HDM exposure enhances subsequent responses to OVA-induced food allergy

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Abstract

HDM (House dust mites) are important environmental trigger factors of airway allergic diseases, the allergens of HDM were detected in the human gut mucosa, which induces local inflammation and increases intestinal permeability. This study tests a hypothesis that house dust mites contribute to the development of OVA (ovalbumin)-induced food allergy. The serum levels of IgE against HDM in patients with food allergy were detected with UniCAP100 (Pharmacia, Uppsala, Sweden); the HDM-induced/the OVA-induced mouse model of food allergy was developed. Difference between 2 groups was determined by Student t test or ANOVA if more than two groups. Compared to the healthy controls, the patients with food allergy have higher levels of serum IgE against HDM. Compared to food allergy alone groups, the levels of IgE against HDM in food allergy with asthma or allergic rhinitis groups were increased significantly. In mouse models, we found that HDM/OVA induced allergy-like symptoms, lower body temperature, and lower body weight. The levels of IgE, IgG1, mMCP-1 (mouse mast cell protease-1), IL-4 and IL-5 in the HDM and HDM + CT (cholera toxin) groups were higher than the control groups, and the levels of IgE, IgG1, IL-4 and IL-5 in the HDM, OVA and HDM + OVA groups were higher than the control groups. The pathology of intestinal tract in the HDM and HDM + CT/the HDM, OVA and HDM + OVA groups were more severe and exhibited more eosinophils than the control groups. Moreover, the prior exposure to HDM induced intestinal barrier dysfunction, and facilitated the development of intestinal allergy in mice. Based on above data and previous researches, we put forward that HDM exposure enhances subsequent responses to OVA-induced food allergy.

Background

Food allergy is estimated to affect 5% of adults and 8% of young children; the prevalence of food allergy has been increasing significantly in the recent decades. Food allergy have become a worldwide health problem, which burdens patients and families by increasing expenses of healthcare and negatively affects the quality of life.

There are 8 most common food allergens proved by the U.S. Food and Drug Administration (FDA), including peanuts, tree nuts, soy, wheat, fish, shellfish, milk and eggs. These food allergens usually go straightly through the mouth into the host, and then induce intestinal sensitization/allergy.

There is a high coincidence between food allergy and other allergic diseases, such as allergic asthma, allergic rhinitis and atopic dermatitis. Asthmatic patients appear to be at significantly increased risk of severe allergic reactions induced by food allergens. The cross-reactivity between aeroallergens and food allergens may induce food allergy in patients with airway allergy. Especially, tropomyosin was involved in cross-reactivity between HDM and shrimp, thus shrimp and house dust mite (HDM) allergies usually occur in the same patient, and consequently, the frequency of HDM sensitization in shrimp allergic people is higher.
There are many seasonal/outdoor aeroallergens, such as dust mites, pollen, cockroaches, fungi and animal feathers. Nearly 80% of asthmatic patients are sensitized to HDM, the predominant sources of aeroallergens. In recent research, HDM allergen was detected in the human intestine, which was able to induce local inflammation and increased intestinal permeability.

Based on the information above, we hypothesized that HDM may be not only an aeroallergens, but also an important role in food allergy. In this study, the levels of specific IgE against HDM in patients with food allergy were analyzed, and a HDM/OVA-induced mouse model of food allergy was developed. Moreover, the exposure to HDM increased the intestinal barrier permeability and facilitated the development of intestinal allergy in mice. The data demonstrate that HDM not only is an aeroallergen, which induces airway allergy, but also enhances subsequent responses to OVA-induced food allergy.

Materials And Methods

Human subjects

In total of 825 food allergic patients’ serum were collected at the First Affiliated Hospital, Guangzhou Medical University (Guangzhou, China) during January 2015 to November 2016. The diagnosis was conducted by the doctors of this hospital. The clinical features of human subjects are presented in Table 1. This study has been approved by the Human Ethic Committee at Shenzhen University and Guangzhou Medical University. Informed consent was obtained from all subjects and if subjects are under 18, from a legal guardian. All experiments were performed in accordance with the relevant guidelines and regulations.
Table 1
Clinical features of human subjects.

|                        | **Food allergy (n = 825)** | **Healthy subjects (n = 25)** |
|------------------------|---------------------------|-------------------------------|
| Sex M/F                | 528/297 (M-64%)           | 14/11 (M-54.5%)              |
| Age mean; range        | 11.46 ± 0.6051 (1–87)     | 17.46 ± 4.761 (1–82)         |
| Personal history of atopy | 332 (40.24%)            | 0                             |
| Total IgE              | 447.7 ± 21.64**           | 109.8 ± 17.76                 |
| Atopic comorbidities   |                           |                               |
| Bronchial asthma       | 223 (27.03%)              | 0                             |
| Allergic rhinitis      | 83 (10.06%)               | 0                             |
| Allergic dermatitis    | 26 (3.15%)                | 0                             |
| Positive sIgE to HDM   | 544 (65.93%)              | 20%                           |
| Allergen distribution  |                           |                               |
| milk                   | 511 (61.93%)              | 0                             |
| egg                    | 419 (50.78%)              | 0                             |
| shrimp                 | 223 (27.03%)              | 0                             |
| crab                   | 114 (13.80)               | 0                             |
| wheat                  | 57 (6.90%)                | 0                             |
| peanut                 | 12 (1.45%)                | 0                             |
| soy                    | 12 (1.45%)                | 0                             |
| fish                   | 2 (0.24%)                 | 0                             |

*Dermatophagoides pteronyssinus* Culture and Extracts Preparation

*Dermatophagoides pteronyssinus* mites were cultured as reported previously,11 dust mites were cultured at 25 °C with 70% relative humidity. Subsequently, mites were isolated from the medium using a modified heat-escape method and the dust mites purity was evaluated by checking mite morphology. Mite bodies were washed with PBS, weigh 2 gram sample adding 1 ml lysate (9M urea, 4% CHAPS, 60 mM DTT, 2% IPG buffer) and homogenized in liquid nitrogen, centrifuged at 15,000 rpm for 20 min under refrigeration. The supernatant was termed HDM extract.

Mice
6 to 8 weeks old female BALB/c mice (weight: 18–20 g), obtained from the Guangdong Experimental Animal Center (Guangzhou, China), were maintained in specific pathogen-free conditions according to standard guidelines for the care and use of animals. The experimental procedures were approved by the Institutional Ethics Committee at Shenzhen University (Shenzhen, China). The study was carried out in compliance with the ARRIVE guidelines.

Induction of experimental food allergy

As shown by Fig. 2A, 18 mice were randomly divided into 3 groups: HDM + cholera toxin (CT) group, HDM group and Control group. Mice were sensitized intraperitoneally with PBS (Control group), HDM extract (1 mg/mouse) and CT (20 µg/mouse) (HDM + CT group), or HDM extract (1 mg/mouse) (HDM group) on day 0 and day 3 respectively. From day 5 on, challenge was performed every other day for 10 days, mice were challenged with PBS (Control group), HDM extract (1 mg/mouse) and CT (20 µg/mouse) (HDM + CT group), or HDM extract (1 mg/mouse) (HDM group) by intra-gastric (i.g) gavage. The body weight of each mouse was recorded every other day. An OVA food allergy followed by HDM exposure were induced in Balb/c mice. Mice were continually exposed to HDM (1 mg, i.g)/PBS for a week. Combined HDM exposure and food allergy was obtained by applying the two protocols successively (Fig. 4A). Control mice were sensitized with PBS alone and challenged with PBS.

Enzyme-linked immunosorbent assay (ELISA)

The levels of specific IgE and IgG1 for HDM were determined by ELISA as described previously. Briefly, the ELISA microtiter plates were coated with HDM with at 1 µg/well in 100 µl Carbonate buffered solution (CBS, 15 mM Na2CO3 and 35 mM NaHCO3, pH9.5). After incubation (overnight, 4 °C), plates were washed 3 times with PBST (PBS/0.05% Tween 20) and blocked with 3% bovine serum albumin in PBS (3% BSA-PBS) (1 hour, 37 °C). The serum (1:10 diluted with 3% BSA-PBS) or BSA (using as a negative control) were then added to each well and incubated (2 hours, 37 °C). Subsequently, 100 µL of peroxidase-labeled goat anti-mouse IgE (1:2000) was added to each well. The plates were incubated (2 hours, 37 °C). Following 3 washings, the reactions were developed with TMB (tetramethylbenzidine, 100uL/well) for 20 min and stopped by 50 µl 2 M H2SO4. The plates were read by an ELx808 absorbance microplate reader (BioTek, Shanghai, China) at 450 nm. The splenocytes culture supernatant levels cytokines IL-4 (Boster, Wuhan, China), IL-5 and IFN-γ (Sino Biological Inc, Beijing, China) were determined by ELISA with commercial reagent kits following the manufacturer's instruction.

Flow cytometry

Spleen cells were prepared according to Gunzer M et al’s report. Splenocytes (2x10^6/well) were labeled with CFSE (5,6-carboxyfluorescein diacetate, succinimidyl ester) in dark, incubated with 50 µg/ml HDM or culture medium and 2 µl/ml cell stimulation cocktail (a cocktail of phorbol 12-myristate 13-acetate (PMA) and ionomycin, ebioScience) in 96-well plate (72 h, 37 °C). Following washing with PBS, the cells were stained with CD4-APC (1 h, room temperature). After washing, the cells were analyzed by flow cytometry. The data were analyzed with the software Flowjo.
Assessment of the Intestinal permeability in vivo

This measure is based on the intestinal epithelial barrier permeability to 4,000-Da fluorescent-dextran (Sigma-Aldrich).\textsuperscript{14} 6-h-fasted mice were fed with fluorescein isothiocyanate (FITC)-dextran at 600 mg/kg body weight (125 mg/ml). After 1 h, the mice were sacrificed. The blood was collected from the tip of the tail vein. The blood was centrifuged t 5000 rpm (3 min, 4 °C.) Plasma was diluted in an equal volume of PBS (pH 7.4) and the FITC-dextran concentration in the plasma was determined with a fluorescence spectrophotometer at the excitation wavelength of 485 nm and the emission wavelength of 535 nm. Standard curves for calculating the FITC-dextran concentration in the plasma were obtained by diluting FITC-dextran in nontreated plasma diluted with PBS (1:2 [vol/vol]).

Histology

For assessment of pathologic alterations, jejunum samples were fixed in 4% formalin overnight and embedded in paraffin. The tissues were cut into 4-µm thick sections and stained with hematoxylin and eosin (HE). The eosinophils and mononuclear cells in jejunum were counted under a light microscope. To avoid the observer bias, the observers were not aware of the code.

Statistics

Data are presented as mean ± SD. Difference between 2 groups was determined by Student t test or ANOVA if more than two groups. P < 0.05 was set as a significant criterion.

Results

Patients with food allergy have higher levels of IgE against HDM in the serum

We collected 825 patients at allergy clinic. As shown by Figure 1A, compared to the healthy controls, the patients with food allergy have higher levels of specific IgE against HDM. Compared to the food allergy alone group (FA), the levels of specific IgE against HDM in the food allergy with bronchial asthma group (FA+BA) or allergic rhinitis group (FA+AR) were increased significantly, while there was no significant difference between FA and bronchial asthma alone group (BA) or allergic rhinitis alone group (AR). Those with HDM-sIgE levels >0.35IU/ml were set as the IgE positive. Thus in healthy control group (HS), IgE reactivity with HDM had approximately 20.0% , while in FA, BA, AR, FA+BA and FA+AR, IgE reactivity with HDM were 65.9%, 74.1%, 84.35%, 77.5%, and 85.5% (Figure 1B).

Establishment of a mouse model of food allergy with HDM

As illustrated in Figure 2A, mice were sensitized and challenged with HDM following the procedures we previously reported.\textsuperscript{15} After sensitization, the mice were challenged intragastrically with HDM. Systemic anaphylactic symptoms were evaluated within 30 to 40 minutes. All the mice in the HDM+CT group and HDM group developed anaphylaxis (median anaphylactic score 3.3 and 2.83 respectively). On the contrary, the control mice showed no anaphylactic reactions (Figure 2B). There is a decrease in body
temperature during systemic anaphylaxis. Twenty-five minutes after HDM challenge, rectal temperature was measured. As shown in Figure 2C, mice in HDM+CT group and HDM group showed significant reductions in rectal temperature than that of control group (P < 0.001). Allergic mice presented a metabolic change that leads to significant body weight loss compared with the control group. Consequently, we observed that HDM challenged mice presented body weight loss when compared with control mice (Figure 2D). What’s more, the contour of the jejunum from the control group mice were clear and there was less inflammatory cell infiltration in the submucosa, whereas the inflamed jejunum of mice with HDM-treated revealed high levels of inflammatory cells infiltration and sloughing of enterocytes at the tips of the villi (Figure 2E-F).

**HDM facilitates Th2 immune response**

Spleen cells labeled with CFSE were cultured in the presence of HDM or saline for 72 h. The result showed that CD4+CSFE- cells in the HDM+CT group and HDM group were more abundant than that in the control group, which indicated that CD4+T cells markedly proliferated after stimulating with HDM (Figure 3A). To further investigate whether HDM can enhance Th2 immune response, splenocyte cytokine profiles were analyzed in the present study. The result demonstrated that splenocytes from HDM-treated mice produced significantly high levels of Th2 cytokines (IL-4 and IL-5), but the levels of Th1 cytokine (IFN-γ) were not different (P>0.05) (Figure 3B-D). The specific immune response to HDM was also measured by testing the serum specific immunoglobulin levels. As shown in Figure 3E-F, HDM-specific IgE and IgG1 were significantly increased in the HDM+CT group and HDM group. The mouse mast cell protease-1 (mMCP-1) is a marker of mast cell activation. As shown in Figure 3G, the mMCP-1 concentration in the serum was higher in HDM-sensitized mice than that of the control group (P < 0.001).

**Exposure to HDM facilitates development of OVA-induced intestinal allergy.**

Prior HDM exposure aggravate allergy-like symptoms, increases permeability of intestinal epithelial barrier, lower body temperature, and lower body weight in OVA-induced intestinal allergy (Figure 4B-E). The levels of sIgE, sIgG1, IL-4 and IL-5 in the HDM + OVA groups were higher than the PBS + OVA groups and control groups (Figure 4F-I). Moreover, the pathology of intestinal tract in HDM +OVA groups were more severe and exhibited more eosinophils than PBS + OVA groups and control groups (Figure 4J-K).

**Discussion**

A large amount of studies have been conducted to highlight the critical role of HDM allergen exposure particularly in respiratory allergic diseases. In contrast, HDM were known as aeroallergen and little attention was attracted in the study of food allergy. Tulic et al reported recently that HDM allergen was detected in the human gastrointestinal tract, and the intestinal barrier function was affected directly by the cysteine protease activity of HDM allergen without prior sensitisation. Nevertheless, the contribution of HDM in the pathogenesis of food allergy remains unknown. We carried out this study to elucidate that HDM is a crucial environmental trigger factor for developing food allergy.
Specific IgE antibodies play an important role in mediating type 1 allergic reaction in human.\textsuperscript{19} Specific IgE that have already bound to the surface of mast cells or basophils can be bound by food allergens to cause the secretion of the allergy-related mediators such as histamines. Subsequently, with the second exposure to the specific allergens, symptoms were usually triggered soon. In this study, we have observed that among 825 food-allergic patients, 65.9\% exhibited IgE reactivity to HDM. Compared to FA, the levels of IgE against HDM in FA + BA or FA + RA were increased significantly, while there was no significant difference between FA and BA or RA. These data indicate that HDM may be an important contributor to food allergy.

A HDM-induced mouse model of food allergy was developed successfully in the present study, and hypersensitivity was evaluated by using some well established parameters, including allergy-like symptoms scores, decreased body temperatures, heavy infiltration of inflammatory cells in the jejunal mucosa,\textsuperscript{20–21} increased serum mMCP-1 levels\textsuperscript{22} and decreased body weights,\textsuperscript{23} which demonstrate HDM induce an obvious intestinal allergy in mouse model.

Cytokines, secreted by T helper type 2 (Th2) cells, such as IL-4 and IL-5, are the major pathological feature of allergic disease, including food allergy.\textsuperscript{23} Especially, IL-4 promotes the production of allergen-specific IgE and activates mast cells to mediate type 1 inflammation in food allergy.\textsuperscript{24} Meanwhile, IL-5 plays an important role in the proliferation, recruitment and activation of eosinophils, and then promotes the development of type 1 inflammation.\textsuperscript{25} In this study, we found that mice exposed to HDM showed significantly higher levels of Th2 cytokine IL-4 and IL-5, but not T helper type 1 (Th1) cytokine IFN-\gamma, indicating that HDM facilitates Type 2 inflammation in mice.

CT, secreted by Vibrio cholera, which is a potent mucosal adjuvant for stimulating allergen-specific immune response,\textsuperscript{26} is also considered as a potent Th2 adjuvant because it stimulates production of Th2 cytokines and promotes specific IgE and IgG1 production.\textsuperscript{27} In the present study, we fed the mice with HDM (1 mg/mouse) in the presence of CT as an adjuvant. The group 1 allergen of Dermatophagoides pteronyssinus (Der p1) has been proven as the major allergen, the proteolytic activity of Der p1 resulted in a significant reduction in IL-12 production in dendritic cells (DCs), and that DCs induced naive T cells (Th0) to secret less Th1 cytokine and more Th2 cytokine.\textsuperscript{28} It is reported that T cell immunoglobulin mucin domain (TIM)4 expressed by antigen-presenting cells (APCs) that ligates TIM1 on Th2 cells, and TIM-1-TIM-4 interaction promote Th2 cells polarization.\textsuperscript{29} Mo LH et al reported that exposure to Der p1 induce the TIM4 gene transcription and expression in DCs,\textsuperscript{30} indicating that Der p1 can conduce DCs to produce more TIM4 and induce Th2 polarization subsequently. Tulic et al showed that Der p1 was present in the human intestine, the proteolytic activity of Der p1 resulted in disrupted of TJ proteins, reduced integrity of the mucus barrier, as well as increased permeability of epithelial cells.\textsuperscript{10} Therefore, there is a hypothesis that some HDM proteins like Der p1 can act as mucosal adjuvant, which facilitate Th2 polarization, contribute to intestinal barrier dysfunction, and increase the allergen transportation across the intestinal epithelial barrier. In this study, mice were sensitized and challenged without any adjuvant, and the result show that HDM alone induce an intestinal allergy in mice obviously. To further
test our hypothesis, an OVA food allergy followed by HDM exposure were induced in mice. Consequently, the result show that the prior exposure to HDM is able to induce intestinal barrier dysfunction, and facilitate the development of intestinal allergy in mice.

**Conclusion**

In conclusion, based on above data and previous researches, we put forward that HDM contribute to the development of food allergy.

**Abbreviations**

HDMs  
House dust mites  
HDM  
House dust mite  
mMCP-1  
mouse mast cell protease-1  
CT  
cholera toxin  
ELISA  
Enzyme-linked immunosorbent assay  
CBS  
Carbonate buffered solution  
BSA  
bovine serum albumin  
CFSE  
5,6-carboxyfluorescein diacetate, succinimidyl ester  
PMA  
horbol 12-myristate 13-acetate  
FITC  
fluorescein isothiocyanate  
HE  
hematoxylin and eosin  
Th  
T helper type  
APCs  
antigen-presenting cells  
DCs  
dendritic cells  
Der p1
The group 1 allergen of *Dermatophagoides pteronyssinus*

**Declarations**

**Ethics approval and consent to participate**

The ethics approval number from Laboratory animal ethics committee, Shenzhen Research Institute, Hong Kong Polytechnic University was #161201, and 201540 from Medical ethics committee, The First Affiliated Hospital, Guangzhou Medical University.

**Consent for publication**

Not applicable.

**Availability of data and materials**

All data generated or analysed during this study are included in this published article and its additional files.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors' contributions**

Jianli Lin and Desheng Chen designed and performed experiments, Kexin Chang analyzed data and interpreted the results. Dan Li and Lvxin Guan performed experiments. Baoqing Sun, Pingchang Yang and Zhigang Liu interpreted the results, supervised the study and edited the manuscript.

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**Figures**

**Figure 1**

All patients’ data were collected from the First Affiliated Hospital of Guangzhou Medical University. Including HS (healthy control) without allergic disease, 25 cases; FA (food allergy) without asthma, allergic dermatitis and allergic rhinitis, 403 cases; BA (bronchial asthma), 61 cases; AR (allergic rhinitis), 59 cases; FA+BA (food allergy with asthma), 223 cases; FA+AR (Food allergy with allergic rhinitis), 83 cases. (A) the levels of IgE against HDM. (B) the percentage of IgE against HDM. Those with HDM-sIgE level >0.35IU/ml were judged to be IgE positive. * p < 0.05, **p < 0.01, ***p < 0.001, (ANOVA) compared with the HS group, and &p < 0.05, &&p < 0.01 compared with the FA group.
Figure 3

HDM facilitates Th2 immune response. (A) the histograms show the proliferation of CD4+ T cells. The bars show the levels of IL-4 (B), IL-5 (C) and IFN-γ (D) in the splenocytes culture supernatant. The level of serum HDM specific IgE (E), IgG1 (F) and mMCP-1 (G) were detected using EILSA. Each group consists of six mice, with *p < 0.05, **p < 0.01, ***p < 0.001, (ANOVA) compared to the control group.
Figure 4

Exposure to HDM facilitates development of OVA-induced intestinal allergy. (A) The protocol for mouse model of HDM facilitates OVA-induce food allergy. (B) the score of allergy of mouse. no symptoms, 0; scratch, scratch head and nose, 1; eye and mouth swelling, diarrhea, reduced activity and/or reduced activity with breathing emergency urge, 2; asthma, slow breathing, mouth and tail cyanosis, 3; After stimulate or shake, there is no activity, 4; death, 5. (C) Permeability to dextran (4,000-Da) of intestinal epithelial barrier in vivo. (D) the body temperature of mouse. (E) body weight of mouse. (F-G) The bars show the levels of serum OVA specific IgE, IgG1. (H-I) The bars show the levels of IL-4, IL-5 in the splenocytes culture supernatant. (J) HE staining of jejunum. (K) the number of eosinophils in jejunum. Each group consists of six mice, with *p < 0.05, **p < 0.01, ***p < 0.001, (ANOVA) compared to the control group. &p <0.05, &&p <0.01, &&&p <0.01 (t test) was compared to the PBS + OVA group.