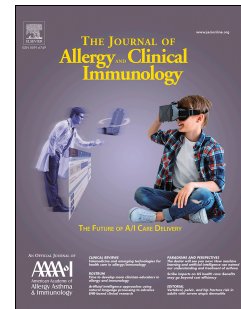


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The airway as a route of sensitization to peanut: an update to the dual allergen exposure hypothesis

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Keywords: food allergy, peanut allergy, dual allergen exposure hypothesis, airway, cutaneous

Abbreviations:

BREATHE: Biology of Respiratory Exposure to Allergens in the Household Environment

CLA: Cutaneous lymphocyte antigen

HBEC: Human bronchial epithelial cell

LEAP: Learning Early About Peanut Allergy

TEWL: Transepidermal water loss

Tfh: T follicular helper

Th: T helper

TLR: Toll-like receptor

Abstract:

Food allergies have increased at an alarming rate over the past two decades, indicating environmental factors are driving disease progression. It has been postulated that sensitization to foods, in particular peanut, occurs through impaired skin. Peanut allergens have been quantified in household dust and may be the culprit source. Indeed, Th2-skewing innate cytokines can be driven by application of food antigens on both intact and impaired skin of mice, resulting in antigen-specific IgE production, and anaphylaxis on allergen exposure. However, allergy induction through the skin can be prevented by oral tolerance induction prior to skin exposure. These observations led to the dual allergen exposure hypothesis, wherein oral exposure to food antigens leads to tolerance, and antigen exposure on impaired skin leads to allergy. Here, we propose the airway as an alternative route of sensitization in the dual allergen exposure hypothesis that leads to food allergy. Specifically, we will provide evidence from mouse models and human cell-based studies that together implicate the airway as a plausible route of sensitization.

Food allergy is a potentially fatal disease affecting approximately 4-8% of children and 10% of adults in the US (1, 2). Peanut allergy affects over 1% of the general population in the US and Europe, and often persists lifelong (3, 4). Classically, food allergy is thought to originate in the gut, through a failure in oral tolerance mechanisms (5). However, an interesting clinical observation that the majority of allergic individuals react on their first oral exposure to peanut led researchers to hypothesize that sensitization occurred through non-oral routes (6). Over the past decade, cutaneous sensitization to food allergens has become well-accepted (7). Here we propose the airway as an additional route of food allergen sensitization by highlighting data from murine models and human cell-based studies (Figure 1).

Cutaneous sensitization has been proposed through the dual allergen exposure hypothesis. This theory evolved from observations that early introduction of peanut into the diet in Israel led to lower prevalence compared to genetically similar cohorts in the UK (8). Conceptually, oral exposure to peanut allergens leads to oral tolerance, whereas exposure through the skin in the absence of oral exposure leads to allergy. Impaired skin barrier, caused by atopic dermatitis, has long been associated with the development of peanut allergy (9). An important study further supporting this hypothesis demonstrated that household peanut protein quantities significantly increased the risk for peanut sensitization in children with filaggrin mutations, which indicate an impaired skin barrier (10). A landmark clinical trial, Learning Early About Peanut Allergy (LEAP), involving 640 infants aged 4-11 months clearly demonstrated that early and regular oral feeding of peanut proteins dramatically decreased the incident of food allergy at 5 years of age compared to infants avoiding oral ingestion of peanuts (11). These findings support the dual allergen exposure hypothesis, and emphasize the importance of early oral exposure to peanut before sensitization can occur through alternate routes.

Transcutaneous sensitization to allergens

A dysregulated skin barrier is postulated to be an induction site of food allergy in humans, especially in individuals with atopic dermatitis. An association has been demonstrated between severity of atopic dermatitis and peanut-specific IgE (7, 12). Two epithelial cytokines, TSLP and IL-33, are increased in the skin of atopic dermatitis patients, and are known to play an important role in inflammation following allergen exposure (13). Additionally, transepidermal water loss (TEWL) is increased in individuals with atopic dermatitis, and is associated with sensitization to food allergens and aeroallergens (14, 15). Despite the mounting evidence from observational studies in humans showing associations between inflamed eczematous skin and environmental peanut leading to peanut allergy (6, 7), there is no direct evidence that application of peanut protein to impaired or intact human skin directly leads to peanut allergy. The ethical constraints of human clinical trials have led investigators to utilize mouse models of food allergy to directly prove that sensitization can occur through the skin.

Impaired skin barrier can be induced in mice by removal of the stratum corneum by tape stripping, which results in inflammation. This method was used and peanut was applied to the damaged skin leading to production of Th2 cytokines and serum IgE (16). Interestingly, another study utilizing a model of atopic dermatitis induced by tape-stripping showed an IL-33-dependent mechanism of mast cell expansion and activation in the GI tract, suggesting an interactive role between the skin and gut barriers (17). An additional study used mice with filaggrin mutations to demonstrate food sensitization after co-exposure to peanut and environmental allergens on neonatal skin and subsequent anaphylaxis following challenge (18). Finally, application of peanut proteins on intact skin following depilation led to sensitization through the IL-33 receptor and anaphylaxis upon

challenge (19). Our group attempted to replicate these mouse findings in a non-human primate model using African green monkeys (20). Skin was shaved and peanut applied on the back of the neck for four weeks. Interestingly, peanut-specific IgG was detected in the serum but not IgE, indicating that application of peanut on the skin led to an immune response. Together, these studies support the transcutaneous route of sensitization to food antigens, and implicate a key role for IL-33.

Airway sensitization to allergens

The dual allergen exposure hypothesis implicates non-oral routes of exposure that cause sensitization and allergy, with the primary focus on cutaneous exposure. Our team at UNC (Biology of Respiratory Exposure to Allergens in the Household Environment, BREATHE) hypothesized that exposure to food allergens in the airway would also lead to food allergy. Given that sensitization to environmental allergens (e.g. pollens, pet dander, dust mite) occurs through the airway, and can lead to pollen food allergy syndrome (21), it is plausible that systemic sensitization to food antigens occurs through the same route. Furthermore, several case reports suggested potential sensitization to millet, buckwheat, lupine seed flour, egg and sunflower seed through the airway and subsequent anaphylaxis upon oral ingestion of the food (22-26). These reports represent individual instances of sensitization to foods by inhalation, but we believe they are indicative of a broader paradigm of non-oral sensitization. Here we will provide evidence from mouse models of food allergy and cellular data from human biospecimens to argue that exposure to food allergens in the airway is a viable route of sensitization.

Importantly, there is an environmental source of peanut in household dust that could be inhaled by infants. Several reports have found peanut proteins in vacuumed dust from parental and infant bedrooms, living rooms, and kitchens (27-30). The peanut proteins within dust appear to be structurally intact, containing IgE-binding epitopes, as they can cause degranulation of basophils in *ex vivo* assays (30). Another epidemiologic study demonstrated the presence of peanut proteins in school cafeterias and classrooms (31). These findings establish that peanut proteins are present in environments that children spend a majority of their time, and suggest the potential for airway exposure to peanut.

We recently reported a model of airway sensitization to peanut with household dust as an adjuvant (32). Exposure to peanut extract or dust alone did not lead to sensitization, but combined exposure over a two week period led to peanut-specific IgE production and anaphylaxis upon peanut challenge. Importantly, very low, environmentally-relevant quantities of peanut were used for sensitization (50 ng) (33). Investigations into the mechanism demonstrated uptake of peanut antigen by cDCs migrating to lung-draining lymph nodes (32). Furthermore, increases in IL-33, IL-1 α and IL-1 β were observed in lung homogenates after exposure to dust, while TSLP was increased after peanut exposure.

Since household dust contains many immunostimulatory agents, we next aimed to understand which components most influence the immune response. Using the same mouse model, we demonstrated that neither protease activity nor beta-glucan activation through Dectin-1 were necessary for the adjuvant activity (33). Dust also contains endotoxin and bacterial DNA, which signal through toll-like receptors (TLRs) and the MyD88 pathway. Therefore, we tested MyD88^{-/-} mice and found that signaling through the MyD88 pathway is required for the adjuvant activity. Interestingly, co-administration of endotoxin or bacterial DNA with peanut and dust did not protect against sensitization. However, pre-treatment with endotoxin or bacterial DNA for two weeks prior to peanut and dust exposure significantly suppressed anaphylaxis. Together these results indicate that TLR

ligands are an important component in the adjuvant activity of dust and that timing of exposure relative to peanut may determine allergy outcomes.

Other investigators have developed a model using peanut flour exposure in the airways. Briefly, mice were exposed to 100 µg peanut flour for four weeks, and subsequently produced peanut-specific IgE and reacted upon challenge (34). Mechanistically, T follicular helper (Tfh) cells were critical for IgE production and there was increased production of IL-1 α , IL-1 β , IL-33 and TSLP in lung homogenates after peanut exposure. Specifically IL-1 receptor depletion led to decreased Tfh cells and therefore decreased IgE production (34). In a separate study by this group, IL-13 was found to be critical for IgE production after airway exposure to peanut (35). The predominant source of IL-13 was ILC2s in the lung. These studies convincingly demonstrate that airway exposure to peanut in the absence of any adjuvant can drive IgE production, leading to anaphylaxis. While there are notable differences between these mouse models of airway sensitization, these results provide convincing evidence that airway exposure to peanut is a viable route of sensitization.

Several human T cell studies have been performed that support non-oral routes of sensitization, including the skin and airway. In peanut allergic subjects, peanut-specific T helper (Th) cell proliferation was greater for Th cells expressing the skin-homing receptor cutaneous lymphocyte antigen (CLA) than the gut-homing integrin $\alpha 4\beta 7$, suggesting that peanut sensitization had occurred through the skin (36). Furthermore, gut-homing Th cells were skewed toward Th1 cytokine production, consistent with possible tolerance, whereas skin-homing Th cells were skewed toward a Th2 phenotype, consistent with sensitization (36). An additional study found that peanut-specific CD4⁺ T cells from peanut allergic subjects had increased expression of the skin- and lung-homing chemokine receptor CCR4 compared with nonallergic subjects (37). Interestingly, the authors found that pathogenic peanut-specific Th2 cells from peanut-allergic subjects expressed CCR4 but not CLA, leading them to speculate that these cells may have originated in the airways rather than the skin (37). In a separate study using HLA class II/Ara h 1 tetramers, Ara h 1-specific CD4⁺ T cells from peanut-allergic subjects highly expressed CCR4 but not $\beta 7$, indicating that these cells may have been initially primed outside of the gastrointestinal tract (38). Finally, our group used primary human bronchial epithelial cells (HBECS) and demonstrated expression of IL-1 α and β following exposure to peanut plus household dust (32). Since IL-1 was shown to be critical in mouse models for production of IgE via Tfh cells (34), we present a potential link between IL-1 production from human airway cells and Th2 cell-priming that drives allergy in humans.

Gaps in knowledge in non-oral routes of sensitization

While there is accumulating evidence to support sensitization to food through the airway and skin, important questions remain. Although eczema and impaired skin barrier are associated with the development of peanut allergy, all eczematous individuals do not develop food allergy, and conversely, all individuals with food allergy do not have eczema. Therefore, it is plausible that these individuals are sensitized through an alternate route, like the airway. Interestingly, despite the evidence demonstrating a crucial role for skin inflammation during sensitization, the role of inflammation in the airway has yet to be explored in the context of sensitization to food.

In addition to eczema, the presence of environmental peanut in the home has also been identified as a risk factor for developing peanut allergy. Previous studies have interpreted this to support transcutaneous sensitization, with limited focus on any alternate mucosal route, including the airway. It is currently unknown whether sensitization is occurring exclusively through one route or a combination of routes, although our hypothesis is that this differs between individuals. Furthermore, it is unclear if non-oral exposure to environmental peanut may promote tolerance in some individuals.

Indeed, animal studies have shown that airway (39) and cutaneous (40) exposure to antigens under noninflammatory conditions can induce tolerance, and epicutaneous immunotherapy is being investigated as a treatment for peanut allergy (41). We suspect that co-exposure to environmental adjuvants, including TLR ligands and possibly pollutants, is a key determinant of whether non-oral exposure to peanut results in allergy or tolerance. Whether early-life interventions aimed at reducing exposure to environmental adjuvants can mitigate peanut allergy risk in children is unknown and warrants further study.

Conclusions and future directions

The studies presented here suggest a role for airway sensitization in the dual allergen exposure hypothesis. Based on these data, it is plausible for infants to be exposed to peanut from the household environment via the airway. Data from our group specifically implies a role for immunostimulatory agents in the household dust, providing further context for the potential exposure route. Several studies suggest a critical role for IL-33 in airway sensitization, which is consistent with findings from skin sensitization studies. Importantly, this addition to the dual allergen exposure hypothesis does not negate the role of the skin in sensitization, but instead provides an additional route of exposure that, in the absence of previous oral exposure, leads to sensitization.

Future studies should focus on the role of airway exposure and sensitization to other foods, as other food proteins (e.g. milk and egg) have also been detected in household dust (42, 43). Additionally, other environmental factors including air pollutants may play a role in promoting airway sensitization, because urban rates of food allergy are higher compared to rural areas (44, 45). Furthermore, investigating the microbiome, metabolome, and proteome of the household environment may help identify correlations between specific components and the likelihood of inducing sensitization. Modulating these environmental components, including removing peanut from the environment before oral exposure, may lead to the prevention of food allergy.

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Figure Legend:

Figure 1. An update to the dual allergen exposure hypothesis with the airway as a potential route of exposure. After exposure through the airway with an adjuvant or through skin with atopic dermatitis, in the context of increased innate cytokine production, peanut is taken up by dendritic cells, which migrate and present to naïve $CD4^+$ T cells in the draining lymph nodes. These T cells become activated, produce Th2 cytokines and signal to B cells to produce peanut-specific IgE. In contrast, after oral exposure, peanut is taken up by dendritic cells in the tolerogenic environment of the GI tract, which migrate and present to naïve $CD4^+$ T cells in the mesenteric lymph nodes. These T cells become programmed into Tregs, leading to tolerance. Created with BioRender.com.

Dual Allergen Exposure Hypothesis: An Update

