Published in final edited form as:

Pediatr Allergy Immunol. 2014 May ; 25(3): 257–263. doi:10.1111/pai.12205.

Transforming growth factor beta (TGFβ₁) in breast milk and indicators of infant atopy in a birth cohort

Christine LM Joseph, PhD^{a,b}, Suzanne Havstad, MA^{a,b}, Kevin Bobbitt, PhD^{a,b}, Kimberley Woodcroft, PhD^{a,b}, Edward M. Zoratti, MD^{b,c}, Christian Nageotte, MD^{b,c}, Rana Misiak, MD^{b,c}, Robert Enberg, MD^c, Charlotte Nicholas, MPH^a, Jerel M. Ezell, MPH^{a,b}, Dennis R. Ownby, MD^{b,d}, and Christine Cole Johnson, PhD^{a,b}

^aDepartment of Public Health Sciences, Henry Ford Hospital, Detroit, MI

^bCenter for Allergy, Asthma and Immunology Research, Henry Ford Hospital, Detroit MI

^cDivision of Allergy and Immunology, Department of Internal Medicine, Henry Ford Hospital, Detroit, MI

^dDivision of Allergy and Immunology, Department of Pediatrics, Georgia Regents University, Augusta, GA

Abstract

Background—The infant gut's ability to suppress immunologic reactions to food proteins could be influenced by levels of TGFβ in breast milk. We hypothesized that lower levels of TGFβ₁ in the breast milk (BM) of mothers in the WHEALS birth cohort are associated with atopy at infant age 2–3 years.

Methods—We used data collected during infancy in addition to results of skin prick tests (SPT+) and measures of specific IgE>0.35 IU/ml (sIgE) to milk, egg, and peanut at infant age 2–3 years. Infants were classified as food allergic (FA) based on parental report of infant symptoms/diagnoses and information from clinical assessments.

Results—Data for 304 cohort members was analyzed. Among non-black infants, BM-TGFβ₁ was lower for those classified as FA (versus no FA) and those SPT+ (versus SPT-), geometric mean=1100pg/ml versus 1417pg/ml, $p=0.081$; and 1100pg/ml versus 1415pg/ml, $p=0.064$, respectively. Among infants of non-atopic mothers, BM-TGFβ₁ was lower for those with elevated (vs. not elevated) sIgE, geometric mean=1347pg/ml versus 1651 pg/ml, $p=0.047$. Using logistic regression, adjusted odds ratios describing the association of BM-TGFβ₁ to the presence of atopic indicators in the infant were in the hypothesized direction only for non-black infants of non-atopic mothers: aORs for FA, sIgE and SPT+ were 0.08, 0.34, and 0.26 respectively; $p=0.091$, 0.13, and 0.23.

Conclusion—Immune benefit of BM-TGFβ₁ could inform prevention strategies. Evidence of an association appears greatly influenced by infant race and maternal atopy. More research can

determine if these relationships represent a modifiable risk factor for development of food allergy in certain subgroups.

Keywords

Food allergy; food-specific IgE; milk; egg; peanut; expert panel; health disparities

Introduction

Much is unknown about the development of food allergies during infancy.¹ Current research suggests that variation in the risk of developing food allergy is linked to the infant gut's ability to develop oral tolerance.² Oral tolerance refers to the immune system's self-suppression of immunologic reactions to food proteins.³ Breast milk is often the first food to which infants are exposed.^{4, 5} The benefits of breast feeding include transmission of Immunoglobulin A (IgA).⁶ IgA works in conjunction with TGF β and other cytokines postulated to play an important role in the development of oral tolerance.⁷ TGF β is a polypeptide that regulates cell growth through exerting both stimulatory and inhibitory effects on a variety of cell types.⁸ Three isoforms of this cytokine are expressed in humans, two of which are found in human milk (TGF β_1 and TGF β_2).⁸ TGF β and interleukin 10 (IL10) have been shown to affect IgA production (3) and mediate other parts of the mucosal immune system. Production of TGF β in gut lymphoid tissue acts to maintain tolerance to benign gut pathogens and food allergens by downregulating inflammatory cytokines, and working with IL10 to promote IgA production.³ Because TGF β is integral to infant gut immunity, it has been suggested that levels of this and other cytokines in breast milk may explain the lack of consistent findings in studies investigating the association of breast feeding to risk of food allergy, i.e., some studies have reported a reduced risk of atopy with breast feeding, while others have reported no association or increased risk.⁹⁻¹¹

One hypothesis is that lower levels of these cytokines in breast milk may confer a greater risk of atopy developing in the infant.^{12, 13} To our knowledge, this hypothesis has not been explored by race. We explored the relationship between atopic manifestations and levels of TGF β_1 in the breast milk of mothers who participated, along with their infants, in the WHEALS birth cohort. We hypothesized that lower levels of TGF β_1 in the breast milk of mothers enrolled in the cohort would be associated with the following in the infant (1) prevalence of egg, milk, or peanut allergy; (2) specific IgE to egg, milk, or peanut; and/or (3) a positive skin prick test to egg, milk, or peanut.

Methods & Materials

Eligibility

All aspects of this research were approved by institutional review boards responsible for the conduct of research in human subjects at Henry Ford Health System (HFHS) and Georgia Regents University. The overall objective of the WHEALS cohort is to identify environmental factors, especially pet exposures, related to the development of allergy and asthma in infancy and childhood.¹⁴ The WHEALS cohort comprises pregnant women aged 21-45 years who were seen for prenatal care in one of five HFHS obstetric clinics between

9/1/2003 and 11/26/2007, resided in the city of Detroit or Detroit western suburbs as defined by zip code, spoke English well enough to provide written informed consent, and had plans to stay in the Detroit area for two years after delivery.

Recruitment

Recruitment for the WHEALS birth cohort is described in previous publications.¹⁵ Potentially eligible women were identified using a centralized automated appointment scheduling system. Each woman was sent a study introduction letter within two weeks of her mid-third trimester appointment, and was approached in the waiting room prior to her appointment by a trained recruiter, who obtained written informed consent if the woman was interested in participating. Eligibility was not based on maternal or family history of allergy or asthma.

Home and clinic visits

Home visits were conducted at infant age 1, 6, and 12 months by research staff. At child age 2-3 years, clinic visits were conducted by HFHS staff physicians and nurses trained in the study protocol. During this visit, a venous blood sample was collected from the child for measuring total and specific IgE to milk, egg, and peanut allergen.

Collection of information on breast feeding and measurement of TGF β in breast milk

At the 1, 6, and 12-month home visits, mothers were asked about breast feeding practices and duration of breast feeding. At the one-month visit, breast milk specimens were collected by those participants that were currently breast feeding.

A sterilized disposable breast pump kit (Medela Classic) was used to collect 2 ounces of breast milk from each participant. For each 2 ounce sample, 3 $\times$ 1 ml aliquots of breast milk were transferred into 3 sterile cryovials using a sterile pipette. The breast milk samples were placed on ice until delivery to the Department of Public Health Sciences lab where the remaining breast milk was centrifuged at 4°C to separate the lipid and aqueous fractions. After removal of the lipid layer, 3 $\times$ 1 ml aliquots of the aqueous fraction were transferred into cryovials and the whole milk, lipid layer and aqueous fractions were stored at -80°C. The aqueous fraction was utilized for assay of cytokines. TGF β ₁ was assayed using commercial ELISA kits (R&D Systems, Minneapolis, MN) according to manufacturer instructions.

Concentration of Allergen-specific IgE

Total and allergen specific IgE (sIgE) measurements in plasma collected at 2-3 years of age were performed using the Phadia UniCAP system (Phadia, Thermo Fisher Scientific, Inc., Waltham, MA). Total serum IgE concentrations were measured using the UniCAP low range protocol (0.1-100 IU/ml). Samples with IgE > 70 IU/ml were diluted as necessary and reassayed to obtain a valid measurement. As this paper focuses on food allergy, samples included in this analysis were tested for IgE antibodies to milk, egg, and peanut allergens. Infant serotopy to food allergens was defined as sIgE $\geq$ 0.35 IU/ml to egg, milk or peanut. Mother's blood samples were analyzed for specific IgE to 8 allergens: egg white, *Dermatophagoides farinae*, cat, dog, cockroach, ragweed, timothy grass, and *Alternaria*

alternata. Mother's seratology was defined as a specific IgE $\geq$ 0.35 IU/ml to one or more of these allergens.

Skin prick test

Skin prick testing was performed in children without contraindications by the puncture method on the volar surfaces of the forearm using a Duotip-test® device (Lincoln Diagnostics, Inc., Decatur, IL) and using commercial extracts. Allergens tested included egg, milk, peanut, dog, cat, cockroach, short ragweed, grass, *Alternaria*, and two dust mite species (*D. pteronyssinus* and *D. farinae*). A negative saline control and a positive histamine control (1 mg/ml) were included with all tests. Fifteen minutes after the skin tests were placed; the resulting wheal and flare responses were measured at the longest diameter and recorded. A positive skin prick test (SPT+) was defined as one producing a wheal with a diameter of at least 3mm larger than the negative control.¹⁶

Determination of food allergy

We used a physician panel to classify cohort participants as to the presence or absence of IgE-mediated food allergy (FA) to milk, peanut and egg. The physician panel, two board-certified allergists, classified infants as “Highly Probable”, “Likely” and “Unlikely” for IgE-mediated food allergy. A third allergist was consulted for discordant reviews. The physician panel was presented with data collected from the parent interview (including information on infant gastrointestinal illnesses suggestive of food intolerance or food allergy, other potential allergic reactions such as rash or colic, and any report of food avoidance) as well as information from clinical assessments (serum total and sIgE and SPT results). Infants were selected for physician panel review if they met at least one of the following three criteria: 1) possessed at least one food-specific IgE $\geq$ 0.35 IU/ml; 2) possessed a positive SPT (i.e., wheal 3mm or greater than the control); or 3) possessed food allergy-related symptoms/food avoidance (i.e., occurrence of rash, wheeze, hives, vomiting, diarrhea, pruritus, abdominal pain etc., attributed to food ingestion) *plus* at least one food-specific IgE $\geq$ 0.10 IU/ml. The latter criterion was set low purposefully to ensure that the records of all participants with a remote possibility of food allergy were included for physician review. Panelists reviewed (1) cord blood IgE measures; (2) parent interviews at 1, 6, 12, and 24 months, including family history of asthma/allergy; (3) a 36-month parent interview including items on food avoidance, gastrointestinal symptoms, onset and duration of reactions to food; (4) infant medical records; and (5) allergen-specific IgE and skin prick test results at 24 months. Children classified as “Highly Probable” or “Likely” for IgE-mediated FA were included in this analysis.

Statistical Analysis

The study population for these analyses includes cohort children with 1-month breast milk samples, 6 or 12-month interview data, data from the clinic visit at 2-3 years, and specific IgE measures at 2-3 years. For this analysis, atopic phenotypes included FA as determined by physician panel, allergen-specific IgE to egg, milk, or peanut (spIgE) and SPT+ to egg, milk, or peanut. Chi-square tests were used to make subgroup comparisons of participant

characteristics for binary and categorical variables, and Student's t-tests were used for comparison of continuous variables.

Due to skewness of the data, cytokine levels were compared with the Wilcoxon Rank Sum (WRS) test. Geometric means (GM) and 95% confidence intervals (95% CI) are presented for descriptive purposes. TGF β_1 values were log-transformed prior to inclusion in any model. To assess interaction, we used a logistic regression model for each atopic phenotype where the log-transformed TGF β_1 values and the variable of interest (infant race or mother's atopic status), along with the interaction term, was entered. A p-value < 0.10 was used to indicate the presence of an interaction.¹⁷ Logistic regression was used to calculate odds ratios (OR) and corresponding 95% CIs to describe the association between TGF β_1 concentrations (pg/ml) and atopic phenotype after controlling for potential confounders. Independent variables reported at predelivery included yearly household income < \$40,000, maternal education – High school, and marital status. In addition, from the 1 and 6 month survey, we collected mother's report of infant race, infant gender, breastfeeding < 6 months, infant exposure to environmental tobacco smoke during mother's pregnancy, and current smoking in child's household. Independent variables were retained in the final model if their inclusion changed the estimate of effect between atopic phenotype and TGF β_1 by more than 15%.¹⁸

Results

Of the 800 participants completing the 2 year clinic visit, 620 reported breast-feeding, of which 304 had data sufficient for analysis (Figure 1). Prevalence of FA in the cohort was 7.9% (n=24) according to classification by physician panel review. Table 1 is a comparison of cohort members included and excluded from the study sample. Those included in the analysis did not significantly differ in terms of maternal history of asthma, but did differ with regard to factors already shown to be related to breast feeding, including race,¹⁹ maternal age,²⁰ maternal education,²⁰ socioeconomic status,¹⁹ and smoking.²¹ Table 2 shows the characteristics of the study population by mother's report of infant race. Black mothers were significantly younger, less likely to be married, had less education, lower incomes, were less likely to breastfeed for < 6 months, and were more likely to reside with a smoker (Table 2).

Table 3 shows TGF β_1 levels in breast milk, by infant race and maternal atopy, and by whether or not the infant met criteria for FA, spIgE, or was SPT+ to egg, milk, or peanut. TGF β_1 levels in the breast milk of mothers were significantly higher for Black infants (geometric mean = 2027pg/ml vs. 1394pg/ml, p<0.001) and atopic mothers (1813 pg/ml vs. 1509 pg/ml, p=0.007). Overall, levels of TGF β_1 in breast milk were not significantly different for infants who were determined by physician panel review to have FA, had specific IgE to egg, milk or peanut < 0.35 IU/ml, or had a positive skin prick test to any of these three food allergens.

Table 4 shows the relationship of TGF β_1 levels in breast milk and atopic phenotype stratified by infant race/ethnicity and maternal atopy. Among non-Black infants, TGF β_1 levels in breast milk were lower for those classified as FA (p=0.081) and for SPT+ to food

allergens ($p=0.064$). Maternal atopy also seemed to modify the relationship between breast milk TGF β_1 level and infant risk of atopy (Table 4). Among mothers with atopy, TGF β_1 levels in breast milk were positively related to her child having specific IgE to egg, milk or peanut ($p=0.001$). However, among non-atopic mothers, levels of TGF β_1 in breast milk were lower for sensitized infants compared to non-sensitized infants ($p=0.047$). Although not significant, this pattern was also observed for infants who were classified as FA and who had positive skin prick tests.

Multivariate analyses

We observed interactions between infant race and TGF β_1 ($p=0.046$) and maternal atopy and TGF β_1 ($p=0.064$) on FA; also, between mother's atopy status and TGF β_1 ($p=0.001$) on spIgE. Based on this assessment, we created strata that included both mother's atopy status and child's race and ran logistic regression models for each of the atopic indicators (Table 5). For the three atopic indicators, a pattern emerged similar to that observed for bivariate analyses. Elevated odds ratios for the association between levels of TGF β_1 in breast milk and indicators of atopy in the infants of atopic, Black mothers were observed (adjusted OR=2.30 and 2.22, respectively, for FA and spIgE, $p=0.12$ and $p=0.067$.) If mother was atopic, but infant was non-Black, results suggested an inverse relationship between TGF β_1 levels in breast milk and FA, but a positive relationship between TGF β_1 and spIgE. For non-atopic mothers of non-Black infants, results suggest that TGF β_1 levels in breast milk may be inversely related to food sensitization in the infant at age 2-3 years. Adjusted ORs were consistently less than 1.0 for FA, spIgE, and SPT+, although ORs did not reach conventional levels of statistical significance. Using the BM TGF β_1 values categorized as tertiles, as done in a previously published paper by Oddy et al, did not change the results of this analysis.¹³

Discussion

We explored the association of TGF β_1 in breast milk to risk of atopic manifestations in the breast-fed infant by age 2-3 years. We hypothesized that low TGF β_1 levels in breast milk would be positively associated with evidence of atopy by age 2-3 years. We found that race and mother's atopy status were strong effect modifiers of this relationship. Evidence in support of our hypothesis was found only among non-atopic mothers with non-Black infants. Consistent differences by race for risk factors of asthma and allergy have been reported.²²⁻²⁶ Clearly, identifying the social or biological factors associated with infant race that can impact immune function and regulation will be key to determining the mechanisms by which the risk of asthma and allergy is modulated in Black individuals.

Currently, there is no consensus on the relationship of breast feeding and risk of atopy in the infant.¹ The composition of breast milk varies considerably between mothers and is influenced by maternal atopy.^{27, 28} In a systematic review published by Oddy et al., allergic status of the mother was one of several factors that influenced the measured concentrations of TGF β_1 , in addition to maternal diet, local infection, and genetic factors.¹

Some patterns observed in this analysis, to our knowledge, have not appeared in the literature. In 2003, Oddy et al. examined the relationship between infant wheeze and measures of TGF β and IL10, among other cytokines, in the breast milk of 243 mothers

enrolled in the Infant Immune Study, and found an inverse relationship between TGF β ₁ and risk of infant wheeze ($p=0.006$, trend X^2).¹³ Similar results have been reported in Finland high-risk cohorts,^{5, 29} although Bottcher et al found that composition of cytokines, chemokines and sIgA in mothers' breast milk did not influence the risk of atopy by age 2 years.³⁰ According to the Oddy review, of 8 of 12 human studies investigating TGF β in breast milk demonstrated an inverse association between TGF β ₁ or TGF β ₂ and allergic manifestations in early childhood or infancy.¹

In our study, breast milk TGF β ₁ was significantly higher in atopic mothers, but this is in contrast to some previous reports. Rigotti et al., found that TGF β ₁ was significantly less secreted in mature milk of allergic mothers.³¹ Laiho, in 2003, reported lower concentrations of TGF β ₂ in mothers with atopic dermatitis than in mothers without atopic dermatitis.³² A study done in the Czech Republic did not find significant differences in TGF β by mother's allergic status.³³ All of these studies had relatively small sample sizes and contained only White subjects.

There are several limitations to our analysis. We included only participants who reported breast feeding and for whom a breast milk sample was available for processing. Race, education, smoking and income are highly related to the decision to breast feed. For this reason, our results apply only to those mother-infant pairs with characteristics similar to the cohort participants in this analysis. These relationships should be explored in other birth cohorts by race and maternal atopy status. In addition, we did not collect colostrum. In the few studies that have included colostrum, trends have not been found to be significantly different from those observed in mature breast milk.^{29, 33} We were also unable to examine associations by individual foods due to small cell sizes. We know from previous analyses that risk of food sensitization may vary by allergen,^{25, 26} and therefore variations in exposure to breast milk cytokines may influence risk of atopy differently for different allergens. Finally, a larger sample would have allowed better point estimates, given that we identified maternal atopy and Black infant race as effect modifiers of the associations of interest.

In summary, we explored an association between the level of TGF β ₁ in breast milk and several atopic phenotypes in infants. Certainly, more research is needed in this area especially with regard to infant race/ethnicity and mother's atopic status. Exploring level of TGF β ₁ in breast milk and infant atopy may elucidate mechanisms for incidence of FA. Knowledge of an immune benefit to infants of TGF β ₁ in breast milk could contribute to the development of protective strategies or interventions designed to prevent atopy in children.

Acknowledgments

We extend our thanks and appreciation to the participating families for their continued commitment to this research, and to the research staff who helped carry out the collection and analysis of data for this study.

Declaration of all sources of funding: This work was supported by the National Institutes of Health (#1R21A1080066-01A1), HL068971, #AI050681-06A2, and #AI050681-06A2S1

References

1. Oddy WH, Rosales F. A systematic review of the importance of milk TGF β on immunological outcomes in the infant and young child. *Pediatric Allergy and Immunology*. 2010; 21:47–59. [PubMed: 19594862]
2. Brandtzaeg P. Update on mucosal immunoglobulin A in gastrointestinal disease. *Curr Opin Gastroenterol*. 2010; 26:554–63. [PubMed: 20693891]
3. Chehade M, Mayer L. Oral tolerance and its relation to food hypersensitivities. *J Allergy Clin Immunol*. 2005; 115:3–12. [PubMed: 15637539]
4. Letterio JJ, Roberts AB. Regulation of immune responses by TGF-beta. *Annu Rev Med*. 1998; 16:137–61.
5. Kalliomaki M, Ouwehand A, Arvilommi H, Kero P, Isolauri E. Transforming growth factor-[beta] in breast milk: A potential regulator of atopic disease at an early age. *Journal of Allergy and Clinical Immunology*. 1999; 104:1251–7. [PubMed: 10589009]
6. Eigenmann PA. Mechanisms of food allergy. *Pediatr Allergy Immunol*. 2009; 20:5–11. [PubMed: 19154253]
7. Chehade M, Mayer L. Oral tolerance and its relation to food hypersensitivities. *J Allergy Clin Immunol*. 2005; 115:3–12. quiz 3. [PubMed: 15637539]
8. Yoshimura, A.; Muto, G. TGF-beta Function in Immune Suppression. In: Ahmed, R.; Honjo, T., editors. *Negative Co-Receptors and Ligands*. 350. Springer Berlin; Heidelberg: 2011. p. 127-47.
9. Ando T, Hatsushika K, Wako M, Ohba T, Koyama K, Ohnuma Y, et al. Orally administered TGF-beta is biologically active in the intestinal mucosa and enhances oral tolerance. *Journal of Allergy & Clinical Immunology*. 2007; 120:916–23. [PubMed: 17606291]
10. Organization WH. The WHO Global Data bank on Breastfeeding and Complementary Feeding. World Health Organization; 2011.
11. Srivastava MD, Srivastava A, Brouhard B, Saneto R, Groh-Wargo S, Kubit J. Cytokines in human milk. *Research Communications in Molecular Pathology & Pharmacology*. 1996; 93:263–87. [PubMed: 8896040]
12. Wold AE, Adlerberth I. Does breastfeeding affect the infant's immune responsiveness? *Acta Paediatr*. 1998; 87:19–22. [PubMed: 9510441]
13. Oddy WH, Halonen M, Martinez FD, Lohman IC, Stern DA, Kurzius-Spencer M, et al. TGF-[beta] in human milk is associated with wheeze in infancy. *Journal of Allergy and Clinical Immunology*. 2003; 112:723–8. [PubMed: 14564350]
14. Aichbhaumik N, EM Z, Strickler R, GR W, DR O, SL H, et al. Prenatal exposure to household pets influences fetal immunoglobulin E production. *Clin Exp Allergy*. 2008; 38:1787–94. [PubMed: 18702655]
15. Johnson C, Havstad S, Wegienka G, Zoratti E, Ownby D. The impact of Caesarian Section on the relationship between allergen exposure and allergen-specific IgE at age 2 years. *JACI*. 2013
16. Ownby DR, Anderson JA. An improved prick skin-test procedure for young children. *J Allergy Clin Immunol*. 1982; 69:533–5. [PubMed: 7076995]
17. Hosmer, DW.; Lemeshow, S. *Applied Logistic Regression*. Second. New York, N.Y.: Wiley & Sons; 2000.
18. Rothman, K.; Greenland, S. *Modern Epidemiology*. 2nd. Lippincott-Raven Publishers; 1998.
19. Jones JR, Kogan MD, Singh GK, Dee DL, Grummer-Strawn LM. Factors associated with exclusive breastfeeding in the United States. *Pediatrics*. 2011; 128:1117–25. [PubMed: 22123898]
20. Chertok IR, Luo J, Culp S, Mullett M. Intent to breastfeed: a population-based perspective. *Breastfeeding Medicine: The Official Journal of the Academy of Breastfeeding Medicine*. 6:125–9. [PubMed: 20958106]
21. Weiser TM, Lin M, Garikapaty V, Feyerharm RW, Bensyl DM, Zhu BP. Association of maternal smoking status with breastfeeding practices: Missouri, 2005. *Pediatrics*. 2009; 124:1603–10. [PubMed: 19917583]

22. Nelson DA, CC J, Divine GW, Strauchman C, CLM J, DR O. Ethnic differences in the prevalence of asthma in middle class children. *Ann Allergy Asthma Immunol.* 1997; 78:21–6. [PubMed: 9012615]
23. Yang JJ, Burchard EG, Choudhry S, CC J, DR O, Favro D, et al. Differences in allergic sensitization by self-reported race and genetic ancestry. *J Allergy Clin Immunol.* 2008; 122:820–7. [PubMed: 19014772]
24. Havstad S, Wegienka G, Zoratti EM, Lynch SV, Boushey HA, Nicholas C, et al. Effect of prenatal indoor pet exposure on the trajectory of total IgE levels in early childhood. *J Allergy Clin Immunol.* 2011
25. Wegienka G, Havstad S, Joseph CLM, Zoratti E, Ownby D, Woodcroft K, et al. Racial disparities in allergic outcomes in African Americans emerge as early as age 2-years. *Clinical & Experimental Allergy.* 2012; 42:909–17. [PubMed: 22909162]
26. Joseph CLM, Ownby DR, Havstad SL, Woodcroft KJ, Wegienka G, MacKechnie H, et al. Early complementary feeding and risk of food sensitization in a birth cohort. *Journal of Allergy and Clinical Immunology.* 2011; 127:1203–10. [PubMed: 21458850]
27. Rudloff S, Niehues T, Rutsch M, Kunz C, Schroten H. Inflammation markers and cytokines in breast milk of atopic and nonatopic women. *Allergy.* 1999; 54:206–11. [PubMed: 10321555]
28. Bottcher MF, Jenmalm MC. Breastfeeding and the development of atopic disease during childhood. *Clin Exp Allergy.* 2002; 32:159–61. [PubMed: 11929473]
29. Saarinen KM, Vaarala O, Klemetti P, Savilahti E. Transforming growth factor-beta1 in mothers' colostrum and immune responses to cows' milk proteins in infants with cows' milk allergy. *J Allergy Clin Immunol.* 1999; 104:1093–8. [PubMed: 10550758]
30. Bottcher MF, Bjurstrom J, Mai XM, Nilsson L, Jenmalm MC. Allergen-induced cytokine secretion in atopic and non-atopic asthmatic children. *Pediatr Allergy Immunol.* 2003; 14:345–50. [PubMed: 14641603]
31. Rigotti E, Piacentini GL, Ress M, Pigozzi R, Boner AL, Peroni DG. Transforming growth factor-beta and interleukin-10 in breast milk and development of atopic diseases in infants. *Clin Exp Allergy.* 2006; 36:614–8. [PubMed: 16650046]
32. Laiho K, Lampi AM, Hamalainen M, Moilanen E, Piironen V, Arvola T, et al. Breast milk fatty acids, eicosanoids, and cytokines in mothers with and without allergic disease. *Pediatr Res.* 2003; 53:642–7. [PubMed: 12612204]
33. Prokesova L, Lodinova-Zadnikova R, Zizka J, Kocourkova I, Novotna O, Petraskova P, et al. Cytokine levels in healthy and allergic mothers and their children during the first year of life. *Pediatric Allergy & Immunology.* 2006; 17(3):175–83. [PubMed: 16672003]

Abbreviations used

95% CI	95% Confidence Interval
FA	Food Allergy
GM	Geometric mean
HFHS	Henry Ford Health System
IgA	Immunoglobulin A
IgE	Immunoglobulin E
OR	Odds Ratio
spIgE	Allergen-specific IgE to egg, milk, or peanut
SPT	Skin prick test
TGFβ	Transforming growth factor beta

WHEALS	Wayne County Health, Environment Allergy and Asthma Longitudinal Study
WRS	Wilcoxon Rank Sum Test

Figure 1. Sample used for analysis of TGF β ₁ in breast milk and indicators of infant atopy in the WHEALS birth cohort

*Declined blood draw at 2 years (n=177); insufficient blood sample for measurement of IgE (n=29).

Table 1
Characteristics of the study population taken from the WHEALS predelivery, 1 month and 6 month questionnaires

A	B	C	D	
	Included n=304	No breast milk sample n=316	Did not breastfeed n=180	B vs. C p
Characteristic				p
Maternal age, mean (s.d.)	30.6 (5.1)	29.3 (5.1)	29.7 (5.1)	0.001 0.064
Black race, n (%)	146 (48.0)	230 (72.8)	124 (68.9)	<0.001 <0.001
Currently married, n (%)	242 (79.6)	178 (56.3)	109 (60.6)	<0.001 <0.001
Bachelor's degree, n (%)	147 (48.4)	88 (27.8)	29 (16.1)	<0.001 <0.001
Maternal history asthma/allergy, n (%)	118 (39.3)	126 (40.7)	118 (39.3)	0.74 0.20
HH ¹ income < \$40,000, n (%)	68 (25.1)	122 (45.5)	72 (45.9)	<0.001 <0.001
ETS ² during pregnancy, n (%)	44 (14.5)	92 (29.1)	60 (33.3)	<0.001 <0.001
Current smoker in household, n (%)	21 (6.9)	56 (17.7)	50 (27.8)	<0.001 <0.001

¹ Household;

² Environmental tobacco smoke

Table 2
Characteristics of the study population taken from the WHEALS predelivery, 1 month and 6 month questionnaires

Characteristic	All n=304	Black n=146	Non Black ¹ n=158	p value
Maternal age, mean (s.d.)	30.6 (5.1)	29.8 (5.5)	31.4 (4.5)	0.004
Currently married, n (%)	242 (79.6)	92 (63.0)	150 (94.9)	<0.001
Bachelor's degree, n (%)	146 (48.4)	52 (35.6)	95 (60.1)	<0.001
Mother history of asthma/allergy, n (%)	118 (39.3)	63 (43.8)	55 (35.3)	0.13
HH ² income < \$40,000, n (%)	68 (25.1)	39 (31.7)	29 (19.6)	0.022
ETS ³ during pregnancy, n (%)	44 (14.5)	25 (17.1)	19 (12.0)	0.21
Current smoker in household, n (%)	21 (6.9)	14 (9.6)	7 (4.4)	0.076
Breastfed < 6 months, n (%)	126 (42.1)	74 (51.7)	52 (33.3)	0.001

¹ Non-Black includes 62% White/Caucasian; 12.7% Hispanic/Latino; 9.5% Asian; 13.9% Arab/Middle Eastern; 1.9% Other;

² Household;

³ Environmental tobacco smoke

Table 3
Geometric means for TGFβ₁ levels in breast milk samples of breast feeding mothers in the WHEALS birth cohort by atopic phenotypes

	n	Geo. Mean (pg/ml) ¹	95% CI ²	p value
Black				
Yes	146	2027	1823 2254	<0.001
No	158	1394	1288 1509	
Mother atopic				
Yes	174	1813	1658 1983	0.007
No	127	1509	1358 1667	
Food Allergy				
Yes	24	1781	1355 2340	0.82
No	280	1660	1546 1782	
Allergen-specific IgE				
Yes	103	1805	1600 2036	0.15
No	134	1579	1439 1732	
SPT ⁺ ³				
Yes	46	1709	1414 2066	0.99
No	238	1650	1527 1784	

¹ Geometric mean;
² Confidence Interval;
³ Positive skin prick test

Table 4
TGFβ₁ levels in breast milk samples by infant atopic phenotypes for participants of the WHEALS birth cohort, stratified by infant race and mother's atopy status

	n	Geo. Mean (pg/ml) ¹	Ci ²	p	n	Geo. Mean (pg/ml)	Ci	p
		Black				Non Black		
Food Allergy	Yes 14	2512	1769 3567	0.19	Yes 10	1100	882 1372	0.081
	No 132	1982	1772 2217		No 148	1417	1304 1540	
Allergen-specific IgE	Yes 58	2184	1845 2586	0.3	Yes 45	1412	1219 1635	0.7
	No 60	1894	1642 2185		No 74	1362	1216 1525	
SPT³	Yes 31	2115	1676 2669	0.98	Yes 15	1100	895 1352	0.064
	No 102	2025	1775 2309		No 136	1415	1298 1543	
		Atopic Mother				Non-Atopic Mother		
Food Allergy	Yes 17	2160	1554 3004	0.30	Yes 7	1114	782 1585	0.12
	No 157	1779	1621 1954		No 120	1533	1377 1714	
Allergen-specific IgE⁴	Yes 66	2161	1868 2499	0.001	Yes 36	1347	1134 1600	0.047
	No 76	1525	1347 1726		No 57	1651	1427 1910	
SPT⁵	Yes 30	1927	1540 2411	0.66	Yes 15	1463	1034 2070	0.54
	No 133	1756	1581 1951		No 103	1529	1359 1719	

¹ Geometric mean;
² Confidence Interval;
³ Any positive skin prick test;
⁴ Data unavailable for n=2 persons;
⁵ Data unavailable for n=3 persons

Table 5
Results of logistic regression in the analysis of TGFβ₁ levels measured in mother's breast milk and atopic phenotypes at 2-3 years of age in breastfed infants

Strata	n	Covariate	aOR ¹	95%CI ²	p
Atopic Mother/Black	253	-			
Infant Food Allergy	92	Male	2.30	(0.80-6.64)	0.12
Allergen-specific IgE	77	None	2.22	(0.95-5.20)	0.067
SPT+ ³	84	None	1.12	(0.50-2.47)	0.79
Atopic Mother/Non-Black	226	-			
Infant Food Allergy	82	None	0.56	(0.10-3.08)	0.5
Allergen-specific IgE	65	GA ⁴	2.62	(0.78-8.84)	0.12
SPT+	79	None	0.55	(0.13-2.38)	0.42
Non-Atopic Mother/Black	144	-			
Infant Food Allergy	54	GA	1.15	(0.15-8.96)	0.89
Allergen-specific IgE	41	BS ⁵	0.47	(0.14-1.63)	0.24
SPT+	49	None	1.00	(0.36-2.78)	0.99
Non-Atopic Mother/Non-Black	194	-			
Infant Food Allergy	73	None	0.08	(0.004-1.51)	0.091
Allergen-specific IgE	52	None	0.34	(0.08-1.40)	0.13
SPT+	69	None	0.26	(0.03-2.29)	0.23

¹ Adjusted odds ratio;

² 95% Confidence Interval;

³ Any positive skin prick test,

⁴ Gestational age entered in model as a continuous variable (number of weeks); aOR represents change in "risk" per unit change in gestational age (i.e. 1 week);

⁵ Mother educated at or beyond Bachelor's degree