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Deserters on the atopic march:
Risk factors, immune profile and clinical outcomes of food sensitized-tolerant infants

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All the authors have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; and been involved in drafting the manuscript or revising it critically for important intellectual content; and given final approval of the version to be published. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content; and have agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Conflicts of interest

None

Abstract

Background

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Few studies have investigated the antecedents and outcomes of infants who demonstrate IgE sensitization to foods that they clinically tolerate. Improved understanding of this sensitized-tolerant phenotype may inform strategies for the prevention of food allergy.

Methods

In an Australian birth cohort (n=1074), assembled using an unselected antenatal sampling frame, participants were categorised as non-sensitized (NS), sensitized-tolerant (ST) or food allergic (FA) based on skin-prick testing and food challenge at 12 months of age. Environmental exposures were recorded throughout. Cord blood regulatory T-cell populations were measured at birth. Subsequent childhood allergic disease was assessed by parent report, clinical examination and repeat skin-prick testing.

Results

The covariates of interest varied between NS(n=698), ST(n=27) and FA(n=61) groups as follows, suggesting that across these measures the ST group was more similar to the NS than the FA group: family history of eczema NS 44.6%, ST. 44.6%, FA 65.6%; pet ownership at 12 months: NS 71.5%, ST 81.5%, FA 45.8%; eczema during the first 12 months: NS 19%, ST 32%, FA 64%; and aeroallergen sensitization at 4 years: NS 19.1%, ST 28.6%, FA 44.4%. At birth a higher proportion of activated regulatory T cells was associated with ST (OR=2.89, 95%CI 1.03–8.16, *P*=0.045).

Conclusion

Food sensitized-tolerance in infancy appears to be associated with a similar pattern of exposures, immunity and outcomes to non-sensitized infants. In addition, we found some evidence that an elevated proportion of activated regulatory T cells at birth was specific to the sensitized-tolerant infants, which may be relevant to suppression of clinical disease.

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Graphical abstract

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Highlights

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Key words
Allergy
Food allergy
Immune programming
Regulatory T-cell
Sensitized-tolerant

Abbreviations

BIS – Barwon Infant Study

DAG – directed acyclic graph

Ig – Immunoglobulin

PBS – phosphate buffered saline

SCORAD – scoring atopic dermatitis

SEIFA – Socio-Economic Indexes for Areas

SPT – skin prick testing

Treg – regulatory T-cell

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Introduction

The “atopic march” of childhood allergic disease describes a putative causal pathway linking eczema in infancy to subsequent allergic sensitization to food, food allergy, hayfever, atopic wheeze and asthma.(1-5) Allergic sensitization to a food in infancy is common, occurring in up to 16% of infants at 12 months, but fewer than half the infants who are sensitized to foods are clinically allergic.(6) Many remain tolerant, able to ingest the food(s) without symptoms, despite having generated specific immunoglobulin E antibodies against the implicated food(s)(7, 8) and these infants are termed “sensitized-tolerant”.(9) Limited information exists on the protective factors and early immune profile that may prevent sensitized-tolerant infants from progression to food allergy.

Genetic risk factors for increased likelihood of allergic sensitization and food allergy include male sex and family history of allergic disease(10), but the genetic and demographic factors associated with sensitized-tolerance determined by food challenge remain unknown. Evidence from studies of aeroallergen-mediated allergic disease indicates that progression from allergic sensitization to clinical expression of allergy may be influenced by environmental factors. For example, a study of children from urban vs. rural areas in Ethiopia found that rural children had an increased incidence of allergic sensitization to dust mite, but greatly decreased risk of wheeze and asthma compared to urban children.(11) This intriguing finding suggests a rural microbial and antigenic environment may promote the sensitized-tolerant phenotype, which is consistent with the notion of benign sensitization.(12)

Maternal exposure to environmental microbes influences antenatal immune programming, altering patterns of early immune response and associated clinical manifestations of allergic disease.(13-20) We, and others, have reported that infants who subsequently develop food allergy have a lower proportion of naïve regulatory T-cells (Treg) at birth(20-22) than non-allergic infants. However, few studies have examined Treg populations in relation to the sensitized-tolerant phenotype.(23-26)

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Currently, there is limited knowledge of the risk factors, environmental exposures, immune profile at birth and subsequent allergic disease outcomes of sensitized-tolerant infants. Indeed, the existing evidence is based largely on studies performed in cohorts at high risk of food allergy, with food allergy status defined by parent report rather than formal food challenge.(27-30)

The aim of this study was to investigate, in a pre-birth cohort incorporating both skin-prick testing and oral food challenge, the environmental factors, cord blood immune profile and subsequent allergic disease outcomes of food sensitized-tolerant infants.

Methods

Enrolment

The Barwon Infant Study (BIS) is a birth cohort study (n=1074) conducted in south-eastern Australia. Details of the study have been reported previously.(31) In brief, mothers were recruited during pregnancy using an unselected antenatal sampling frame. The eligibility criteria included: (i) residents of the defined geographical region in the Barwon area of Victoria, (ii) less than 32-weeks gestation at the time of enrolment, and (iii) planning to give birth at a local hospital. The final inception birth cohort constituted 1064 mothers and 1074 infants (10 sets of twins). Data were also collected on baseline characteristics of those mothers who chose not to participate in the study. Ethics approval (10/24) for this study was obtained from the Barwon Health Human Research Ethics committee.

Determination of food sensitization phenotype at 12 months

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At the 12-month review, infants underwent a skin-prick test (SPT) to 10 food and aeroallergens: cow's milk, egg, peanut, cashew, sesame, house dust mite, cat, dog, rye grass and the fungus *Alternaria tenuis,* with a positive and negative control. A food allergen SPT wheal size of at least 2mm greater than the negative control in the presence of a positive histamine control was defined as food-sensitized. Food-sensitized infants and all participants with food SPT wheals 1mm or greater than the negative control were offered an in-hospital open food challenge. Food challenges were not performed on non-sensitized participants. Participants with a positive oral food challenge were classified as food allergic. In addition, those regularly ingesting the sensitized food at the time of SPT were defined as sensitized-tolerant without formal challenge and included in the sensitized-tolerant group for analysis. If, on clinical review, the participant had a clinical history and reaction consistent with a diagnosis of IgE-mediated food allergy within 2 months either side of the 12-month review and a positive SPT, they were defined as food-allergic without proceeding to food challenge and included in the food allergic group for analysis.

Demographics and Risk Factors

Birth record data and questionnaires administered during pregnancy were used to obtain demographic information. Exposure to known or predicted risk factors for allergic disease during the first 12 months was determined from clinical data and questionnaires completed by parents at several timepoints during pregnancy and up to the 12-month review.

Cord blood lymphocyte populations

Blood sampling and isolation of mononuclear cells

Umbilical cord blood was collected at birth by syringe and immediately diluted in 10IU/mL preservative-free sodium heparin (Pfizer) in 10ml of RPMI 1640 (Gibco, Life Technologies). Mononuclear cells were isolated by density gradient centrifugation (Lymphoprep, Axis-Shield), and 2–4 x 10^4 cells immediately used for flow cytometric measurement of Treg cells.

Measurement of regulatory T-cell subsets by flow cytometry

All blood samples were stained for flow cytometric analysis within 12 hours of collection. Isotype controls were used to set up the instrument for positive gating, and, once established, these settings were maintained throughout. Mononuclear cells were stained

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with anti-CD4-PE, and anti-CD45RA-PECy5 and then washed in PBS and formalin fixed. After overnight fixation, cells were permeabilized (0.5% Tween in PBS) and stained with anti-FOXP3-Alexa Fluor488 followed by analysis on a 3-channel flow cytometer. Gating of naïve Tregs ($CD4^+/FOXP3^+/CD45RA^+$) and activated Tregs ($CD4^+/FOXP3^{++}/CD45RA^-$) was performed as previously described (32) and reported as a proportion of the total $CD4^+$ T-cell population.

Eczema status during the first 12 months

Data on eczema were collected by questionnaires administered at 1, 3, 6, 9 and 12 months, and clinical assessments conducted at 1, 6 and 12 months. Eczema was defined according to the modified UK working party criteria.(33) The Scoring Atopic Dermatitis Scale (SCORAD) was used to quantify eczema severity.(34, 35)

Allergic sensitization at age 4 years

Participants were assessed at 4-year review intended shortly after the 4^{th} birthday (Mean age 4.28 years, Standard deviation (SD) 0.35). At the 4-year review infants underwent SPT to the same 10 food and aeroallergens using identical equipment and technique. Allergic sensitization to an allergen at 4-years was defined as a wheal size of 3mm greater than the negative control, in the presence of a positive control ≥3mm and a negative saline control ≤3mm. Aeroallergen sensitization was defined as allergic sensitization to an aeroallergen (any of house dust mite, cat, dog, rye grass or the fungus *Alternaria tenuis*).

Wheeze, hayfever, atopic wheeze and doctor-diagnosed asthma at age 4 years

At the 4-year review parents were asked if their child had wheezed or suffered from hayfever symptoms in the past 12 months. Atopic wheeze was defined as allergic sensitization to any allergen at age 4 years plus parent-reported wheeze in the preceding 12 months. At the 2 and 4-year reviews, parents were asked if a doctor had ever diagnosed their child with asthma.

Statistical analysis

In order to avoid misclassification, analysis was restricted to those infants who could be confidently classified as either non-sensitized (n=698), sensitized-tolerant (n=27) or food

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allergic (n=61) (Table 1). To estimate odds ratios for the effect of cord blood Treg populations, and infant eczema during the first 12 months, on infant food sensitization phenotype, we fitted multinomial logistic regression models adjusted for relevant covariates. These models differ from our previously reported analysis(21) by including a third group, the sensitized-tolerant infants. Given the low baseline risk of sensitized tolerance, the estimated odds ratios are interpretable as risk ratios (RR). To estimate risk ratios for the effect of infant food sensitization phenotype on aeroallergic disease outcomes to age 4 years we fitted logarithmic binomial regression models adjusted for relevant covariates. Covariates included in analysis models were those known or potential risk factors for allergic disease included in causal models represented by directed acyclic graphs (DAGs)(Supplementary Figures 1-3). Data analysis used the statistical software Stata/SE version 15.1 (Statacorp, TX, USA). See supplementary methods for further details of methods.

Results

Table 1 lists demographic details for participants with food sensitization phenotype determined in infancy (n=786). As previously reported(36), 845 infants were included in the 12-month review. Of these 93/845 (11.0%) were sensitized to one or more foods at 12 months. Following food challenge, 61/845 (7.2%) infants were food allergic and 27/845 (3.2%) were sensitized-tolerant. A further 59/845 (7.0%) were either: sensitized to aeroallergens only (17/845), had a non IgE-mediated food allergy (1/845) or had inconclusive results (41/845). These participants were excluded from the analysis in order to specifically focus on the sensitized-tolerant and food allergic groups.

Table 1.

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Genetic and environmental exposures

Environmental exposures did not appear to distinguish sensitized-tolerant from non-sensitized individuals. By contrast, in comparison to food allergic infants, both non-sensitized and sensitized-tolerant infants appeared to be less likely to have a family history of eczema and asthma, and have higher rates of household pet ownership during gestation and infancy (Table 1). A particularly strong difference was seen with respect to pet exposure during infancy which was more frequent in sensitized-tolerant infants than food allergic infants (RR 3.32, 95%CI 1.38-7.99, *P*=0.007). We did not find evidence that known risk factors for increased allergic disease, such as male sex, no labour prior to delivery and reduced household size, differed between groups.

Cord blood regulatory T cells

Figure 1. (attached separately)

Lower cord blood naïve regulatory T-cells are associated with subsequent food allergy

We previously reported that food allergic infants in our cohort had a lower proportion of umbilical cord blood naïve Treg cells than non-sensitized participants (Odds ratio (OR) 0.63, 95% CI 0.44 – 0.90, *P*= 0.010).(20, 21) By contrast, there was no evidence of a difference in the proportion of naïve Treg cells between the sensitized-tolerant and non-sensitized groups (OR 0.89 95% CI 0.59 – 1.36, *P*= 0.619) (Figure1).

Higher cord blood activated Tregs may be associated with subsequent sensitized-tolerance

In comparison to non-sensitized infants, there was some evidence that sensitized-tolerance was associated with a higher proportion of umbilical cord blood activated Treg cells (OR = 2.89 95% CI 1.03 – 8.16, *P*= 0.045). There was however no evidence of a difference in the proportion of activated Treg cells between the food allergic and non-sensitized groups (OR 0.72 95% CI 0.24 – 2.18, *P*= 0.566) (Figure 1).

Eczema during the first 12 months

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As previously reported(36), at the 1-month review none of the participants reported eczema. The cumulative prevalence of eczema up to the 3, 6, 9 and 12 month review was 9/763 (1.2%); 65/737 (8.8%); 126/685 (18.4%); and 162/701 (23.1%) respectively. Eczema during infancy appeared to be strongly predictive of food allergy, and perhaps weakly predictive of sensitized-tolerance (Table 2)(Supplementary figures 4 and 5)

Table 2.

Aeroallergen sensitization and allergic disease to age 4 years

We next investigated the relationship between food sensitization status at 12 months and aeroallergen sensitization, hayfever, atopic wheeze, and doctor diagnosed asthma to age 4 years. SPT was performed in 546 participants at 4-year review and 156/546 (28.6%) children were sensitized to aeroallergens. We did not find evidence that in comparison to non-sensitized infants, sensitized-tolerance at 12 months were at increased risk of subsequent aeroallergen sensitization, hayfever, or doctor-diagnosed asthma to age 4 years. However, there was a weak indication that sensitized-tolerance might be associated with atopic wheeze, with the 95% CI not excluding large effects. By contrast, in comparison to non-sensitized infants, food allergy at 12 months strongly predicted subsequent aeroallergen sensitization, hayfever, atopic wheeze at age 4 years and doctor-diagnosed asthma to age 4 years (Table 3). Further, food allergic infants were sensitized to a greater number of aeroallergens at age 4 years (Supplementary Figure 6) and had a higher average aeroallergen wheal size (Supplementary Figure 7) than either sensitized-tolerant infants or non-sensitized infants.

Table 3.

Discussion

In this pre-birth cohort study, incorporating SPT and oral food challenge at 1 year, food sensitized-tolerance during infancy appeared to appears to be associated with a similar

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pattern of exposures and outcomes to non-sensitized infants. In addition, we found some evidence that a regulatory immune profile at birth was associated with subsequent sensitized-tolerance.

Genetic factors may influence the progression from food sensitization to clinically expressed food allergy. A family history of allergic disease, in particular eczema and asthma, was strongly associated with food allergy but not sensitized-tolerance. There is conflicting evidence regarding associations between pet ownership and infant allergic disease.(37-39) This is the first study to address the relationship between pet ownership and sensitized-tolerance. Amongst sensitized infants, pet ownership at 12 months was strongly associated with an increased incidence of sensitized-tolerance, and by contrast, a reduced incidence of food allergy. This suggests that greater postnatal microbial exposure promotes a sensitized-tolerant, rather than sensitized-allergic, phenotype; which is consistent with high levels of sensitized-tolerance among children from rural versus urban Africa.(11) At 4 years of age there was a very high proportion of dog ownership (422/559, 75.5%) but very low incidence of dog sensitization (4/546, 0.7%). There was no evidence of concordance between dog ownership and dog sensitization (p = 0.474). Cat ownership was less common than dog ownership (204/559, 36.5%) and cat sensitization was more frequent than dog sensitization (25/545, 4.6%). However, there was no evidence of concordance between cat ownership and cat sensitization (p = 0.281). It is therefore unlikely that a relationship between dog/cat ownership and dog/cat sensitisation is confounding the relationship between pet ownership and aeroallergen sensitisation overall.

Associations between genetic and environmental factors, and food sensitization phenotype, may reflect differences in immune function at birth. Previous studies have found associations between a lower proportion of Tregs at birth and subsequent allergic disease.(22, 24, 40). As far as we are aware, this is the first study to find this deficit is not apparent among infants with sensitized-tolerance.

Interestingly, we found some evidence that sensitized-tolerant infants had an increased proportion of umbilical cord blood activated Tregs in comparison to non-sensitized infants.

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Both naïve and activated Tregs are equally suppressive but activated Tregs have a memory (CD45RA^{neg}) phenotype(41) and are more proliferative(42). It has been recently reported that sensitized-tolerant infants exhibit an increased capacity to produce and maintain activated Tregs after oral food challenge.(26) There is mounting evidence regarding the impact of the maternal microbial environment, microbiome and diet on foetal immune development and Treg populations(13), although little is reported with specific reference to activated Tregs. Our findings are consistent with an increase in activated Tregs during fetal immune development in infants with subsequent sensitized-tolerance. Increased activated Tregs are likely to provide sensitized-tolerant infants with a greater Treg response and suppressive capacity, which may limit progression to food allergy.

Differences in the clinical expression of allergic disease among food sensitized-tolerant versus food allergic infants were evident from early infancy and persisted throughout early childhood. The association between eczema and food allergy, which was not apparent between eczema and food sensitization, may reflect either causation or shared antecedent factors. The dual-allergen-exposure model proposes that deficits in skin barrier function in infancy are causally related to subsequent food allergy.(43) Alternatively, immune phenotype in early infancy may underlie both eczema and food allergy. Infants with eczema have been reported to have reduced Treg, with blunted responses to stimulation from microbial components(23), however the relationship between the activated Treg cell population and eczema has not been reported. It is plausible that an enhanced proliferative response of activated Tregs reduces the risk of eczema and promotes sensitized-tolerance by enabling more effective induction of tolerance following early allergen exposures. (42) Antenatal exposure to allergen may augment this mechanism by promoting the production of memory activated Tregs.(41)

Differences in the clinical expression of allergic disease by early food sensitization phenotype persisted to age 4 years. In keeping with previous studies(44-46), food allergy was strongly associated with subsequent aeroallergen sensitization, including the number of aeroallergens sensitized and wheal size and was strongly associated with hayfever, atopic wheeze and asthma. By contrast, sensitized-tolerant infants appeared to have a similar risk of each of these outcomes to non-sensitized infants.

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The strengths of this study include the longitudinal design, immune profiling at birth, and determination of food allergy by formal food challenge. Food challenges provide robust delineation of food sensitization phenotype in comparison to doctor diagnosis or parent report which are often inaccurate (47, 48) but relied upon in previous studies.(27-30) A potential limitation is the SPT wheal cut-offs chosen to define cases. In clinical practice food sensitization at 12 months of age is defined as a SPT wheal size 3mm or greater than the negative control(49), however recent studies have used a definition of 2mm or greater than the negative control in infants.(50) This change in definition is supported by evidence that a high proportion of 12 month old infants with a 2-3mm SPT response demonstrate clinically apparent food allergy on formal challenge.(50) *A priori*, we therefore selected 2mm as an appropriate definition of allergic sensitization at 12 months of age. Additionally, we adopted a lower cut-off (1mm) to screen for infants who should undergo a formal food challenge in order to optimize detection of clinically apparent food allergy in the cohort. We did not have sufficient resources to conduct food challenges in the complete cohort at 12 months, nor to conduct formal food challenges at 4 years. Another important limitation is the relatively small number of sensitized-tolerant infants. There are substantial challenges associated with performing food challenge in sufficiently large cohorts of infants to identify enough children with sensitized-tolerance. Nonetheless, further delineation and investigation of the sensitized-tolerant phenotype may well provide crucial insights.

Conclusion

Food sensitized-tolerance in infancy appears to be associated with a similar pattern of exposures and outcomes to non-sensitized infants. In addition, an elevated proportion of activated regulatory T cells at birth was specific to the sensitized-tolerant infants, and may

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be relevant to suppression of clinical disease. Further understanding of the mechanisms underlying the sensitized-tolerant phenotype may inform prevention of allergic disease.

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Table 1. Participant demographics for participants with known infant food sensitization phenotype

		Non-sensitized	Sensitized-tolerant	Food allergic	P-value for test of difference across all groups[#]
n	Total = 786	698	27	61	
Child sex	Male	346 (49.6%)	17 (63.0%)	34 (55.7%)	0.27
	Female	352 (50.4%)	10 (37.0%)	27 (44.3%)	
Plurality	Singleton	686 (98.3%)	27 (100.0%)	59 (96.7%)	0.53
	Twin	12 (1.7%)	0 (0.0%)	2 (3.3%)	
Maternal country of birth	Australia	627 (89.8%)	26 (96.3%)	56 (91.8%)	0.82
	Other	69 (9.9%)	1 (3.7%)	5 (8.2%)	
	Unknown	2 (0.3%)	0 (0.0%)	0 (0.0%)	
Paternal country of birth	Australia	605 (86.7%)	24 (88.9%)	49 (80.3%)	0.72
	Other	61 (8.7%)	2 (7.4%)	8 (13.1%)	
	Unknown	32 (4.6%)	1 (3.7%)	4 (6.6%)	
Family history of hayfever	Yes	438 (64.2%)	20 (76.9%)	47 (77.0%)	0.061
	No	244 (35.8%)	6 (23.1%)	14 (23.0%)	
Family history of eczema	Yes	303 (44.6%)	12 (44.4%)	40 (65.6%)	**0.007**
	No	376 (55.4%)	15 (55.6%)	21 (34.4%)	
Family history of asthma	Yes	331 (48.2%)	14 (51.9%)	43 (70.5%)	**0.004**
	No	356 (51.8%)	13 (48.1%)	18 (29.5%)	
Maternal age		31.93 (4.54)	31.64 (3.78)	32.21 (4.36)	0.83

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at conception, mean (SD)					
Paternal age at conception, mean (SD)		33.91 (5.65)	33.20 (5.02)	34.35 (5.67)	0.62
Maternal highest education	Less than year 10	6 (0.9%)	0 (0.0%)	0 (0.0%)	0.85
	Year 10 or equivalent	35 (5.0%)	0 (0.0%)	2 (3.3%)	
	Year 12 or equivalent	93 (13.4%)	3 (11.1%)	12 (19.7%)	
	Trade certificate or Diploma	177 (25.5%)	7 (25.9%)	12 (19.7%)	
	Bachelor degree	249 (35.8%)	10 (37.0%)	23 (37.7%)	
	Postgraduate degree	135 (19.4%)	7 (25.9%)	12 (19.7%)	
Paternal highest education	Less than year 10	16 (2.3%)	0 (0.0%)	0 (0.0%)	0.51
	Year 10 or equivalent	44 (6.4%)	1 (4.0%)	5 (8.3%)	
	Year 12 or equivalent	107 (15.6%)	2 (8.0%)	14 (23.3%)	
	Trade certificate or Diploma	270 (39.5%)	11 (44.0%)	19 (31.7%)	
	Bachelor degree	175 (25.6%)	6 (24.0%)	17 (28.3%)	
	Postgraduate degree	72 (10.5%)	5 (20.0%)	5 (8.3%)	
SEIFA* disadvantage tertile	Low SEIFA (most disadvantaged)	219 (31.8%)	7 (26.9%)	18 (29.5%)	0.92
	Medium SEIFA	233 (33.8%)	8 (30.8%)	20 (32.8%)	
	High SEIFA (least	237 (34.4%)	11 (42.3%)	23 (37.7%)	

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	disadvantaged)				
Household size during pregnancy	1 person	8 (1.1%)	0 (0.0%)	2 (3.3%)	0.21
	2 people	267 (38.4%)	13 (48.1%)	22 (36.1%)	
	3 people	240 (34.5%)	8 (29.6%)	28 (45.9%)	
	4 or more people	181 (26%)	6 (22.2%)	9 (14.8%)	
Sibling number at 12 months	No siblings	287 (41.1%)	13 (48.1%)	22 (36.1%)	0.50
	One sibling	244 (35.0%)	9 (33.3%)	28 (45.9%)	
	Two siblings	130 (18.6%)	3 (11.1%)	10 (16.4%)	
	Three or more siblings	37 (5.3%)	2 (7.4%)	1 (1.6%)	
Any maternal smoking during pregnancy	Any	84 (12.2%)	5 (18.5%)	9 (14.8%)	0.54
	None	607 (87.8%)	22 (81.5%)	52 (85.2%)	
Any maternal passive smoke exposure during pregnancy	Yes	77 (11.4%)	2 (7.4%)	6 (9.8%)	0.77
	No	601 (88.6%)	25 (92.6%)	55 (90.2%)	
Pet ownership during pregnancy	Yes	522 (75.0%)	21 (77.8%)	34 (55.7%)	**0.004**
	No	174 (25.0%)	6 (22.2%)	27 (44.3%)	
Pet ownership at 12 months	Yes	487 (71.5%)	22 (81.5%)	27 (45.8%)	**<0.001**
	No	194 (28.5%)	5 (18.5%)	32 (54.2%)	
Livestock exposure during pregnancy	Yes	56 (8.1%)	1 (3.7%)	1 (1.7%)	0.14
	No	635 (91.9%)	26 (96.3%)	59 (98.3%)	
Hospital type	Public hospital	477 (68.3%)	16 (59.3%)	41 (67.2%)	0.61
	Private hospital	221 (31.7%)	11 (40.7%)	20 (32.8%)	
Any labour prior to delivery	Yes	550 (78.9%)	24 (88.9%)	50 (82.0%)	0.40
	No	147 (21.1%)	3 (11.1%)	11 (18.0%)	
Birthweight, kg, mean		3.53 (0.53)8	3.72 (0.53)	3.51 (0.46)	0.34

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(SD)					
Birthweight, Z-score (SD)		0.38 (0.91)	0.54 (0.97)	0.17 (0.88)	0.11
Any breastfeeding	Yes	687 (98.4%)	27 (100.0%)	59 (96.7%)	0.48
	No	11 (1.6%)	0 (0.0%)	2 (3.3%)	

665

666 # P-values were calculated using Pearson's chi-squared test for binary or categorical
667 outcomes and a Kruskal-Wallis test for continuous outcomes.
668 * SEIFA - Socio-Economic Indexes for Areas. Lower score indicates greater relative socio-
669 economic disadvantage.

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690 **Table 2. Eczema during infancy and subsequent food sensitization phenotype at 1 year in**
691 **comparison to non-sensitized infants**

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Time point	Cumulative incidence of eczema			Adjusted risk ratio (aRR) † (95% Confidence interval)	
	NS	ST	FA	Sensitized-tolerant	Food allergic
3 months	4/678 (0.6%)	1/26 (3.9%)	4/59 (6.8%)	4.86 (0.49-48.11) p = 0.176	5.62 (0.83-38.00) p = 0.076
6 months	45/655 (6.9%)	3/26 (11.5%)	17/56 (30.3%)	1.58 (0.45-5.60) p = 0.478	4.18 (2.05-8.52) p < 0.001
9 months	96/610 (15.7%)	6/25 (24.0%)	24/50 (48.0%)	1.67 (0.62-4.47) p = 0.308	3.76 (1.97-7.16) p < 0.001
12 months	117/617 (19.0%)	8/25 (32.0%)	38/59 (64.4%)	2.05 (0.83-5.07) p = 0.119	5.81 (3.15-10.72) p < 0.001

†Adjusted for sex, family history of eczema, any siblings during pregnancy, pet ownership and livestock exposure during pregnancy

Table 3. Food sensitization phenotype at 1 year and subsequent aeroallergen sensitization and disease in comparison to non-sensitized infants

Allergic disease outcome	Incidence			Adjusted risk ratio (aRR) † (95% Confidence interval)	
	NS	ST	FA	Sensitized-tolerant	Food allergic
Aeroallergen sensitization at age 4 years	81/425 (19.1%)	4/14 (28.6%)	20/45 (44.4%)	1.39 (0.59-3.30) p = 0.449	3.84 (2.94-5.02) p < 0.001
Current	54/604	1/21	11/53	0.80	2.02

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hayfever at age 4 years	(8.9%)	(4.8%)	(20.8%)	(0.21-3.02) p = 0.740	(1.17-3.50) p = 0.012
Current atopic wheeze at age 4 years	24/423 (5.7%)	2/14 (14.3%)	18/45 (40.0%)	2.95 (0.76-11.45) p = 0.117	5.97 (3.34-10.68) p < 0.001
Doctor-diagnosed asthma by age 2 years	36/551 (6.5%)	2/22 (9.1%)	8/45 (17.8%)	1.41 (0.35-5.59) p = 0.627	2.90 (1.42-5.86) p = 0.004
Doctor-diagnosed asthma by age 4 years	81/658 (12.3%)	3/24 (12.5%)	22/57 (38.6%)	0.92 (0.32-2.70) p = 0.883	2.80 (1.92-4.08) p < 0.001

[†] Adjusted for sex, family history of eczema, any siblings at 12 months and pet ownership at 12 months

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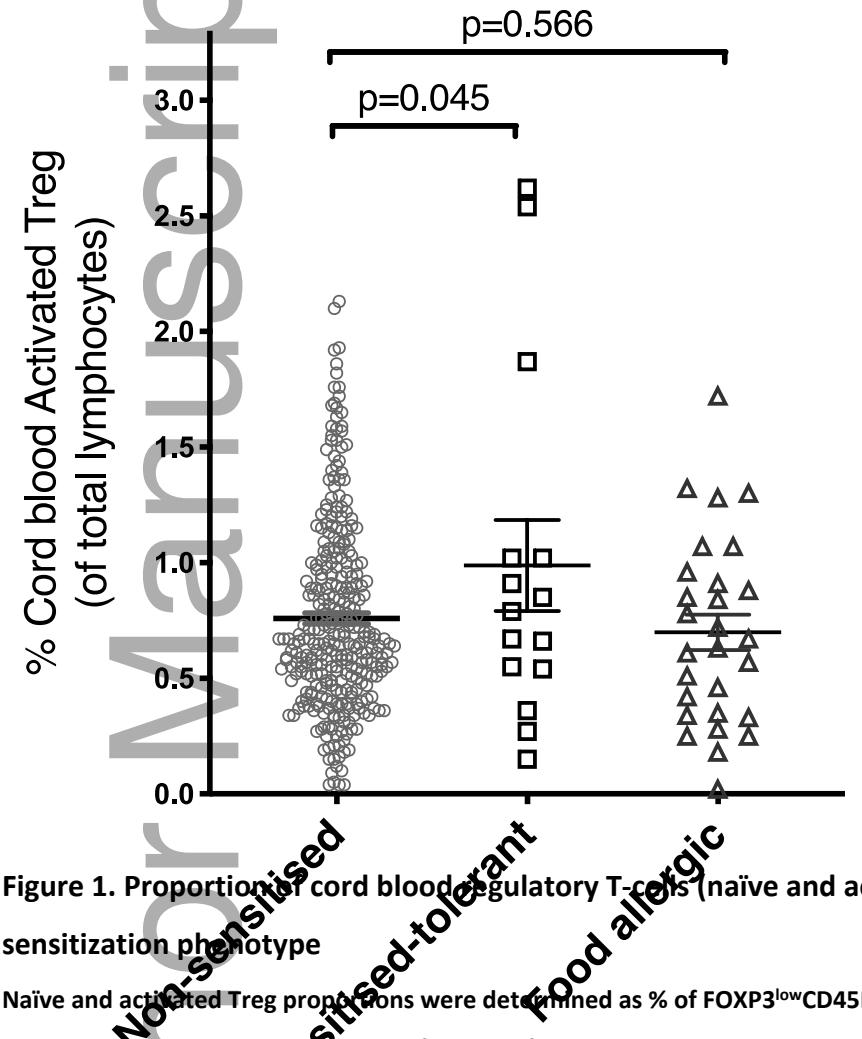

Figure 1. Proportion of cord blood regulatory T-cells (naïve and activated) by food sensitization phenotype

Naïve and activated Treg proportions were determined as % of FOXP3^{low}CD45RA^{+} cells or FOXP3^{high}CD45RA^{-} cells respectively within the CD4^{+} cell fraction of cord blood mononuclear cells.

A. Reduced proportion of cord blood naïve Tregs in food allergic infants (n=28) compared to non-sensitized (n=313) (Odds ratio (OR) 0.63 per % change in proportion of naïve Treg, 95% confidence interval (CI) 0.44 – 0.90 , *P*= 0.010).(20, 21) B. Increased proportion of cord blood activated Tregs in the sensitized-tolerant group (n=15) compared to non-sensitized (n=313) (OR 2.89 per % change in proportion of activated Treg 95% CI 1.03 – 8.16, *P*= 0.045). Horizontal bar indicates mean. Whiskers indicate 95% CI of the mean.

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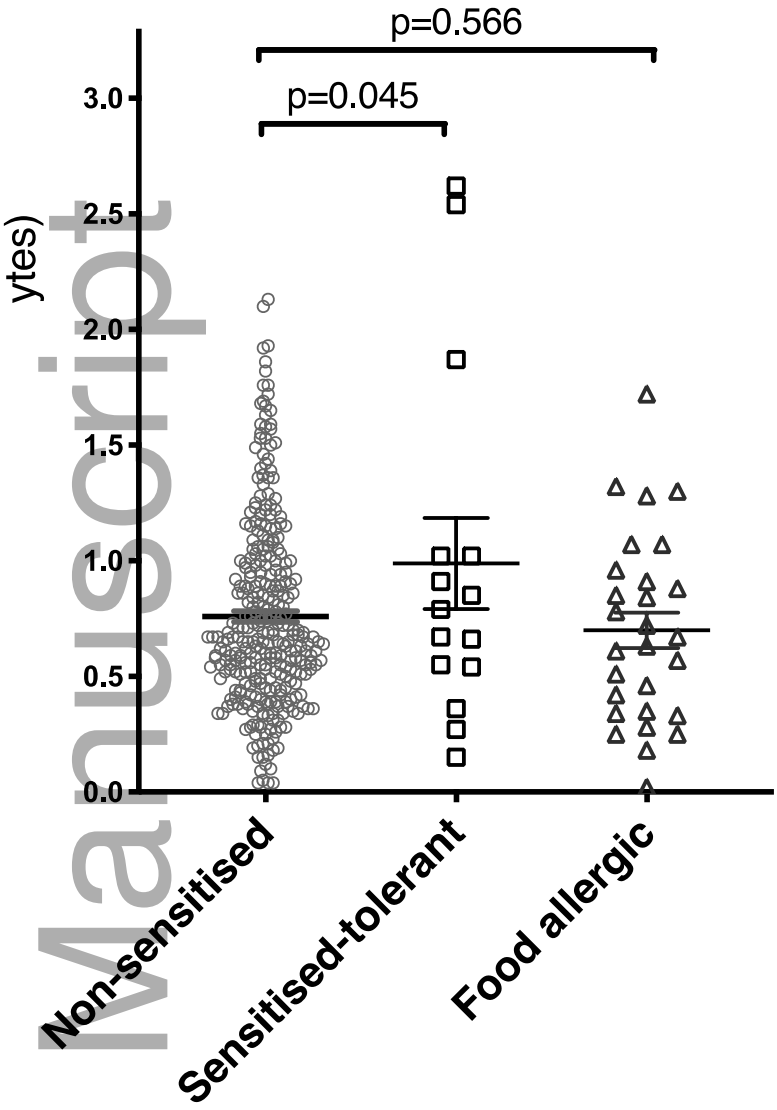

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