Maternal allergy increases susceptibility to offspring allergy in association with T_H2-biased epigenetic alterations in a mouse model of peanut allergy

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Background: Although maternal atopy is a risk factor for the development of peanut allergy, this phenomenon has not been well characterized experimentally, and the mechanisms underlying offspring risk are unclear.

Objective: We sought to determine whether offspring of mothers with peanut allergy (O-PAM mice) are more susceptible to peanut allergy than offspring of naive mothers (O-NM mice) in a murine model and, if so, whether the susceptibility is linked to T_H2-biased epigenetic alterations.

Methods: Five-week-old O-PAM and O-NM mice were intragastrically sensitized to and challenged with peanut. Serum peanut-specific IgE levels, plasma histamine levels, anaphylactic reactions, and splenocyte and MLN cell cytokine production were measured. DNA methylation levels of the *Il4* gene promoter from splenocytes and MLN cells from sensitized offspring and splenocytes from unsensitized neonatal offspring were determined by means of pyrosequencing.

Results: O-PAM mice exhibited 3-fold higher peanut-specific IgE levels after peanut sensitization, as well as 5-fold higher histamine levels and significantly higher anaphylactic symptom scores after challenge than O-NM mice ($P < .05$-$.01$). Cultured splenocytes and MLNs from O-PAM mice produced significantly more T_H2 cytokines than cells from O-NM mice ($P < .05$-$.01$). Cells from O-PAM mice exhibited significantly reduced DNA methylation at CpG sites of the *Il4* gene promoter than cells from O-NM mice. DNA methylation levels were inversely correlated with IL-4 and IgE production. O-PAM neonatal splenocyte hypomethylation of the *Il4* gene promoter was also present.

Conclusion: This study is the first to demonstrate that increased susceptibility to peanut allergy in O-PAM mice is associated with epigenetic alteration of the *Il4* gene promoter. This finding might provide insight into preventing the development of early-life allergy. (J Allergy Clin Immunol 2014;134:1339-45.)

Key words: *Peanut allergy, anaphylaxis mice, offspring, IgE, IL-4, DNA methylation*

Food allergy, a growing public health concern in the United States, affects up to 8% of children and 4% of adults and is a major cause of anaphylaxis.[1-3] Among these food allergies, peanut allergy has attracted great public attention because of its prevalence, severity of reactions, and frequent lifelong persistence.[4-7] In 1996, Hourihane et al[8] reported that the prevalence of peanut and other allergies in the families of persons with peanut allergy was increased in successive generations in maternal but not paternal relatives. Additional clinical observational studies also show that maternal peanut allergy and other allergies increase the risk of a child having peanut allergy.[9-11] The reason for this is not known, and although allergy-associated genes have been identified, none are strongly associated with peanut or other food allergy.[12]

Peanut allergy, like other allergies, is a T_H2-mediated immune disorder characterized by increased expression of IL-4, IL-5, and IL-13 in human subjects[13] and animal models.[14-16] IL-4 is a key T_H2 cytokine required for B cells switching to IgE production, mast cell activation, and T_H2 cell differentiation.[17] Higher IL-4/IFN-γ ratios were found in patients with persistent peanut allergy than in patients who naturally outgrew peanut allergy.[18]

Increasing evidence shows that epigenetic mechanisms are involved in regulating T-cell differentiation, cytokine expression, allergic sensitization, and allergic asthma development.[19-21] CpG DNA methylation is a well-known epigenetic modification that affects chromatin remodeling and can drive cytokine expression in the absence of alterations in DNA sequences.[22] DNA hypomethylation activates cytokine expression, whereas hypermethylation represses cytokine expression.[23-25] DNA methylation status is dynamic and influenced by both external and internal factors. A study investigating the effect of inhalation of diesel exhaust particles and allergen on DNA demethylation status at several CpG sites of the *Il4* gene promoter showed that the level of DNA CpG^{-408} demethylation was inversely

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Abbreviations used
Con A: Concanavalin A
CPE: Crude peanut extract
CT: Cholera toxin
foxp3: Forkhead box P3
MLN: Mesenteric lymph node
NM: Naive mothers
O-NM: Offspring of naive mothers
O-PAM: Offspring of mothers with peanut allergy
PAM: Mothers with peanut allergy
PBL: Peripheral blood leukocyte

correlated with increased IgE production in a murine model of asthma.[26] Although it has been suggested that association of maternal atopy with increased offspring susceptibility to food allergy is linked to epigenetic changes,[27,28] direct experimental evidence of such an association is lacking.

We hypothesized that maternal peanut allergy would increase propensity for peanut-specific IgE production, hypersensitivity reactions, T_H2 cytokine production, and T_H2 cytokine gene promoter hypomethylation in offspring. To test this possibility, we used a mouse model to compare IgE levels and anaphylactic reactions after suboptimal active peanut sensitization and challenge in offspring of mothers with peanut allergy (O-PAM) with those in offspring of naive mothers (O-NM). We also determined cytokine production and methylation status of the T_H2 signature cytokine *Il4* gene promoter at CpG sites in DNA from mesenteric lymph node (MLN) cells and splenocytes of offspring.

METHODS

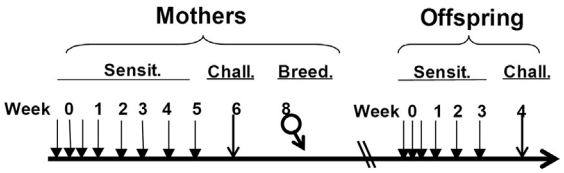
Mice and reagents

Six-week-old female and male C3H/HeJ mice purchased from the Jackson Laboratory (Bar Harbor, Me) were maintained on peanut-free chow under specific pathogen-free conditions according to standard guidelines for the care and use of animals.[29] Freshly ground whole roasted peanut and crude peanut extract (CPE) were prepared as described previously (see the Methods section in this article's Online Repository at www.jacionline.org).[30-32] Sources of reagents used in this study are provided in the Methods section in this article's Online Repository.

Experimental protocols

Maternal protocol. To establish maternal peanut allergy, female C3H/HeJ mice on a peanut-free diet were orally sensitized with peanut, as previously described,[15,16] with slight modification. Briefly, 6-week-old female mice were sensitized with peanut (10 mg) and cholera toxin (CT; 20 μg) intragastrically weekly for 5 weeks and challenged at week 6 (200 mg of peanut per mouse). Our sensitization protocol differed from our previous standard protocol in that mice did not receive an additional boost (50 mg of peanut plus 10 mg of CT). Mice treated with the protocol used in our current study are termed mothers with peanut allergy (PAMs). As control animals, naive female mice were PBS sham sensitized and challenged; these mice are termed naive mothers (NMs). One week after peanut challenge, mice in both groups were bred with naive male subjects. All mice were fed peanut-free chow during gestation and lactation.

Determination of peanut protein levels in milk. During lactation, milk was collected from PAMs or NMs when their offspring were 10 to 15 days old with a mouse milking machine modified in our laboratory, as previously described.[33] An additional group of PAM lactating mice was administered 10 mg of peanut protein intragastrically to establish a positive control. Milk was collected 2 hours later. Levels of peanut protein in milk from these 3 groups were determined with a Veratox Peanut commercial kit (Neogen, Lansing, Mich), according to the manufacturer's instruction and as previously described.[34]



Groups	Mothers		Offspring	
	Sensitization i.g.	Challenge i.g.	Sensitization i.g.	Challenge i.g.
O-NM	PBS	PBS	5mgPN+CT	PN
O-PAM	10mgPN+CT	PN	5mgPN+CT	PN
Naive	None	None	None	PN

FIG 1. Experimental protocols. Peanut-sensitized female mice were orally challenged with peanut and then mated with naive male mice 1 week later. Five-week-old O-PAM and O-NM mice were sensitized weekly for 3 weeks and challenged at week 4. Offspring of PBS-challenged naive mothers served as normal control animals. *Breed.*, Breeding; *Chall.*, challenge; *i.g.*, intragastric; *PN*, peanut; *Sensit.*, sensitization.

TABLE I. Primers used for PCR amplification and pyrosequencing experiments

Il4	
PCR forward	GTTTTTAAGGGGTTTTTATAGTAGGAAGT
PCR reverse	Biotin-AATTACCACTAACTCTCCTCCTACA
Pyrosequencing	AGATTTTTTTGATATTATTTTGTTT

Offspring protocol. O-PAM and O-NM, weaned at 4 weeks of age, were sensitized 1 week later with 3 weekly intragastric suboptimal doses of peanut (5 mg) and CT (10 μg). A third group of age-matched peanut-naive offspring of peanut-free mothers (naive) served as normal control mice (Fig 1). All offspring were orally challenged at week 4 with 200 mg of ground peanut.

Measurement of peanut-specific antibodies. Sera were obtained from blood collected weekly during sensitization and 1 day before challenge by means of submandibular venipuncture to monitor antibody responses to peanut sensitization. Serum IgG antibodies were first depleted by using protein G–Sepharose (BioVisio, Milpitas, Calif) centrifugation to measure serum peanut-specific IgE levels.[35,36] In brief, equal volumes of protein G–Sepharose and undiluted mouse serum were mixed and incubated at room temperature for 10 minutes and then centrifuged at 1200 rpm for 10 minutes at room temperature. Supernatants were collected, and the process was repeated twice. Peanut-specific IgE levels in protein G–depleted sera were measured by using previously described ELISA methods.[37,38] Serum peanut-specific IgG_1 and IgG_{2a} levels were determined, as previously described.[37,38]

Assessment of hypersensitivity reactions. Anaphylactic symptoms were evaluated 30 minutes after oral challenge by using the scoring system described previously (see the Methods section in this article's Online Repository at www.jacionline.org).[15] In this scoring system 0 is no reaction, 1 is mild, 2 is moderate, 3 is severe, 4 very severe, and 5 is death.[39] Core body temperatures were measured with a rectal probe (Harvard Apparatus, Holliston, Mass). To confirm that anaphylactic reactions were not IgG mediated, peanut-sensitized mice were pretreated with anti-FcγRIIB/FcγRIII mAb (mAb 2.4G2, 500 μg per mouse) or isotype control antibody 24 hours before challenge, as previously described.[40,41]

Histamine measurement. Histamine levels were measured with an enzyme immunoassay kit (ImmunoTECH, Marseille, France), as described by the manufacturer.

Cell culture and cytokine measurements. Offspring splenocytes and MLNs were prepared, as previously described,[33] and cultured in 24-well plates (4×10^6/well/mL) in the presence or absence of CPE (200 μg/mL) or concanavalin A (Con A; 2.5 μg/mL). Seventy-two hours later,