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Naïve regulatory T cells in infancy: Associations with perinatal factors and development of food allergy

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Author Contributions

FC instigated, designed and performed the measurement of the samples, data acquisition and analysis, interpretation, and writing of the manuscript. ALP, SB & PV were involved in the study design, the interpretation and writing of the manuscript. MO'H and LEG contributed to analysis and review of the manuscript. MT contributed to conception and design of the Treg measures and review of the manuscript. JM undertook the assessment of food allergy status and review of the manuscript. DB, RS, SR & KA contributed to review of the manuscript.

Abstract

Background: In previous studies, deficits in regulatory T-cell (Treg) number and function at birth have been linked with subsequent allergic disease. However longitudinal studies, that account for relevant perinatal factors, are required. The aim of this study was to investigate the relationship between perinatal factors, naïve Treg (nTreg) over the first postnatal year, and development of food allergy.

Methods: In a birth cohort (n=1074), the proportion of nTreg in the $CD4^+$ T-cell compartment was measured by flow cytometry at birth (n=463), six (n=600) and twelve (n=675) months. IgE-mediated food allergy was determined by food challenge at one year. Associations between perinatal factors

(gestation, labour, sex, birth size), nTreg at each time point and food allergy at 1 year were examined by linear regression.

Results: A higher proportion of nTreg at birth, larger birth size and male sex were each associated with higher nTreg in infancy. Exposure to labour, as compared to delivery by pre-labour Caesarean section, was associated with a transient decrease nTreg. Infants that developed food allergy had decreased nTreg at birth, and the labour-associated decrease in nTreg at birth was more evident among infants with subsequent food allergy. Mode of birth was not associated with risk of food allergy and there was no evidence that nTreg at either six or twelve months were related to food allergy.

Conclusion: The proportion of nTreg at birth is a major determinant of the proportion present throughout infancy, highlighting the importance of prenatal immune development. Exposure to the inflammatory stimulus of labour appears to reveal differences in immune function among infants at risk of food allergy.

Introduction

Regulatory T cells (Treg) are heterogeneous in relation to their developmental origin, functional activity and activation status (1). Deficits in Treg number and function during early life have been associated with autoimmune disease (2), eczema (3, 4), and asthma (5), and the role of Treg in the pathogenesis of allergic disease has been widely examined (2, 6, 7). The thymus-derived naive $CD4^+$ Treg (nTreg) subset has an immunoregulatory role that is critical in early life for control of allergic, autoimmune and antimicrobial responses (8), with nTreg reported to regulate both the adaptive immune response by suppression of Th2 mediated reactions (9) and the immediate hypersensitivity response by mast cells (10). Treg inhibit these pathways by production of a variety of suppressive cytokines and factors. When their number or function is impaired, as observed in genetic mutations (11) or animal Treg depletion experiments (12) this results in severe allergic disease. In addition, the acquisition of food tolerance among previously allergic children appears to be associated with greater frequency of circulating Treg (13). We recently replicated evidence of a univariate association between a decreased proportion of Treg at birth and subsequent food allergy (14). However little is known regarding the interplay between perinatal factors, nTreg over the first year of life and allergic outcomes.

Naïve Treg, produced in the thymus, are characterised by expression of FOXP3 and the naïve T cell marker, CD45RA (15). Their strong immunosuppressive properties are conferred by a combination of

demethylation of the *FOXP3* gene in the 5' flanking and STAT5-responsive gene region and stable expression of *FOXP3* (15, 16). We previously showed that nTreg make up the majority of FOXP3 positive $CD4^+$ T cells at birth (17). Studies suggest that birth-related factors, including gestation and mode of delivery, influence the proportion of Treg in cord blood but the methods used to identify Treg vary, and results have been conflicting (18-22). Importantly, few studies have considered the impact of perinatal factors on the relationship between cord blood immune phenotype and allergic disease.

The aim of this study was to examine the influence of key birth and infant-related perinatal variables on nTreg proportions over the first postnatal year, and relate the findings to the development of food allergy.

Methods

Study design

Study participants were enrolled in the Barwon Infant Study (BIS), a cohort assembled during pregnancy using an unselected sampling frame in the south east of Australia (23). Exclusion criteria included birth before 32 weeks of gestation, major congenital malformation and/or genetically determined disease, and/or serious illness. Blood samples were collected from cord blood at birth and from peripheral blood at six months and twelve months. The study was approved by Barwon Health Human Research and Ethics Committee (HREC 10/24).

Data Collection

Data on gestational age, mode of delivery, infant sex, and birthweight, were collected from participant questionnaires and hospital records. Exposure to labour was categorised according to either (a) no labour = infants born by planned Caesarean section (C-section) prior to the onset of labour, or (b) exposure to labour = remaining infants, including those born by C-section following the onset of labour. Absence of labour was used as the reference in exposure to labour analysis. To investigate the influence of birth size we calculated a birthweight z-score using British growth charts (24, 25), adjusted for gestation and sex; factors that are both associated with birthweight (26).

Blood Sampling and Isolation of Mononuclear Cells

Umbilical cord blood was collected by syringe and immediately diluted in 10IU/mL preservative-free sodium heparin (Pfizer) in 10ml of RPMI 1640 (Gibco, Life Technologies). Venous peripheral blood was collected at six and twelve months of age and added to a 15ml tube containing 10IU/mL preservative-free sodium heparin (Pfizer). Mononuclear cells were isolated by density gradient centrifugation (Lymphoprep, Axis-Shield), and 2-4 x 10^4 cells used for flow cytometric measurement of nTreg.

Measurement of Treg 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 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 (FACSCalibur, Becton Dickinson). All antibodies were sourced from BD Biosciences, San Jose, California. Gating of nTreg ($CD4^+$/$FOXP3^+$/$CD45RA^+$) was performed as previously described (17) (Supplementary Figure S1), and reported as a proportion of the total $CD4^+$ T cell population.

Assessment of Food Allergy Status

At the 12 month review, infants underwent a skin prick test (SPT) to five foods: cow's milk, egg, peanut, cashew and sesame (ALK-Abelló, Madrid, Spain) with a positive (10 mg/ml histamine) and negative control (saline). Quintip® lancets (Hollister-Stier Laboratories, Spokane, WA) were used to perform SPT's on infant's backs. Allergic sensitisation was defined as a wheal of 2mm or greater than the negative control (27, 28). All participants with SPT wheal size 1mm or greater than the negative control were offered an in-hospital open food challenge (29). Open food challenges were performed under clinical supervision using validated protocols from the HealthNuts study (29) and described in detail previously (30, 31). A positive challenge was defined by one or more of the following (within 2 hours of ingesting a dose of challenge food); three or more concurrent non-contact urticaria for five minutes or longer; vomiting or diarrhoea; angioedema; anaphylaxis (circulatory or respiratory compromise). If, on clinical review, the participant had a clinical history and reaction consistent with a diagnosis of IgE-mediated food allergy within 2 months of the 1 year review and a positive SPT of 2mm or greater than the negative control, they were defined as food allergic without proceeding to food challenge. Infants with a positive histamine wheal and either an antigen SPT wheal size of 0mm, or ≤1mm with a negative food challenge, were classified as non food allergic and non-sensitised (31).

Statistical Analysis

The nTreg data were not transformed as the sample size at each time point (birth, six and twelve months of age) was large (>450) and the distribution approximated normality (32). To assess the influence of birth size, a birthweight z-score was calculated using British growth charts (24, 25). The birthweight z-score (birth size) was used as a single determinant factor in all analyses. Univariate and multivariate linear regression was used to determine the influence of sex, gestational age, labour and birth size on nTreg proportions at each time point. Differences in the birth nTreg proportions in the food allergic group were determined using a Mann Whitney test. The relative risk (RR) for development of food allergy at 12 months was determined using the general linear model (GLM). Linear regression was also used to determine the relationship between cord blood nTreg (starting proportion) with nTreg proportions at later time points (in infants that had nTreg measures at all three time points). These longitudinal data were adjusted for variables that had cross sectional associations with nTreg. Statistical analysis was performed using Stata 13.1 (Stata Corp, College Station, TX).

Results

Perinatal characteristics of infants with nTreg measurements and subsequent food allergy

The proportion of nTreg (as a percentage of $CD4^+$ T cells) was analysed in 463/974 freshly collected cord blood samples, 600/789 six month infant peripheral blood samples, and in 675/744 twelve month infant peripheral blood samples. A total of 191 infants had measures at all three time points. There were no differences in gestational age, exposure to labour, birth size (based on birthweight z-score) or sex, between the full cohort and the subcohorts for whom nTreg measurements were performed (Table 1A). There were also no differences in these perinatal characteristics between the infants with or without subsequent food allergy (Table 1B).

Influence of perinatal factors on the proportion of nTreg during the first year of life

Greater gestational age was associated with a lower proportion of nTreg at birth (Figure 1A, Table 2), but this was attenuated by adjustment for exposure to labour, sex and birth size (Table 2) and no longer apparent by six months of age (Figure 1B, Table 2).

Exposure to labour is associated with physiological stress for mother and infant. Consistent with this, exposure to labour was associated with an increase in granulocyte proportion, hsCRP, soluble CD14 and IL-6 in cord blood (Supplementary Figure S2), in combination with a decrease in nTreg proportion (-0.70% (95%CI -0.99, -0.42) p<0.0001, Figure 1C), which persisted following adjustment for gestational age, sex and birth size (-0.59% (95%CI -0.89, -0.29) p<0.0001, Table 2). The difference associated with exposure to labour was evident in both vaginal delivery (-0.67% (95%CI -0.96, -0.37) p<0.0001) and emergency C-section (-0.85% (95%CI -1.29, -0.42) p<0.0001). In a subsample (n=193), the number of white blood cells in the cord blood were counted and the absolute number of nTreg enumerated (in addition to proportion of nTreg). Labour was also associated with a decrease in the absolute count of nTreg at birth (Supplementary Figure S3B). In contrast, the proportion and absolute number of activated Treg, a population derived from the nTreg (15), were similar and not affected by labour (Supplementary Figure S3C & D). This indicates that the Treg do not move from a naive to activated phenotype in response to labour.

Male sex was associated with a higher proportion of nTreg at birth, six and twelve months of age (Figure 2A, Table 2) in unadjusted analyses. This association persisted at six and twelve months following adjustment for gestational age, labour and birth size (Table 2). Two groups that represented either high (>1SD above the mean) or low (>1SD below the mean) birth size categories were generated (33). A low birth size was associated with slightly lower nTreg proportion at birth and six months of age (Figure 2B, Table 2). These associations persisted in adjusted analyses. There was also a weak positive cross-sectional association between body weight and nTreg proportion at twelve months (Supplementary Figure S4).

Longitudinal pattern of nTreg over the first year of life

Measurements of nTreg were completed in 191 infants at all three time points. The proportion of cord blood nTreg was positively associated with the proportion of nTreg at both six (p<0.0001), and twelve months (p=0.013) (Supplementary Table 1). These associations persisted following adjustment for sex and birth size.

To investigate longitudinal trends, the proportion of cord blood nTreg was divided into tertiles. Neonates with cord blood nTreg in the lower tertile (1.4 - 3.6% nTreg at birth) had an equivalent proportion to the middle tertile (3.6% - 4.7% nTreg at birth) by six months of age, while infants with

cord blood nTreg in the upper tertile (>4.7% nTreg at birth) sustained a higher proportion of nTreg over the entire first year (Figure 3).

Infant nTreg and development of food allergy

The proportion of cord blood nTreg was lower in children that subsequently developed food allergy compared with non-food allergic infants (3.75% compared to 4.41% of total $CD4^+$ T-cells, a difference of -0.66% (95% CI -1.15, -0.17), p=0.009) (Figure 4A & C, Supplementary Table 2) (14). This association persisted following adjustment for gestational age, labour, sex, and birth size (-0.57% (95% CI -1.05, -0.09, p=0.020). The mean cord blood nTreg in those that went on to develop food allergy was 3.75% (of $CD4^+$ T cells) compared with 4.41% in non-food allergic infants (Figure 4A). A higher proportion of cord blood nTreg was associated with a decreased risk for development of food allergy at twelve months (RR 0.65 (95% CI 0.47, 0.89) per 1% increase in proportion of nTreg, p=0.008) (Supplementary Table 2). This risk was unchanged after adjusting for exposure to labour, sex, gestational age and birthweight z-score (RR 0.66 (95% CI 0.48, 0.93) per 1% increase in proportion of nTreg, p=0.016) (Supplementary Table 2).

The decrease in cord blood nTreg in infants exposed to labour was almost 3-fold greater among infants with subsequent food allergy compared to among infants without food allergy (proportion of nTreg at birth decreased by -1.9% versus -0.7%, p=0.03 for difference of effect by food allergy status in a generalised linear model, Figure 4B). Mode of birth was not associated with food allergy among offspring and there was no difference in proportion of nTreg at six or twelve months and the relative risk of food allergy at one year (Figure 4C, Supplementary Table 2).

Discussion

Consistent with the importance of prenatal immune programming, this study demonstrates that the proportion of nTreg in cord blood at birth, as well as the gender and size of the neonate, are major determinants of the proportion of nTreg present in peripheral blood throughout the first year of life. The physiological response to labour is associated with a decrease in cord blood nTreg but this is transient. In addition, we have now confirmed that the previously reported univariate association

between decreased birth nTreg and subsequent allergy (14) is independent of relevant perinatal covariates. This association was primarily evident among infants exposed to labour, suggesting labour-associated immune activation reveals differences in infants at increased risk of allergic disease.

Prenatal immune development is likely to have important implications for long term immune function and health (34). In mice, the maternal microbiome has a key role in stimulating foetal immunity (35), but the relevance of these findings to humans remains uncertain (36). Larger birth size is linked to decreased autoimmune and infectious diseases (37), and in the current study, was associated with greater birth nTreg. Maternal and infant nutritional status may be relevant, particularly given the close alignment between maternal and infant Treg status (38). Male sex was also associated with greater nTreg, consistent with sex patterns in adults (39) but inconsistent with previous measures of cord blood Treg measures as determined by the Treg-specific demethylated region (TSDR) (3). Potentially relevant differences of the current study include measurement of Treg by flow cytometry rather than via an epigenetic marker, as well adjustment for exposure to labour. The mechanisms by which sex influences in Treg development are poorly characterised but may include genetic factors, particularly as the FOXP3 gene is on the X chromosome; or the influence of sex hormones.

In other studies, both decreased suppressive activity of cord blood Treg ($CD4^+CD25^+CD127^{low}$) and lower Treg numbers have been associated with subsequent egg allergy (40), and atopic dermatitis and food sensitisation (3) respectively. Consistent with these, we have previously reported a univariate association between cord blood nTreg and food allergy at 1 year (14). Here we have extended these studies by confirming, for the first time, that the association between low birth Treg and allergy is independent of important covariates including sex, birth size and gestation.

In relation to the labour-related decrease in nTreg (both proportion and absolute number), we showed that the nTreg do not move from a naive to activated phenotype. Other possibilities for the decrease in nTreg are that labour-related inflammatory mediators either induce apoptosis or initiate differentiation to an alternate $CD4^+$ subtypes (eg Th2, Th17) (41). It is unlikely that there is apoptosis without an increase in Treg activation. However consistent with cellular differentiation inflammatory cytokines suppress FOXP3 and induce IL-4 expression in cord blood nTreg, indicative of a switch to a Th2 adaptive immune phenotype (14). It has been proposed that absence of a labour-related inflammatory stimulus has a detrimental impact on perinatal immune development, contributing to increased immune related disease among infants born by planned C-section (21). Although C-section

appears to be a risk factor for asthma development (42), we have replicated the previously reported absence of association between mode of birth and IgE mediated food allergy (43). It is important to recognise that the association between exposure to labour and decreased nTreg at birth appears to be transient in that it was no longer evident in blood collected in later infancy.

The labour-related decrease in nTreg is likely very important immunologically immediately following birth. We propose that rather than providing long term immune programming, the labour-associated inflammation merely unmasks differences in the immune systems of infants at risk for allergic disease. It may be appropriate to consider whether measures of Treg at birth could be used to identify at-risk infants for whom preventive strategies may be relevant. For example, it would be of interest to investigate whether promotion of skin barrier function and timely introduction of allergenic foods is particularly important among infants with low Treg at birth. Perhaps more importantly, the current study highlights the need for further work regarding the role of foetal immune programming in the prevention of allergic disease.

The strengths of this study include the large number of participants with longitudinal nTreg measures, detailed investigation of relevant perinatal covariates and determination of food allergy status by in-hospital food challenge. The relatively large number of infants born via planned C-section (unexposed to labour) enabled identification of an exaggerated labour-related decrease in cord blood nTreg in infants with subsequent food allergy. Limitations include the lack of data on Treg function, the absence of postnatal factors in analyses, and failure to elucidate the mechanistic basis of association between male sex, larger birth size and increased nTreg during the first postnatal year.

Further studies are now required to inform strategies to promote healthy in utero immune development, and to understand the antenatal mechanisms associated with an exaggerated immune response to labour among infants at risk of allergic disease.

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Table 1A. Cohort Characteristics among infants with nTreg Measurements

	Total Cohort	Birth nTreg	6 mth nTreg	12 mth nTreg	nTreg measures at all 3 time points
n (%)	1074 (100.0)	463 (43.1)	600 (55.9)	675 (62.9)	191 (17.8)
Gestational Age, weeks, mean (SD) (range)	39.4 (1.5) (32.0-42.0)	39.5 (1.3) (34.0-41.9)	39.5 (1.5) (32.0-41.9)	39.5 (1.5) (32.0-42.0)	39.6 (1.2) (36.0-41.7)
No Labour / Exposure to Labour, n (%)	200 (18.7) / 871 (81.3)	91 (19.7) / 372 (80.3)	114 (19.0) / 485 (81.0)	139 (20.6) / 535 (79.4)	39 (20.4) / 152 (79.6)
Time of Labour, hr, mean (SD) (range)	7.5 (5.4) (0.5-41.1)	7.3 (5.7) (0.7-41.1)	7.5 (5.9) (0.7-41.1)	7.6 (5.4) (0.7-41.1)	7.2 (6.7) (0.7-41.1)
Birth Size (Birthweight z-score), mean (SD) (range)	0.4 (0.9) (-2.2 – 3.6)	0.4 (0.9) (-2.2 – 3.6)	0.3 (0.9) (-2.2 – 3.6)	0.3 (0.9) (-2.2 – 3.6)	0.3 (0.9) (-2.2 – 3.6)
Sex - Female (%) - Male (%)	 518 (48.2) 556 (51.8)	 229 (49.5) 234 (50.5)	 299 (49.8) 301 (50.2)	 333 (49.3) 342 (50.7)	 94 (49.2) 97 (50.8)

Table 1B. Cohort Characteristics among infants with Food Allergy

	Non-food Allergic	Food Allergic
Of those tested at 12 mth and not inconclusive, n (%)	698 (82.6)	61 (7.2)
Gestational Age, weeks, mean (SD) (range)	39.4 (1.5) (32.0-42.0)	39.7 (1.3) (37.0-41.9)
No Labour / Exposure to Labour, n (%)	147 (21.1) / 550 (78.9)	11 (18.0) / 50 (82.0)
Time of Labour, hr, mean (SD) (range)	7.4 (5.3) (0.5-38.4)	8.0 (6.4) (2.0-33.0)
Birth Size (Birthweight z-score), mean (SD) (range)	0.4 (0.9) (-2.2-3.6)	0.2 (0.9) (-1.9-2.7)
Sex - Female (%) - Male (%)	 352 (50.4) 346 (49.6)	 27 (44.3) 34 (55.7)

Table 2. Association of birth- and infant-related factors with the proportion of nTreg (as % of CD4 T cells) at birth, 6 and 12 months of age

	Birth-Related Factors				**Infant-Related Factors**			
	Exposure to Labour		Gestational Age (weeks)		Sex		Birth Size (z-score)	
Reference Group	No Labour				Female			
	Unadjusted	*Adjusted	Unadjusted	*Adjusted	Unadjusted	*Adjusted	Unadjusted	*Adjusted
Birth %nTreg (n=463)								
Mean Diff (%) (95% CI)	-0.70 (-1.00, -0.42)	-0.59 (-0.89, -0.29)	-0.13 (-0.21, -0.04)	-0.06 (-0.15, -0.03)	0.27 (0.04, 0.50)	0.23 (-0.00, 0.45)	0.15 (0.02, 0.28)	0.11 (-0.02, 0.23)

p value	**<0.0001**	**<0.0001**	**0.004**	0.177	**0.024**	**0.047**	**0.019**	0.089
6 mth %nTreg (n=600)								
Mean Diff (%) (95% CI)	-0.13 (-0.42, 0.16)	-0.10 (-0.41, 0.21)	-0.03 (-0.10, 0.05)	-0.00 (-0.09, 0.08)	0.25 (0.02, 0.48)	0.24 (-0.01, 0.47)	0.14 (0.01, 0.26)	0.14 (0.01, 0.26)
p value	0.365	0.515	0.513	0.901	**0.030**	**0.039**	**0.032**	**0.035**
12 mth %nTreg (n=675)								
Mean Diff (%) (95% CI)	-0.17 (-0.47, 0.13)	-0.18 (-0.50, 0.14)	-0.00 (-0.08, 0.08)	-0.03 (-0.06, 0.12)	0.30 (0.05, 0.53)	0.30 (0.06, 0.54)	0.11 (-0.03, 0.24)	0.12 (-0.02, 0.25)
p value	0.260	0.277	0.944	0.495	**0.016**	**0.015**	0.114	0.089

*Adjusted refers to the coefficient estimated in a single regression including all four factors.

Table entries are mean difference, (95%CI), p-value (bold for <0.05)

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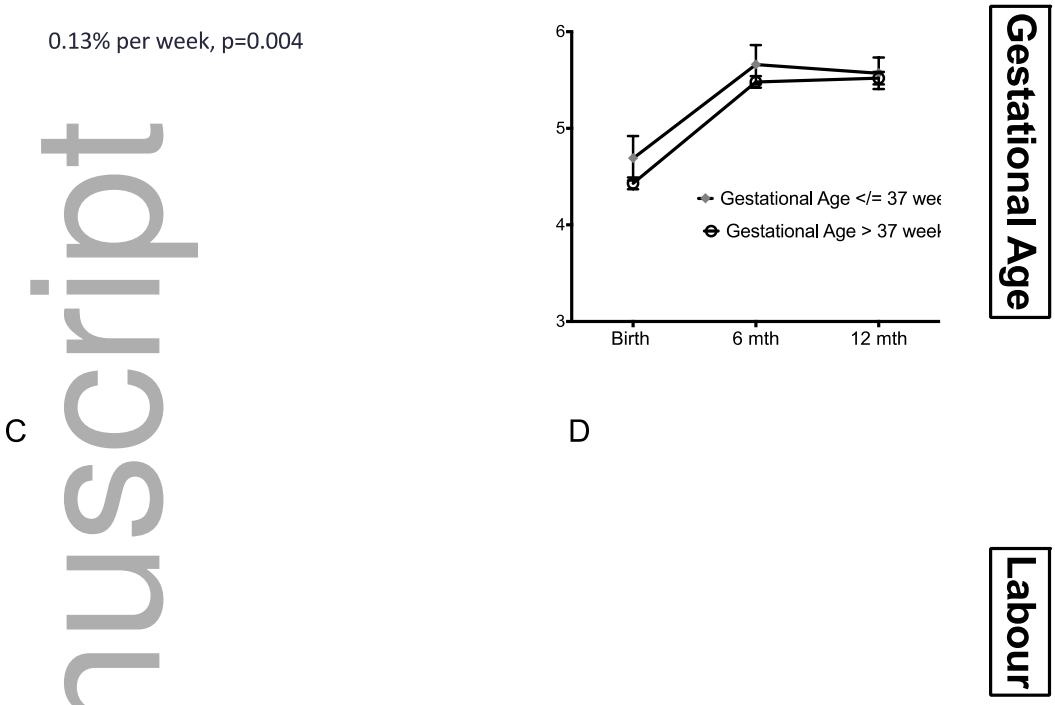

Figure 1. Influence of birth-related factors on the proportion of nTreg in the first year of life.

The nTreg proportion was determined as % of $FOXP3^{+}CD45RA^{+}$ cells within the $CD4^{+}$ cell fraction at birth, 6 and 12 months of age. (A) There is a decrease in proportion of cord blood nTreg in response to increasing gestation, unadjusted linear regression, p=0.004. (B) There is no difference in the proportion of nTreg at 6 and 12 months in relation to gestational age (classified according to pre-term and term, ≤37 weeks and >37 weeks) (mean±SEM). (C) There is a decrease in proportion of cord blood nTreg in response to labour, unadjusted linear regression, p<0.0001. (D) There is no difference in the proportion of nTreg at 6 and 12 months in response to labour (mean±SEM).

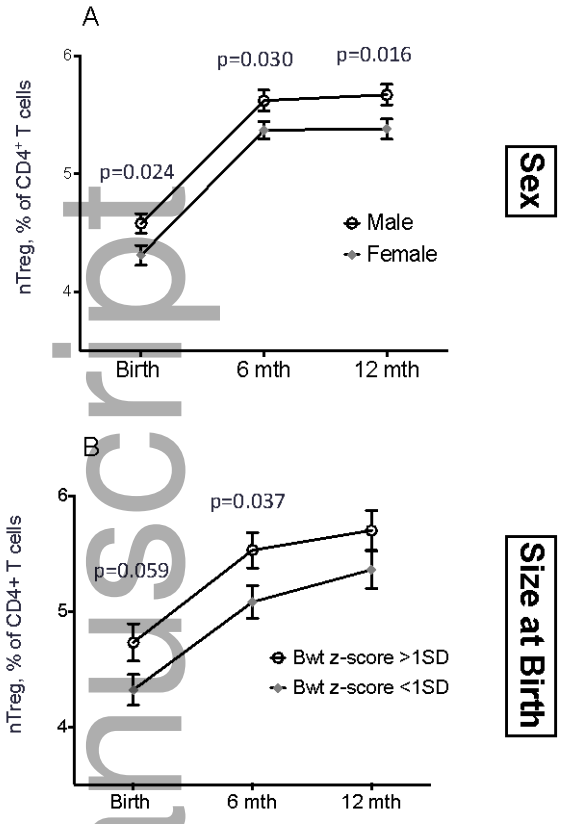

Figure 2. Influence of infant-related factors on the proportion of nTreg in the first year of life. The nTreg proportion was determined as % of $FOXP3^{+}CD45RA^{+}$ cells within the $CD4^{+}$ cell fraction at birth, 6 and 12 months of age (mean±SEM). (A) The proportion of nTreg is higher in male infants from the time of birth to 12 months of age. (B) The low birthweight z-score infants (<1SD) were compared to infants with high birthweight z-score (>1SD). The proportion of nTreg is higher in infants that had high birthweight z-score (large birth size) at birth and 6 months (p values from unadjusted linear regression analysis).

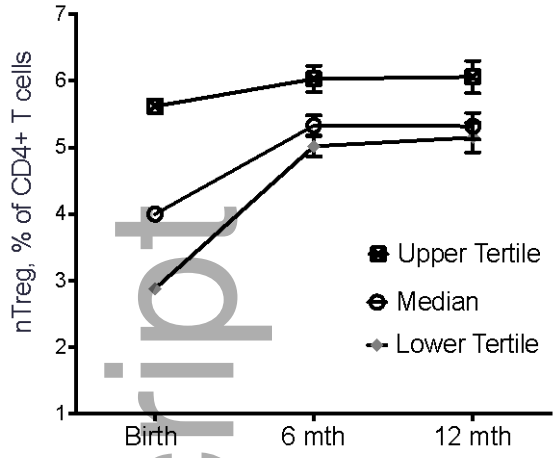

Figure 3. Higher proportion of cord blood nTreg relates to higher proportion at 6 and 12 months

Data from infants that had nTreg measures at all three data points was analysed (n=191). Cord blood nTreg proportions (as % of $CD4^+$ T cells) were divided into tertiles; and designated as: Lower, 1.45-3.57%, n=63; Middle, 3.58-4.66%, n=64 and Upper, 4.67-8.54%, n=64. The nTreg proportion at 6 mth and 12 mth were plotted according to their tertile grouping at birth (mean ± SEM). A higher nTreg proportion at both 6 mth and 12 mth was predicted by a higher tertile at birth (6mth, mean difference 0.70% (95% CI 0.23, 1.17) compared to the middle tertile, unadjusted linear regression, p=0.004; 12mth, mean difference 0.74% (95% CI 0.12, 1.36) compared to the median tertile, unadjusted linear regression, p=0.020).

A

c

p=0.008

B

p=0.008

C

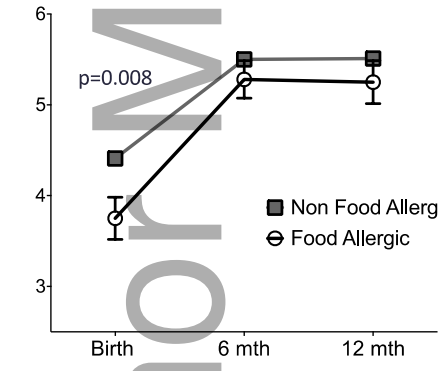

Figure 4. The proportion of nTreg (as % of CD4+ T cells) throughout the first year of life in relation to food allergy at one year of age.

A. Distribution of cord blood nTreg in infant that develop food allergy compared to non-food allergic infants. There is a shift to the left in the distribution of cord blood nTreg proportion in infants that develop food allergy at one year of age (mean proportion of nTreg, 3.75% (of $CD4^+$ T cells) compared with 4.41% in non-food allergic infants, p=0.008, Mann Whitney test). B. Differences in cord blood nTreg with exposure to labour and stratified by food allergy. The decrease in cord blood nTreg in infants exposed to labour was almost 3-fold greater among infants with subsequent food allergy compared to among infants without food allergy (proportion of nTreg at birth decreased by -1.9% versus -0.7%, p=0.03 for difference of effect by food allergy status in a generalised linear model). C. Proportion of nTreg during the first year of life. There was no difference in proportion of nTreg at six or twelve months in infants that develop food allergy vs non-food allergic infants (mean±SEM).