

Epicutaneous Exposure to Peanut Oil Induces Systemic and Pulmonary Allergic Reaction in Mice

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Keywords

Asthma · Atopic dermatitis · Epicutaneous sensitization · Peanut allergy · Peanut oil

Abstract

Background: The prevalence of peanut allergy (PA) is constantly on the rise. Atopic dermatitis (AD) is a major risk factor for developing food allergy. Some bath oils and skin creams used for treating AD contain peanut oil, and it has been suggested that exposure to peanut allergens through a disrupted skin barrier is a potential cause of PA. Our aim was to investigate whether application of peanut oil to irritated skin causes a systemic or respiratory allergic response to peanuts in an animal model. **Methods:** BALB/c mice underwent epicutaneous sensitization with either peanut oil (PM, $n = 9$) or phosphate buffered solution (controls, $n = 9$) daily for 5 consecutive days. Ten days after the last exposure the mice were challenged with intranasal peanut protein for 5 consecutive days. Bronchial alveolar lavage fluid was collected for cellular studies and measurement of cytokine levels. Sera were collected for immunoglobulin E (IgE) measurement. **Results:** Epicutaneous peanut oil sensitization in-

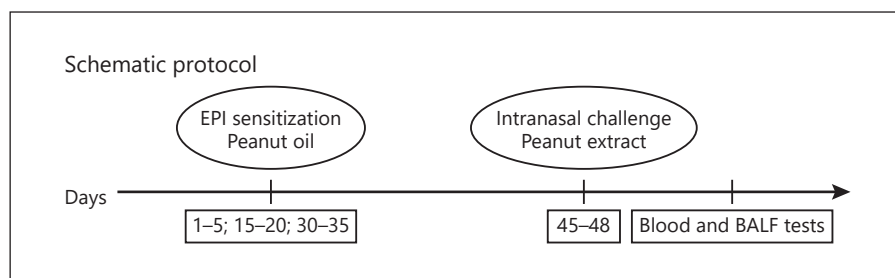
creased leukocyte and eosinophil counts and interleukin-13 levels ($p = 0.003$, $p = 0.0006$ and $p = 0.03$, respectively), in addition to increasing total serum IgE ($p = 0.03$). **Conclusions:** The results suggest that topical application of peanut oil may play a role in the etiology of PA. © 2019 S. Karger AG, Basel

Introduction

The worldwide prevalence of peanut allergy (PA) among children has increased in the last 20 years, reaching rates of 0.6–4.6% [1–5]. PA develops early in life and is a life-threatening condition. Family history of atopy and the presence of atopic dermatitis (AD) are risk factors for the development of food allergy. The early introduction of peanuts into the diet significantly decreases the frequency of PA among high-risk children, and modulates the immune responses to peanuts [6, 7]. Interestingly, early peanut sensitization is observed in infants

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Fig. 1. Epicutaneous exposure of peanut oil in mice. Schematic protocol. Abdominal skin was tape stripped and immediately exposed to peanut oil or PBS. The mice underwent 3 series of sensitization for 5 consecutive days followed by 10-day intervals, for a total of 15 exposures. Challenge: Ten days after the last exposure, all mice were intranasally challenged with peanut extract daily for 4 days.



with no history of dietary peanut consumption [6]. The skin of those infants may have been exposed to peanuts through the application of peanut oil-containing ointments and creams [8]. An association between PA and early epicutaneous exposure to peanuts in creams and oils among children was demonstrated in a longitudinal study [9], and the effect of epicutaneous peanut exposure was reported to be augmented in children with AD [10].

PA was also reported in children and infants fed with infant formula containing peanut oil [11, 12]. Olszewski et al. [13] demonstrated the presence of allergenic proteins large enough to bind to immunoglobulin E (IgE) in crude, neutralized, and refined peanut oils. Eight protein bands were observed by Western blot analysis of serum from allergic patients. The protein extracts bound specific IgE from patient sera, stimulated histamine release in vitro and were associated with positive skin-prick tests [13, 14]. Oral food challenge with crude peanut oil resulted in a positive allergic reaction in 10% of peanut allergic subjects [15].

Anaphylactic reactions to eggs can be induced by epicutaneous sensitization to ovalbumin in mice [16]. Epicutaneous sensitization to peanut protein has also been demonstrated in a mouse model, and the effect was augmented by the use of Triclosan, an antiseptic additive included in many cosmetic preparations [17]. The effect of epicutaneous peanut oil application on sensitization to peanut protein has not been thus far reported in an animal model. The aim of this study was to determine whether topical application of peanut oil to irritated skin can induce both a systemic and a pulmonary allergic response to the peanut protein.

Methods and Materials

Mice

BALB/c female 7-week-old mice were purchased from Harlan Laboratory, Israel. All the animals were maintained in the animal facility at Tel Aviv Sourasky Medical Center. Standard guidelines

for laboratory animal care set down by the Israeli Ministry of Health were followed, and institutional permission for animal handling in our animal facilities was approved.

Sensitization and Challenge

Epicutaneous sensitization of mice. The abdominal fur of each mouse was removed by shaving. The abdominal skin was tape stripped 3 times and immediately exposed to 50 μ L of either peanut oil (peanut mice, PM; Olitalia-Italy) or phosphate buffered solution (PBS, Sigma, USA). The mice were exposed daily for 5 consecutive days, followed by a 10-day interval: this schedule was repeated 2 more times for a total of 15 exposures (illustrative protocol Fig. 1).

Allergen challenge. Peanut extract was obtained from peanut flour (Byrd Mill, USA; Fig. 1). Ten days after the last epicutaneous exposure, mice were subjected to intranasal challenge to peanut extract (100 μ g/mL in 50 μ L of PBS) once daily for 4 days.

Bronchoalveolar Lavage Fluid

In order to collect bronchoalveolar lavage fluid (BALF), the trachea of the anesthetized mouse was surgically exposed, cannulated with a syringe, and flushed twice with 1 mL of PBS. Cells in the lavage fluid were counted by hemacytometer, and BALF cell differentials were determined on slide preparations stained with May-Grünwald-Giemsa and counted in 15–20 high-power fields under light microscopy (Leica DM 4000B). The supernatants were stored at -20°C for cytokine analysis.

Measurement of Serum Total IgE Antibodies

Blood was obtained from each group of mice via the retro-orbital vein immediately after the last sensitization point and 24 h after challenge. Sera were separated and stored at -20°C . Serum IgE levels were measured using commercial ELISA according to the manufacturer's instructions (Bethyl Laboratories, Inc., USA). Sera samples were diluted at 1:20 for the analysis of the IgE levels.

Cytokine Quantification

Concentrations of interleukin-4 (IL-4) and IL-13 in BALF were determined using commercially available ELISA kits (DuoSet e-bioscience Kit, USA). ELISA kits were used according to the manufacturer's instructions.

Statistical Analysis

Statistical analyses were performed using the Mann Whitney U test (non-parametric data). A p value <0.05 was considered statistically significant. All statistical analyses were performed using Prism Graph Software.

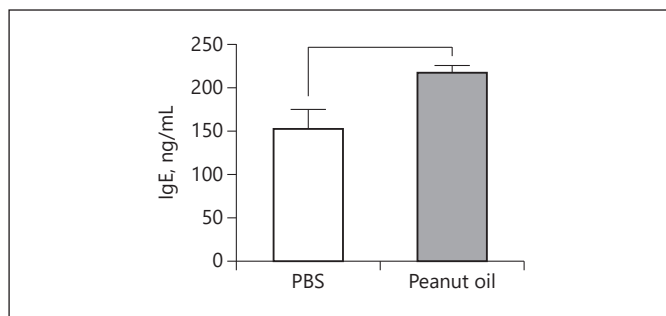


Fig. 2. Exposure of mouse skin to peanut oil increased immunoglobulin E (IgE) levels. Serum IgE was obtained from PM and controls groups 24 h after the last challenge. The results were obtained from 2 experiments (9 animals per group).

Results

Peanut Oil Exposure Increased Serum IgE

The effect of epicutaneous peanut oil exposure on systemic allergic responses was measured by total serum IgE levels 24 h after the last antigen exposure. The PM group ($n = 9$) showed significantly higher levels of total IgE compared to the control group ($n = 9$; $p = 0.03$; (Fig. 2). No peanut-specific IgE was detected.

Peanut Oil Exposure Increased Pulmonary Leukocyte and Eosinophil Counts

Pulmonary allergic inflammation following epicutaneous sensitization of peanut oil and intranasal challenge of peanut protein were measured by BALF leukocyte and eosinophil counts, both of which were significantly higher in the PM group ($p = 0.003$ and $p = 0.0006$, respectively; Fig. 3a–c).

Peanut Oil Exposure Increased BALF IL-13 Levels

In order to better characterize lung inflammation, we examined type 2 cytokine release in the BALF. Increased levels of IL-13 were observed in PM compared to controls ($p = 0.035$; Fig. 4). There were only trace amounts of IL-4 in BALF of both groups.

Discussion

Irritated or inflamed skin is an important route of antigen penetration and sensitization. We demonstrated that the application of peanut oil onto irritated skin followed by peanut extract challenge induced a systemic allergic response as well as a subsequent airway inflamma-

tory response in mice. AD is a common condition in childhood, often associated with IgE-mediated sensitization to environmental antigens. It is considered the initial step of the “atopic march,” progressing to food allergy, allergic rhinitis, and asthma [18].

The pathophysiology of atopic asthma is characterized by elevated serum IgE levels, airway eosinophilia, bronchial hyper-responsiveness, inflammatory cell infiltration, and goblet cell metaplasia in the airways. Th-2-type cytokines, IL-4, IL-5, and IL-13 are thought to play a pivotal role in this allergic process [19].

Previous studies have shown that epicutaneous exposure of mice to allergens results in sensitization when the skin was even slightly damaged. Kang et al. [20] and Yu et al. [21] demonstrated that epicutaneous sensitization with ovalbumin (OVA) induced a strong allergic response and pulmonary asthma in mice as expressed by elevated levels of serum OVA-specific IgE and the BALF eosinophils IL-4, IL5, and IL-13. Noti et al. [22] showed that sensitization with OVA and peanut protein on stripped skin induced symptoms of food allergy in both food allergens, but only OVA-specific IgE and not peanut-specific IgE were reported. Similarly in our study, we could not detect elevations in peanut-specific IgE. This could be because peanut-specific IgE was a small fraction of total IgE elevation and sensitivity was lost [22].

The allergic response in the current study was characterized by elevated total IgE levels and airway inflammation manifested by increased BALF leukocyte counts and eosinophil and IL-13 levels. We observed that exposure to the peanut oil induced a nonsignificant increase in IL-4 levels in 2 out of the 5 PM, while IL-13 levels were significantly increased in all of them. IL-13 is a critical mediator of allergic inflammation, which is expressed in acute and chronic lesions of AD [23, 24]. Studies using a skin-specific IL-13 transgenic mouse model demonstrated increased inflammation and fibrosis in the skin, supporting a critical role for IL-13 signaling pathways in AD [25]. Furthermore, a distinct and critical role of IL-13 in comparison to IL-4 has been reported in AD [26]. Despite the 20–25% sequence homology and shared effector functions between those Th2 cytokines, IL-13 was significantly upregulated in AD lesions in 27 of 28 patients, whereas IL-4 expression was seen in only 3 patients [26].

Current treatment guidelines for PA focus on elimination diet but there are no guidelines regarding skin contact avoidance. Our results demonstrate that topical application of peanut oil may induce PA. They suggest that PA can appear without any previous dietary exposure. Peanut oil has been used as an ingredient in various skin

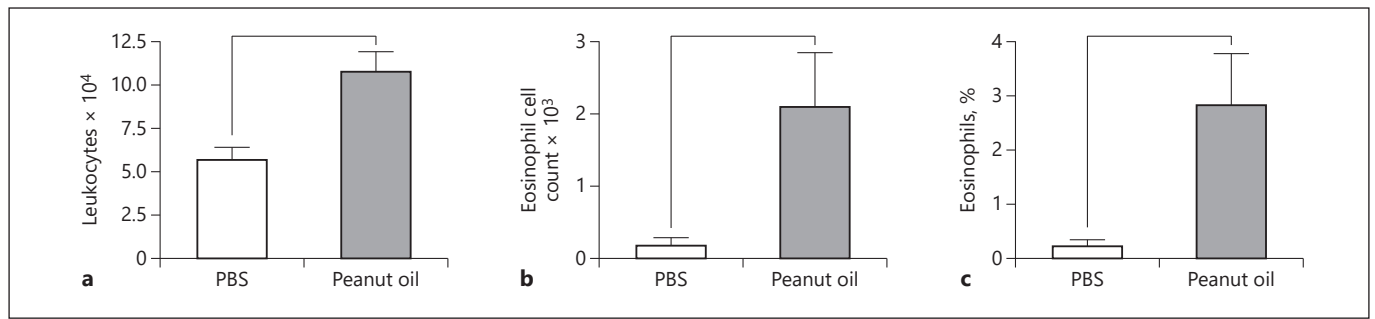


Fig. 3. Peanut oil epicutaneous exposure induces pulmonary inflammation in mice. **a** Leukocytes. **b** Eosinophil counts. **c** Percent of eosinophil infiltration. Results obtained from two experiments in a total of 10 animals. PBS, phosphate buffered solution.

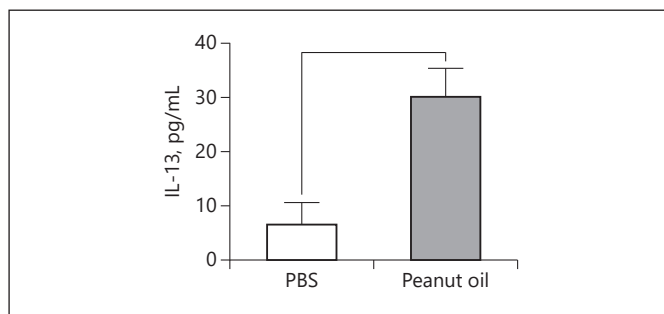


Fig. 4. Exposure of mouse skin to peanut oil increased Serum IL-13 levels. Serum IgE was obtained from PM and controls groups 24 h after the last challenge. The results were obtained from 2 experiments (9 animals per group). IL-13, interleukin.

care products for the treatment of AD, including creams, lotions, oils and ointments. In the “Health Nuts” cohort of 4,453 infants, the authors demonstrated that at 12 months of age, infants with AD were 11 times more likely to develop PA compared with healthy infants [27]. Lack et al. [9] demonstrated a significant independent relationship between PA and the use of skin preparations containing peanut oil. Since diagnostic allergy testing is based on aqueous extracts of the respective allergenic

source, potential allergenic proteins of lipophilic nature may be underrepresented as putative allergens [28]. Wheat is another food allergen widely used in skin care products for atopic skin. A recently published work by Ballegaard demonstrated that sensitization to wheat was possible through cutaneous exposure [29]. On the other hand, cutaneous application of food allergens such as peanut and eggs to intact skin may promote tolerance as has been shown in another murine model [30]. Chemical analysis of refined peanut oil demonstrated trace amounts of peanut protein in the oil, sufficient to induce IgE production upon exposure [31].

In summary, our data suggest that topical application of peanut oil to irritated skin may play a role in the etiology of PA. The increasing popularity of “natural” vegetable oil-based topical treatment of AD may explain the increasing incidence of peanut and other food allergies. These results are clinically relevant to the prevention of PA.

Disclosure Statement

The authors declare that they have no conflicts of interest to disclose.

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