

REGULAR ARTICLE

Interleukin-10 and transforming growth factor- β 1 in cord blood: relationship with paternal allergy and cesarean section

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Abstract

Aim: To measure Interleukin-10 (IL-10) and transforming growth factor- β 1 (TGF- β 1) in cord blood and assess their relationship with parental allergy and perinatal characteristics.

Methods: In a neonatal care unit 212 consecutive full-term and appropriate for gestational age newborns were recruited. IL-10 and TGF- β 1 levels were determined in cord blood by high sensitivity ELISA. Perinatal characteristics, mode of delivery and presence of allergy in parents were recorded.

Results: Out of 212 newborns, 136 were of non-allergic parents and 76 (35.8%) of one or both allergic parents. In newborns of allergic fathers median IL-10 levels tended to be lower (0.67 vs. 1.06 pg/mL, $p = 0.07$) and TGF- β 1 levels were significantly lower (40.9 vs. 45.3 ng/mL, $p = 0.008$) than in newborns of non-allergic parents. Multiple general regression analysis showed that presence of paternal allergy ($\beta = -0.19$, $p = 0.003$) to be born by cesarean section ($\beta = -0.21$, $p = 0.03$) and younger gestational age ($\beta = 0.14$, $p = 0.04$) independently contributed to decrease TGF- β 1 levels (multiple $R = 0.38$, $p < 0.0001$).

Conclusion: Paternal allergy and cesarean section are associated to decreased TGF- β 1, which might be the mediator of the increased risk of atopy development. Cord blood IL-10 and TGF- β 1 levels of our newborn series could be used as reference values for further studies on these relationships.

INTRODUCTION

Prevalence of allergic disorders such as asthma, atopic dermatitis and rhinoconjunctivitis is rising in children from industrialized countries in the last decades. Both family history of atopy and environmental factors can be involved although causes and mechanisms remain to be determined. A deregulation of the mucosal immune system, which is the first route of interaction between exogenous antigens and T helper (Th) lymphocytes, is currently the most accepted hypothesis. The possibility that modern life environment may hinder T regulatory (T_{reg}) lymphocytes maturation, one subtype of Th cells, has been proposed (1). Transforming growth factor- β 1 (TGF- β 1) and Interleukin-10 (IL-10) are the two main mediators of T_{reg} and are thought to induce a 'clonal anergy' both towards Th1 and Th2 cells (2). Both IL-10 and TGF- β 1 down-regulate cellular immunity and allergic inflammation (3). Decreased secretion of these cytokines could contribute to the development of a milieu where allergic inflammation and asthma can develop (4). The number of TGF- β 1 producing T cells is reduced in children with food allergies (5) and TGF- β 1 concentration in maternal colostrum is inversely related with atopy in their infants (6). IL-10 has shown potent anti-inflammatory properties in an *in vitro* model on

human cord blood mast cells (7) and TGF- β 1 production is reduced in cord blood mononuclear cells of newborns of atopic mothers (8,9). Moreover, cord blood is a major source of T_{reg} precursor cells capable of potent suppressor function (10). T_{reg} cytokine pattern at time of delivery are influenced both by maternal and paternal genetic background and by maternal immune status through the feto-placental unit, and may be crucial for the development of neonatal immune system (11).

Therefore, the primary aim of our study was to measure in a large series of Italian newborns IL-10 and TGF- β 1 cord blood levels, which may become possible reference values for further investigations. A secondary aim was to assess the relationship between IL-10 and TGF- β 1 and parental allergy and perinatal characteristics.

METHODS

Newborn recruitment

From November 2005 to May 2007, cord blood samples were obtained in our paediatric unit from 222 consecutive full-term and appropriate for gestational age newborns whose parents had consented to the study. Data on gestational age, birth weight, length, cranial circumference, mode of delivery, amniotic fluid appearance, time to membranes rupture and Apgar index were recorded for each case. Full-term birth was defined by gestational age between 37 and 42 weeks. Appropriate for gestational age was defined as birth weight between 10th and 90th percentile for gestational age and sex. In addition, the following parameters

Abbreviations

95%CI, 95% confidence intervals; IL-10, Interleukin-10; IQR, interquartile range; TGF- β 1, transforming growth factor- β 1; Th, T helper; T_{reg} , T regulatory.

were recorded: mothers' nationality, age, manual or non-manual occupation, number of previous pregnancies, premature deliveries, miscarriages and living children. Father's age, manual or non-manual occupation and nationality were also recorded. All newborns of parents born outside Italy were excluded at recruitment.

Parents were screened for allergies by administering a questionnaire about any allergic symptom, type of allergen and result of allergic tests and were considered allergic only where results of allergic laboratory tests were positive. Newborns whose parents received immunotherapy or immunomodulatory treatments were excluded. Out of the 222 newborns initially selected only 212 completely fulfilled the inclusion criteria. In four newborns cord blood samples were insufficient for cytokine determinations and in six newborns parental history of allergic reaction was not proven by laboratory tests. The study has been approved by the Regional Ethical Committee and informed consent was signed by the parents.

Sample collection and measurements

Cord blood was collected immediately after delivery and centrifuged at 3500 rpm for 15 min. Sera were stored at -20°C until tested for IL-10 and TGF- β 1 levels. Determinations were performed after a median of 6 months (range 2 weeks–16 months) of storage at -20°C .

IL-10 serum levels were determined using a high sensitivity quantitative ELISA test (Quantikine[®] HS, R&D Systems Inc, Minneapolis, MN, USA). Sera were processed according to manufacturer instructions. Optical density was measured at dual wavelength of 490/650 nm in an automated microplate reader (SpectraCount, Packard, Meriden, USA). The minimum detectable level of IL-10 being 0.5 pg/mL.

For the determination of TGF- β 1 serum levels, a procedure of activation was used to detach TGF- β 1 antigen from circulating protein complexes in order to enable its recognition from specific antibodies. This was obtained by adding to serum samples a solution of 2.5N acetic acid/10M urea and by neutralizing them with a 2.7N NaOH/1M HEPES solution after a 10-min incubation. A 15-fold dilution of activated samples was used in the ELISA kit specific for TGF- β 1 (Quantikine[®] HS, R&D Systems Inc, Minneapolis, MN, USA). Optical density was measured at dual wavelength of 450/570 nm in an automated microplate reader. The minimum detectable level of TGF- β 1 being 7 pg/mL.

Statistical analysis

Anthropometric data were expressed as mean $\pm$ SD and compared by Student *t*-test. Since IL-10 and TGF- β 1 variables were not normally distributed (i.e. all values were skewed towards low values) all analyses were performed by non-parametric tests and their values expressed as median and interquartile range (IQR). IL-10 and TGF- β 1 levels were compared by ANOVA Kruskal Wallis test and by the Mann–Whitney *U*-test with post hoc Bonferroni adjustment. Correlations between IL-10 and TGF- β 1 and neonatal parameters were evaluated by Spearman rank correlation. The chi-square test or Fisher test – when appropriate – were

used to evaluate prevalence differences. General multiple regression analysis was used to test the independent contribution of paternal allergy, cesarean section, gestational age and male gender to TGF- β 1 levels. Statistical analysis was performed with STATISTICA software v.6.1 (Statsoft Inc., Tulsa, OK, USA).

RESULTS

Out of the 212 cord blood samples, 136 were from newborns of non-allergic parents and 76 (35.8%) from newborns of one or both parents with a laboratory proven allergy. The two groups were similar for sex, gestational age, weight, length, cranial circumference, mode of delivery, amniotic fluid appearance, time to membranes rupture and Apgar index (Table 1). Out of 424 parents, a history of allergy was present in 84 (19.8%, 95%CI: 16–23.6%) 54 mothers and 30 fathers (8 newborns had both parents with allergy).

IL-10 levels were similar in cord blood of newborns of non-allergic and allergic parents (Table 2). Median IL-10 levels tended to be lower in newborns of allergic fathers than in newborns of non-allergic parents (0.67 vs. 1.06 pg/mL, $p = 0.07$) and were even lower in eight newborns where both parents were allergic (0.49, IQR 0–4.2 pg/mL) but the decrease was not significant for the small number of newborns in this group. IL-10 was undetectable in 55 (25.9%) cord blood samples and no difference was found in prevalence of undetectable IL-10 according to time of serum sample

Table 1 Neonatal characteristics in 212 newborns of non-allergic parents and of one or both parents with laboratory proven allergy

Neonatal characteristics	Newborns of non-allergic parents N = 136	Newborns of one or both allergic parents N = 76
Sex, N (%)		
Males	69 (51)	38 (50)
Females	67 (49)	38 (50)
Gestational age (weeks)		
mean $\pm$ SD	39.3 $\pm$ 1.2	39.2 $\pm$ 1.3
Weight (g) mean $\pm$ SD	3276 $\pm$ 402	3323 $\pm$ 496
Length (cm) mean $\pm$ SD	50.2 $\pm$ 1.8	50.5 $\pm$ 2.1
Cranial circumference (cm)		
mean $\pm$ SD	34.3 $\pm$ 1.2	34.3 $\pm$ 1.2
Mode of delivery, N (%)		
Eutocic	56 (41)	38 (50)
Cesarean	67 (49)	34 (45)
Induced	8 (6)	3 (4)
Dystocic	5 (4)	1 (1)
Complications at birth, N (%)		
None	123 (91)	63 (83)
LT	6 (4)	7 (9)
PROM	4 (3)	4 (5)
Low Apgar	3 (2)	2 (3)

LT = liquidum tintus.

PROM = premature membranes rupture > 18 h.

Low Apgar = Apgar score < 8 at 1 min.

Table 2 Median (IQR) IL-10 and TGF- β 1 levels according to family history of allergy (maternal allergy in 54 newborns, paternal allergy in 30 newborns and both maternal and paternal allergy in 8 newborns), newborn gender and mode of delivery

	IL-10 pg/mL	TGF- β 1 ng/mL
Newborns of non-allergic parents (N = 136)	1.06 (0–1.81)	45.3 (37.8–52.1)*
Newborns of allergic parents (N = 76)	0.97 (0–1.92)	43.8 (33.0–50.9)
of allergic mothers (N = 54)	1.29 (0.5–2.36)	44.6 (33.4–53.2)
of allergic fathers (N = 30)	0.67 (0–1.36)	40.9 (29.5–46.2)*
Newborns of non-allergic parents (N = 136)	1.06 (0–1.81)	45.3 (37.8–52.1)
males (N = 69)	1.11 (0.5–1.82)	46.5 (39.6–53.4)†
females (N = 67)	1.0 (0–1.81)	42.9 (37.1–51.4)
Newborns of allergic parents (N = 76)	0.97 (0–1.92)	43.8 (33.0–50.9)
males (N = 38)	0.94 (0–1.98)	41.5 (31.6–48.6)†
females (N = 38)	1.24 (0.5–1.85)	45.7 (34.5–51.2)
Cesarean delivered newborns (N = 105)	1.06 (0–1.63)	39.8 (35–48)‡
Vaginal delivered newborns (N = 107)	1.02 (0.5–2.29)	48.8 (41.7–55.1)‡

* $p = 0.008$, † $p = 0.02$, ‡ $p < 0.0001$, by Mann–Whitney U -test.

storage or according to maternal or paternal allergic status. IL-10 values were also similar according to mode of delivery and gestational age.

TGF- β 1 levels were similar in cord blood of newborns of non-allergic and allergic parents (Table 2). However, median TGF- β 1 levels were significantly lower in newborns of allergic fathers – whether or not mothers were also allergic – than in newborns of non-allergic parents (40.9 vs. 45.3 ng/mL, $p = 0.008$) and were even lower in 8 newborns from both allergic parents, although not significantly (40.2, IQR 29.7–45.8 ng/mL). Moreover, TGF- β 1 was significantly lower in sons of allergic parents than in sons of non-allergic ones (41.5 vs. 46.5 ng/mL, $p = 0.02$) while in daughters no difference was found (Table 2). TGF- β 1 was detectable in all cord blood samples and its levels were positively correlated with gestational age in newborns of non-allergic parents (Spearman $R = 0.32$, $p = 0.0002$) but this correlation was lost in newborns of allergic parents. TGF- β 1 levels significantly changed according to the mode of delivery ($p < 0.0001$) being lowest in cesarean delivered newborns (39.8, IQR 35–48 ng/mL) and highest in dystocic delivered newborns (50.1, IQR 48–57 ng/mL). Median TGF- β 1 was significantly decreased in cesarean delivered newborns as compared to vaginal delivered ones (i.e. eutocic, induced and dystocic delivery, Table 2). Multiple general regression analysis in all newborns showed that presence of paternal allergy (β coefficient = -0.19 , 95%CI = -0.32 to -0.07 , $p = 0.003$) to be born by cesarean section ($\beta = -0.21$, 95%CI = -0.39 to -0.02 , $p = 0.03$) and younger gestational age ($\beta = 0.14$, 95%CI = 0.01 to 0.28 , $p = 0.04$) all independently contributed to a decrease of TGF- β 1 levels, while male gender did not (multiple $R = 0.38$, $p < 0.0001$).

IL-10 and TGF- β 1 levels were similar in males and females both in newborns of allergic and non-allergic parents. No correlation was found between IL-10 and TGF- β 1 and any of perinatal characteristics (birth weight, length and cranial circumference, amniotic fluid appearance, time to membranes rupture, Apgar index) parental occupation, type of allergen causing parental allergy and mothers' obstetric his-

tory (number of previous pregnancies, premature deliveries, miscarriages and number of living children). No correlation between IL-10 and TGF- β 1 levels was found.

DISCUSSION

In the present study we evaluated IL-10 and TGF- β 1 cord blood levels in full-term newborns and found that TGF- β 1 levels were significantly lower in newborns of allergic fathers, in cesarean delivered newborns and in those with a younger gestational age.

The immunological events that drive Th memory development are initiated early in life and possibly even in uterus (12). Cytokine production within the feto-placental unit, as measurable in cord blood, may influence susceptibility to subsequent atopy and asthma development (12). IL-10 and TGF- β 1 secretions are the main effectors of the suppressor mechanisms of T_{reg} cells (3). In our series IL-10 and TGF- β 1 were decreased in newborns of allergic fathers, however only the decrease of TGF- β 1 was significant. IL-10 and TGF- β 1 are reported to skew the antibody production from IgE towards the non-inflammatory isotypes IgG4 and IgA, respectively (3). Therefore, newborns of allergic fathers, having lower levels of cord blood regulatory cytokines, could have an immunoglobulin pattern skewed towards IgE production making them more liable to atopic diseases later in life.

Little is known about the relationship between cord blood cytokine profile and risk to develop allergy later in life in newborns with family history of atopy. We found TGF- β 1 levels significantly lower in newborns of allergic fathers, but not in newborns of allergic mothers. And this finding seems to be at variance with other reports: TGF- β secreting mononuclear cells were significantly lower in cord blood of newborns of atopic mothers (8) where, however, paternal history of atopy was not taken into account. Up to now most studies investigating feto-placental unit in relation to development of pre-natal and early post-natal immune system (12) disregarded paternal influence on the process.

Moreover, influence of parental allergy was usually analysed only in relation to cord blood IgE and not to cord blood cytokines. Cord blood IgE levels were shown to be influenced by maternal – but not paternal – history of allergy and by maternal IgE values (13,14) but later a multivariate analysis revealed that only maternal IgE values were significantly associated with cord blood IgE (15). Only recently paternal IgE levels were reported to be related to their boys', but not girls', IgE levels (16) and paternal asthma to wheezing in preschool children (17). The importance of male gender was highlighted not only by the paternal allergy influence, but also by the finding of significantly reduced TGF- β 1 levels in sons of allergic parents but not in daughters, thus suggesting a sex-related heritability of atopic predisposition. Linkage studies have attempted to explain genetically sex-related effects and both maternal and paternal imprinting has been suggested for allergic sensitization. Genomic imprinting refers to differential expression of genes depending on maternal or paternal origin (18). Therefore, in our series imprinting is a plausible mechanism for paternal influence on atopic predisposition. Further investigations are required to better assess the extent of genetic background influence on newborn immunological status, as shown by the role of paternal allergy and male gender or the extent of immunity factors transferred by mothers during pregnancy, where cord blood cytokine patterns may play a role.

Cord blood IL-10 levels in full-term newborns were quite near – hardly twofold – the lower sensitivity limit of the commercially available ELISA kits, therefore high sensitivity kits had to be used. The ELISA kit we used has such a characteristic, and indeed IL-10 was detectable in 74% of our samples, quite higher than previously reported data obtained by not-high sensitivity kits where IL-10 was detectable only in 15% of samples (12). So we were able to confirm – in a large series – data on IL-10 levels reported in 31 Spanish (19), 26 English (20) and 398 Australian newborns (12).

TGF- β 1 levels in our samples were similar to those reported in 20 appropriate for gestational age newborns (21) and they seem a bit higher than those reported in a group of 26 full-term newborns (20). In our series cord blood TGF- β 1 levels were significantly lower after cesarean section, where labour is not completed and stimulation by oxytocin, hydrocortisone and progesterone, involved in the parturitional process, is missing. Indeed, it has been shown that these hormones increase TGF- β 1 mRNA expression and release *in vitro* (22). The reduced cord blood TGF- β 1 after cesarean section may be associated to the increased risk to develop atopy. Indeed in children born by cesarean section an increased prevalence of allergic rhinoconjunctivitis, asthma, hay fever and hospitalization for asthma has been reported in most studies (23–26), although one did not confirm this correlation (27). Therefore, further studies are needed to better clarify it and TGF- β 1 levels in cord blood may be used as an intermediate marker for such studies.

In conclusion, we found decreased TGF- β 1 levels in cord blood of newborns of allergic fathers, suggesting that paternal allergy might influence neonatal cytokine produc-

tion more than expected. And TGF- β 1 was also decreased after cesarean section, suggesting that higher risk to develop atopy in cesarean delivered children could be mediated by a decrease in TGF- β 1 at birth. Long-term clinical monitoring of infants of this series will be performed to evaluate the potential prognostic significance of these changes. The allergic status of our infants cannot be assessed yet, because they are still too young.

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