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Maternal peanut consumption provides protection in offspring against peanut sensitization that is further enhanced when co-administered with bacterial mucosal adjuvant

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Abstract

The aims of the present study were to assess whether protection against peanut (PN) sensitization can be conferred by maternal PN consumption alone and if so, whether protection was increased by mucosal adjuvant co-administration. Mice were fed with low dose of either PN or PN with cholera toxin (CT) preconceptionally, and during pregnancy and lactation. Offspring serum PN-specific immunoglobulins and cellular responses by splenocytes and mesenteric lymph node (MLN) cells were determined after an active PN sensitization protocol. Milk was collected from lactating mothers of 11–21-day-old pups for evaluation of PN-specific immunoglobulin levels. We found that offspring of PN fed mothers exhibited lower PN-specific IgE levels and reduced PN-stimulated splenocyte and MLN cells cytokine secretion than offspring of non PN fed mothers. CT co-administration with PN enhanced these responses.. Milk from mothers fed PN and CT, but not PN alone preconceptionally and during pregnancy and lactation contained markedly and significantly increased levels of both peanut-specific IgG2a and IgA. Our study demonstrated that maternal feeding of PN alone had a protective effect against PN sensitization of the progeny, which was enhanced by co-administration of a mucosal adjuvant. Increased levels of PN-specific IgG2a and/or IgA in milk were seen when PN and CT were administered together, suggesting that transmission of maternal immunoglobulins may play a role in the observed protection.

Keywords

Peanut allergy; pregnancy; lactation; IgE suppression; IgG2a

1. Introduction

Peanut allergy (PNA) affects approximately 1% of Americans and is the leading cause of food allergy death in the United States (US) (Bock, Muñoz-Furlong & Sampson, 2007). Unlike most food allergies, which appear in children but resolve with age, PNA usually

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Conflict of Interest

All the authors have no competing financial interests to disclose.

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persists into adulthood and can reappear in individuals who have become peanut (PN) tolerant (Skolnick, Conover-Walker, Koerner, Sampson, Burks & Wood, 2001). In addition, PN is difficult to avoid because of its frequent presence in manufactured products. The prevalence of childhood PNA has been increasing, currently being 1.4%, compared to 0.8% in 2002 and 0.4% in 1997 (Sicherer, Munoz-Furlong, Godbold & Sampson, 2010). This increase has been speculated to be due to either early introduction of PN to the immature immune system, or delayed dietary introduction of PN, non-ingestion environmental exposures, and/or insufficient exposure to pathogens (Pali-Scholl, Renz, & Jensen-Jarolim, 2009). It is well-recognized that in addition to genetic factors, the *in utero* environment may strongly influence a child's immune system (Hubeau, Apostolou & Kobzik, 2006; Prescott et al., 1998). Maternal avoidance of PN during pregnancy and lactation was recommended for many years in the U.S. and the U.K. Recently, this recommendation has been revised due to lack of conclusive evidence of benefit (Greer, Sicherer, & Burks, 2008) and concerns that this approach may indeed increase the risk of development of PNA (Burks, 2008). Hourihane et al (2007) reported PNA outcomes in a cohort of children born after the U.K. government's advice to mothers of high-risk infants to follow maternal avoidance during pregnancy and lactation, and to avoid introduction of PN to their children until 3 years of age. The rate of PNA in this cohort was 1.8%, the highest recorded at that time. In addition, several recent studies indicated that early introduction of PN to infants may be beneficial (Burks, 2008; Wennergren, 2009). The latest epidemiologic data suggests that earlier, more frequent and larger consumption of PN during the first year of life was associated with a low prevalence of PNA as seen in Jewish Israeli children (0.17%) compared to Jewish children in the U.K. (1.85%) (du Toit et al., 2008). In the U.K., avoidance of PN was significantly more common in mothers during breastfeeding than in Israel, and avoidance during pregnancy had a similar trend. These findings raise the question whether introduction of PN during infancy, or even antenatally might be associated with development of tolerance to PN.

Several rodent studies found that maternal exposure to certain allergens during pregnancy and lactation prevented offspring from developing allergic asthma (Fusaro et al., 2007; Polte, Hennig, & Hansen, 2008; Verhasselt et al., 2008). Although most of these studies employed ovalbumin (OVA) together with an adjuvant, one study showed that preconceptional feeding of OVA alone significantly prevented OVA-induced allergic airway response in the offspring, which was associated with maternal transmission of IgG (Hennig, & Hansen, 2008). However, a recent study showed that co-administration of the adjuvants pertussis toxin or aluminum hydroxide was required to protect offspring against OVA sensitization (Ellertsen, Nygaard, Melkild & Lovik, 2010). We previously reported that 5 week old offspring of PN-sensitized mothers exhibited IgG1-mediated anaphylaxis upon first exposure to PN, which was prevented by maternal ingestion of PN co-administered with CT at doses below the clinical threshold (induction of symptoms) throughout pregnancy and lactation (Lopez-Expósito, Song, Jarvinen, Srivastava & Li, 2009). Protected offspring had higher levels of protective PN-specific IgG2a antibody and lower PN-specific IgG1 to IgG2a ratios than unprotected offspring that reflected maternal serum concentrations. We also found that offspring of mothers fed PN with CT throughout pregnancy and lactation showed significantly reduced PN-IgE production in response to 6 weeks of sensitization (Lopez-Expósito, Song, Jarvinen, Srivastava & Li, 2009). However, whether maternal feeding of PN alone can prevent offspring from PN sensitization was not investigated. The mechanisms underlying the protection induced by maternal dietary PN exposure against PN sensitization in the progeny also remain unknown.

The objective of this study was to assess whether maternal feeding of PN alone protects against PN active sensitization in offspring compared to the effect of a mucosal adjuvant co-administration with PN in mothers who are PN-allergic or PN-immunized. In this study, the

potential protective role of PN-specific antibodies transferred from mothers to offspring is also discussed.

2. Materials and Methods

2.1 Animals and reagents

Six-week-old female and male C3H/HeJ mice purchased from the Jackson Laboratory (Bar Harbor, ME) were maintained on PN-free chow under specific pathogen-free conditions according to standard guidelines for the care and use of animals (Institute of Laboratory Animal Resources Commission of Life Sciences NRC). Freshly ground whole roasted PN (Pathmark®, New York, NY) prepared as previously described (Li et al., 2000) was used as an antigen. Endotoxin levels in ground PN were tested using the Pyrogen Plus assay kit (Lonza, Basel, Switzerland) as previously described (Srivastava, Qu, Zhang, Goldfarb, Sampson & Li, 2009) and were below detection. Crude peanut extract (CPE) was prepared as described previously (Burks et al., 1991). Cholera toxin (CT) was purchased from List Biological Laboratories Inc (Campbell, CA). Concanavalin A (ConA), avidin-peroxidase and dinitrophenyl-phosphate (DNP) were purchased from Sigma Aldrich (St. Louis, MO). Dioxigenin (DIG) and HRP-labeled Anti-DIG antibody were purchased from Roche Diagnostics (Indianapolis, IN). The rest of antibodies for ELISA were purchased from Accurate Scientific (Westbury, NY) or Pharmingen (San Diego, CA). ABTS (2, 2'-azino-di(3-ethylbenzthiazoline-6-sulfonate) was purchased from KPL (Gaithersburg, MD). Tetramethylbenzidine was purchased from BD Biosciences (San Jose, CA).

2.2 Maternal experimental protocol

Female C3H/HeJ mice divided in groups were subjected to different immunization protocols. Mice in PN+CT group received PN and CT intragastrically (i.g.) (10 mg PN/mouse; 20 µg CT/mouse); PN group received PN only i.g. (10 mg/mouse). The CT group received CT i.g. (20 µg/mouse). A group of mothers without any PN or CT feeding were employed as a control (unimmunized). All groups were sensitized 5 times at weekly intervals (Fig 1), and then mated with naïve males. The same feeding regimen continued during pregnancy and lactation. Mothers were sacrificed after weaning, and blood and spleens were collected for measurement of humoral and cellular responses as described below.

2.3 Offspring experimental protocol

To determine the effect of maternal PN, CT and PN+CT exposure, as compared to unexposure, on active PN sensitization in the offspring, 5-week-old offspring from all groups were sensitized with PN+CT i.g. (10 mg PN/mouse; 20 µg CT/mouse) 5 times at weekly intervals, followed by two boosting doses at weeks 8 and 11 (50 mg PN/mouse; 20 µg CT/mouse) (Fig 1). Five groups of offspring were thereby attained: mothers fed PN+CT/offspring sensitized with PN+CT (PN+CT/PN+CT), mothers fed PN/offspring sensitized with PN+CT (PN/PN+CT), mothers fed CT/offspring sensitized with PN+CT (CT/PN+CT), unimmunized mothers/offspring sensitized with PN+CT (unimmunized/PN+CT) and naïve mice without PN+CT sensitization (Naïve). Offspring were sacrificed at week 15, 4 weeks after the last sensitization dose, and blood, spleens, and mesenteric lymph nodes (MLN) were collected for measurement of humoral and cellular responses as described below.

2.4. Milk extraction

Mothers were separated from the pups for 2 hrs and anesthetized intraperitoneally (i.p.) with pentobarbital (80 mg/kg). One unit oxytocin was given i.p. 5 min prior to milking using a mouse milking machine modified from Bednar-Tantscher et al (Bednar-Tantscher, Mudde &

Rot, 2001) Continuous vacuum of 7 mmHg was created using electrical pump and carefully designed teat cups connected to the collection vessel were applied to the nipples. Lactating mothers of 11–21-day-old pups yielded 50–500 μL /milking/mouse. Fat layer was discarded and milk was diluted 1:2 in PBS and stored at -80°C until PN-specific IgE, IgG1, IgG2a, and IgA levels in milk were measured using ELISA as described below.

2.5 Measurement of serum and milk peanut-specific antibodies

Retro-orbital venous blood was collected from mothers 5 and 11 weeks after initial immunization and at weeks 0, 2, 5, 8, 11 and 15 from offspring after initial PN sensitization. Sera was collected and stored at -80°C until analyzed. PN-specific IgE, IgG1, IgG2a and IgA levels were measured in pooled samples from sera from offspring and individual samples from mother's milk as previously described (López-Expósito et al., 2009). Additionally, individual serum titrations for PN-specific IgE, IgG1 and IgG2a were also carried out at the end of the protocol, at week 15 in order to compare the individual variations in the immunoglobulin levels of the offspring as a final endpoint. IgE at week 15 was measured according to van Wijk et al. (2007).

2.6 Cell culture and cytokine measurements

Splenocytes (SPCs) derived from mothers and SPCs and mesenteric lymph node cells (MLN) from their offspring were isolated using standard techniques. After lysis of red blood cells, they were resuspended in RPMI 1640 supplemented with fetal bovine serum. Cells were cultured in 24-well plates (4×10^6 /well/mL) in the presence or absence of CPE (200 μg /mL) or ConA (2.5 μg /mL). Supernatants were collected after 72 h. IL-4, IL-13, IL-10, IL-17, IFN- γ and TGF- β levels were determined according to manufacturer's instructions (R&D Systems, Minneapolis, MN for IL-13, IL-17 and TGF- β (BD-Pharmingen, San Jose, CA for all others).

2.7 Statistical analysis

Statistical significance ($P < 0.05$) was determined by *t*-test for all pair-wise comparisons of data that passed the normality test. For those data which failed to pass test for normality, Mann-Whitney *U* test (rank-sum test) was employed. All statistical analyses were performed with GraphPad Prism Software, Inc. (San Diego, CA).

3. Results

3.1 PN+CT feeding preconceptionally and during pregnancy and lactation induced the presence of peanut-specific immunoglobulins in maternal milk and serum

To determine the effect of adjuvant on the presence of specific antibodies in milk, we measured PN-specific antibodies in milk from mothers during 11–21 days of lactation. Milk from mothers fed PN+CT contained high levels of PN-specific IgG2a ($350,000 \pm 174,983$ ng/ml; Fig 2A). PN-specific IgG1 was also present in milk ($90,000 \pm 59,612$ ng/ml; Fig 2B), but the levels were markedly less than those of IgG2a. Indeed, the ratio of PN-specific IgG2a to IgG1 was 5.54 ± 1.29 . PN-specific IgA was also present in milk of PN+CT mothers (1100 ± 166.4 ng/ml; Fig 2C). Milk from mothers fed PN or CT alone and that from unimmunized mothers contained no detectable PN-specific antibodies (Fig 2A–C). PN-specific IgE was undetectable in milk from all groups (data not shown).

We also determined serum PN-specific antibody levels and found that mothers fed PN+CT showed significantly elevated serum levels of PN-specific IgG2a (3.9×10^5 ng/mL $\pm 1.5 \times 10^5$ vs 0; $P < 0.001$), IgG1 (1.9×10^6 ng/mL $\pm 1.0 \times 10^6$ vs 0; $P < 0.001$), and IgE (260.8 ng/mL ± 49.6 vs 0; $P < 0.05$) immediately after weaning than unimmunized mothers. Similar pattern of PN-specific antibodies was observed before mating. Sera from mothers fed PN

alone contained negligible levels of PN-specific IgE, IgG2a and IgG1 at all time points. Sera from CT-fed mothers, like unimmunized mothers, contained no PN-specific antibodies. PN-specific IgA was detected only in the PN+CT group ($1873 \text{ ng/mL} \pm 272.7$).

3.2 Splenocytes from PN+CT mothers produced higher levels of cytokines in response to peanut stimulation

Immediately after weaning, mothers were sacrificed and spleens removed to determine the SPC cytokine profiles. SPCs from PN+CT mothers showed significantly increased secretion of the cytokines IL-4, IL-13, IL-10 and IL-17 after PN stimulation, when compared to SPCs from mothers fed PN or CT or unimmunized mothers (Fig 3A–C and E; $P < 0.001$ for IL-4 and IL-13; $P < 0.01$ for IL-10 and $P < 0.05$ for IL-17). Among cytokines, IL-10 levels were the highest. SPCs of mothers fed PN alone also secreted more IL-13 and IL-10 than unimmunized mothers; however the difference was not statistically significant. IFN- γ levels were comparable in all four groups. TGF- β levels were negligible in all the groups (Fig 3D and F).

3.3 Offspring of PN+CT mothers exhibited reduced peanut-specific IgE production and enhanced peanut-specific IgG2a production in response to active peanut sensitization

Five-week-old offspring were sensitized 10 times over 11 weeks with PN+CT, and their levels of serum PN-specific IgE, IgG1 and IgG2a at weeks 0, 2, 5, 8, 11 and week 15 (4 weeks after the last sensitization dose) were determined from pooled samples to show a trend and from individuals samples at week 15, the final endpoint to account for individual variability.

One day before PN sensitization, sera from all offspring contained no detectable PN-specific IgE. However, by 2 weeks, PN-specific IgE levels were significantly elevated in PN/PN+CT, CT/PN+CT and Unimmunized/PN+CT offspring as compared to PN+CT/PN+CT offspring ($P < 0.001$, Fig 4A). PN-specific IgE levels were 13-fold less in sera of PN+CT/PN+CT offspring than in sera from Unimmunized/PN+CT offspring at 2 wks following PN sensitization. Significant suppression of PN-specific IgE in response to repeated PN sensitization in PN+CT/PN+CT offspring persisted until week 15, the latest time point tested ($P < 0.001$, Fig 4A–B). PN/PN+CT offspring showed a significant reduction in PN-specific IgE levels at week 15, although the reduction was less than in PN+CT/PN+CT offspring (Fig. 4B; $P < 0.05$).

Both PN-specific IgG1 and IgG2a were present before sensitization in sera from PN+CT/PN+CT offspring, but were undetectable in the other groups ($P < 0.001$, Fig 4C and 4E). Interestingly, from the second week of sensitization, the PN-specific IgG1 and IgG2a levels were significantly lower in the PN+CT/PN+CT, PN/PN+CT and CT/PN+CT offspring than in the Unimmunized/PN+CT offspring ($P < 0.001$) (Fig 4C and 4E). At the end of the sensitization protocol there were no significant differences in PN-specific IgG1 levels between the different groups. However, PN-specific IgG2a levels were significantly lower in PN/PN+CT offspring (Fig. 4D and 4F).

Table 1 shows offspring PN-specific IgG2a/IgG1 ratios during sensitization. Throughout sensitization PN+CT/PN+CT offspring showed a significantly higher PN-specific IgG2a/IgG1 ratio when compared to Unimmunized/PN+CT offspring ($P < 0.001$). Between weeks 5 and 8 PN/PN+CT offspring also displayed a transiently higher PN-specific IgG2a/IgG1 ratios when compared with Unimmunized/PN+CT offspring ($P < 0.001$).

3.4 Feeding mothers PN+CT inhibited cytokine production by SPCs in their offspring

Our previous studies showed that prior to sensitization no PN-stimulated cytokine production was detectable in offspring's SPC culture supernatants (López-Expósito et al., 2009). In the present study, we determined offspring SPC production of Th1 (IFN- γ), Th2 (IL-4 and IL-13), Th3 (TGF- β and IL-10) and Th17 (IL-17) cytokines at week 15, the end point of the experiment, in response to CPE stimulation as shown in Table 2. CPE-stimulated SPCs from PN+CT/PN+CT offspring showed significant reduction in IL-17 and IFN- γ production compared to Unimmunized/PN+CT offspring. There was also a trend towards reduction of IL-13, IL-10 and TGF- β . Interestingly, PN/PN+CT SPCs showed significantly reduced CPE-stimulated IL-13, IL-17 and TGF- β production when compared to that by SPCs from Unimmunized/PN+CT offspring ($P < 0.05$).

3.5 Mesenteric lymph node cells from offspring of PN+CT mothers showed reduced cytokine production in response to peanut stimulation

PN+CT/PN+CT offspring MLN cell cultures showed dramatically less Th1, Th2, Th3 and Th17 cytokine production as compared to Unimmunized/PN+CT offspring MLN cultures ($P < 0.05$ for IL-13, $P < 0.001$ for all other cytokines, Fig 5A–E). Similar reductions were also found in PN/PN+CT offspring MLN cultures ($P < 0.001$ for IL-10 and IFN- γ , $P < 0.01$ for IL-17 and $P < 0.05$ for IL-4 and IL-13, Fig 5A–E), although the reduction of IL-10 and IFN- γ levels was significantly less than in PN+CT/PN+CT offspring cultures, ($P < 0.01$ for IL-10 and $P < 0.001$ for IFN- γ ; Fig 5C–D). Surprisingly, a significant reduction in IL-10, IFN- γ and IL-17 levels were also found in CT/PN+CT offspring MLN cultures ($P < 0.05$ for IL-10 and $P < 0.001$ for IL-17 (Fig 5C–E).

4. Discussion

The prevalence of pediatric PNA continues to increase (Sicherer, Munoz-Furlong, Godbold & Sampson, 2010). Currently, there is no preventive approach to alter this trend, due, at least partially, to incomplete understanding of the mechanisms of PNA. It has been speculated that increased PNA prevalence is due to either too early or too late dietary introduction of PN (Sicherer, Munoz-Furlong, Godbold & Sampson, 2010). Our recent publication reported that feeding PN allergic mothers low doses of PN and CT throughout pregnancy and lactation reduced offspring's PN-specific IgE production in response to 6 weekly sensitizations (López-Expósito et al., 2009). However, whether CT is required to produce this result was not determined. In this communication, we report that protection did occur in offspring of mothers fed PN alone, but that co administration of CT and PN gave offspring the greatest protection against PN sensitization for up to 15 weeks of oral sensitization. These findings reemphasize that maternal PN feeding can induce protection against PN sensitization in the offspring and may have important implications for identifying optimal maternal dietary regimens in sensitized individuals by combination of a PN dose below the threshold for clinical reactions and suitable bacterial adjuvant as a potential intervention for preventing offspring's peanut sensitization. The current study does not address whether maternal feeding of PN affords protection against PN sensitization also in the progeny of mothers who are not allergic nor immunized to PN.

The important role of an adjuvant in providing protection against peanut sensitization was demonstrated in this study. Feeding PN alone was less effective than PN plus mucosal adjuvant in the suppression of PN-specific IgE or cytokine responses. CT enhances Th2 driven (IgE) and some Th1 driven responses (IGg2a and IgA) in adult mice (F0) (Li et al., 2000; Snider, Marshall, Perdue & Liang, 1994). In addition, oral CT administration induces selective dendritic cells (DC) migration, maturation, and T cell priming activity in the MLN (Blazquez & Berin, 2008) as well as generation of regulatory T cells to the bystander

antigen (Lavelle et al., 2003). These phenomena may play a role in the observed protective effects. We for the first time have demonstrated that CT as an adjuvant together with a low dose of PN protected offspring of PN-allergic mice against PN sensitization, which was associated with higher PN-specific IgG2a to IgG1 ratios than those in offspring of unimmunized mothers. Development of IgG1 and IgE in mice is characteristic of Th2-type responses and IgG2a is considered a Th1-type antibody as IgG4 in humans. The mechanisms underlying this effect are unknown, but protection may be mediated by maternal transmission of protective antibodies such as IgG2a (Srivastava, Qu, Zhang, Goldfarb, Sampson & Li, 2009; Cortes-Perez et al., 2007; Martinez-Gomez et al., 2007). It is known that IgG1 and IgG2a but not IgE cross the placenta, but also they can be transferred through breast milk (Matson, Thrall, Rafti, Lingenheld & Puddington, 2010; Avrech et al., 1994). We found that serum PN-specific IgG2a was present in high levels in mothers fed PN+CT, whereas only negligible amounts were present in PN-fed mothers. Milk from mothers fed PN+CT, but not PN or CT alone contained PN-specific IgG2a and IgA antibodies. Secretory IgA is a crucial component of first-line immune mechanisms at mucosal surfaces and has many anti-inflammatory functions that might suppress allergy (Corthesy, Gaskins & Mercenier, 2007; Pilette, Durham, Vaerman & Sibille, 2004). A recent study showed that elevated antigen-specific IgA in airway luminal secretions, induced by OVA- and cholera toxin subunit B-treated dendritic cells suppressed airway Th2 inflammation in allergic mice (Smits et al., 2009). Furthermore, higher levels of secretory IgA have also been detected in milk of mothers with healthy infants when compared to those with cow's milk- allergy (Jarvinen, Laine, Jarvenpaa & Suomalainen, 2000). Therefore, we speculate that maternal milk transmission of PN-specific IgG2a and IgA may be involved in the protection against PN sensitization seen in the progeny when mucosal adjuvant is co-administered. It has been shown that milk-borne IgG is transferred to neonates via the Fc receptor (FcRn) and responsible for protection against asthma. This effect was associated with induction of antigen-specific FoxP3(+) CD25(+) regulatory T cells (Mosconi et al., 2010) and lack of protection against asthma seen in FcRn-deficient mice (Matson, Thrall, Rafti, Lingenheld & Puddington, 2010). Secretory IgA on the other hand is thought to have a role in immune exclusion. Further studies are required to determine the role for IgG2a and IgA, and milk vs placental transmission of IgG2a in protection against PN sensitization.

Unlike maternal cytokines, which showed overall increases in PN-stimulated cytokine production by SPCs in mothers fed PN+CT, there was an overall trend of suppression of cytokine production by SPCs in the protected offspring. Also MLN cell cytokine (Th1, Th2, Th17 and Treg) responses to PN stimulation were nearly completely suppressed in the protected PN+CT/PN+CT offspring. Cytokine production by SPCs and MLN cells from offspring of PN-fed mothers was also inhibited, but the suppression was less than those from offspring of mothers fed PN+CT. It is unknown, how maternal oral immunization with PN +CT mediated such robust suppression of offsprings' T cell responses, but there are several possible explanations. First, maternally transmitted IgG2a may mediate negative signaling through the T cell IgG receptor, FcγRIIb (Ravetch & Bolland, 2001), or by IgG2a depletion. Second, because we found significantly elevated IL-10 production by SPCs from mothers fed PN+CT, trans-placental transfer of maternal IL-10 may mediate T cell suppression in the offspring *in utero*. Third, maternally transmitted low doses of antigen may cause T cell anergy because immature DCs expressing little or no co-stimulatory factors induce anergic T cells when encountering antigen, whereas mature DCs, that encounter antigen induce T cell activation (Mahnke, Schmitt, Bonifaz, Enk, & Jonuleit, 2002). Further studies are required to test these possibilities. Lastly, it cannot be excluded that milk-borne leukocytes could be responsible for the effects seen, as suggested before (Jarvinen & Suomalainen, 2002). In fact, we detected few leukocytes in mouse milk (Jarvinen KM, unpublished data), though their numbers were too few to study further. In animal studies, milk leukocytes have been shown to enter the circulation, spleen, and lymphoid tissue of the newborn (Seelig, &

Head, 1987; Tuboly, Bernath, Glavits, Kovacs & Megyeri, 1995; Jain et al, 1989; Ma, Walter, Deguzman, Muller & Walker, 2008; Siafacas, Anderson, Walker & Xanthou, 1997). In recipients, immunity to tetanus toxoid was transmitted (Tuboly, Bernath, Glavits, Kovacs & Megyeri, 1995) and delayed-type hypersensitivity modified (Ma, Walter, Deguzman, Muller & Walker, 2008) suggesting transmission of viable milk lymphocytes to the suckling neonate.

In conclusion, this study provides evidence that maternal PN feeding can induce protection against sensitization to PN in the offspring of PN immunized mothers, which is further enhanced by co-administration of a mucosal adjuvant CT. Although knowledge of the mechanisms underlying this effect is still limited, maternal transmission of PN-specific antibodies IgG2a and IgA may be, at least in part, responsible for this protection. Although murine models are not identical to human disease, this work provides a rationale for further studies on maternal dietary interventions for reducing PNA in children. Investigation whether maternal PN exposure of unimmunized/unsensitized mothers during pregnancy and lactation prevents offspring from sensitization is underway. Ultimately, such approach could potentially alter the trend of increasing prevalence of PNA in childhood.

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Abbreviations

CPE	crude peanut extract
CT	cholera toxin
DC	dendritic cell
MLN	mesenteric lymph nodes
OVA	ovalbumin
PN	peanut
PNA	peanut allergy
SPC	spleen cells
CT/PN+CT	mothers fed cholera toxin during pregnancy and lactation/ offspring sensitized with peanut and cholera toxin
PN+CT/PN+CT	mothers fed peanut and cholera toxin during pregnancy and lactation/offspring sensitized with peanut and cholera toxin
PN/PN+CT	mothers fed peanut during pregnancy and lactation/offspring sensitized with peanut and cholera toxin
unimmunized/PN+CT	unimmunized mothers/offspring sensitized with peanut and cholera toxin

References

Avrech OM, Samra Z, Lazarovich Z, Caspi E, Jacobovich A, Sompolsky D. Efficacy of the placental barrier for immunoglobulins: correlations between maternal, paternal and fetal immunoglobulin levels. *Int Arch Allergy Immunol.* 1994; 103:160–5. [PubMed: 8292903]

- Bednar-Tantscher E, Mudde GC, Rot A. Maternal antigen stimulation downregulates via mother's milk the specific immune responses in young mice. *Int Arch Allergy Immunol.* 2001; 126:300–8. [PubMed: 11815737]
- Blazquez AB, Berin MC. Gastrointestinal dendritic cells promote Th2 skewing via OX40L. *J Immunol.* 2008; 180:4441–50. [PubMed: 18354165]
- Bock SA, Moñoz-Furlong A, Sampson HA. Further fatalities caused by anaphylactic reactions to food, 2001–2006. *J Allergy Clin Immunol.* 2007; 119:1016–8.
- Burks AW, Williams LW, Helm RM, Thresher W, Brooks JR, Sampson HA. Identification of soy protein allergens in patients with atopic dermatitis and positive soy challenges; determination of change in allergenicity after heating or enzyme digestion. *Adv Exp Med Biol.* 1991; 289:295–307. [PubMed: 1897398]
- Burks AW. Peanut allergy. *Lancet.* 2008; 37:1538–46. [PubMed: 18456104]
- Cortes-Perez NG, Ah-Leung S, Bermudez-Humaran LG, Corthier G, Wall JM, Langella P, et al. Intranasal coadministration of live lactococci producing interleukin-12 and a major cow's milk allergen inhibits allergic reaction in mice. *Clin Vaccine Immunol.* 2007; 14:226–33. [PubMed: 17202306]
- Corthesy B, Gaskins HR, Mercenier A. Cross-talk between probiotic bacteria and the host immune system. *J Nutr.* 2007; 137:781S–90S. [PubMed: 17311975]
- du Toit G, Katz Y, Sasieni P, Mesher D, Maleki SJ, Fisher HR, et al. Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. *J Allergy Clin Immunol.* 2008; 122:984–91. [PubMed: 19000582]
- Ellertsen LK, Nygaard UC, Melkild I, Lovik M. Maternal allergen immunisation to prevent sensitisation in offspring: Th2-polarising adjuvants are more efficient than a Th1-polarising adjuvant in mice. *BMC Immunol.* 2010; 11:8. [PubMed: 20193059]
- Fusaro AE, Brito CA, Victor JR, Rigato PO, Goldoni AL, Duarte AJS, et al. Maternal-fetal interaction: preconception immunization in mice prevents neonatal sensitization induced by allergen exposure during pregnancy and breastfeeding. *Immunology.* 2007; 122:107–15. [PubMed: 17608811]
- Greer FR, Sicherer SH, Burks AW. Effects of early nutritional interventions on the development of atopic disease in infants and children: the role of maternal dietary restriction, breastfeeding, timing of introduction of complementary foods, and hydrolyzed formulas. *Pediatrics.* 2008; 121:183–91. [PubMed: 18166574]
- Hourihane JO, Aiken R, Briggs R, Gudgeon LA, Grimshaw KE, DunnGalvin A, et al. The impact of government advice to pregnant mothers regarding peanut avoidance on the prevalence of peanut allergy in United Kingdom children at school entry. *J Allergy Clin Immunol.* 2007; 119:1197–202. [PubMed: 17353036]
- Hubeau C, Apostolou I, Kobzik L. Adoptively transferred allergen-specific T cells cause maternal transmission of asthma risk. *Am J Pathol.* 2006; 168:1931–9. [PubMed: 16723708]
- Institute of Laboratory Animal Resources Commission of Life Sciences NRC. Guide for the Care and Use of Laboratory Animals. National Academy Press; 1996.
- Jain L, Vidyasagar D, Xanthou M, Ghai V, Shimada S, Blend M. *In vivo* distribution of human milk leucocytes after ingestion by newborn baboons. *Arch Dis Child.* 1989; 64:930–3. [PubMed: 2774634]
- Jarvinen KM, Laine ST, Jarvenpaa AL, Suomalainen HK. Does low IgA in human milk predispose the infant to development of cow's milk allergy? *Pediatr Res.* 2000; 48:457–62. [PubMed: 11004235]
- Järvinen KM, Suomalainen H. Leucocytes in human milk and lymphocyte subsets in cow's milk-allergic infants. *Pediatr Allergy Immunol.* 2002; 13:243–54. [PubMed: 12390440]
- Lavelle EC, McNeela E, Armstrong ME, Leavy O, Higgins SC, Mills KH. Cholera toxin promotes the induction of regulatory T cells specific for bystander antigens by modulating dendritic cell activation. *J Immunol.* 2003; 171:2384–92. [PubMed: 12928385]
- 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; 106:150–8. [PubMed: 10887318]

- Lopez-Exposito I, Song Y, Jarvinen KM, Srivastava K, Li XM. Maternal peanut exposure during pregnancy and lactation reduces peanut allergy risk in offspring. *J Allergy Clin Immunol*. 2009; 124:1039–46. [PubMed: 19895992]
- Ma LJ, Walter B, Deguzman A, Muller HK, Walker AM. Trans-epithelial immune cell transfer during suckling modulates delayed-type hypersensitivity in recipients as a function of gender. *PLoS One*. 2008; 3:e3562. [PubMed: 18958163]
- Mahnke K, Schmitt E, Bonifaz L, Enk AH, Jonuleit H. Immature, but not inactive: the tolerogenic function of immature dendritic cells. *Immunol Cell Biol*. 2002; 80:477–83. [PubMed: 12225384]
- Martinez Gomez JM, Fischer S, Csaba N, Kündig TM, Merkle HP, Gander B, et al. A protective allergy vaccine based on CpG- and protamine-containing PLGA microparticles. *Pharm Res*. 2007; 24:1927–35. [PubMed: 17541735]
- Matson AP, Thrall RS, Rafti E, Lingenheld EG, Puddington L. IgG transmitted from allergic mothers decreases allergic sensitization in breastfed offspring. *Clin Mol Allergy*. 2010; 8:9. [PubMed: 20626874]
- Mosconi E, Rekima A, Seitz-Polski B, Kanda A, Fleury S, Tissandie E, et al. Breast milk immune complexes are potent inducers of oral tolerance in neonates and prevent asthma development. *Mucosal Immunol*. 2010; 3:461–74. [PubMed: 20485331]
- Pali-Scholl I, Renz H, Jensen-Jarolim E. Update on allergies in pregnancy, lactation, and early childhood. *J Allergy Clin Immunol*. 2009; 123:1012–21. [PubMed: 19249083]
- Pilette C, Durham SR, Vaerman JP, Sibille Y. Mucosal immunity in asthma and chronic obstructive pulmonary disease: a role for immunoglobulin A? *Proc Am Thorac Soc*. 2004; 1:125–35. [PubMed: 16113425]
- Polte T, Hennig C, Hansen G. Allergy prevention starts before conception: maternofetal transfer of tolerance protects against the development of asthma. *J Allergy Clin Immunol*. 2008; 122:1022–30. [PubMed: 19000583]
- Prescott SL, Macaubas C, Holt BJ, Smallacombe TB, Loh R, Sly PD, et al. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. *J Immunol*. 1998; 160:4730–7. [PubMed: 9590218]
- Ravetch JV, Bolland S. IgG Fc receptors. *Annu Rev Immunol*. 2001; 19:275–90. [PubMed: 11244038]
- Seelig LL, Head JR. Uptake of lymphocytes fed to suckling rats: An autographic study of the transit of labelled cells through the neonatal gastric mucosa. *J Reprod Immunol*. 1987; 10:285–97. [PubMed: 3625602]
- Siafakas, C.; Anderson, I.; Walker, WA.; Xanthou, M. Breast milk cells and their interaction with intestinal mucosa. In: Bellanti, JA.; Bracci, R.; Prindull, G.; Xanthou, M., editors. *Neonatal Hematology and Immunology III*. Amsterdam: Elsevier Science Publishers; 1997.
- Sicherer SH, Munoz-Furlong A, Godbold JH, Sampson HA. US prevalence of self-reported peanut, tree nut, and sesame allergy: 11-year follow-up. *J Allergy Clin Immunol*. 2010; 125:1322–6. [PubMed: 20462634]
- Skolnick HS, Conover-Walker MK, Koerner CB, Sampson HA, Burks W, Wood RA. The natural history of peanut allergy. *J Allergy Clin Immunol*. 2001; 107:367–74. [PubMed: 11174206]
- Smits HH, Gloudemans AK, van NM, Willart MA, Soullié T, Muskens F, et al. Cholera toxin B suppresses allergic inflammation through induction of secretory IgA. *Mucosal Immunol*. 2009; 2:331–9. [PubMed: 19404246]
- Snider DP, Marshall JS, Perdue MH, Liang H. Production of IgE antibody and allergic sensitization of intestinal and peripheral tissues after oral immunization with protein Ag and cholera toxin. *J Immunol*. 1994; 153:647–57. [PubMed: 8021502]
- 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; 123:443–51. [PubMed: 19203662]
- Tuboly S, Bernath S, Glavits R, Kovacs A, Megyeri Z. Intestinal absorption of colostral lymphocytes in newborn lambs and their role in the development of immune status. *Acta Vet Hung*. 1995; 43:105–15. [PubMed: 7625282]

- van Wijk F, Wehrens EJ, Nierkens S, Boon L, Kasran A, Pieters R, et al. CD4+CD25+ T cells regulate the intensity of hypersensitivity responses to peanut but are not decisive in the induction of oral sensitization. *Clin Exp Allergy*. 2007; 37:572–81. [PubMed: 17430355]
- Verhasselt V, Milcent V, Cazareth J, Kanda A, Fleury S, Dombrowicz D, et al. Breast milk-mediated transfer of an antigen induces tolerance and protection from allergic asthma. *Nat Med*. 2008; 14:170–5. [PubMed: 18223654]
- Wennergren G. What if it is the other way around? Early introduction of peanut and fish seems to be better than avoidance. *Acta Paediatr*. 2009:1–3.

Highlights

- We assess whether maternal feeding of peanut alone protects against peanut active sensitization in offspring compared to the effect of a mucosal adjuvant co-administration with peanut
- Maternal peanut feeding can induce protection against sensitization to peanut in the offspring of peanut immunized mothers
- The protective effect is enhanced when peanut is delivered together with the adjuvant cholera toxin
- Maternal transmission of peanut-specific antibodies IgG2a and IgA through breast milk may be, at least in part, responsible for this protection.

Fig. 1.

Experimental protocol of mothers' and offsprings' sensitization. Female C3He/J mice were divided into 3 groups according to different sensitization protocols: PN+CT group received peanut and CT i.g., PN group only peanut i.g., and the CT group only CT i.g. for five weekly feedings. An unimmunized group was employed as a control. At 5 weeks they were mated with naïve males and continued on the same feeding regimen during pregnancy and lactation. 5-week old offspring from all the 4 groups were sensitized with PN+CT i.g. weekly for 5 weeks followed by 2 boosting doses. Mothers were sacrificed at weaning and offspring at week 15 for analysis.

Fig. 2. Peanut-specific immunoglobulin levels in mouse milk. Milk (a) peanut-specific IgG2a (ng/mL), (b) peanut-specific IgG1 (ng/mL) and (c) peanut-specific IgA (ng/mL) were measured by antigen-specific ELISA. Data are expressed as means $\pm$ SEM of duplicates for each group (n=3–4) *P<0.05, ***P<0.001 vs Unimmunized.

Fig. 3. Crude peanut extract (CPE)-stimulated cytokine levels in mothers' splenocyte culture supernatants (pg/mL). (a) IL-4, (b) IL-13, (c) IL-10, (d) IFN- γ , (e) IL-17 and TGF- β levels were measured by ELISA. Data are expressed as means $\pm$ SEM of duplicates for each group (n=3-4). *P<0.05, **P<0.01, ***P<0.001 vs Unimmunized.

Fig. 4. Offspring humoral response. Serum (a) peanut-specific IgE, (c) peanut-specific IgG1 and (e) peanut-specific IgG2a measured by antigen-specific ELISA with data points indicating group means $\pm$ SEM of triplicates for each group (pooled samples; $n=7-10$). Serum (b) peanut-specific IgE, (d) specific IgG1, and (f) specific IgG2a titrations at week 15, measured by antigen-specific ELISA with data points indicating group means $\pm$ SEM of individual samples for each group ($n=7-10$). Sensitization was finished at week 11. * $P<0.05$, ** $P<0.01$ *** $P<0.001$ vs Unimmunized/PN+CT.

Fig. 5. CPE-stimulated cytokine levels in MLN culture supernatants. (a) IL-4, (b) IL-13, (c) IL-10, (d) IFN- γ and (e) IL-17 levels were measured by ELISA. Data are expressed as means $\pm$ SEM of triplicates from two sets of pooled samples. Each set included 3–5 mice. * $P < 0.05$, *** $P < 0.001$ vs Unimmunized/PN+CT, ## $P < 0.01$, ### $P < 0.001$ vs PN+CT/PN+CT.

Offspring's ratio of serum PN-specific IgG2a to IgG1. Sera from each group (n=7–10 mice) were pooled, and peanut-specific IgG1 and IgG2a levels were determined by ELISA.

Table 1

Group	PN-specific IgG2a/IgG1 Ratio						
	w0	w2	w5	w8	w11	w15	
PN/PN+CT	0.00±0.00	0.55±0.07	1.46±0.01	1.26±0.14	0.93±0.04	1.36±0.05	
CT/PN+CT	0.00±0.00	0.61±0.01	0.71±0.05	0.66±0.02	1.17±0.28	2.08±0.39	
PN+CT/PN+CT	0.73±0.01	0.98±0.01	1.21±0.03	1.33±0.05	1.74±0.02	2.75±0.02	
Unimmunized/PN+CT	0.00±0.00	0.50±0.02	0.41±0.03	0.32±0.03	0.38±0.02	1.06±0.05	
P value							
PN/PN+CT vs CT/PN+CT	NS	NS	p<0.001	p<0.01	NS	p<0.05	
PN/PN+CT vs PN+CT/PN+CT	p<0.001	p<0.001	p<0.001	NS	p<0.05	p<0.001	
PN/PN+CT vs Unimmunized/PN+CT	NS	NS	p<0.001	p<0.001	NS	NS	
PN+CT/PN+CT vs CT/PN+CT	p<0.001	p<0.001	p<0.001	p<0.001	NS	p<0.05	
PN+CT/PN+CT vs Unimmunized/PN+CT	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	

NS: not significant

Table 2

CPE-stimulated cytokines levels in offsprings' splenocyte culture supernatants (pg/mL). IL-4, IL-13, IL-10, IFN- γ , IL-17 and TGF- β levels were measured by ELISA. Data are expressed as means $\pm$ SEM of duplicates from three to four sets of pooled samples. Each set included 2–3 mice.

Group	CPE-stimulated cytokine levels (pg/mL)					
	IL-4	IL-13	IL-10	IFN- γ	IL-17	TGF- β
PN/PN+CT	0.0 $\pm$ 0.0 [#]	344.7 $\pm$ 193.7 [*]	4171 $\pm$ 1267	33166 $\pm$ 32053	3701 $\pm$ 1206 [*]	49.4 $\pm$ 24.7 [*]
CT/PN+CT	23.2 $\pm$ 23.2	1054 $\pm$ 436.1	5794 $\pm$ 2118	59769 $\pm$ 14258	4917 $\pm$ 1354	244.2 $\pm$ 18.3
PN+CT/PN+CT	76.7 $\pm$ 5.3	564.6 $\pm$ 190.4	2956 $\pm$ 470.1	12711 $\pm$ 4447 [*]	971.8 $\pm$ 59.91 [*]	98.6 $\pm$ 57
Unimmunized/PN+CT	21.2 $\pm$ 17.4	872.3 $\pm$ 12.4	4202 $\pm$ 270.1	80412 $\pm$ 6437	10330 $\pm$ 2091	267.7 $\pm$ 49.8

* P<0.05 vs Unimmunized/PN+CT,

[#] P<0.05 vs PN+CT/PN+CT.