema. A baseline venipuncture was performed, and infants had a test dose of the study medication and were discharged after 2 hours of observation. Parents were taught how to administer the IMP twice daily for the following 12 months and were advised on potential side effects. Regular contact by means of telephone occurred at least monthly, and study visits occurred every 3 months, when repeat assessments were carried out, including SPTs to all 6 allergens. In addition, infants were assessed for any adverse events, and used IMP vials were counted for adherence. Venipuncture was repeated at the final visit for measurement of serum IgE levels.

Primary and secondary outcomes

The *a priori* coprimary outcomes were cumulative sensitization to HDM and sensitization to any of the tested allergens, both of which were assessed by using SPTs. The secondary outcomes were prevalence of eczema, wheeze, and food allergy. Additionally, levels of total and specific IgE to HDM and common food allergens and aeroallergens (fx5 and Phadiatop IgE; Thermo Fisher, Milton Keynes, United Kingdom) in serum were measured at baseline and final visits.

Adverse events

Adverse events were collected throughout the study as spontaneous reports from parents/guardians of the participants who, additionally, were asked at each assessment about any events associated with administration of the study medication. A serious adverse event was defined as one that was life-threatening, required hospitalization, or resulted in significant incapacity. Serious adverse events were reported to the research and development departments of the 2 institutions and sponsors according to good clinical practice guidelines. All adverse events were reviewed by an independent data-monitoring committee at regular intervals.

Statistical analysis

The sample size required to detect the correct ordering of the primary outcomes (sensitization) for preliminary proof of efficacy was calculated as 60 subjects per group, with 85% probability of demonstrating the correct ordering of the 2 treatment groups,[11] while assuming a sensitization rate in the placebo group of 0.27, as observed in 2-year-old children from our previous study.[12] A 10% reduction was considered clinically important because this was shown previously to translate later into a significant reduction in asthma during childhood.[5,6] The study was not powered for sensitization demonstrated by specific IgE levels or for clinical end points.

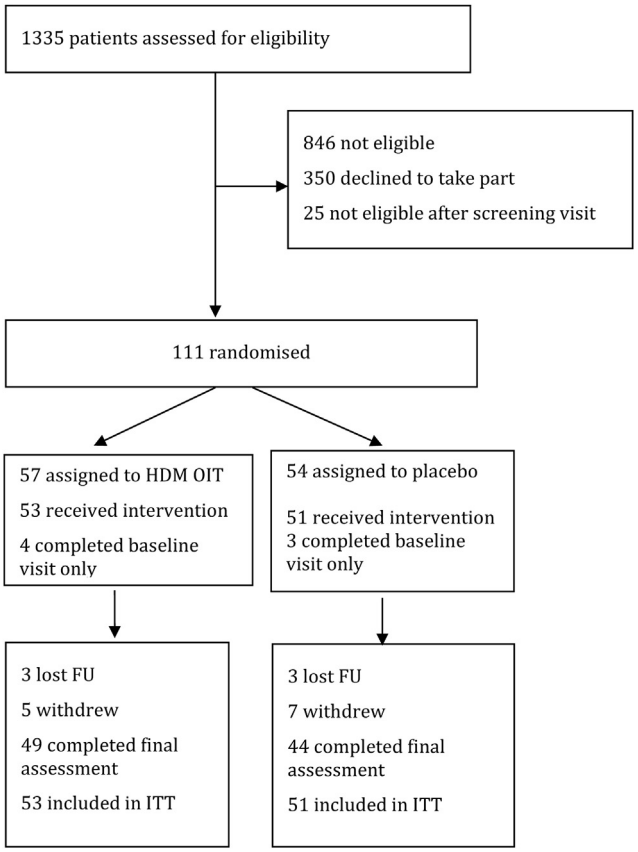

FIG 1. Flow diagram of the study process. *FU*, Follow-up; *ITT*, intention to treat; *OIT*, oral immunotherapy.

An intention-to-treat analysis was undertaken in which all randomized participants attended at least 1 postrandomization assessment. Time-to-event analysis was undertaken to compare the ordering of proportions in the 2 groups over time, and Kaplan-Meier curves were generated.

Data were censored at the time of last follow-up or at 450 days, whichever was sooner, because a minimum 12-month follow-up was planned. Differences in proportions of participants with primary and secondary outcome events at the end of the study were calculated with 95% CIs. Percentage adherence was estimated on the basis of used vials as percentages of prescribed vials. Summaries of continuous variables are presented as means ± SDs for normally distributed data; categoric variables are presented as frequencies (percentages). Every attempt was made to ensure that missing data were minimized by following up participants and continuing to collect outcome data regardless of adherence or withdrawal from treatment. As a consequence, no imputation for missing data was needed, and participants were included until their last study visit. Logistic regression model–fitted and adjusted risk differences were estimated by using the Δ method to estimate the SE of difference implemented by using the Stata command *adjrr* command.[13] Statistical analyses were performed in Stata software, version 11 (StataCorp, College Station, Tex).

RESULTS

Participants

A total of 1335 infants were screened; 846 were not eligible, and a further 350 declined to take part (CONSORT Fig 1). Of 136 participants who attended a screening visit, 25 were not eligible, leaving 111 participants randomized into the study. Fifty-seven (51.4%) infants were randomly assigned to the active group, and 54 (48.6%) were randomly assigned to the placebo group.

TABLE I. Baseline characteristics

Characteristic	HDM OIT (n = 53)	Placebo (n = 51)
Male sex	29 (54.7)	24 (47.0)
Eczema	8 (15.1)	3 (5.9)
Reported wheeze	18 (34.0)	13 (25.5)
Reported allergic reaction to a food	0 (0.0)	0 (0.0)
Age (mo)	6.7 (1.3 [53])	6.9 (1.2 [51])
Height (cm)	68.1 (4.2 [49])	68.2 (3.2 [48])
Weight (kg)	8.2 (1.1 [50])	8.2 (1.0 [50])
Gestation (wk)	40.2 (1.2 [41])	40.0 (1.1 [38])
Birth weight (kg)	3.48 (0.55 [41])	3.58 (0.49 [41])
Pet ownership		
Any	32 (60.4)	22 (43.1)
Dog	18 (34.0)	10 (19.6)
Cat	18 (34.0)	10 (19.6)
Other	7 (13.2)	10 (19.6)
Feeding		
Did you ever breast-feed your child, yes	32 (60.4)	27 (52.7)
Ever received any infant formula, yes	46 (86.8)	43 (84.3)
How old was child when breast-feeding stopped (wk)	20 (6.36 [37])	30 (11.44 [42])
Maternal smoking		
Yes, daily	4 (7.7)	6 (12.0)
Yes, occasionally	0 (0.0)	1 (2.0)
No	48 (92.3)	43 (86.0)
Anyone else smoke inside home		
Yes, daily	8 (15.4)	4 (8.0)
Yes, occasionally	0 (0.0)	3 (6.0)
No	44 (86.4)	43 (86.0)
Family		
Attends day care or nursery	10 (18.9)	5 (10.2)
Total family income		
<£12,000	7 (17.7)	5 (13.2)
£12,000-£17,999	4 (9.8)	5 (13.2)
£18,000-£29,999	6 (14.6)	6 (15.8)
£30,000-£41,999	10 (24.4)	13 (34.2)
>£42,000	14 (34.2)	9 (23.7)
Home		
Owned privately	25 (61.0)	23 (59.0)
Rented privately	8 (19.5)	10 (25.6)
Rented council/housing association	6 (14.6)	5 (12.8)
Other	2 (4.9)	1 (2.6)
Mother		
Asthma	26 (55.3)	20 (45.5)
Hay fever	43 (91.5)	39 (86.7)
Eczema	24 (51.1)	27 (60.0)
Father		
Asthma	23 (48.9)	24 (51.1)
Hay fever	39 (83.0)	33 (75.0)
Eczema	10 (21.7)	16 (36.4)

Numbers represent frequencies (percentages), means (SDs [numbers of data points]), or medians (25th-75th percentiles [numbers of data points]) depending on data distribution.
OIT, Oral immunotherapy.

The 2 groups were generally well balanced at baseline (Table I) and similar for demographics, growth, and family history. However, 32 (60.4%) households in the active group had pets compared with 22 (43.1%) households in the placebo group. Moreover, children in the active group were more likely to have eczema and reported wheeze.

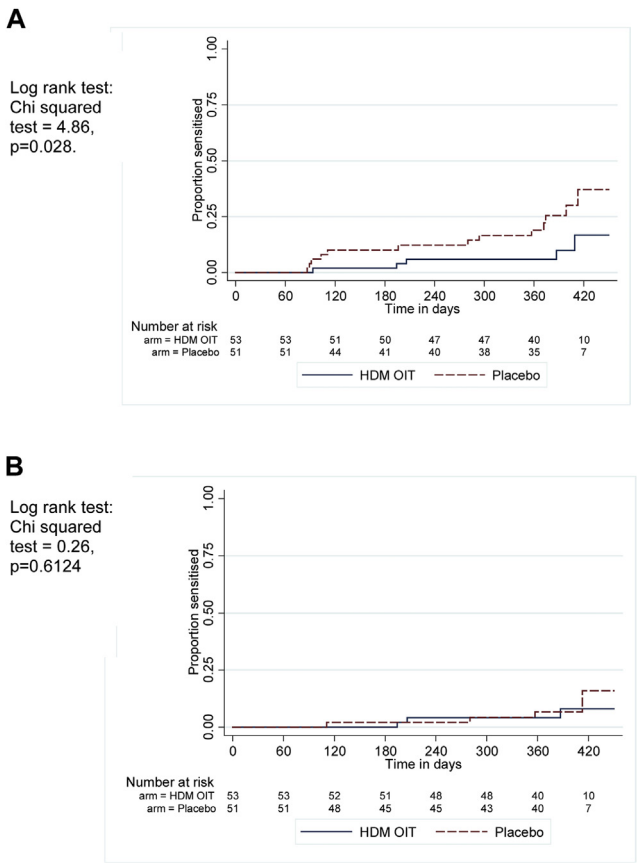

FIG 2. Cumulative sensitization to any common allergen **(A)** and HDM sensitization **(B)**. *OIT,* Oral immunotherapy.

A total of 49 participants in the active group and 44 in the placebo group completed the final assessment, with 8 and 10 participants, respectively, being lost to follow-up or withdrawn from the study by their parents (Fig 1). A total of 53 and 51 participants were included in the intention-to-treat analysis from the active and placebo groups, respectively. The active and placebo groups were followed up for a median of 379 days (interquartile range, 363-406 days) and 378 days (interquartile range, 363-406 days), respectively.

Allergic sensitization

For the coprimary outcome of cumulative sensitization to any allergen (Fig 2, *A*), there was a significant ($P = .03$) reduction in the active group after 12 months of treatment (5 [9.4%] compared with 13 [25.5%], amounting to a 16.0% difference [95% CI, 1.7% to 30.4%]; ie, >50% reduction in relative terms; Table II). Sensitization to individual allergens in the 2 groups is provided in Table E1 in this article's Online Repository at www.jacionline.org. There was no significant difference in the cumulative proportion specifically sensitized to HDM between the 2 groups (Fig 2, *B*). At the end of 1 year of follow-up, 3 (5.7%) and 4 (7.8%) subjects of the active and placebo groups, respectively, were sensitized to HDM (difference, 2.2% [95% CI, −7.5% to 11.8%]; Table II). There were no statistically significant differences in baseline variables between treatment arms. However, given the potential protective effect of pets (especially dog exposure) and observed differences in pet ownership between the 2 groups, we performed additional analysis adjusted for exposure to dogs, cats, and any pets. This did not change the direction or size of the estimated differences (see Table E2 in this article's Online Repository at www.jacionline.org).

Clinical allergic diseases

There was no statistically significant difference in the proportion of participants in the active and placebo groups with eczema. More infants had eczema and wheeze in the active group at baseline (Table I). After randomization, 9 (17.0%) children had eczema in the active group compared with 14 (27.5%) in the placebo group, with no statistically significant difference ($P = .23$; Table II and see Fig E2 in this article's Online Repository at www.jacionline.org). Similarly, there was no statistically significant difference whether participants with wheeze (see Fig E3 in this article's Online Repository at www.jacionline.org) or food allergy (see Fig E4 in this article's Online Repository at www.jacionline.org) at baseline were excluded (Table II). Two of 4 children in the active group reported symptoms to the index foods to which they were sensitized compared with 5 of 7 children in the placebo group (see Table E3).

Treatment adherence

The active group was recorded to have used 56% of the active preparations compared with an average of 65% for the placebo group. Stratification by adherence (>50% or >80%) for sensitization to either HDM or any allergen suggested that restricting to these levels of adherence did not markedly alter the study results (see Table E4).

Immunology

Serum samples were available for a subset of participants (see Table E5 in this article's Online Repository at www.jacionline.org). Serum concentrations of total IgE, specific IgE, and IgG to HDM, fx5 (for common food allergens), and Phadiatop inhalant (for common aeroallergens) screens demonstrated no significant differences between the active and placebo groups after 12 months of treatment (see Table E5).

Adverse events

The active and placebo interventions were equally tolerated, with 6 reported serious adverse events in the active group and 5 in the placebo group (see Table E6 in this article's Online Repository at www.jacionline.org). None were interpreted as being related to the study intervention but represented typical illnesses at this age (eg, viral wheeze, gastroenteritis, seizures, cellulitis, and croup). A total of 169 adverse events were noted in 48 participants in the active group and 186 events were noted in 51 participants in the placebo group; none were judged to be related to the study intervention and again represented the typical illnesses experienced by children of this age (see Table E7 in this article's Online Repository at www.jacionline.org).

DISCUSSION

In this first ever primary prevention study of allergen immunotherapy in infancy, preliminary evidence for a protective effect of high-dose HDM allergen extract on the development of atopy

TABLE II. Difference in the proportion of participants with a positive SPT response and each clinical outcome by treatment group

Outcome	HDM OIT (n = 53)	Placebo (n = 51)	Difference in proportion* (95% CI)
Positive HDM SPT response	3 (5.7)	4 (7.8)	2.2 (−7.5 to 11.8)
Positive SPT response to any common allergens	5 (9.4)	13 (25.5)	16.0 (1.7 to 30.4)
Eczema	17 (32.1)	17 (33.3)	1.3 (−16.8 to 16.8)
New-onset eczema	9 (17.0)	14 (27.5)	10.5 (−5.5 to 26.4)
Wheeze	36 (67.9)	35 (68.6)	0.7 (−18.6 to 17.2)
New-onset wheeze	18 (34.0)	22 (43.1)	9.2 (−9.5 to 27.8)
Food allergy	2 (3.8)	5 (9.8)	6.0 (−3.6 to 15.7)

Figures represent frequencies (percentages). Some participants already had eczema and reported wheeze at randomization, and therefore results for those with new-onset eczema or reported wheeze during the intervention period are presented. Differences (95% CIs) represent the difference in the proportion between the 2 groups. A difference that is positive favors the new intervention arm.

OIT, Oral immunotherapy.

*Difference might vary by 1 dp from (Placebo – HDM OIT) because of a rounding error.

(as defined by a positive SPT response to any allergen), but not on the coprimary outcome of sensitization to HDM, was observed. The treatment was safe, with no adverse effects related to intervention. Therefore this study provides early indication that prophylactic immunotherapy might be effective in preventing sensitization in children at hereditary risk of atopy. However, given the small size of the study, these findings require confirmation in larger phase IIb trials. Reassuringly, the recorded adherence and adverse event data suggest that it is an acceptable and safe prevention strategy.

Allergic sensitization in early childhood is a precursor of later, clinically apparent allergic disease.[14] Although we did not observe a significant effect on clinical outcomes, the effects observed on allergen sensitization at this early age might translate into a reduction in clinical manifestations of atopy (asthma, eczema, rhinoconjunctivitis, and food allergy) as the child grows, as we have seen in other preventive approaches.[15] We have previously shown that multiple atopy phenotypes exist and that clinical expression of asthma depends on the pattern of allergic sensitization over time.[16] Further follow-up of these children will clarify the endotypes of atopy and asthma that could be influenced by using oral immunotherapy. Although there were no statistically significant differences in clinical outcomes, the correct ordering of the outcomes (less in the active group), which was our original hypothesis, was achieved for both coprimary and 3 secondary outcomes. Because more children in the active group had pet cats and dogs, we adjusted the analysis for pet exposure, but this did not change either the direction or size of the effects (see Table E2).

HDM allergen was not selected for its biological effect on HDM allergy because the intervention was not directed at those infants who are allergic to HDM but rather for its potent allergenic and enzymatic properties and its capacity to engage with the immune system.[17] This was because the intervention was aimed at influencing the developing immune system in a more generic way. The combined effect of the adaptive and innate immune reactions makes HDM allergens potent immune reactors.[18] Components that can activate the immune system include not only proteases and immunogenic epitopes but also the structural polysaccharide chitin from the exoskeleton, microbial adjuvant compounds, and ligands originating from mite-associated compounds.[17] We expected a bystander effect and hence had sensitization to any allergen as a coprimary variable. In addition, in early childhood sensitization to HDM is rare, although sensitization to foods is common, which reverses by age 3 years.[19] If the effect of oral immunotherapy in our study is sustained beyond early childhood, we might see a difference in HDM sensitization at 3 years (assessment is ongoing).

For primary prevention of food allergy, it has been suggested that early introduction of allergenic food might induce immune tolerance rather than allergy.[20,21] In a randomized controlled trial we have recently confirmed that early introduction of peanut into infants' diets can prevent peanut allergy, which is supportive of the concept of induction of tolerance after early allergen exposure.[22] Our hypothesis that active HDM treatment will result in tolerance induction was based on evidence from a murine model in which oral administration of HDM extract inhibited the production of HDM-specific IgE while inducing the production of HDM-specific IgG.[23] In another murine model prophylactic sublingual immunotherapy with HDM extract prevented the development of airway hyperreactivity, eosinophil recruitment, allergen-specific IgE, and systemic T_H2 cytokine production.[24]

To our knowledge, there has been only one other study investigating the effects of exposing children to aeroallergens to prevent the development of allergy.[25] In a pilot study Holt et al[25] randomized 50 children aged 18 to 30 months with a positive atopic family history and personal history of atopic dermatitis and food allergen sensitization to HDM, cat, and timothy grass pollen mixture or placebo administered sublingually daily for a year. This study showed no significant differences in sensitization or asthma between the groups at 4 years of follow-up. However, the 2 studies are different in several respects: (1) the intervention in the study by Holt et al commenced in the second year of life, (2) Holt et al included only those children who had atopic dermatitis and were already sensitized to at least 1 food allergen, (3) higher amounts of *D pteronyssinus*/*D farinae* allergen (11 vs 7.5 μg) were used in our study, and (4) we used twice daily administration as opposed to once daily administration in the previous study. Therefore we would suggest that the window of opportunity is in the first year of life, before allergic sensitization had developed, which is why we chose our intervention to commence before the infants reached 12 months of age. Importantly, however, Holt et al demonstrated the safety of this treatment in a group of preschool children who already had sensitization, which gave us confidence to start immunotherapy in this very young population.

Although we found a significant treatment-related reduction in cutaneous sensitization, this was not mirrored in serum specific IgE results, which can be explained by a number of factors. First, the study was not powered to detect an order effect for this

outcome. Second, asymptomatic transient IgE sensitization is seen during infancy, which might also explain the discordance in cutaneous and serum sensitization.[26] Third, in young children a substantial disagreement between SPT responses and specific IgE levels has been previously demonstrated, suggesting that SPT responses and IgE levels are dissociated.[27] The mechanisms underlying this dissociation are unclear, but SPT responses are reported to be more closely associated with clinical disease.[28] Interestingly, significant differences were seen in SPT sensitization, but not in specific IgE levels, in our successful peanut prevention study.[22]

Allergen immunotherapy is the only known treatment that alters the natural history of allergic disease,[29] induces long-term remission,[30] prevents the onset of new sensitizations,[31] and possibly abrogates the development of asthma in patients with allergic rhinitis.[32] It acts at least partly through induction of Treg cells and suppression of T_H2-type cytokine responses.[33] Both the subcutaneous immunotherapy and sublingual modes of delivering immunotherapy are effective in patients with allergic diseases.[29,30] However, subcutaneous immunotherapy is inconvenient for patients, causes discomfort in children, and places significant demand on health care resources, making it impractical for large-scale prophylactic treatment, especially in this young population.[34] It also carries a small but definite risk of anaphylaxis. In contrast, sublingual immunotherapy is relatively safe, with no inconvenience or discomfort, except minor oral pruritus.[35] The growing confidence in the safety and efficacy of sublingual immunotherapy and some indication of a possible preventive effect on sensitization and asthma provide an opportunity to prevent the development of atopy in young children, in whom it is likely to have long-term prophylactic effects. However, there are important differences in how prophylactic immunotherapy influences the relatively immature immune system of an infant from a situation in which immunotherapy is administered in a child who is already sensitized to that allergen. Furthermore, in-depth investigations at cellular and molecular levels are required to understand the immunologic mechanisms underlying any potential preventive effect of prophylactic immunotherapy.

Several epidemiologic studies have shown that allergen sensitization in early life is the most important risk factor for development of asthma later in childhood.[36] The Isle of Wight primary prevention study would suggest that if early development of allergic sensitization can be prevented in the first 2 years, the development of childhood asthma at 10 and 18 years, especially atopic asthma, can also be prevented.[5,6] We plan to assess the participants of the current trial at 3 and 6 years of age to evaluate an effect on such clinical outcomes as asthma, rhinitis, and food allergy.

Our study is a small, single-center proof-of-concept study. As such, it has a number of limitations that need to be addressed in a follow-up confirmatory study. The sample size was small so that we could only assess correct ordering of the outcomes and could not estimate the size of the difference. Future studies need to be adequately powered. Furthermore, immunotherapy is usually administered for 2 to 5 years. It is possible that a longer duration of treatment might be more effective.

Another potential issue that might have affected the outcome is the allergen dose. Because this was the first such study and safety needed to be established, we used a dose that we believe was large enough to be immunogenic but likely to be safe. Results of serum IgG measurements are available in a small subgroup, but these did not show any differences. It is possible that a larger dose is needed to have a detectable immune effect measured by IgG, and now that the safety of this strategy is established, future studies could safely use a larger dose. It could be argued that adherence to treatment was not high enough to evoke a significant IgG response. However, we chose a very strict definition of adherence, "used vials as percentages of prescribed vials," which means that the 56% and 65% adherence rates for the active and placebo groups are an underestimate because many parents did not remember to bring used vials back to follow-up visits. Furthermore, we did not expect adherence to be perfect in this age group and chose a twice daily dose in the expectation that many children would only take the treatment once a day. This level of adherence is similar to that reported for other long-term therapies that seem to be effective.[37]

In summary, this is the first proof-of-concept study providing early evidence of the safety and possibly efficacy of this approach. Because the sample size is relatively small, further proof of efficacy of early intervention requires replication in larger multicenter studies. Further studies are also required to elucidate the mechanisms of action of oral immunotherapy in infants at risk of sensitization and to evaluate the long-term efficacy of the treatment to prevent clinical atopic diseases, such as asthma.

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Clinical implications: This study provides evidence suggestive of early proof of concept for a feasible, safe, and effective strategy to prevent allergic sensitization by using high-dose HDM oral immunotherapy.

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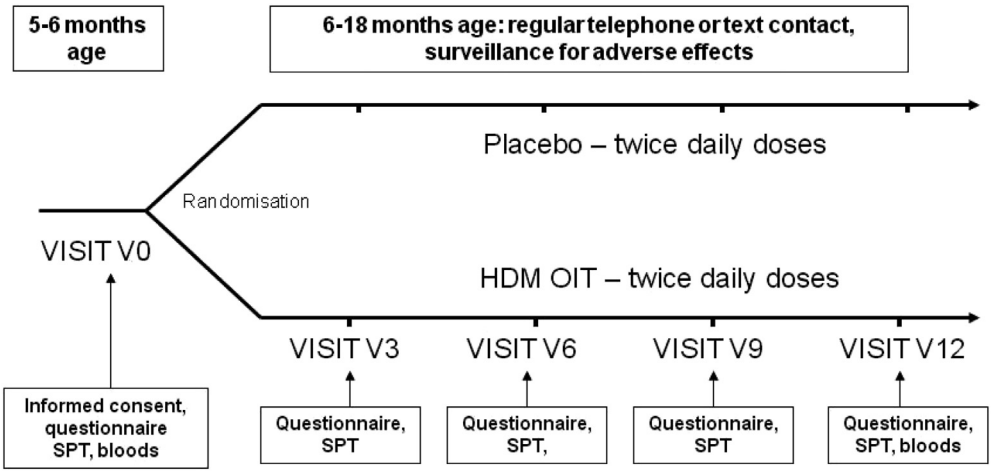

FIG E1. Overview of study design. *OIT,* Oral immunotherapy.

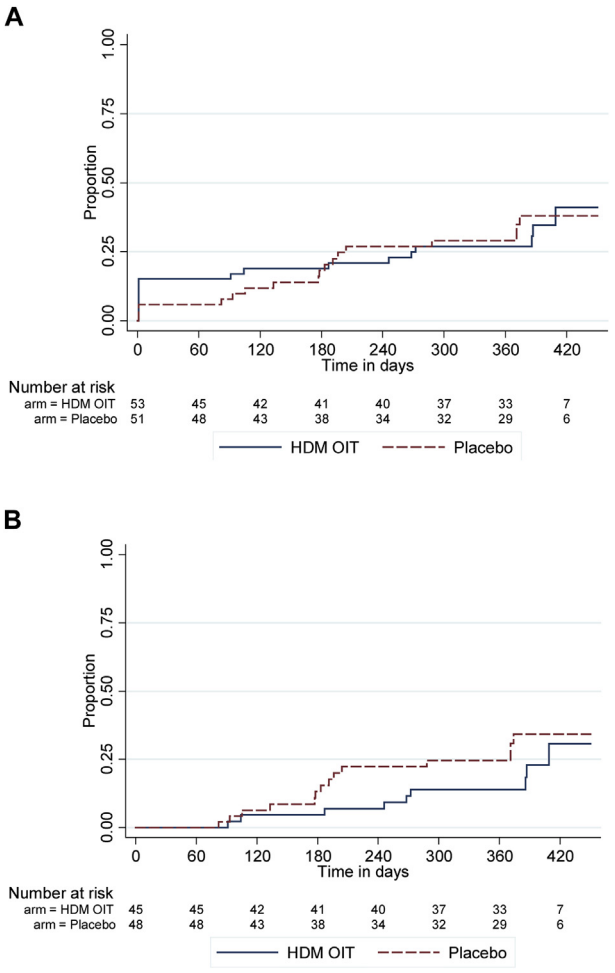

FIG E2. Cumulative proportion with eczema for all participants **(A)** and excluding those with eczema at baseline **(B)**. *OIT*, Oral immunotherapy.

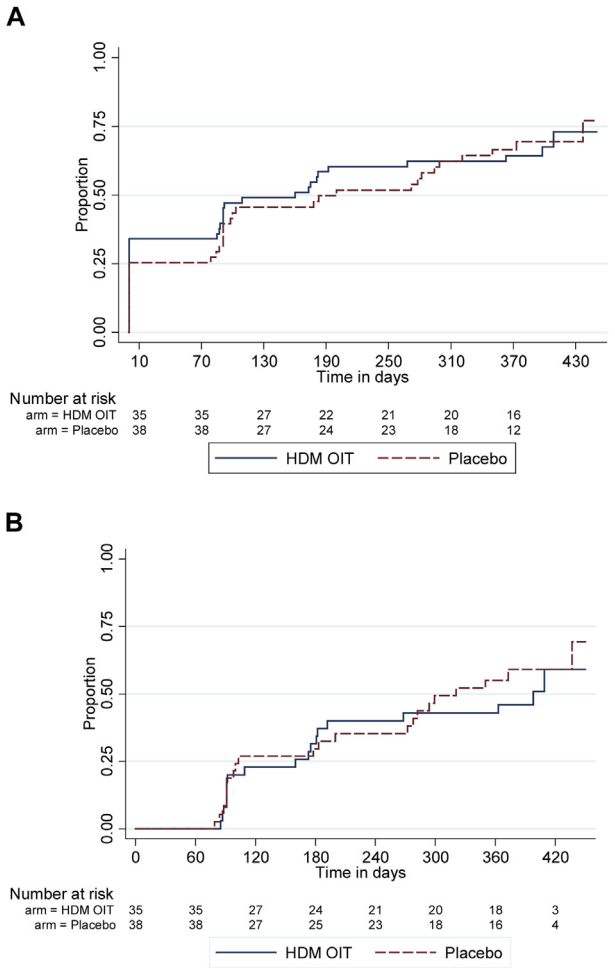

FIG E3. Cumulative proportion with reported wheeze symptoms for all participants **(A)** and excluding those with wheeze at baseline **(B)**. *OIT*, Oral immunotherapy.

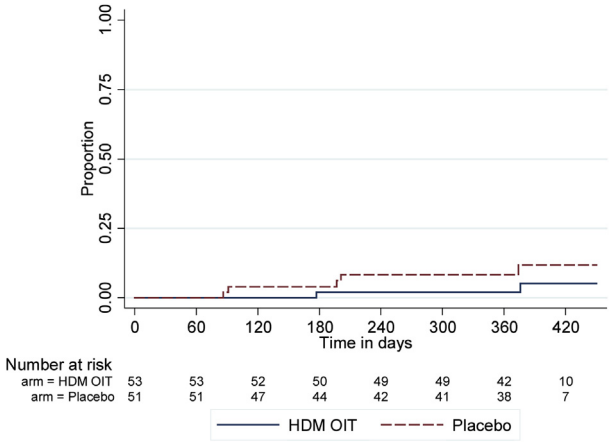

FIG E4. Cumulative proportion with food allergy. *OIT*, Oral immunotherapy.

TABLE E1. Sensitization SPT to each allergen by treatment group

Allergen	HDM OIT	Placebo
HDM	3	4
Egg	2	4
Peanut	1	2
Milk	1	2
Cat	1	2
Grass pollen	2	1
Total allergens	10	15

Overall, 5 participants in the HDM oral immunotherapy group were sensitized to 10 allergens (1 child was sensitized to all 6 allergens, and the rest were monosensitized). In the placebo group 13 children were sensitized to 15 allergens (1 child was sensitized to both milk and egg, and another child was sensitized to both egg and peanut). The rest of the children were monosensitized.
OIT, Oral immunotherapy.

TABLE E2. Differences in sensitization to any allergen and main clinical outcomes adjusted for dogs, cats, and any pets

Outcome	Adjusted for:	Difference (%)	Lower 95% CI	Upper 95% CI
HDM	Too few events for which to be able to adjust			
Any allergen	Unadjusted	16.0	1.7	30.4
	Dog	16.6	2.2	31.0
	Cat	15.5	1.1	29.8
	Any pet	15.8	1.4	30.2
Eczema	Unadjusted	1.3	−16.8	16.8
	Dog	3.4	−14.3	21.2
	Cat	1.8	−16.3	20.0
	Any pet	1.7	−16.4	19.7
New-onset eczema	Unadjusted	10.5	−5.5	26.4
	Dog	12.2	−3.9	28.4
	Cat	11.7	−4.6	28.0
	Any pet	11.0	−5.5	27.3
Wheeze	Unadjusted	0.7	−18.6	17.2
	Dog	1.1	−16.9	19.1
	Cat	0.8	−17.2	18.9
	Any pet	0.6	−17.3	18.6
New-onset wheeze	Unadjusted	9.2	−9.5	27.8
	Dog	10.5	−8.1	29.1
	Cat	9.7	−9.1	28.4
	Any pet	9.8	−8.8	28.4
Food allergy	Too few events for which to be able to adjust			

A logistic regression model was fitted and adjusted risk differences were estimated by using the Δ method to estimate the SE of difference implemented with the Stata command adjrr command.[13]

TABLE E3. Participants with a positive SPT response to food and concomitant reported food allergy

	HDM OIT		Placebo	
Allergen	Sensitization with reported symptoms	Sensitization with no reported symptoms	Sensitization with reported symptoms	Sensitization with no reported symptom
Positive egg SPT response	1	1	4	0
Positive peanut SPT response	1	0	1	1
Positive milk SPT response	0	1	0	1

OIT, Oral immunotherapy.

TABLE E4. Primary outcomes at 12 months of follow-up stratified by adherence

Group	Result	HDM OIT	Placebo
Sensitization to HDM			
All	Negative	50 (94)	47 (92)
	Positive	3 (6)	4 (8)
>50% Adherence	Negative	24 (96)	34 (97)
	Positive	1 (4)	1 (3)
>80% Adherence	Negative	16 (100)	15 (100)
Sensitization to any common allergen			
All	Negative	48 (91)	38 (75)
	Positive	5 (9)	13 (25)
>50% Adherence	Negative	23 (92)	27 (77)
	Positive	2 (8)	8 (23)
>80% Adherence	Negative	15 (94)	11 (73)
	Positive	1 (6)	4 (27)
	Positive	0 (0)	0 (0)

Figures represent frequencies (percentages).
OIT, Oral immunotherapy.

TABLE E5. Levels of serum specific and total IgE by treatment group

	Baseline	12 mo Follow-up
Median HDM-specific IgE (kU_A/L)		
HDM OIT	0.005 (0.005-0.005 [43])	0.005 (0.005-0.010 [33])
Placebo	0.005 (0.005-0.005 [33])	0.005 (0.005-0.010 [27])
P value	.992	.958
Median fx5-specific IgE (kU_A/L)		
HDM OIT	0.050 (0.040-0.100 [40])	0.050 (0.040-0.120 [33])
Placebo	0.050 (0.040-0.070 [32])	0.050 (0.040-0.125 [28])
P value	.375	.798
Positive Phadiatop IgE result		
OIT group	0/43 (0.0%)	4/34 (11.8%)
Placebo group	0/33 (0.0%)	2/28 (7.1%)
P value	—	.540
Median total IgE (IU/L)		
OIT group	4.04 (2.61-9.14 [36])	10.10 (4.94-26.30 [30])
Placebo group	4.66 (2.16-8.07 [27])	11.00 (4.79-13.20 [23])
P value	.776	.760
Median total IgG (IU/L)		
OIT group	—	326 (106.00-625.00 [36])
Placebo group	—	403.00 (284.00-622.00 [28])
P value	—	NS

There were no differences between the oral immunotherapy and placebo groups for any of the immunologic outcomes at either the baseline or 12-month follow-up time points. Fx5 and Phadiatop represent screening tests for food allergens (wheat, egg, cow's milk, soya, peanut, and fish) and aeroallergens (grasses, trees, weeds, cat, dog, mites, and molds), respectively (Thermo Fisher, Uppsala, Sweden). Results of less than the limit of detection were recoded as half the lower limit of detection (0.005 kU_A/L). Figures are medians (25th-75th percentiles [numbers]), except for Phadiatop, where they represent the number of positive results (n/N [%]). *P* values comparing groups at specific time points represent 2 sample Wilcoxon rank-sum or Fisher exact tests depending on the type of data.
NS, Not significant; *OIT*, oral immunotherapy.

TABLE E6. Serious adverse events by treatment group

	HDM OIT		Placebo	
Serious adverse event	**No.**	**Causality**	**No.**	**Causality**
Viral wheeze	1	Unlikely	3	All unlikely
Gastroenteritis	1	Unlikely	1	Unlikely
Seizure	0	NA	1	Not related
Cellulitis	1	Unlikely	0	NA
Croup	1	Unlikely	0	NA
Surgery	2	All not related	0	NA

NA, Not applicable; *OIT*, oral immunotherapy.

TABLE E7. All nonserious adverse events reported during the study period

	HDM OIT		Placebo			HDM OIT		Placebo	
Adverse event	**Events**	**Participants**	**Events**	**Participants**	**Adverse event**	**Events**	**Participants**	**Events**	**Participants**
Allergic reaction			1	1	Laryngitis	1	1	2	1
Angioedema			2	2	LRTI	9	8	8	7
Bronchiolitis	4	3			Missed IMP	2	2		
Chicken pox	3	3	6	6	Otitis media	4	4	8	4
Colic			1	1	Pyrexia	2	2	1	1
Conjunctivitis			3	3	Pneumonia	1	1	0	
Cough			2	2	Periorbital edema	1	1		
Cow's milk allergy	2	2			Rash	5	4	4	2
Coxsackie infection	4	4	2	2	Respiratory tract infection			1	1
Croup	2	2	1	1	Rhinitis	2	2	1	1
Crying, unknown cause	1	1			Rhinoconjunctivitis			1	1
Diarrhea	10	7	4	3	Positive SPT response to HDM	1	1	1	1
Drug allergy, penicillin			1	1	Stopped IMPs	2	2	3	3
Eczema	6	6	11	10	Teething	1	1		
Egg allergy			3	3	Tonsillitis	11	4	4	4
Fall, accidental	1	1			URTI	13	10	19	13
Gastroenteritis	14	13	20	16	Urticaria	4	4	1	1
Gastroesophageal reflux	1	1			Viral gastritis	2	2	5	4
Head lice			1	1	Viral rash	2	2		
Headache	1	1			Viral URTI	41	25	48	20
Heat rash			1	1	Viral urticaria	1	1	2	2
Impetigo (skin infection)	1	1	2	1	Viral wheeze	12	9	11	7
Infected eczema			1	1	Vomiting	1	1	4	4

LRTI, Lower respiratory tract infection; *OIT*, oral immunotherapy; *URTI*, upper respiratory tract infection.