

BRIEF COMMUNICATION

Respiratory allergen from house dust mite is present in human milk and primes for allergic sensitization in a mouse model of asthma

P. Macchiaverni^{1,*}, A. Rekima^{2,*}, M. Turfkruyer², L. Mascarelli³, S. Airouche³, P. Moingeon³, K. Adel-Patient⁴, A. Condino-Neto¹, I. Annesi-Maesano^{5,6}, S. L. Prescott^{7,8}, M. K. Tulic^{2,8} & V. Verhasselt^{2,8}

¹Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil; ²University Nice Sophia Antipolis, TIM, Nice; ³Research and Development, Stallergenes SA, Antony; ⁴INRA, UR496 Immuno-Allergie Alimentaire, CEA/IBiTeC-S/SPI, CEA de Saclay, F-91191 Gif sur Yvette cedex; ⁵EPAR UMR-S 707 INSERM; ⁶EPAR UMR-S 707 UPMC Paris6, Medical School Saint-Antoine Paris, Paris, France; ⁷School of Pediatrics and Child Health, University of Western Australia; ⁸The International Inflammation 'in-FLAME' Network, Worldwide Universities Network (WUN), Perth, Australia

To cite this article: Macchiaverni P, Rekima A, Turfkruyer M, Mascarelli L, Airouche S, Moingeon P, Adel-Patient K, Condino-Neto A, Annesi-Maesano I, Prescott SL, Tulic MK, Verhasselt V. Respiratory allergen from house dust mite is present in human milk and primes for allergic sensitization in a mouse model of asthma. *Allergy* 2013; DOI: 10.1111/all.12332.

Keywords

allergic sensitization; allergy primary prevention; house dust mite; human breast milk; neonatal oral immune tolerance.

Correspondence

Valérie Verhasselt, MD, PhD, EA 6302
'Tolérance Immunitaire'-Université de Nice
Sophia-Antipolis Hôpital de l'Archet, Route
Saint Antoine de Ginestière – BP3079.
06202 Nice Cedex 03, France.
Tel.: +33-(0)4-92-15-77-06
Fax: +33-(0)4-92-15-77-09
E-mail: verhasselt@unice.fr

*These authors contributed equally.

Abstract

There is an urgent need to identify environmental risk and protective factors in early life for the prevention of allergy. Our study demonstrates the presence of respiratory allergen from house dust mite, Der p 1, in human breast milk. Der p 1 in milk is immunoreactive, present in similar amounts as dietary egg antigen, and can be found in breast milk from diverse regions of the world. In a mouse model of asthma, oral exposure to Der p through breast milk strongly promotes sensitization rather than protect the progeny as we reported with egg antigen. These data highlight that antigen administration to the neonate through the oral route may contribute to child allergic sensitization and have important implications for the design of studies assessing early oral antigen exposure for allergic disease prevention. The up-to-now unknown worldwide presence of respiratory allergen in maternal milk allows new interpretation and design of environmental control epidemiological studies for allergic disease prevention.

Accepted for publication 23 October 2013

DOI:10.1111/all.12332

Edited by: Angela Haczku

It has been proposed that, on the contrary to allergen avoidance, early allergen exposure may be necessary for allergic disease prevention to allow immune tolerance induction and prevent the development of allergen sensitization as the first step for development of allergic disease [reviewed in (1–3)]. In this regard, early oral administration of allergens in the context of food allergy prevention has received much interest

(2, 3) as the oral route is a well-known way to induce tolerance both in animal models and in humans (4).

We and others have previously demonstrated that breast-milk-mediated oral transfer of an antigen may prevent antigen-specific immune responses and allergic disease development in rodents (5–10). Although numerous papers have described the presence of food antigens in breast milk (11), none have previously considered the possibility of the presence of respiratory antigen in human milk and its impact on allergy development in the breastfed child. We have set out to investigate whether Der p 1, a major allergen from house dust mite (HDM) *Dermatophagoides pteronyssinus*, can be found in

Abbreviations

Der p, *Dermatophagoides pteronyssinus*; HDM, house dust mite; OVA, ovalbumin.

human colostrum and 1- and 6-month milk samples from mothers distributed in three continents, Oceania (Australia, $n = 32$), South America (Brazil, $n = 76$), and Europe (France, $n = 97$) (see the Supplementary Methods in the Supporting Information). Using a sensitive ELISA (Supplementary Methods), we were able to detect Der p 1 in 58, 70.1, and 78.1% of colostrum samples from Brazil, France, and Australia, respectively, and in 52.1 and 55.2% of 1- and 6-month Australian milk samples, respectively. As shown in Fig. 1A and supplementary Table S1, Der p 1 levels were heterogeneous in colostrum and milk samples. There was no statistical difference in colostrum Der p 1 levels from the three countries. When analyzed altogether ($n = 205$), median Der p 1 concentration in colostrum was 96 pg/ml (IQR 50.2–201.6 pg/ml) and highest value was 1293 pg/ml. Concentration of Der p 1 in 1- and 6-month milk samples was not statistically different, and median Der p 1 concentration in all milk samples was 65.9 pg/ml (IQR 41.5–172 pg/ml). Der p 1

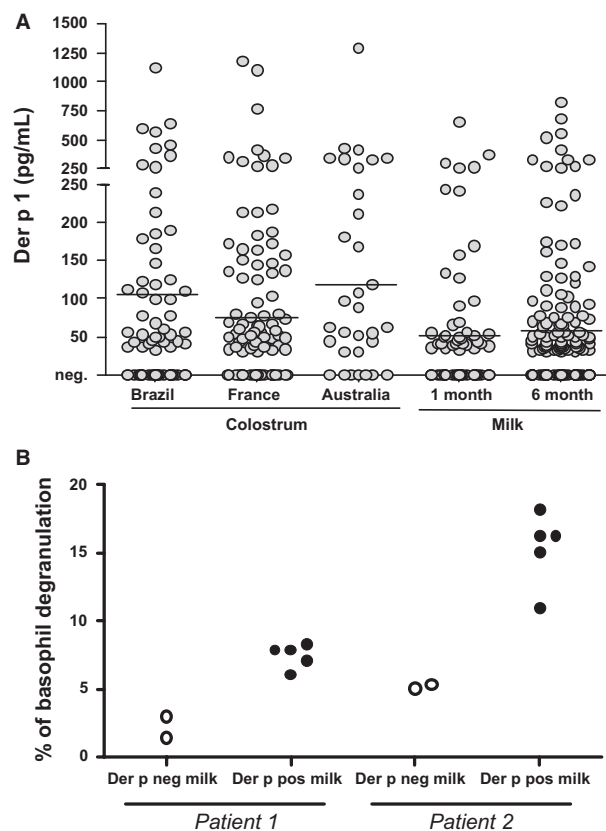


Figure 1 House dust mite *Dermatophagoides pteronyssinus* major allergen Der p 1 is found worldwide in human colostrum, and Der p 1-containing milk is able to induce human IgE-mediated basophil degranulation. (A) Concentration of Der p 1 in human colostrum and milk samples. Bars indicate median values of positive samples. (B) Percentage (%) of degranulation of basophils coated with immunopurified IgE from serum of two patients allergic to Der p (patients 1 and 2) and incubated with seven milk samples: 5 Der p1 positive (pos) and two negative (neg). One hundred percentage corresponds to degranulation induced by anti-human IgE.

levels in milk were statistically lower than Der p 1 concentration in colostrum ($P = 0.0001$). Interestingly, as shown in supplementary Fig. S2 and Table S2, the levels of Der p 1 in milk and colostrum are in the same magnitude as levels found for a maternal dietary egg antigen (12, 13) (OVA, median 133.6 pg/ml IQR 78.5–290.4 pg/ml and median 127.9 pg/ml IQR 77.2–159 pg/ml in milk and colostrum samples, respectively).

To determine whether Der p 1 detected in milk by ELISA technique is immunologically relevant, we tested the ability of Der p 1-containing milk to induce degranulation of basophils coated with human Der p-specific IgE (Supplementary Methods). As shown in Fig. 1B, only milk containing detectable levels of Der p by ELISA were able to induce significant basophils cell degranulation. Importantly, these data indicate that Der p found in human milk is recognized by the immune system and is immunologically active.

Previously, we showed in a mouse model the beneficial impact of milk OVA-specific IgG and TGF- β on tolerance induction upon OVA transfer through milk (9, 10). Interestingly, Der p-specific IgG was detected in almost all colostrum and milk samples (supplementary Fig. S3 and Table S3), confirming and extending our previous observations using Brazilian human colostrum samples (14). TGF- β was also found in all samples, and there was no difference found in its content between countries. Levels were higher in colostrums than in milk as described by others (15) (supplementary Fig. S4 and Table S4).

We next set up studies to investigate whether oral transfer of such ubiquitous respiratory allergen through breast milk will affect progeny's susceptibility to allergic asthma. To do this, we established a unique mouse model of HDM allergen milk transfer, which closely mimics the human situation. This model does not involve the use of adjuvant and importantly allows us to explore the specific impact of HDM allergen transfer in breastfed pups on allergic disease susceptibility excluding environmental and genetic factors (supplementary methods and supplementary Fig. S1).

When assessing Der p 1 levels in mouse milk, we found undetectable levels in unexposed mothers, while in Der p-exposed mothers, levels were in the same order of magnitude as found in human milk (513 pg/ml $\pm$ 105 pg/ml; median $\pm$ SD; $n = 6$). Milk TGF- β levels in unexposed and exposed mothers were comparable (1292 pg/ml $\pm$ 375 pg/ml vs 1122 pg/ml $\pm$ 420; $n = 6$). As in humans, maternal exposure to Der p also resulted in detection of Der p-specific IgG1 in mouse milk (200 AU/ml $\pm$ 114 AU/ml; $n = 5$), while they were undetectable in unexposed mothers. We then compared allergic sensitization and airway disease susceptibility in 6-week-old mice challenged with Der p extracts, which only differed by their early exposure to Der p allergens and specific antibodies through breast milk. We observed that mice breastfed by Der p-exposed mothers were primed for allergic sensitization as demonstrated by their fivefold increase in serum Der p-specific IgE (Fig. 2A) and IgG1 (Fig. 2B) levels compared with mice breastfed by naïve mothers. Lung function analyzed by both invasive (Fig. 2D) and noninvasive methods (supplementary Fig. S5d), as well as

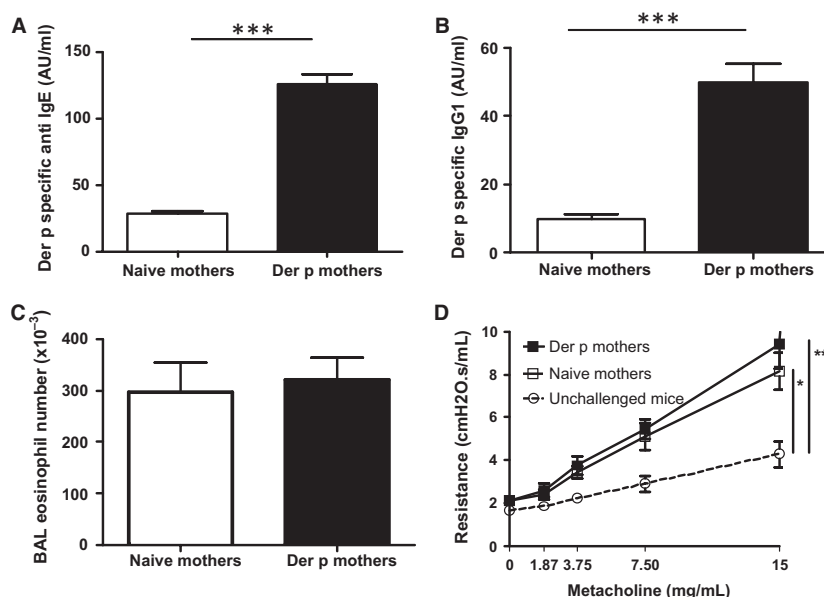


Figure 2 Der p allergen transfer through breast milk increases allergic sensitization and has no impact on allergic airway disease in a mouse model. Mice breastfed by Der p-exposed (■/filled bars) or unexposed (□/empty bars) naïve mothers were tested for allergic sensitization (A and B) and airway disease (C and D) after Der p challenge when 6–8 weeks old. (A) Serum levels of Der p-specific

lung inflammation (Fig. 2C, supplementary Fig. S5a, b, c), was not significantly affected by maternal transfer of Der p through breast milk. TGF- β in milk had no impact on breast-milk-mediated allergic increased sensitization (supplementary Fig. S6).

This study demonstrates that neonates and infants can be exposed to ubiquitous respiratory allergens through breastfeeding during a critical window of immune development. This original observation gives new interpretation of epidemiological studies on environmental control in early life [reviewed in (16)]. Discrepancy between studies on environmental control in early life and risk of allergic sensitization may rely on the fact that in most studies, focus was predominantly on the child's and not maternal exposure and possible HDM allergen transfer through milk has not been considered. The design of new studies for allergic disease prevention may benefit from our observation, and more attention may be paid to maternal environmental control in case of breastfeeding. In addition, our data obtained in a mouse model mimicking the human situation indicate that early-life oral exposure to Der p allergen through milk increases the post-natal susceptibility to allergic sensitization in the offspring rather than protect it as we reported with egg antigen. This observation highlights the importance of the nature of the allergen on immunological outcome upon oral exposure in early life, and our ongoing research is assessing whether such priming is observed with other food and respiratory allergens. There are important biological and functional differences between OVA and Der p allergens, which may explain our observations. In particular, a large body of evidence indi-

cates that Der p allergens have intrinsic adjuvant activity through their protease activity or molecular mimicry with molecules involved in TLR signaling, which induce innate immune system activation and are known to interfere with tolerance induction [reviewed in (17)]. Our experimental data raise important issue around the possible risk to induce allergic sensitization upon early life oral exposure to natural allergens and suggest that recent recommendation of early introduction of complementary foods needs to be carefully considered. This study is an important prelude to future studies aimed to identify which additional factors, including those in breast milk, may help to prevent allergic sensitization.

Acknowledgments

We thank Maternidade de Campinas Hospital and Silvana Severino Dalgé for colostrum sample collections; Christina Arslanian for technical assistance; Stallergenes for provisions of HDM extract; Véronique Corcelle and the animal facility staff for their excellent care of mice; and Francis Hautem for excellent laboratory management.

Funding

This work was supported by Institut National de la Santé et Recherche Médicale (INSERM), Université de Nice Sophia-Antipolis, Société Française d'Allergologie (SFA), Fond de Recherche en Santé Respiratoire, Fondation Princesse Grace, grant # 2012/51290-6 São Paulo Research Foundation (FAPESP), and all the funding sources for the EDEN study.

Conflict of interest

All authors declare no financial and personal relationships with other people or organisations that could inappropriately influence this work.

Author contributions

PM, AR, and MKT set up and performed human milk ELISA; PM, AR, and MT set up and performed mouse experiments; KAP designed and performed basophil assay; LM contributed to the design of mouse experiment; LM and SA performed lung function assessments (both invasive and noninvasive); PhM supervised LM and SA work; IAM, ACN, and SLP provided colostrum and milk samples from France, Brazil, and Australia, respectively; PM, AR, MKT, and VV designed and analyzed the experiments; PM, MKT, and VV wrote the paper. VV designed and supervised the whole project. All authors read, commented on the manuscript, and approved the final version.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. A mouse model to assess impact of maternal Der p exposure on progeny susceptibility to allergic asthma to Der p.

Figure S2. Egg food antigen ovalbumin (OVA) concentration in human colostrum from France and Australia and 1

and 6 months postpartum Australian milk samples. Bars indicate median values of positive samples.

Figure S3. Der p-specific IgG are found in human colostrum from Brazil, France and Australia and 1 and 6 months postpartum Australian milk samples. Bars indicate median values of positive samples. * $P = 0.003$; ** $P = 0.006$; *** $P < 0.0001$.

Figure S4. TGF- β levels in human colostrum from Brazil, France and Australia and 1 and 6 months postpartum Australian milk samples. Bars indicate median values of positive samples. *** $P < 0.0001$.

Figure S5. Der p allergen transfer through breast milk increases allergic sensitization but not allergic airway disease in a mouse model.

Figure S6. TGF- β in milk has no impact on allergic sensitization of the progeny upon Der p transfer through breast milk.

Table S1. Der p 1 concentration in human colostrum from Brazil, France and Australia and 1 and 6 months postpartum Australian milk samples.

Table S2. OVA concentration in human colostrum from France and Australia and 1 and 6 months postpartum Australian milk samples.

Table S3. IgG-Der p concentration in human colostrum from Brazil, France and Australia and 1 and 6 months postpartum Australian milk samples.

Table S4. TGF- β levels in human colostrum from Brazil, France and Australia and 1 and 6 months postpartum Australian milk samples.

References

- Holt PG, Thomas WR. Sensitization to airborne environmental allergens: unresolved issues. *Nat Immunol* 2005;**6**:957–960.
- Lack G. Epidemiologic risks for food allergy. *J Allergy Clin Immunol* 2008;**121**:1331–1336.
- Prescott SL, Smith P, Tang M, Palmer DJ, Sinn J, Huntley SJ et al. The importance of early complementary feeding in the development of oral tolerance: concerns and controversies. *Pediatr Allergy Immunol* 2008;**19**:375–380.
- Mayer L, Shao L. Therapeutic potential of oral tolerance. *Nat Rev Immunol* 2004;**4**:407–419.
- Komatsu T, Okao M, Miyamoto H, Chen T, Shinka S. Effects of early antigen exposure through lactation on later specific antibody responses in mice. *J Immunol* 1988;**141**:2895–2906.
- Korotkova M, Telemo E, Yamashiro Y, Hanson LA, Strandvik B. The ratio of n-6 to n-3 fatty acids in maternal diet influences the induction of neonatal immunological tolerance to ovalbumin. *Clin Exp Immunol* 2004;**137**:237–244.
- Yamamoto T, Tsubota Y, Kodama T, Kageyama-Yahara N, Kadowaki M. Oral tolerance induced by transfer of food antigens via breast milk of allergic mothers prevents offspring from developing allergic symptoms in a mouse food allergy model. *Clin Dev Immunol* 2012;**2012**:721085.
- 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**:1039–1046.
- 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**:461–474.
- 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**:170–175.
- Palmer DJ, Makrides M. Diet of lactating women and allergic reactions in their infants. *Curr Opin Clin Nutr Metab Care* 2006;**9**:284–288.
- Palmer DJ, Gold MS, Makrides M. Effect of maternal egg consumption on breast milk ovalbumin concentration. *Clin Exp Allergy* 2008;**38**:1186–1191.
- Vance GH, Lewis SA, Grimshaw KE, Wood PJ, Briggs RA, Thornton CA et al. Exposure of the fetus and infant to hens' egg ovalbumin via the placenta and breast milk in relation to maternal intake of dietary egg. *Clin Exp Allergy* 2005;**35**:1318–1326.
- Macchiaverni P, Arslanian C, Frazao JB, Palmeira P, Russo M, Verhasselt V et al. Mother to child transfer of IgG and IgA antibodies against Dermatophagoides pteronyssinus. *Scand J Immunol* 2011;**74**:619–627.
- Oddy WH, Rosales F. A systematic review of the importance of milk TGF- β on immunological outcomes in the infant and young child. *Pediatr Allergy Immunol* 2010;**21**:47–59.
- Simpson A, Custovic A. Prevention of allergic sensitization by environmental control. *Curr Allergy Asthma Rep* 2009;**9**:363–369.
- Gregory LG, Lloyd CM. Orchestrating house dust mite-associated allergy in the lung. *Trends Immunol* 2011;**32**:402–411.

Supplementary Methods

Colostrum and milk samples:

Human colostrum samples were collected from birth cohort studies in three different continents including South America (Brazil, n=76), Europe (France, EDEN birth cohort, n=97) and Oceania (Australia, n=32). All colostrum samples were collected within 48h after delivery. In addition, 1 and 6 months postpartum milk samples were collected from the Australian cohort (n=71 and 161 respectively). Exclusion criteria for enrollment of pregnant mothers were hypertension, diabetes, infections, immunodeficiency, and those who had received corticosteroids, blood-derived products transfusion or other drugs related to chronic diseases during pregnancy. Collection of all samples was approved by the local Ethics Committees and an informed consent form was obtained from each participant. Mouse breast milk was collected from the stomach of 2-week-old pups 4 hrs after maternal exposure to Der p extract (Stallergenes, Anthony, France). Female BALB/c mice (Centre d'Élevage Janvier, Le Genest Saint Isle, France) were housed under SPF conditions. Animal experiments were approved by the Ethical Committee CIEPAL (Protocole NCE/2012-81).

Detection of Der p 1 in milk

Der p 1 was measured in human and mouse breast-milk samples by Der p 1 ELISA kit 5H8/4C1 (EL-DP1, Indoor Biotechnologies). Briefly, 96-well plates were coated overnight at 4°C with 2µg/ml anti-Der p 1 mAb 5H8 in carbonate-bicarbonate buffer pH 9.6. The next day, plates were blocked with 150µl 1% BSA in PBS Tween 0.05% for 1 hr at room temperature (RT). Samples were added neat and diluted 2-fold in the same blocking buffer and incubated overnight at 4°C. The next day, samples were incubated for 2 hrs at RT with biotinylated anti-Der p 1 mAb 4C1 (BI-4C1) diluted in Buffer CGS2 (Cosmo Bio, Tokyo,

Japan), followed by streptavidin-HRP (554066, BD Pharmingen) for 30 minutes. TMB (50-65-00, KPL) was used as the chromogenic substrate. Plates were washed in PBS Tween 0.05% between each step. Absorbance was measured at 450 nm and values read from Der p 1 standard curve (Universal allergen standard, Indoor Biotechnologies). Der p 1 detection level was 30 pg/ml.

Basophil degranulation assay

The ability of Der p containing milk to induce degranulation of basophil coated with IgE specific for Der p was assessed using a previously described protocol with some modification²¹. Briefly, a humanized rat leukemia basophilic cell line expressing the high affinity receptor for human IgE, (FcεR1, cell line RBL-SX38) was coated with IgE immunopurified from sera of patient allergic only to Der p and Der f (patient 1) or only to Der p (patient 2) (Plasma Lab, Wa, USA). Degranulation induced by 7 milk samples (5 Der p1 positive and 2 negative) was assessed by analysing β-hexosaminidase released in culture supernatant. Anti-human IgE (clone Le27) induced degranulation was used as reference release and was assigned the value of 100%. Using Der p extract, we found that sensitivity of the test was 10pg/ml of total Der p protein which corresponds to 0.6 pg/ml Der p 1. Saturation of degranulation was observed at levels of Der p 1 above 6pg/ml.

Detection of Der p-specific immunoglobulins

Der p specific IgG were measured in human colostrum and milk, and mice IgG1 and IgE in milk and serum by ELISA. Briefly, 96-well plates were coated overnight at 4°C with 100μl Der p extract (Stallergenes, Paris, France) diluted in PBS containing 5μg/ml of Der p 1. Plates were saturated with 5% non-fat dry milk in PBS-Tween 0.05% for 1 hr at RT. Samples were diluted in duplicate using two serial dilutions of each in the same blocking buffer and

incubated for 3 hrs at RT. As secondary antibody, we used peroxidase-conjugated anti-human IgA (Sigma, St. Louis, MO), biotinylated anti-human IgG (BD Pharmingen), biotinylated anti-mouse IgG1 (BD Pharmingen) and peroxidase-conjugated anti-mouse IgE (Gentaure, Paris, France) diluted in Buffer CGS2 for respective antibody detection. Streptavidin-HRP (BD Pharmingen) was used as a second-step reagent for detection of the biotin-labeled Abs. TMB was used as the chromogenic substrate for all ELISAS. Results were expressed as arbitrary units (AU) per ml, obtained by comparison with a serum for IgG or colostrum pool for IgA and defined to contain 1000 AU/ml.

Mouse model of Der p allergen transfer through breast milk

Six-8 week-old BALB /c mice were anesthetized with isoflurane and 20µl of Der p extract containing 20µg of Der p 1 was delivered intranasally (IN) from day (d)0-d5 and d8-d10 (Sup Fig.1). Naïve mothers were exposed to PBS following the same protocol. Naïve and Der p-exposed mice (Der p mothers) were mated 2 days after the last exposure. To avoid maternal *in utero* effect, all mice were delivered by naïve unexposed mothers and were fostered at birth by Der p exposed or unexposed mothers. Der p mothers continued to be exposed to 20 µg of Der p three times a week during lactation and naïve mothers to PBS.

Assessment of allergic airway disease susceptibility in the offspring

At 6 weeks of age, mice breastfed by naïve or Der p mothers were challenged to Der p extract for 8 days by IN administration of 20µg of Der p extract as described here above (Supplementary Fig.1). Protocol was adapted from Cates et al ²². Allergic sensitization, lung function and inflammation were analyzed two days after the last IN administration.

Lung Function:

Bronchial resistance was measured using a Flexivent® system (Emka Technologies, Falls Church, VA, USA) after exposure to increasing doses of methacholine (1.8, 3.7, 7.5 and 15 mg/ml)^{3,4}.

Plasma collection

Following lung function, mice were euthanized by lethal intraperitoneal injection of pentobarbital. Blood was harvested by intra-cardiac puncture and collected in heparinized tubes. Plasma was prepared by centrifugation (10 000 *g* for 10 min) and stored at – 20 ° C until analysis.

Total and differential cell counts in broncho-alveolar lavages (BAL) fluid:

After blood collection, eosinophilia in BAL was analyzed as previously described^{23,4}.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA). We assessed statistical significance between 2 groups using a two-tailed P value calculated with the Mann-Whitney nonparametric test.

References

1. Blanc F, Adel-Patient K, Drumare MF, Paty E, Wal JM, Bernard H. Capacity of purified peanut allergens to induce degranulation in a functional in vitro assay: Ara h 2 and Ara h 6 are the most efficient elicitors. Clin Exp Allergy 2009; 39:1277-85.
2. Cates EC, Fattouh R, Wattie J, Inman MD, Goncharova S, Coyle AJ, et al. Intranasal exposure of mice to house dust mite elicits allergic airway inflammation via a GM-CSF-mediated mechanism. J Immunol 2004; 173:6384-92.
3. 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.
4. 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.

Supplementary Figures

Fig.1. A mouse model to assess impact of maternal Der p exposure on progeny susceptibility to allergic asthma to Der p

Female mice were exposed to Der p extract containing 20µg of Der p1 intranasally (IN), from day 1-5 and 8-10 (Der p mothers). Two days after the last exposure they were mated. At delivery, pups from unexposed mothers (naïve pups) were adopted and fostered by Der p mothers. Der p mothers continued to be exposed IN to 20 µg of Der p1 containing Der p extract 3 times a week during lactation until weaning. When 6 weeks old, offspring were exposed to Der p extract containing 20µg Der p1 from day 1-5 and 8-10. Mice were analyzed two days after the last IN exposure. Following the same protocol, a group of mice were breastfed by naïve mothers that were exposed IN to PBS (Naïve mothers).

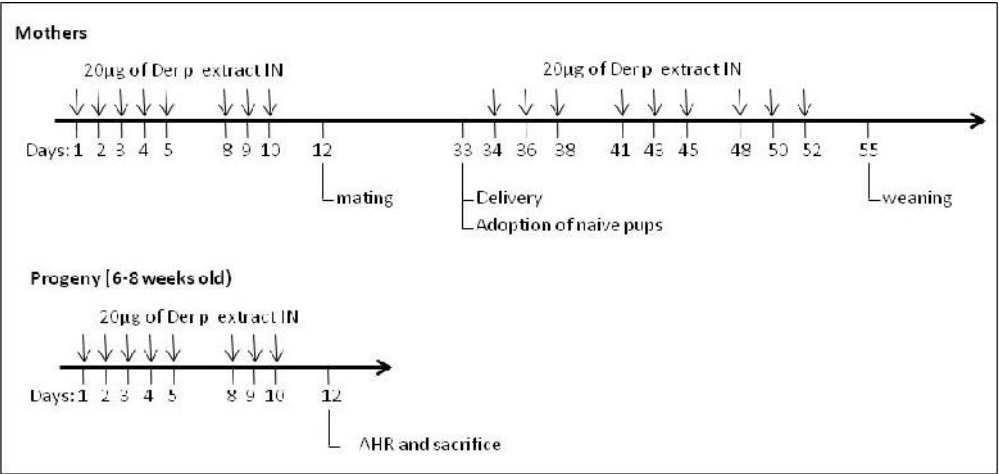


Fig. 2. Egg food antigen ovalbumin (OVA) concentration in human colostrum from France and Australia and 1 and 6 months postpartum Australian milk samples. Bars indicate median values of positive samples

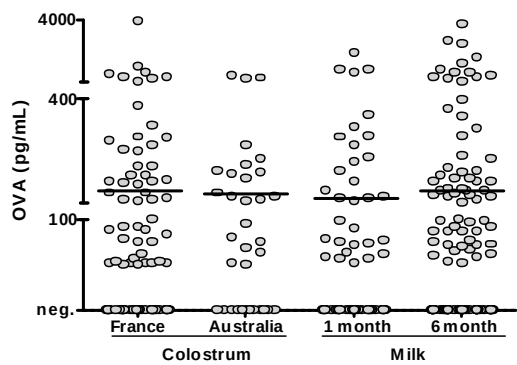


Fig. 3. Der p-specific IgG are found in human colostrums from Brazil, France and Australia and 1 and 6 months postpartum Australian milk samples. Bars indicate median values of positive samples. (*) $p=0.003$; () $p=0.006$; (***) $p<0.0001$**

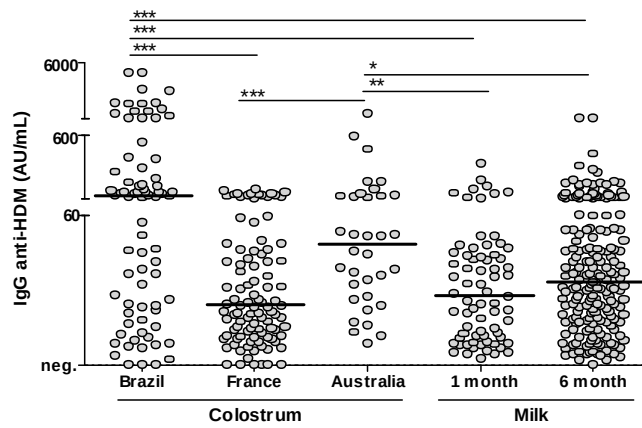


Fig. 4. TGF-beta levels in human colostrum from Brazil, France and Australia and 1 and 6 months postpartum Australian milk samples Bars indicate median values of positive samples. (***) $p < 0.0001$

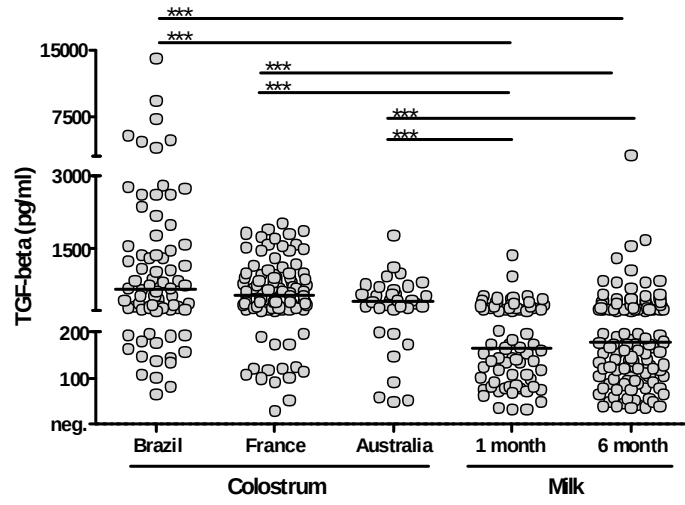


Fig. 5. Der p allergen transfer through breast milk increases allergic sensitization but not allergic airway disease in a mouse model. Mice breastfed by Der p-exposed (■ / filled bars) or unexposed (□ / empty bars) naïve mothers were tested for allergic airway disease after Der p challenge when 6-8 weeks old. (a) lung cells secretion of IL-5 and (b) IL-13 (5 exp, n=33-40); (c) histology of lung sections after Hematoxylin Eosin staining (Biodoxid, Paris, France); (d) airway hyperreactivity assessed by non invasive plethysmography in response to metacholine. Mice breastfed by unexposed mothers and unchallenged with Der p (○) were used as controls. (3 exp, n=21, 26 and 10 for naïve mother, Der p mother and unchallenged groups respectively). (*) p=0.0003; (**) p=0.0002.

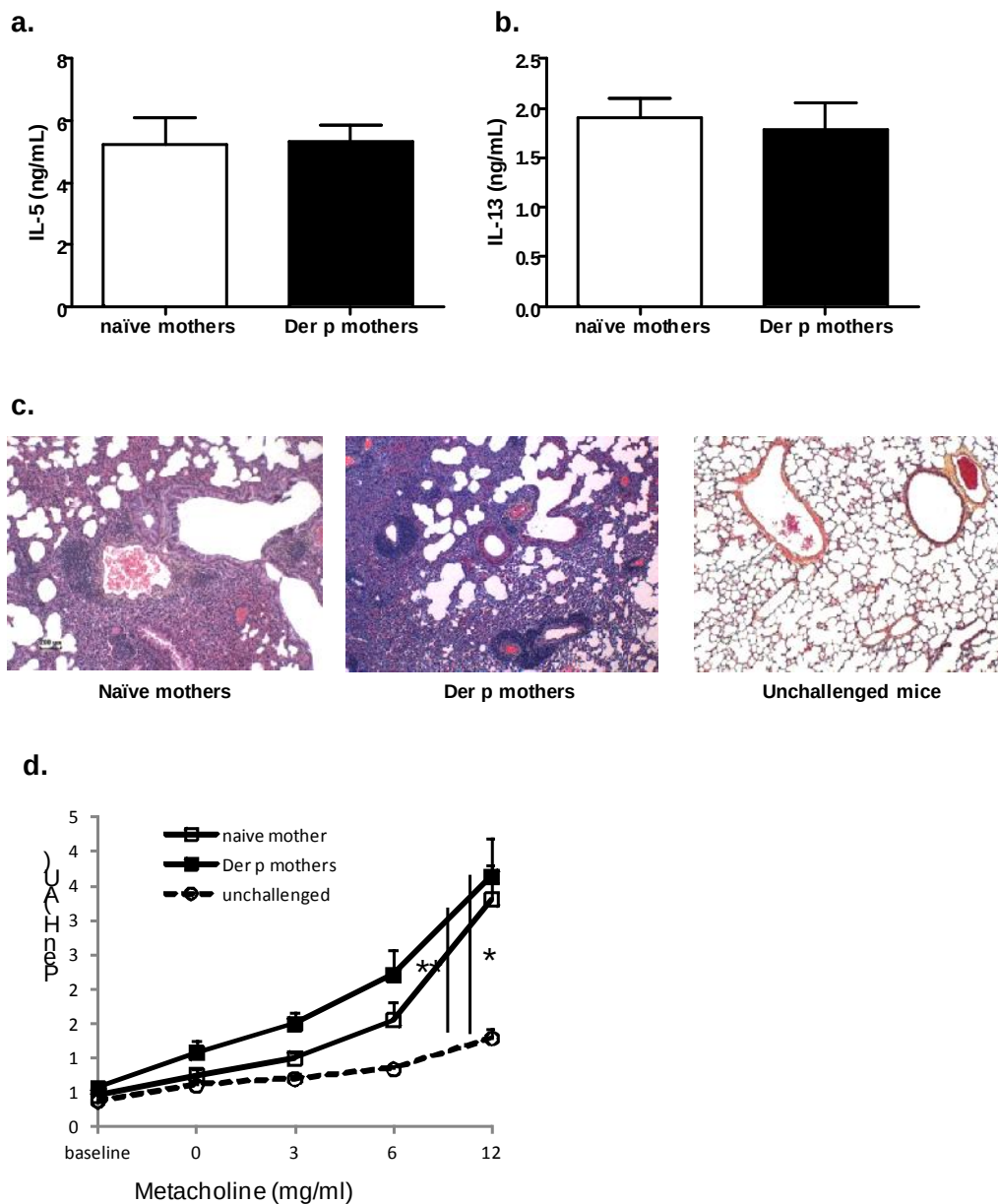
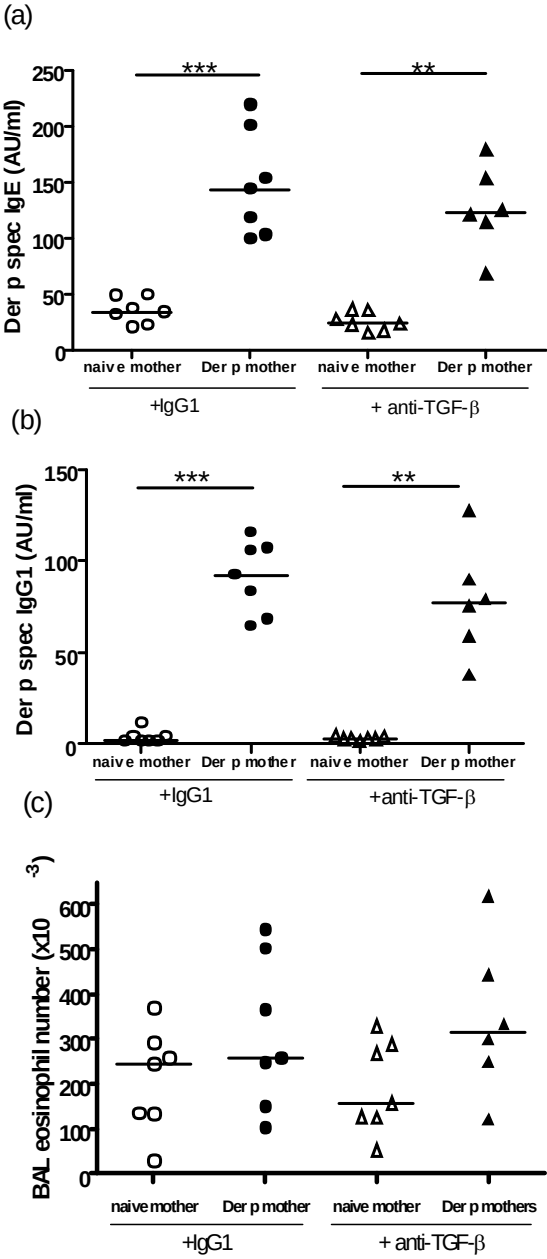


Fig. 6. TGF- β in milk has no impact on allergic sensitization of the progeny upon Der p transfer through breast milk.

Mice were treated as described in Sup Fig1. A subgroup of lactating mothers were treated with 1 mg of anti-TGF- β antibody (1D11 clone, ATCC) or isotype control rat IgG1 (GL113, DNAX) twice a week from delivery until weaning. (a) serum levels of Der p specific-IgE and (b) IgG1 ; (c) broncho-alveolar lavage content in eosinophils . Dots indicate individual mice and bars median values. (***) p=0.0006; (**) p=0.001.



Supplementary Tables

Table 1. Der p 1 concentration in human colostrum from Brazil, France and Australia and 1 and 6 months postpartum Australian milk samples

OVA (pg/mL)	Colostrum		Milk	
	France	Australia	1 month	6 month
Number of samples	97	34	70	136
Number of positive samples	51(52.5%)	22(64.7%)	31(44.3%)	64(47%)
Concentration range	0-3942	0-721.7	0-2062	0-3729
Median*	128.2	122.3	111.7	135.6
25% Percentile*	74.90	78.40	73.55	87.39
75% Percentile*	280	213.5	289.2	338.9

* Analysed only in positive samples

Table 2. OVA concentration in human colostrum from France and Australia and 1 and 6 months postpartum Australian milk samples

OVA (pg/mL)	Colostrum		Milk	
	France	Australia	1 month	6 month
Number of samples	97	34	70	136
Number of positive samples	51(52.5%)	22(64.7%)	31(44.3%)	64(47%)
Concentration range	0-3942	0-721.7	0-2062	0-3729
Median*	128.2	122.3	111.7	135.6
25% Percentile*	74.90	78.40	73.55	87.39
75% Percentile*	280	213.5	289.2	338.9

* Analysed only in positive samples

Table 3. IgG-Der p concentration in human colostrum from Brazil, France and Australia and 1 and 6 months postpartum Australian milk samples

IgG-Der p (AU/mL)	Colostrum			Milk	
	Brazil	France	Australia	1 month	6 month
Number of samples	76	100	34	71	161
Number of positive samples	73(96%)	94(94%)	34(100%)	71 (100%)	160(99.3%)
Concentration range	0-5079	0-143	0-1048	2.7-355.7	0-676.6
Median*	92.03	24.09	48.35	27.1	32.5
25% Percentile*	24.06	14.14	26.93	10.77	17.12
75% Percentile*	404.4	46	84.75	43.88	54.28

* Analysed only in positive samples

Table 4. TGF-beta levels in human colostrum from Brazil, France and Australia and 1 and 6 months postpartum Australian milk samples

TGF-Beta (pg/mL)	Colostrum			Milk	
	Brazil	France	Australia	1 month	6 month
Number of samples	74	96	34	70	127
Number of positive samples	74(100%)	96(100%)	34(100%)	70(100%)	127(100%)
Concentration range	63.95-14006	30.3-2004	49.74-1765	31.65-1348	34.5-3131
Median*	651.6	539.1	393.8	163.9	176.3
25% Percentile*	220.3	266.1	218.2	94.9	111.2
75% Percentile*	1543	931.3	651.8	275.9	296.9

* Analysed only in positive samples