

Experimental food allergy to peanut enhances the immune response to house dust mite in the airways of mice

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Summary

Background Food allergy has been associated with an increased risk for the development of allergic asthma. Asthma is a risk factor for the development of an anaphylactic response to food allergens. An immunological interplay between sensitization to different allergens in different compartments of the body might be involved.

Objective To evaluate the immunological interplay between intragastrical peanut (PE) sensitization and respiratory sensitization to house dust mite (HDM) allergens.

Methods BALB/c mice were intragastrically sensitized to peanut or sham-sensitized and challenged systemically to PE. Between sensitization and challenge, mice were intranasally exposed to HDM extract or PBS, as a control. The response to HDM (eosinophil recruitment, cytokine response, HDM-specific immunoglobulins and airway hyper-reactivity) and to PE (cytokine response, mast cells in gut, mMCP-1 in serum and body temperature) was assessed.

Results A preceding PE sensitization increased HDM-induced production of IL-4, IL-5, IL-13 and IFN γ in lung-draining lymph nodes and total IgE levels in HDM-sensitized mice. However, recruitment of inflammatory cells to the airways or airway hyper-reactivity was not aggravated in PE/HDM double-sensitized mice. Alternatively, HDM-induced airway inflammation did not significantly affect the immune response or the anaphylactic response to a systemic challenge with peanut.

Conclusion and Clinical Relevance Our data show that a preceding peanut sensitization boosted IgE- and HDM-specific Th2 response in the airways in mice. It contributes to the understanding of the underlying immunological mechanism of polysensitization which often occurs in allergic individuals over time.

Keywords food allergy, house dust mite, peanut allergy

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Introduction

Atopic disorders, including food allergy and allergic asthma, have increased tremendously in the last four decades. Studies in experimental animal models have brought significant progress in the elucidation of the immunological mechanisms of the process of sensitization and allergy. Where animal models have been mainly used to study a single sensitization and associated clinical phenotype, it is evident that humans frequently suffer from multiple atopic disorders, often affecting different organs. A possible interplay between different primary sensitizations with their own affected organs has been studied very scarcely.

Affected organs can range from the skin (eczema, urticaria), the upper (runny and/or stuffed nose, sneezing) and lower (wheezing, shortness of breath) airways, the eyes (conjunctivitis, itching) and the gastro-intestinal tract (vomiting, diarrhoea, cramps, angioedema), to the cardiovascular and neurological systems (dizziness, drop in blood pressure, loss of consciousness). The organ of entry does not necessarily overlap with the organ that is affected (e.g. food can cause symptoms of skin), and multiple organs can be affected at the same time (e.g. food can induce allergic symptoms of skin, gastrointestinal tract and cardiovascular system). Allergic individuals often display symptoms in multiple organs and are sensitive to multiple allergen sources. It

is not uncommon that allergic patients have rhinoconjunctivitis and/or asthma caused by house dust mite but also food allergy to, for example, peanut.

The underlying immunological mechanism for polysensitization remains unclear. A phenomenon usually referred to as the 'atopic march' has been described, being the progression from early onset atopic dermatitis with a high comorbidity for food allergy towards allergies of the airways like asthma and rhinoconjunctivitis later in life. Several epidemiological reports suggest an association between sensitization to food allergens and an increased risk for the development of asthma. A longitudinal study in cow's milk sensitized children at the age of 7 months, showed an increased airway inflammation and higher bronchial responsiveness to histamine at 8 years of age compared to control children without cow's milk sensitization [1]. Patelis et al. [2] showed in a population-based study that simultaneous sensitization to food allergens and respiratory allergens strongly increased the risk for the development of asthma compared to sensitization to respiratory allergens exclusively (OR = 18.3 vs. OR = 5.6). Tariq et al. [3] demonstrated that egg allergy in infancy predicts respiratory allergic disease at 4 years of age. The prevalence of food IgE sensitization is up to 45% in asthmatics [4, 5] suggesting an immunological interplay. It is well established that asthma accompanying food allergy is a risk factor for the occurrence of anaphylaxis [6, 7], but the mechanism by which asthma contributes to this systemic response has not been elucidated.

In this study, we aimed to develop a murine model to study the possible interplay between a non-cross-reactive food and respiratory allergy. To that end, mice were intragastrically sensitized with peanut and after one month, systemically challenged i.p. with peanut. Between sensitization and challenge, mice were exposed to house dust mite (HDM) via the airways or to PBS. This order was chosen because food allergy often precedes the development of asthma in humans. Cellular and humoral immune responses to peanut and HDM were examined as well as the subsequent clinical manifestations.

Methods

Animals

Female Balb/c mice (Harlan, Horst, the Netherlands) were housed under specific pathogen-free conditions at the animal facility of the AMC. All experiments were approved by the animal ethics committee of the AMC, the Netherlands.

Peanut extract (PE) preparation

Whole roasted peanuts (O'Lacy International B.V., the Netherlands) were ground, and protein was extracted by

mixing 50 g of peanuts with 500 mL 20 mM Tris buffer. After overnight incubation under agitation at 4°C, the insoluble particulate matter was removed by centrifugation (10.000 g for 20 min), and the supernatant was centrifuged again (2.460 g for 30 min) to remove residual particles. The protein concentration (16.6 mg/mL) was determined using a Pierce BCA protein Assay kit, using bovine serum albumin (BSA) as a standard following the manufacturer's instruction (Pierce, Thermo Scientific, Landsmeer, The Netherlands). The extract contained 0.32 mg Ara h 1/mg total protein and 32.6 µg Ara h 2/mg total protein, as determined by ELISA (Ara h 1 and Ara h 2 ELISA kit, Indoor Biotechnologies, Cardiff, UK).

Sensitization protocol

To co-sensitize mice to peanut and house dust mite (PE/HDM mice), BALB/c mice were first sensitized intragastrically with 100 µg of PE mixed with 10 µg of staphylococcal enterotoxin B (SEB), from *Staphylococcus aureus* (Sigma-Aldrich, Zwijndrecht, the Netherlands) as previously described [8], on days 0, 3, 7, 10, 14, 17 followed by intranasal administration of 100 µg HDM (Greer Laboratories, Lenoir, NC, USA) on days 35, 42, 49, 50 and 51. As controls, one group of mice was sensitized to PE but not to HDM (PE/PBS mice), one group was exposed to HDM and not to PE (PBS/HDM mice) and one group was not sensitized to PE or HDM (PBS/PBS mice). On day 52, all mice were challenged intraperitoneally with 500 µg of PE and core body temperature was determined. On day 53, airway hyperactivity was assessed. For the ex vivo restimulation of splenocytes with PE (Fig. 3b), mice were i.g. sensitized to PE and challenged with PE at day 52 and sacrificed at day 54, as described above.

Core body temperature

The core body temperature was measured with a handheld device with a mouse rectal probe (Physitemp Instruments Inc, Clifton, NJ, USA) every 10 min after intraperitoneal administration of 500 µg PE.

Airway hyperresponsiveness (AHR)

AHR to inhaled methacholine was measured by barometric plethysmography in conscious mice (EMKA Technologies, Paris, France). Nonspecific responsiveness was measured by exposing mice to aerosolized PBS to set a baseline value, followed by increasing concentrations of aerosolized methacholine (3.125, 6.25, 12.5, 25 and 50 mg/mL in PBS for 3 min; Sigma-Aldrich) using ultrasonic nebulizers. PenH (enhanced pause) values were measured during 5 min after each methacholine aerosol.

Broncho-alveolar lavage fluid (BALF)

Inflammatory cells were obtained from the airway lumen by three subsequent washes with 1 mL PBS containing 0.1 mM EDTA after intratracheal cannulation. Cell differentiation was carried out by FACS analysis as described elsewhere [9].

Serum immunoglobulins and murine mast cell protease-1

Serum was analysed for the level of total and HDM or peanut-specific IgE and IgG1 by ELISA (IgE: Opteia, BD, San Diego, CA, USA, IgG1: "Ready-SET-Go!", eBioscience Inc., via Immunosource, Halle-Zoersel, Belgium). In short, Nunc Maxisorp plates were coated with 50 µg PE or 100 µg HDM (equivalent to 1 µg/mL Der p 1) per mL at 4°C overnight. After blocking with 10% fetal calf serum (FCS) in PBS, serum samples were incubated for two hours. Total and allergen-specific immunoglobulins were determined following the manufacturer's instructions. We used a standard curve of murine IgE and IgG1 as reference. MMCP-1 in serum was determined by ELISA (MMCP-1: "Ready-SET-Go!" kit, eBioscience Inc.).

Ex vivo re-stimulation of spleen and lymph node cells

Spleens and lung-draining lymph node cell suspensions were plated in 96-well round-bottom plates at a density of 2×10^5 cells per well and were re-stimulated for 4 days with 100 µg/mL HDM or 200 µg/mL peanut extract. Supernatants were analysed for IL-4, IL-5, IL-13 and IFN γ production by ELISA (Ready-set-go!, eBioscience Inc.).

Histology

Frozen lung sections (6 µm) were stained with periodic acid Schiff's reagent (Sigma-Aldrich). Degree of inflammation and mucus producing goblet cells was semi-quantified as described before [10]. Both small and large intestines were flushed with PBS before they were inflated with OCT Tissue Tek (Sakura Finetek Europe, Zoeterwoude, the Netherlands) before they were snap frozen in liquid nitrogen. Frozen intestinal sections (6 µm) were stained with Toluidine Blue O (Sigma-Aldrich). Number of mast cells (toluidine blue-stained cells) was determined per cm² of tissue using light microscope and software Leica Application Suite (LAS), V3, Heerbrugg, Switzerland.

Statistical analysis

For statistical analysis, Mann-Whitney *U*-test was performed and represented as $\pm$ SEM. All experiments were performed 2–4 times with five animals per group.

Results from all experiments were pooled for statistical analysis. Differences were considered to be significant at $P < 0.05$.

Results

Prior sensitization to peanut enhanced subsequent Th2 response to HDM

To investigate whether sensitization to peanut via the gastrointestinal mucosa would prime sensitization to HDM via the lung mucosa or vice versa, mice were exposed to both allergens as shown in Fig. 1a. Two validated models for respiratory allergy to HDM and gastrointestinal allergy to peanut were applied in the same mouse [8, 11]. Mice sensitized to both HDM and PE had a higher level of total IgE compared with single sensitized mice. However, no significant increase was observed in allergen-specific immunoglobulins in HDM-/PE-sensitized mice compared with mono-sensitized mice, except for a non-significant increase in PE-specific IgE response (Fig. 1b,c). To determine whether the HDM-specific T cell response was altered by the preceding PE sensitization, lung-draining mediastinal lymph node (LLN) cells were re-stimulated *in vitro* with HDM. Production of IL-4, IL-5, IL-13 and IFN γ by LLN cells of mice sensitized to peanut (PE/HDM) was significantly increased in comparison with mice without a peanut allergy (PBS/HDM) (Fig. 2a).

As the mediastinal lymph nodes drain both the lung and the peritoneum, we restimulated LLN cells *ex vivo* with PE to investigate the T cell response to the systemic challenge with PE. As expected, HDM-sensitized mice (PBS/HDM) did not show any cytokine production, while both PE/PBS and PE/HDM mice showed an increase in IL-4, IL-5 IL-13 and IFN γ production compared with PBS/PBS mice (Fig. 2b). Double-sensitized mice (PE/HDM) showed a similar trend for an increase in the level of cytokines induced by PE, as was seen for HDM, in comparison with single sensitized PE/PBS mice, albeit in much lesser extent and significance was not reached. We hypothesized that the intraperitoneal challenge with PE at day 52 activated circulating PE-specific memory T cells in the LLN, after the i.g. priming to PE. To confirm the presence of circulating PE-specific T cells, mice were sensitized i.g. to PE or with PBS as a control and challenged with PE i.p. at day 52. Spleen cells were harvested at day 54 (Fig. 1a) and restimulated with PE. The cytokine production of IL-4, IL-5, IL-13, IFN γ was significantly increased in PE-sensitized mice in comparison with PBS mice (Fig. 3), indicative of circulating memory T cells after i.g. priming and subsequent i.p. challenge.

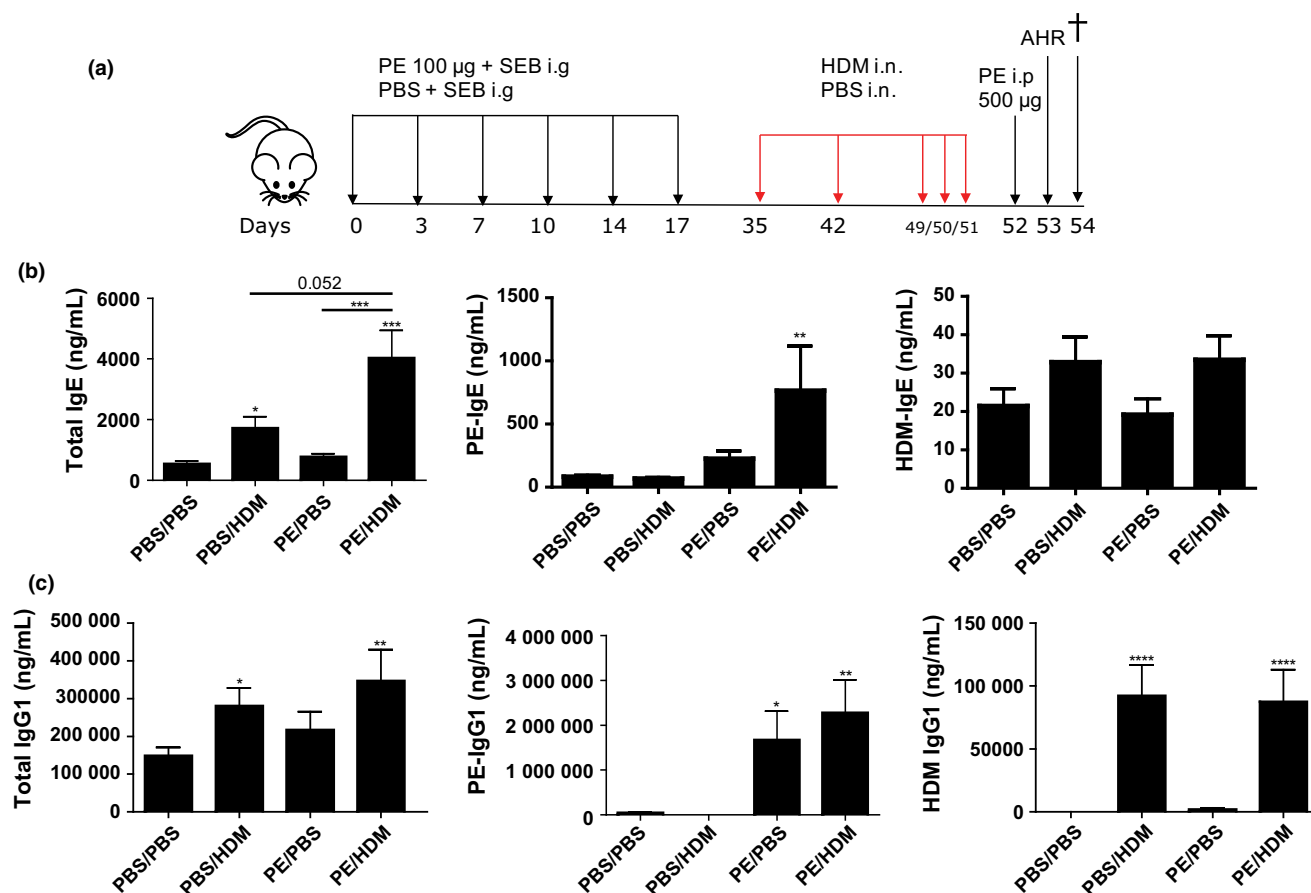


Fig. 1. Immunoglobulin levels in serum after peanut and HDM exposure. BALB/c mice were sensitized i.g. with 100 µg/mouse of PE or PBS mixed with 10 µg of staphylococcal enterotoxin B (SEB). Mice were challenged i.p. with 500 µg of PE at day 52. In between PE sensitization period and PE challenge, the same mice received intranasally, 100 µg/mouse of HDM or PBS (a). Mice were killed at day 54 and total and allergen-specific IgE (b) and IgG1 (c) were determined. Data are presented as means $\pm$ SEM, * P < 0.05, ** P < 0.01, *** P < 0.001 vs. PBS/PBS.

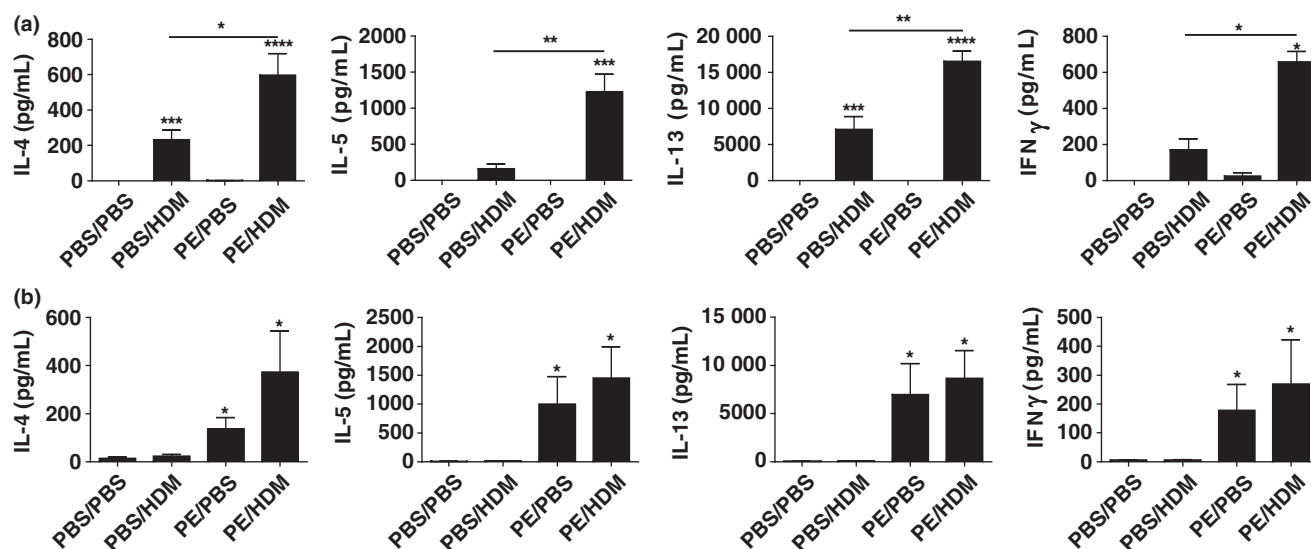


Fig. 2. Allergen induced cytokine production in LLN. Production of IL-4, IL-5, IL-13 and IFN γ in supernatants of lung-draining lymph node cells (LLN) after a) ex vivo restimulation with HDM and b) after ex vivo restimulation with PE. Data are presented as means $\pm$ SEM, * P < 0.05, ** P < 0.01, *** P < 0.001 vs. PBS/PBS.

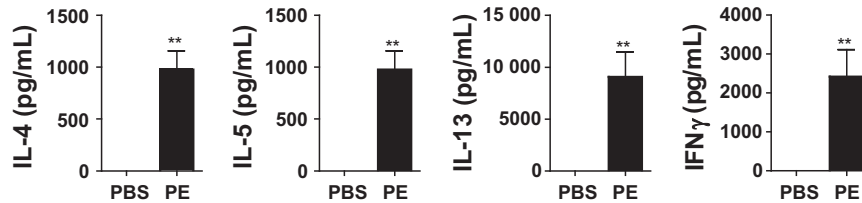


Fig. 3. Peanut induced cytokine production by splenocytes. Production of IL-4, IL-5, IL-13 and IFN γ in supernatants of splenocytes after *ex vivo* restimulation with PE. Data are presented as means $\pm$ SEM, ** $P < 0.01$.

Effect of peanut sensitization on HDM-induced airway inflammation and AHR

Both PBS/HDM and PE/HDM mice showed a clear recruitment of eosinophils, B and T cells to the

broncho-alveolar compartment in response to inhaled HDM while PBS/PBS or PE/PBS mice did not (Fig. 4a). A small and not significant increase was seen in the recruitment of eosinophils in HDM challenged mice with a peanut allergy compared to HDM-sensitized mice

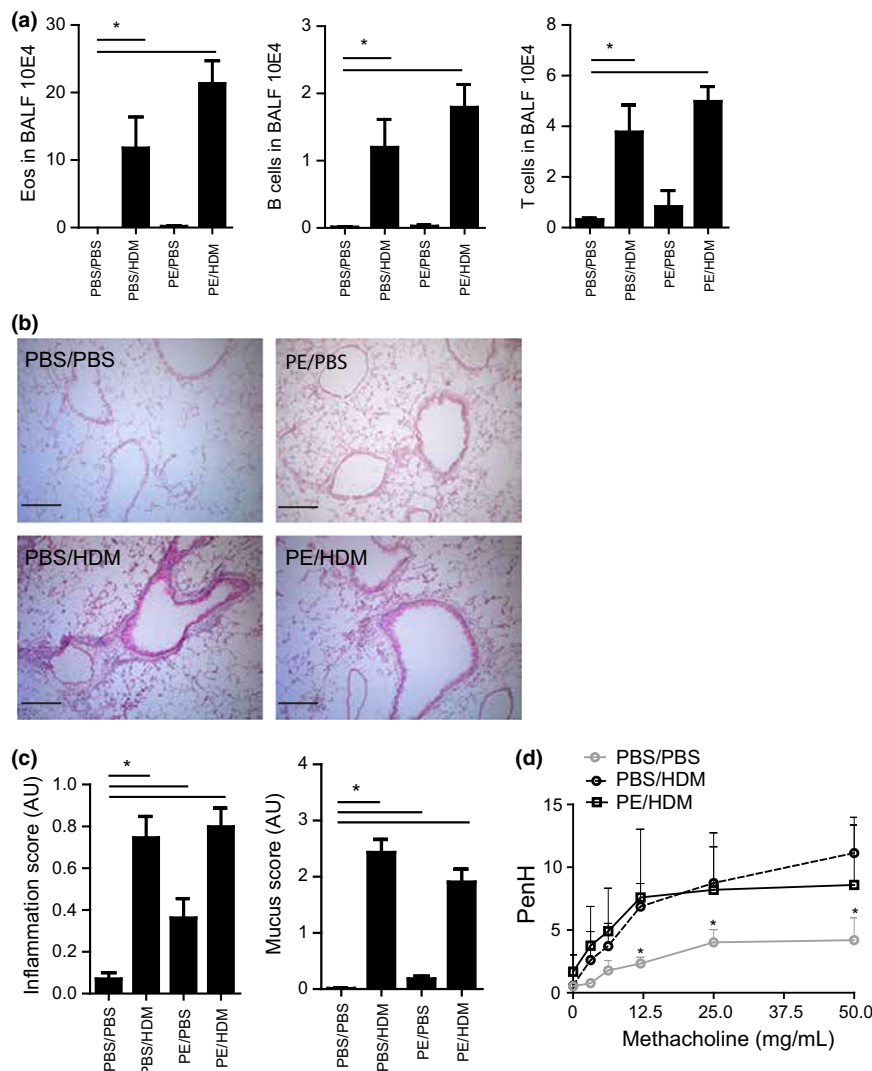


Fig. 4. Lung inflammation and airway hyperactivity. Inflammatory cell recruitment to the airways (a). Lung slides shown at $\times 10$ were stained with periodic acid Schiff's reagent to determine peri-bronchial inflammatory infiltrates and mucus production (b) and quantified (c). Airway hyperactivity to methacholine was determined on day 53 (d). Data are presented as means $\pm$ SEM, * $P < 0.05$.

(PBS/HDM) without a peanut allergy. Although in the PE/PBS group, cell counts in BALF did not show an increase in cell recruitment, analysis of the airway histology revealed a moderate, but significant, increased influx of inflammatory cells and a weak increase in mucus production in the lungs (Fig. 4b,c) compared with PBS/PBS control mice.

To investigate whether airway hyper-reactivity would be increased in PE/HDM mice in comparison with PBS/HDM mice, AHR to methacholine was determined. Despite the increased Th2 cytokine production, preceding peanut sensitization and challenge did not increase PenH values (Fig. 4d).

No effect of HDM-induced airway inflammation on the anaphylactic response to peanut

Because asthma is a risk factor for the development of an anaphylactic response to food which manifests as life-threatening bronchospasm, we investigated in our model whether PE/HDM mice would react more severely to a systemic challenge with 500 µg PE i.p. compared with PE/PBS mice. PE/PBS mice developed a hypersensitivity reaction which is displayed by a severe drop in body temperature reaching an almost fatal temperature (towards 30°C). PE/HDM mice showed a similar drop in temperature (Fig. 5a).

As mast cells in the gut mucosa have been associated with an anaphylactic response [12], the number of mast cells in the gut was determined. No significant differences could be observed, but in PE/HDM mice, mast cell numbers were slightly increased compared with PE/PBS

mice (Fig. 5b). As a marker for mast cell activation, the concentration of murine mast cell protease 1 (mMCP-1) in serum was determined (Fig. 5c). PE/HDM mice had higher levels of mMCP-1 in serum compared with PE/PBS mice. In HDM/PE mice, a significant positive correlation between the level of total IgE and the number of mast cells in the gut was observed, indicating that polysensitization might be associated with an increased number of mast cells in the gut mucosa (Fig. 5d).

Discussion

Several epidemiological studies have suggested that food allergy is associated with an increased risk to develop allergic airway disease [3, 13, 14]. The present study experimentally demonstrated interplay between the adaptive immune response evoked by exposure to peanut via the gastrointestinal mucosal tract and the immune response evoked by HDM via the airway mucosa.

We developed a model in which peanut allergic sensitization was followed by sensitization to HDM. Our model showed that the main hallmarks for both peanut allergy and HDM allergic asthma were successfully induced in the same mouse. PE and HDM co-sensitized mice developed a clear Th2 response to PE and HDM and showed increased PE-specific and HDM-specific IgE and IgG1 in serum. In addition, mice developed anaphylaxis when peanut was systemically injected. At the same time, they also displayed all characteristics of allergic asthma, such as increased airway hyper-reactivity to methacholine, eosinophilic airway inflammation

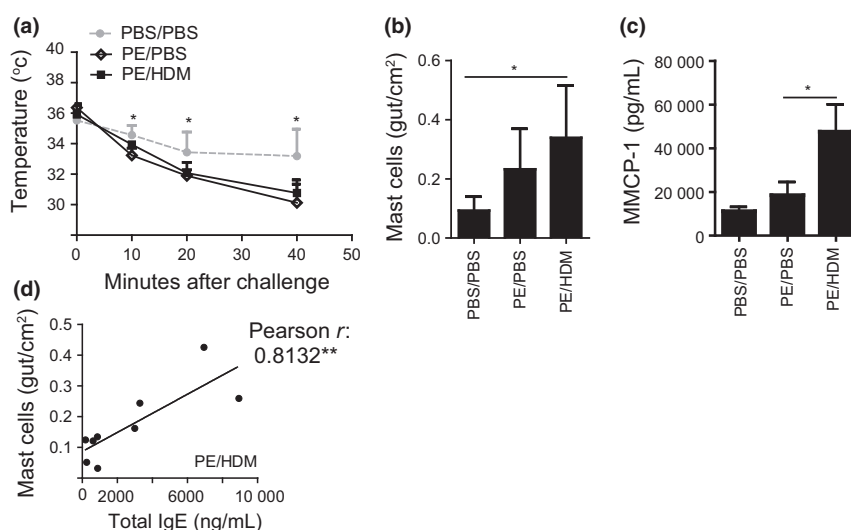


Fig. 5. Anaphylactic response and numbers of mast cells in gut tissue. Core body temperature was measured after i.p. PE challenge on day 52 (* $P < 0.05$: PE/PBS and PE/HDM vs. PBS/PBS) (a). Number of mast cells in the gut was determined by toluidine blue staining intestinal tissue sections and represented as number of cells/per cm² of tissue (b). mMCP-1 levels in serum (c). Correlation between serum level of total IgE and mast cell numbers (number of cells/cm²) in the gut of PE/HDM mice (d). Data are presented as means $\pm$ SEM, * $P < 0.05$, ** $P < 0.01$.

and increased mucus production and peri-bronchial infiltrates. Interestingly, mice with PE and HDM allergy produced higher levels of Th2 cytokines in response to HDM compared to HDM-mono-sensitized mice.

These findings raise the question how PE allergy might have boosted the HDM-specific T cells. We could detect peanut-specific T cells in LLN and spleen after intragastric sensitization to peanut. It is tempting to speculate that unspecific bystander activation of HDM-specific T cells by these circulating PE-specific T cells has led to a higher cytokine production by the HDM-specific T cells. The mechanism of bystander stimulation is a phenomenon primarily described for CD8⁺ T cells [15, 16], but which has also been clearly demonstrated for CD4⁺ T cells [17, 18]. Factors produced during the primary and secondary antigen-specific response, such as IL-2 and IL-15, activate unrelated effector CD4⁺ T cells [18]. As the mediastinal lymph nodes drain both the lung and the peritoneum [19], the i.p. injection with peanut can have activated circulating peanut-specific T cells in the LLN which then consequently contributed to an increased cytokine production by HDM-specific T cells. These data suggest that activation of T cells by exposure to multiple allergens at the same time might have a synergistic effect, which results in an increased cytokine response. Alternatively, we cannot rule out that PE-specific T cells might have been induced in LLN due to unwanted peanut sensitization via the lungs caused by an accidental delivery of PE during gavage. Translating our experimental data to real life, we can speculate that in peanut allergic humans circulating peanut-specific Th2 cells might trigger the activation/expansion of Th2 cells which are specific for a second unrelated allergen, like derived from house dust mite.

Although preceding peanut sensitization increased HDM-specific Th2 cytokine production, this did not translate into aggravated lung inflammation or AHR. Based on the current data set, we can only speculate why the clinical phenotype does not follow the immunological changes. Perhaps the increase in Th2 cytokines is too small to have a clinical impact. Brandt et al. [20] and Bihouée et al. [21] both demonstrated that mice with an OVA alum-induced gastrointestinal allergy developed an increased AHR and eosinophil recruitment after intranasal HDM exposure compared with mice without gastrointestinal allergy. The route of sensitization to the food allergen (gastrointestinal vs. i.p.) and the choice of food allergen (peanut vs. OVA) as well as the type of adjuvant (SEB vs. alum) might have played a role. In contrast, we demonstrated an enhanced Th2 cytokine production after ex vivo restimulation of lung-draining lymph nodes cells with HDM while Brandt et al. did not observe an enhanced Th2

cytokine production. In support of our data, Bihouée et al. observed an increase in IL-4 and IL-5 in BALF but did not determine HDM-induced Th2 cytokines in lymph nodes. The lack of an aggravation of eosinophilia could be explained by our high dose HDM model. This might have induced such a strong innate immune response resulting in recruitment of inflammatory cells and AHR [22] which overwhelmed the boosting effect of the preceding peanut sensitization on the adaptive immune response to HDM. It is important to note that AHR is multifactorial process which does not have a linear correlation with IL-5 or the IgE level. However, future experiments might reveal an effect on AHR when a suboptimal dose of HDM would be used. The preceding peanut allergy might lower the threshold for the induction of a HDM-induced inflammation, allowing to envisage an effect at AHR.

Asthma is a risk factor for the development of an anaphylactic response to food allergens. In our model, PE/HDM mice did have a slight increase in mast cells in the gut which correlated with the level of total IgE. The intestinal recruitment and activation of mast cells has been reported to be dependent on IgE [23, 24]. In support of an enhanced mast cell degranulation, also the level of MMCP-1 in serum was increased compared with PE/PBS mice but this did not result in a more severe drop in temperature. We speculate that the maximal drop in temperature was yet reached and could not be aggravated any further. An alternative explanation might be that hypothermia in mice is not solely dependent on IgE but also on IgG1 [25]. The PE/HDM mice did not show an increased PE-IgG1 level compared to PE/PBS mice.

In conclusion, our data show that allergy to peanut boosted the HDM-specific Th2 cytokine response in the airways. Further research is warranted to clarify the underlying mechanisms of T cell boosting in mice expressing concomitant food and respiratory allergy and whether secondary allergic sensitization in allergic individuals is facilitated by the mechanism of bystander activation of CD4⁺ T cells.

Conflict of interest

The authors declare no conflict of interest.

Research grants/financial disclosure

All the authors declare no competing financial interests.

Authorship credits

LU, RvR and LvR conceived and designed the study. LU, AL and LvR contributed to acquisition of data.

References

- 1 Malmberg LP, Saarinen KM, Pelkonen AS, Savilahti E, Makela MJ. Cow's milk allergy as a predictor of bronchial hyperresponsiveness and airway inflammation at school age. *Clin Exp Allergy* 2010; **40**:1491–7.
- 2 Patelis A, Gunnbjornsdottir M, Malinovschi A *et al*. Population-based study of multiplexed IgE sensitization in relation to asthma, exhaled nitric oxide, and bronchial responsiveness. *J Allergy Clin Immunol* 2012; **130**:397–402.
- 3 Tariq SM, Matthews SM, Hakim EA, Arshad SH. Egg allergy in infancy predicts respiratory allergic disease by 4 years of age. *Pediatr Allergy Immunol* 2000; **11**:162–7.
- 4 Wang J, Visness CM, Sampson HA. Food allergen sensitization in inner-city children with asthma. *J Allergy Clin Immunol* 2005; **115**:1076–80.
- 5 Liu AH, Jaramillo R, Sicherer SH *et al*. National prevalence and risk factors for food allergy and relationship to asthma: results from the National Health and Nutrition Examination Survey 2005–2006. *J Allergy Clin Immunol* 2010; **126**:798–806.
- 6 Gonzalez-Perez A, Aponte Z, Vidaurre CF, Rodriguez LA. Anaphylaxis epidemiology in patients with and patients without asthma: a United Kingdom database review. *J Allergy Clin Immunol* 2010; **125**:1098–104.
- 7 Koplin JJ, Martin PE, Allen KJ. An update on epidemiology of anaphylaxis in children and adults. *Curr Opin Allergy Clin Immunol* 2011; **11**:492–6.
- 8 Ganeshan K, Neilsen CV, Hadsaitong A, Schleimer RP, Luo X, Bryce PJ. Impairing oral tolerance promotes allergy and anaphylaxis: a new murine food allergy model. *J Allergy Clin Immunol* 2009; **123**:231–8.
- 9 van Rijt LS, Kuipers H, Vos N, Hijdra D, Hoogsteden HC, Lambrecht BN. A rapid flow cytometric method for determining the cellular composition of bronchoalveolar lavage fluid cells in mouse models of asthma. *J Immunol Methods* 2004; **288**:111–21.
- 10 van Rijt LS, Vos N, Willart M *et al*. Persistent activation of dendritic cells after resolution of allergic airway inflammation breaks tolerance to inhaled allergens in mice. *Am J Respir Crit Care Med* 2011; **184**:303–11.
- 11 van Rijt LS, Logiantara A, Utsch L, Canbaz D, Boon L, van Ree R. House dust mite allergic airway inflammation facilitates neosensitization to inhaled allergen in mice. *Allergy* 2012; **67**:1383–91.
- 12 Arias K, Chu DK, Flader K *et al*. Distinct immune effector pathways contribute to the full expression of peanut-induced anaphylactic reactions in mice. *J Allergy Clin Immunol* 2011; **127**:1552–61.
- 13 Rhodes HL, Sporik R, Thomas P, Holgate ST, Cogswell JJ. Early life risk factors for adult asthma: a birth cohort study of subjects at risk. *J Allergy Clin Immunol* 2001; **108**:720–5.
- 14 Leung TF, Lam CW, Chan IH, Li AM, Tang NL. Sensitization to common food allergens is a risk factor for asthma in young Chinese children in Hong Kong. *J Asthma* 2002; **39**:523–9.
- 15 Tough DF, Sun S, Sprent J. T cell stimulation *in vivo* by lipopolysaccharide (LPS). *J Exp Med* 1997; **185**:2089–94.
- 16 Tough DF, Borrow P, Sprent J. Induction of bystander T cell proliferation by viruses and type I interferon *in vivo*. *Science* 1996; **272**:1947–50.
- 17 Di GG, Savelyeva N, Suchacki A, Thirdborough SM, Stevenson FK. Bystander stimulation of activated CD4⁺ T cells of unrelated specificity following a booster vaccination with tetanus toxoid. *Eur J Immunol* 2010; **40**:976–85.
- 18 Bangs SC, Baban D, Cattani HJ, Li CK, McMichael AJ, Xu XN. Human CD4⁺ memory T cells are preferential targets for bystander activation and apoptosis. *J Immunol* 2009; **182**:1962–71.
- 19 Kool M, Soullie T, van Nimwegen M *et al*. Alum adjuvant boosts adaptive immunity by inducing uric acid and activating inflammatory dendritic cells. *J Exp Med* 2008; **205**:869–82.
- 20 Brandt EB, Scribner TA, Akei HS, Rothenberg ME. Experimental gastrointestinal allergy enhances pulmonary responses to specific and unrelated allergens. *J Allergy Clin Immunol* 2006; **118**:420–7.
- 21 Bihouee T, Bouchaud G, Chesne J *et al*. Food allergy enhances allergic asthma in mice. *Respir Res* 2014; **15**:142.
- 22 Hammad H, Chieppa M, Perros F, Willart MA, Germain RN, Lambrecht BN. House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat Med* 2009; **15**:410–6.
- 23 Bartnikas LM, Gurish MF, Burton OT *et al*. Epicutaneous sensitization results in IgE-dependent intestinal mast cell expansion and food-induced anaphylaxis. *J Allergy Clin Immunol* 2013; **131**:451–60.
- 24 Galli SJ, Tsai M. IgE and mast cells in allergic disease. *Nat Med* 2012; **18**:693–704.
- 25 Miyajima I, Dombrowicz D, Martin TR, Ravetch JV, Kinet JP, Galli SJ. Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and Fc gammaRIII. Assessment of the cardiopulmonary changes, mast cell degranulation, and death associated with active or IgE- or IgG1-dependent passive anaphylaxis. *J Clin Invest* 1997; **99**:901–14.