Repository materials and methods

Reagents: Cholera toxin (CT) was purchased from List Biological Laboratories Inc (Campbell, CA). Concanavalin A (Con A), collagenase and avidin-peroxidase were purchased from Sigma-Aldrich (St. Louis, MO). DNAse I was purchased from Roche (Branford, CT). Antibodies for ELISA were purchased from BD Pharmingen (San Diego, CA) or R&D Systems (Minneapolis, MN). DNA methylation reagents were purchased from Qiagen (Germantown, MD).

Crude peanut extract preparation: Freshly ground, whole roasted peanut and crude peanut extract (CPE) were prepared as described previously.[1;2] Endotoxin levels in ground peanut were tested using the Pyrogent Plus assay kit (Lonza, Basel, Switzerland) as previously described[3]and were below the detectable level. Briefly, peanuts were ground with a coffee grinder (Krups, Peoria, IL) followed by mortar and pestle to make a smooth paste. The paste was defatted by washing with at least 20 volumes (wt/vol) of cold acetone and air dried overnight at 4°C. Protein was extracted from dried powder by agitating in PBS supplemented with a protease-inhibitor cocktail without EDTA (Roche Diagnostics, IN) overnight at 4°C. After centrifugation at 2500g for 20 minutes at 4°C, the supernatant was collected, filtered, and centrifuged at 12,000g for 3 minutes. Protein concentrations were determined with a Micro BCA Protein Assay Kit (Thermo Scientific, IL). Extract was stored at –80°C.

Evaluation of anaphylaxis: Anaphylactic symptoms were evaluated 30 minutes following oral challenge utilizing the scoring system described previously [4]: 0 - no symptoms; 1 - scratching and rubbing around the snout and head; 2 - puffiness around the eyes and snout, pilarerecti, reduced activity, and/or decreased activity with increased respiratory rate; 3 - wheezing, labored respiration, cyanosis around the mouth and the tail; 4 - no activity after prodding, or tremor and

convulsion; 5 - death. Cage identities were concealed during visual assessment of anaphylactic symptoms.

Determination of global DNA methylation: Genomic DNA from MLN cells was purified as described in "DNA pyrosequencing methylation analysis" in the Methods section. Global DNA methylation was determined using the MethylFlash Methylated DNA Quantification Kit (Colorimetric) (Epigentek Group Inc., New York, NY).[(5)] This kit measures the methyl-cytosine content as a percentage of total cytosine content. Using a DNA concentration of 20 μg/ml, the purified DNA was added to wells in an ELISA plate. The methylated fraction of DNA was quantified using 5-methylcytosine specific antibodies. The amount of methylated DNA was proportional to the OD intensity in an ELISA plate reader at 450 nm. DNA methylation was calculated using the formula: $[(OD_{sample}–OD_{M3})/S]/[((OD_{M4}–OD_{M3})x2)/P]x100$; where OD is optical density; M3 is the negative control, an unmethylated polynucleotide containing 50% of cytosine; S is the amount of input sample DNA in ng; M4 is the positive control, a methylated polynucleotide containing 50% of 5-methylcystosine; P is the amount of input positive control in ng. The amount of methylated DNA was expressed as percentage of total DNA.

Figure Legend

Figure E1: Peanut allergic mothers exhibited hypersensitivity reactions following oral peanut challenge and prior to mating. Blood was collected from peanut allergic mothers (PAM) and naïve mothers (NM) one day prior to challenge. Serum peanut-specific IgE levels (A) were measured by ELISA**.** Anaphylactic scores (B) and core body temperatures (C) were measured, and plasma histamine levels (D) were determined thirty minutes after challenge. **, $p<0.01$; ***, $p<0.001$, vs. NM (N=5/group). Each dot represents an individual mouse. Horizontal bars indicate means. Data shown are representative of two individual experiments.

Figure E2: Following oral challenge, anaphylactic scores and core body temperatures with or without anti-FcγRIIB/RIII monoclonal antibody administration in peanut sensitized offspring of peanut allergic mothers. N=5-6. *, p<0.05.Horizontal lines represent the means of each group.

Figure E3:A: Global methylation of MLN cells in O-PAM, O-NM and normal controls following challenge was measured by the global DNA methylation ELISA kit(Epigentek, Farmingdale, NJ). In this ELISA kit, the methylated fraction of DNA is recognized by 5-methylcytosine antibody and quantified through an ELISA-like reaction. (N=6-7/group). Both p>0.05 for O-PAM vs. O-NM and O-PAM vs. Naïve. B:Purified DNA from sensitized and challenged offspring MLN cells underwent bisulfite treatment, PCR amplification, and pyrosequencing. Percent of DNA methylation of the IFN-γ and Foxp3 promoters in offspring MLN cells (B&C). Data are expressed as means ± SDs of each group. (N=5-6/group).

Figure E4: DNA methylation at CpG-408 and CpG-393 sites of the IL-4 promoter from peripheral blood leucocytes (PBL) of peanut allergic mothers (PAM) and naïve mothers (NM) prior to breeding. Purified DNA from mothers PBL prior to breeding underwent bisulfite treatment, PCR amplification, and pyrosequencing. **p < 0.01; ***p < 0.001 vs.NM (N=5).

Figure E5: Breast milk was collected from lactating peanut allergic mothers (PAM) and naïve mothers (NM) when their offspring were 10-15 day-old, using a mouse milking machine modified in our laboratory. An additional group of PAM lactating mice was inoculated intragastrically with10 mg of peanut protein (PAM-PN). Milk was collected 2 hours following peanut protein feeding. Milk was diluted 1:2 in PBS, and peanut protein levels were detected using a commercial kit (Neogen Corp, Lansing, Mich). N=3-4. ***, p<0.001.

Repository References

(1) Beyer K, Morrow E, Li XM, Bardina L, Bannon GA, Burks AW et al. Effects of cooking methods on peanut allergenicity. J Allergy Clin Immunol 2001; 107(6):1077-81.

(2) Pochard P, Vickery B, Berin MC, Grishin A, Sampson HA, Caplan M et al. Targeting Toll-like receptors on dendritic cells modifies the T(H)2 response to peanut allergens in vitro. J Allergy Clin Immunol 2010; 126(1):92-7.

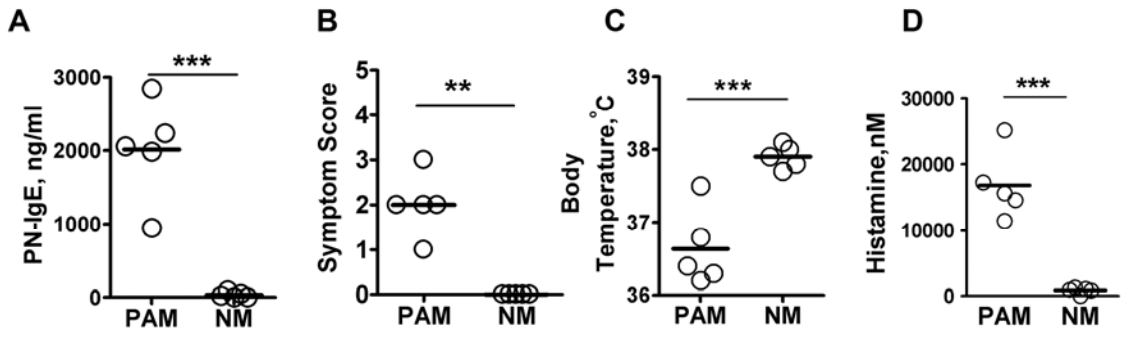
(3) Srivastava KD, Qu C, Zhang T, Goldfarb J, Sampson HA, Li XM. Food Allergy Herbal Formula-2 silences peanut-induced anaphylaxis for a prolonged posttreatment period via IFN-gamma-producing CD8+ T cells. J Allergy Clin Immunol 2009; 123(2):443-51.

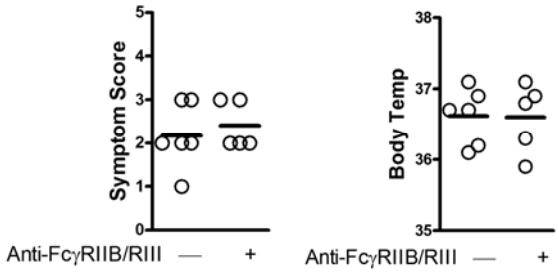
(4) Li XM, Serebrisky D, Lee SY, Huang CK, Bardina L, Schofield BH et al. A murine model of peanut anaphylaxis: T- and B-cell responses to a major peanut allergen mimic human responses. J Allergy Clin Immunol 2000; 106(1 Pt 1):150-8.

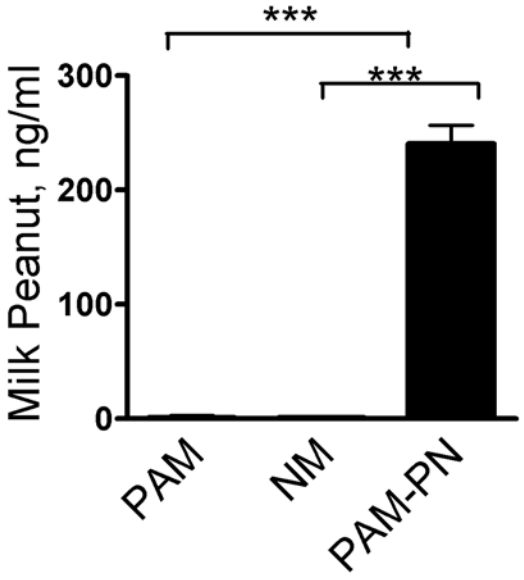
(5) Fernandez-Roig S, Lai SC, Murphy MM, Fernandez-Ballart J, Quadros EV. Vitamin B12 deficiency in the brain leads to DNA hypomethylation in the TCblR/CD320 knockout mouse. Nutr Metab (Lond) 2012; 9:41.

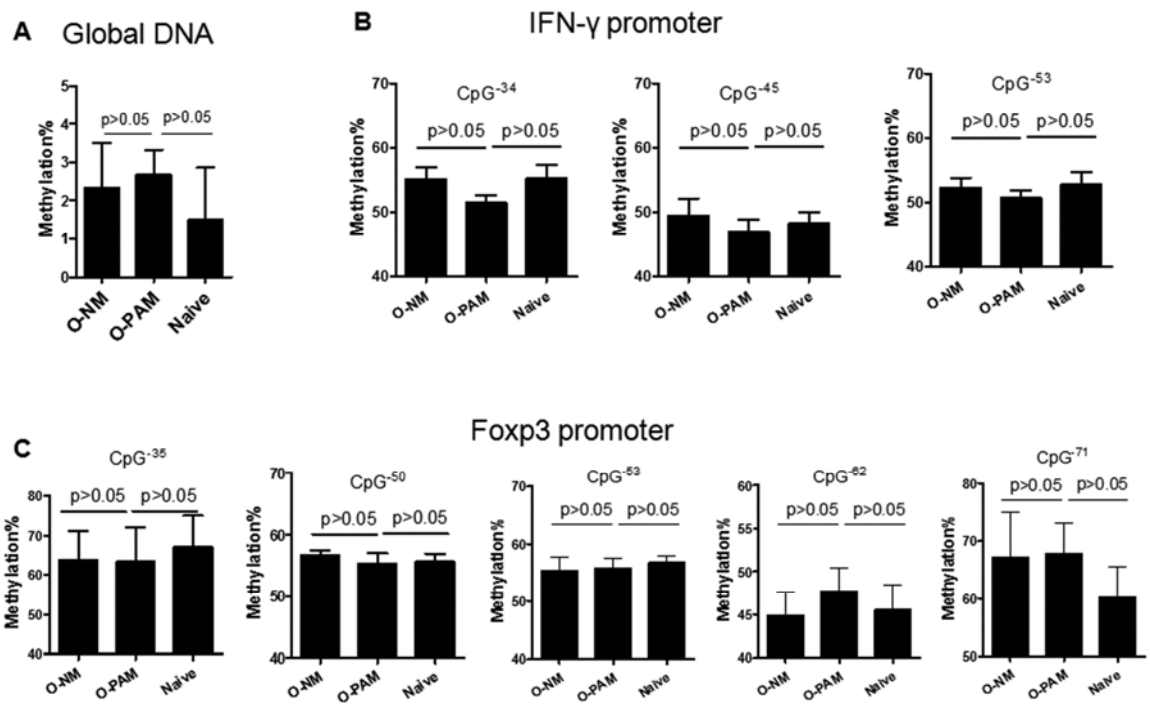
Supplement Table I. Primers used for PCR amplification and pyrosequencing experiments

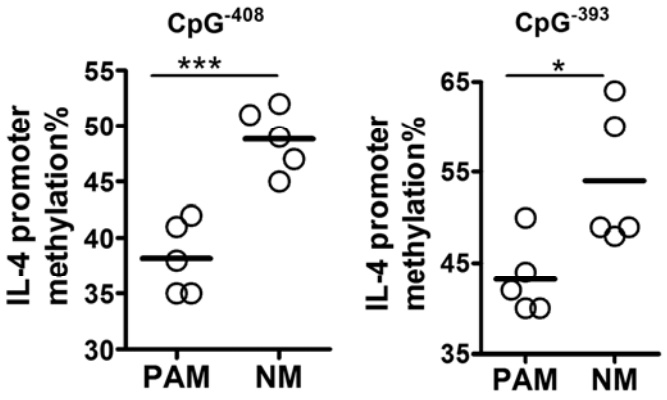
Mouse Foxp3	
PCR forward	TATATTTTTAGATGATTTGTAAAGGGTAAA
PCR reverse	Biotin-TCACCTTAATAAAATAAACTACTA
Pyrosequencing	AAAAAATTGGATTATTAGAA
Mouse IFN-γ	
PCR forward	TGGTGTGAAGTAAAAGTGTTTTTAGA
PCR reverse	Biotin-TACACCTCTCTAACTTCCAATTT
Pyrosequencing 1	AAAAAAAATTTGTGAAAATA
Pyrosequencing 2	GAATGGTATAGGTGGGTA


A
PN-IgE, ng/ml
3000
2000
1000
0
***
PAM
NM
B
Symptom Score
5
4
3
2
1
0
**
PAM
NM
C
Body Temperature, °C
39
38
37
36
***
PAM
NM
D
Histamine, nM
30000
20000
10000
0
***
PAM
NM


Symptom Score
5
4
3
2
1
0
Anti-FcγRIIB/RIII
—
+
Body Temp
38
37
36
35
Anti-FcγRIIB/RIII
—
+


***
***
Milk Peanut, ng/ml
300
200
100
0
PAM
NM
PAM-PN


A
Global DNA
Methylation%
p>0.05
p>0.05
O-NM
O-PAM
Naive
B
IFN-γ promoter
CpG-34
CpG-45
CpG-53
C
Foxp3 promoter
CpG-35
CpG-50
CpG-53
CpG-62
CpG-71


CpG-408
***
IL-4 promoter methylation%
55
50
45
40
35
30
PAM
NM
CpG-393
*
IL-4 promoter methylation%
65
55
45
35
PAM
NM