

EDITORIAL

Cord blood immune status: predicting health or allergy?

DOI:10.1111/j.1398-9995.2012.02800.x

It has become more evident that immune status at birth may be an intrinsic feature of a newborn child that predisposes to the development of allergic disease (1). By now, mainly three predictive factors for later allergy development are evident: cord blood cytokine production, regulatory T cell number/function, and toll-like receptor (TLR) expression/function.

A characteristic feature of atopy is a T helper (Th) 2 skewed immune response and its related cytokines IL-4, IL-5, and IL-13, which are involved in the induction and maintenance of the IgE synthesis. The importance of Th1 cytokines like interferon- γ (IFN- γ) in atopy development is to antagonize the effect of Th2 cytokines. Although published data are not completely consistent in this regard, the most common abnormality detected early in life among children, who go on to have an allergic disease, includes diminished IFN- γ levels (2–4) and altered Th2 responses that are followed by skewing of immune responses toward a Th2 phenotype (5–7). Hagedorens and colleagues (8) found that a significantly lower percentage of IFN- γ -producing stimulated cord blood CD4⁺ T cells was present in children, who developed an atopic dermatitis during the first year of life. Our own data from the LISA study corroborate these findings: children with reduced frequencies of IFN- γ -producing CD4⁺ T cells in the cord blood had a higher risk of developing atopic dermatitis, whereas a high percentage of IL-4-producing T cells in cord blood was associated with an increased risk for atopic dermatitis during the first two years of life (7). Besides Th1/Th2 cytokines, recent publications show the involvement of high levels of cord blood Th2-associated chemokines CCL17 and CCL22 in the prediction of atopic dermatitis, allergic sensitization, and asthma (9–11).

Although different studies have provided evidence of a predictive cord blood cytokine value corresponding to the development of allergic diseases, other birth cohort studies failed to show such associations (12–15). The apparent discrepancy in the reported results might be explained by differences in the subject population, the length of follow-up, and more importantly, the mode of cytokine measurement. Mainly cord blood cytokines measured at the cellular level revealed a correlation to atopy, indicating that the source of cytokines is important in the clarification of allergy development (16). Certainly, by measuring protein concentrations in the supernatant of cord blood cell cultures, cytokines from additional sources than T helper cells are also gathered that may hide/cover possible associations (17).

Recently, there has been emerging evidence that allergic disease or sensitization is also associated to regulatory T cells. These cells downregulate Th2 cells by suppressing their activity and proliferation. Consequently, attenuated neonatal

regulatory T cell (Treg) function or low numbers (18–20) were shown to be associated with later allergy development. Smith et al. (18) demonstrated that children with egg allergy at the age of one had reduced neonatal regulatory T cell function. In our LINA study, reduced maternal Treg frequencies correlated with increased total IgE concentrations in cord blood (20), and, furthermore, it was evident that children developing atopic dermatitis during the first year of life had reduced Treg numbers at birth (19). Cord blood from children born to atopic mothers was characterized by lower numbers and impaired Treg cell function (21).

The defective neonatal T, and in particular IFN- γ response, seems to be a result of the dysfunction of antigen-presenting cells and an altered innate immune responses (22). For instance, cord blood T cells are able to produce markedly increased amounts of IFN- γ when co-cultured with adult macrophages. Vice versa neonatal macrophages can inhibit the IFN- γ response of adult T cells (23). Moreover, when cultured with interleukin 12 (IL-12), a cytokine secreted by macrophages and other antigen-presenting cells that stimulates T cells, neonatal T cells are capable of producing large amounts of IFN- γ (24). Interleukin-12 and other macrophage or dendritic cell-derived cytokines are activated *via* toll-like receptors (TLRs), known as pattern-recognition receptors critically involved in innate immune responses. Thus, deviations in innate immune responses can also contribute to allergic predisposition (25,26). A reduced expression of TLR5 and TLR9 was found in the cord blood of children who later developed an atopic dermatitis (27). Furthermore, children with allergies have shown exaggerated innate cytokine production at birth following activation by TLR ligands (28). However, whether increased or rather decreased expression/function of TLRs may be the basis of allergy development is still a matter of debate.

Besides the genetic background (29–31), it is undoubtedly that environmental exposure also affects the prenatal development of immune cells, hence the allergic predisposition (32–34). Microbial products that stimulate innate immune responses via TLRs can modulate the allergic propensity toward protective effects (28,35,36). On the other hand, maternal chemical exposure during pregnancy can contribute to reduced Th1 responses, therefore increasing the risk of developing allergies (37,38). However, the underlying mechanisms of these additional environmental impacts are far away from being understood.

Collectively, these data suggest a complex interplay of Th1/Th2/Treg as well as factors of the innate immune response in relationship to immune maturation and the development of allergic diseases. An impaired Th1 and Treg func-

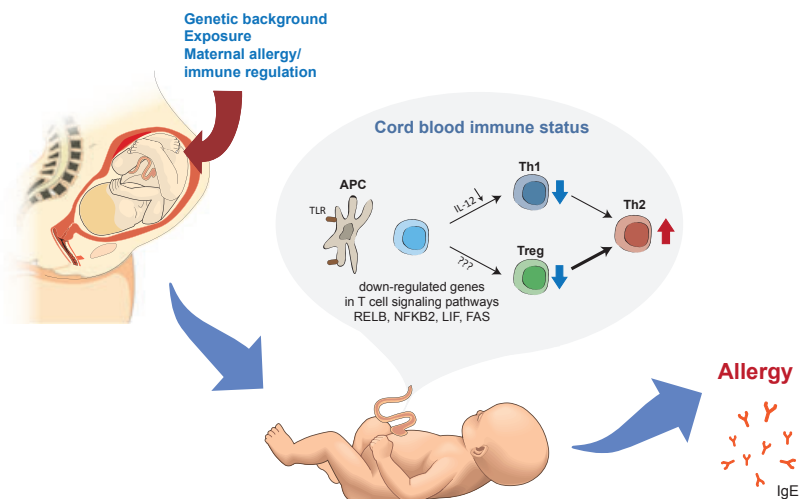


Figure 1 Cord blood immune status in children prone for allergy – known predictive parameters for allergy development. Already at the fetal stage, the developing immune system is influenced by maternal and environmental factors. At birth, down-regulated genes in T cell signaling pathways (RELB, NFkB2, LIF, FAS) in addition to impaired innate immune signals may contribute to altered T helper

cell differentiation. Diminished Th1/Treg activity or numbers contribute to enhanced generation of Th2 cells, which subsequently increases the risk to develop allergic responses. APC, antigen-presenting cell; Th, T helper cell; Treg, regulatory T cell; TLR, toll-like receptors.

tion at birth may contribute to a reduced capacity to suppress Th2 responses in the early postnatal period, increasing the likelihood for the development of an allergic reactivity (Fig. 1).

Recent studies have attributed hypermethylation of CpG sites in the IFN- γ promoter in neonates as causative event responsible for the strong suppression of IFN- γ gene transcription (39–41). However, treatment of cord blood T cells with a demethylating agent did not lead to a significant increase in IFN- γ response (42). In addition, atopy development by age 2 was not found to be associated with variations in methylation patterns in cord blood T cells (40). These data suggest that abnormalities in neonatal T cell function are regulated at the transcriptional level by so far unknown mechanisms.

A study by Martino et al. (43) recently published in *Allergy* provided a deeper insight into the regulatory cascade in neonatal T cells. To characterize the signaling cascades induced downstream of the T cell receptor, the group adopted a genome-wide approach measuring the gene expression profile of stimulated cord blood CD4⁺ T cells. Thereby, in a case-control study, data from children with early allergic outcomes (IgE-mediated food allergy in the first year of life) were compared with the ones from children who remained nonallergic. Furthermore, between these two groups, the allergen-specific responses to ovalbumin were compared. The production of IFN- γ , IL-13, IL-5, and IL-10 was significantly lower at birth in children developing an atopy at age one compared with the nonallergic group, reflecting the immaturity of T cell responsiveness in that group. However, at age one, Th2 as well as Th1 cytokine

responses were increased in the allergic group. At birth, also at molecular level differences between the two children groups were observed. The microarray analyses revealed that anti-CD3 antibody-induced gene responses of isolated CD4⁺ T cells were markedly reduced in the allergic children. Particularly, genes belonging to the NF- κ B family were involved, including RELB, NFkB2, LIF, and FAS. Because of the fact that the proliferative response of T cells depends on signals through the NF- κ B/Rel complex, the observed impaired lymphoproliferation in allergic children may be explained by the lower expression of these genes. In line with other studies, Martino et al. (43) demonstrated that the differences in gene expression detected at birth are only transient (28,44). By 12 month of age, differentially expressed genes in unstimulated CD4⁺ T cells of allergic infants included TCR pathway, the MAL T cell differentiation pathway and as expected the master regulator of Th2 differentiation GATA3. These findings clearly confirm the data of other studies reporting a Th2 bias in allergic children (45–47).

In spite of the recent progress, open questions remain. For instance, it is still unclear how the different regulated gene sets at birth and age one are linked to the identified signaling pathways, which result in impaired T cell function. However, rather imbalanced regulatory networks involved in T cell activation and differentiation than single factors seem to be responsible for priming toward an allergic reactivity. Therefore, to predict allergic outcomes from cord blood-derived factors, a pattern of different factors, including molecular and cellular markers instead of single factors, might be relevant. Furthermore, a deeper understanding of the

differentially regulatory events in neonates at risk may support future allergy prevention strategies.

Conflict of interest

Both authors have declared that they have no conflict of interest.

References

1. Renz H, Conrad M, Brand S, Teich R, Garn H, Pfefferle PI. Allergic diseases, gene–environment interactions. *Allergy* 2011;**66**(Suppl. 95):10–12.
2. Warner JA, Miles EA, Jones AC, Quint DJ, Colwell BM, Warner JO. Is deficiency of interferon gamma production by allergen triggered cord blood cells a predictor of atopic eczema? *Clin Exp Allergy* 1994;**24**: 423–430.
3. Kondo N, Kobayashi Y, Shinoda S, Takenaka R, Teramoto T, Kaneko H et al. Reduced interferon gamma production by antigen-stimulated cord blood mononuclear cells is a risk factor of allergic disorders – 6-year follow-up study. *Clin Exp Allergy* 1998;**28**:1340–1344.
4. Tang ML, Kemp AS, Thorburn J, Hill DJ. Reduced interferon-gamma secretion in neonates and subsequent atopy. *Lancet* 1994;**344**:983–985.
5. Wright RJ, Visness CM, Calatroni A, Grayson MH, Gold DR, Sandel MT et al. Prenatal maternal stress and cord blood innate and adaptive cytokine responses in an inner-city cohort. *Am J Respir Crit Care Med* 2010;**182**:25–33.
6. Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Loh R et al. Reciprocal age-related patterns of allergen-specific T-cell immunity in normal vs. atopic infants. *Clin Exp Allergy* 1998;**28**(Suppl. 5):39–44.
7. Herberth G, Heinrich J, Roder S, Figl A, Weiss M, Diez U et al. Reduced IFN-gamma- and enhanced IL-4-producing CD4 + cord blood T cells are associated with a higher risk for atopic dermatitis during the first 2 yr of life. *Pediatr Allergy Immunol* 2010;**21**:5–13.
8. Hagendorens MM, Ebo DG, Bridts CH, Van de WL, De Clerck LS, Stevens WJ. Prenatal exposure to house dust mite allergen (Der p 1), cord blood T cell phenotype and cytokine production and atopic dermatitis during the first year of life. *Pediatr Allergy Immunol* 2004;**15**:308–315.
9. Abenius MS, Ernerudh J, Berg G, Matthiesen L, Nilsson LJ, Jenmalm MC. High cord blood levels of the T-helper 2-associated chemokines CCL17 and CCL22 precede allergy development during the first 6 years of life. *Pediatr Res* 2011;**70**:495–500.
10. Miyahara H, Okazaki N, Nagakura T, Korematsu S, Izumi T. Elevated umbilical cord serum TARC/CCL17 levels predict the development of atopic dermatitis in infancy. *Clin Exp Allergy* 2011;**41**:186–191.
11. Yang KD, Chang JC, Chuang H, Liang HM, Kuo HC, Lee YS et al. Gene–gene and gene–environment interactions on IgE production in prenatal stage. *Allergy* 2010;**65**:731–739.
12. Rothers J, Halonen M, Stern DA, Lohman IC, Mobley S, Spangenberg A et al. Adaptive cytokine production in early life differentially predicts total IgE levels and asthma through age 5 years. *J Allergy Clin Immunol* 2011;**128**:397–402.
13. Neville WA, Tisler C, Bhattacharya A, Anklam K, Gilbertson-White S, Hamilton R et al. Developmental cytokine response profiles and the clinical and immunologic expression of atopy during the first year of life. *J Allergy Clin Immunol* 2003;**112**:740–746.
14. Halonen M, Martinez FD. A deficient capacity to produce interferon-gamma: is it a risk for asthma and allergies? *Clin Exp Allergy* 1997;**27**:1234–1236.
15. Rindsjo E, Joerink M, Johansson C, Bremme K, Malmstrom V, Scheynius A. Maternal allergic disease does not affect the phenotype of T and B cells or the immune response to allergens in neonates. *Allergy* 2010;**65**:822–830.
16. Cosmi L, Liotta F, Maggi E, Romagnani S, Annunziato F. Th17 cells: new players in asthma pathogenesis. *Allergy* 2011;**66**:989–998.
17. Allakhverdi Z, Delespesse G. Hematopoietic progenitor cells are innate Th2 cytokine-producing cells. *Allergy* 2012;**67**:4–9.
18. Smith M, Tourigny MR, Noakes P, Thornton CA, Tulic MK, Prescott SL. Children with egg allergy have evidence of reduced neonatal CD4(+)CD25(+)CD127(lo/-) regulatory T cell function. *J Allergy Clin Immunol* 2008;**121**:1460–1466.
19. Hinz D, Bauer M, Roder S, Olek S, Huehn J, Sack U et al. Cord blood Tregs with stable FOXP3 expression are influenced by prenatal environment and associated with atopic dermatitis at the age of one year. *Allergy* 2012;**67**:380–389.
20. Hinz D, Simon JC, Maier-Simon C, Milkova L, Roder S, Sack U et al. Reduced maternal regulatory T cell numbers and increased T helper type 2 cytokine production are associated with elevated levels of immunoglobulin E in cord blood. *Clin Exp Allergy* 2010;**40**:419–426.
21. Schaub B, Liu J, Hoppler S, Haug S, Sattler C, Lluís A et al. Impairment of T-regulatory cells in cord blood of atopic mothers. *J Allergy Clin Immunol* 2008;**121**:1491–1499.
22. Mrabet-Dahbi S, Maurer M. Does allergy impair innate immunity? Leads and lessons from atopic dermatitis. *Allergy* 2010;**65**:1351–1356.
23. Taylor S, Bryson YJ. Impaired production of gamma-interferon by newborn cells in vitro is due to a functionally immature macrophage. *J Immunol* 1985;**134**:1493–1497.
24. Wu CY, Demeure C, Kiniwa M, Gately M, Delespesse G. IL-12 induces the production of IFN-gamma by neonatal human CD4 T cells. *J Immunol* 1993;**151**:1938–1949.
25. Allam JP, Duan Y, Winter J, Stojanovski G, Fronhoffs F, Wenghoefer M et al. Tolerogenic T cells, Th1/Th17 cytokines and TLR2/TLR4 expressing dendritic cells predominate the microenvironment within distinct oral mucosal sites. *Allergy* 2011;**66**:532–539.
26. Novak N, Allam JP. Mucosal dendritic cells in allergy and immunotherapy. *Allergy* 2011;**66**(Suppl. 95):22–24.
27. Roduit C, Wohlgensinger J, Frei R, Bitter S, Bieli C, Loeliger S et al. Prenatal animal contact and gene expression of innate immunity receptors at birth are associated with atopic dermatitis. *J Allergy Clin Immunol* 2011;**127**:179–185.
28. Tulic MK, Hodder M, Forsberg A, McCarthy S, Richman T, D'Vaz N et al. Differences in innate immune function between allergic and nonallergic children: new insights into immune ontogeny. *J Allergy Clin Immunol* 2011;**127**:470–478.
29. Liu X, Wang G, Hong X, Wang D, Tsai HJ, Zhang S et al. Gene-vitamin D interactions on food sensitization: a prospective birth cohort study. *Allergy* 2011;**66**:1442–1448.
30. Reijmerink NE, Bottema RW, Kerkhof M, Gerritsen J, Stelma FF, Thijs C et al. TLR-related pathway analysis: novel gene-gene interactions in the development of asthma and atopy. *Allergy* 2010;**65**:199–207.
31. Tesse R, Pandey RC, Kabesch M. Genetic variations in toll-like receptor pathway genes

- influence asthma and atopy. *Allergy* 2011;**66**:307–316.
32. Bozzetto S, Carraro S, Giordano G, Boner A, Baraldi E. Asthma, allergy and respiratory infections: the vitamin D hypothesis. *Allergy* 2012;**67**:10–17.
33. Stenius F, Swartz J, Lilja G, Borres M, Bottai M, Pershagen G et al. Lifestyle factors and sensitization in children – the ALADDIN birth cohort. *Allergy* 2011;**66**:1330–1338.
34. Prescott SL. The influence of early environmental exposures on immune development and subsequent risk of allergic disease. *Allergy* 2011;**66**(Suppl. 95):4–6.
35. Schaub B, Liu J, Hoppler S, Schleich I, Huehn J, Olek S et al. Maternal farm exposure modulates neonatal immune mechanisms through regulatory T cells. *J Allergy Clin Immunol* 2009;**123**:774–782.
36. Joerink M, Oortveld MA, Stenius F, Rindsjo E, Alm J, Scheynius A. Lifestyle and parental allergen sensitization are reflected in the intrauterine environment at gene expression level. *Allergy* 2010;**65**:1282–1289.
37. Lehmann I, Thoeke A, Weiss M, Schlink U, Schulz R, Diez U et al. T cell reactivity in neonates from an East and a West German city—results of the LISA study. *Allergy* 2002;**57**:129–136.
38. Lehmann I, Thoeke A, Rehwagen M, Rolle-Kampczyk U, Schlink U, Schulz R et al. The influence of maternal exposure to volatile organic compounds on the cytokine secretion profile of neonatal T cells. *Environ Toxicol* 2002;**17**:203–210.
39. White GP, Watt PM, Holt BJ, Holt PG. Differential patterns of methylation of the IFN-gamma promoter at CpG and non-CpG sites underlie differences in IFN-gamma gene expression between human neonatal and adult CD. *J Immunol* 2002;**168**:2820–2827.
40. White GP, Hollams EM, Yerkovich ST, Bosco A, Holt BJ, Bassami MR et al. CpG methylation patterns in the IFNgamma promoter in naive T cells: variations during Th1 and Th2 differentiation and between atopics and non-atopics. *Pediatr Allergy Immunol* 2006;**17**:557–564.
41. Martino DJ, Prescott SL. Silent mysteries: epigenetic paradigms could hold the key to conquering the epidemic of allergy and immune disease. *Allergy* 2010;**65**:7–15.
42. Gupta AK, Rusterholz C, Holzgreve W, Hahn S. Constant IFNgamma mRNA to protein ratios in cord and adult blood T cells suggests regulation of IFNgamma expression in cord blood T cells occurs at the transcriptional level. *Clin Exp Immunol* 2005;**140**:282–288.
43. Martino DJ, Bosco A, McKenna KL, Hollams E, Mok D, Holt PG et al. T-cell activation genes differentially expressed at birth in CD4(+) T-cells from children who develop IgE food allergy. *Allergy* 2012;**67**:191–200.
44. Smillie FI, Elderfield AJ, Patel F, Cain G, Tavenier G, Brutsche M et al. Lymphoproliferative responses in cord blood and at one year: no evidence for the effect of in utero exposure to dust mite allergens. *Clin Exp Allergy* 2001;**31**:1194–1204.
45. Tang ML, Kemp AS. Spontaneous expression of IL-4 mRNA in lymphocytes from children with atopic dermatitis. *Clin Exp Immunol* 1994;**97**:491–498.
46. Ng TW, Holt PG, Prescott SL. Cellular immune responses to ovalbumin and house dust mite in egg-allergic children. *Allergy* 2002;**57**:207–214.
47. Renz H, von Mutius E, Illi S, Wolkers F, Hirsch T, Weiland SK. T(H)1/T(H)2 immune response profiles differ between atopic children in eastern and western Germany. *J Allergy Clin Immunol* 2002;**109**:338–342.