

CORRESPONDENCE

Neonatal IL-10 production and risk of allergy development

D. M. A. Bullens¹, A. Kasran², E. Dilissen² and J. L. Ceuppens²¹Pediatric Immunology and ²Clinical Immunology, Department of Microbiology and Immunology, KU Leuven, Leuven, Belgium

We read with interest the recent report of Belderbos and coworkers in this journal showing that low neonatal TLR-4-mediated IL-10 production is associated with subsequent development of atopic dermatitis [1]. The authors stimulated whole blood of 1-month-old neonates with ultrapure lipopolysaccharide (LPS) and Interferon (IFN)- γ , and measured IL-10 in the culture supernatants. Neonates who developed physician-diagnosed atopic dermatitis at age 1 year had 1.8-fold lower TLR-4-mediated IL-10 production at the age of 1 month [1]. In a birth cohort 'Follow-up of Newborn's Immune development in relation to Allergic outcome' (FONIA) study, we obtained data on the association between allergen-induced IL-10 production by cord blood cells and prospective risk of allergy development. The diagnosis of 'clinical allergy' was based on the presence of symptoms (skin, gastrointestinal or respiratory) in association with relevant IgE sensitization [2]. Cord blood

mononuclear cells (CBMC) ($n = 67$) isolated immediately after uncomplicated vaginal delivery were stimulated by either ovalbumine (OVA) or by recombinant(r) Der p 2 (a kind gift from JM-Saint Rémy, KU Leuven, Belgium). r IL-4 was added because we have previously found that rIL-4 increases *in vitro* allergen-induced IL-10 production by T cells from non-allergic subjects, but not by those from allergic subjects [3].

As shown in Fig. 1, IL-10 was significantly lower in OVA/IL-4 stimulated CBMC cultures from children who had developed clinical allergy at age 6 ($n = 19$) compared with the others ($n = 45$). IL-10 above 0.5 pg/mL was present in OVA/IL-4 stimulated cultures from 19 of 45 non-allergics and predicted a non-allergic outcome at age 6 (Relative Risk (RR) (in fact 'relative benefit') 1.49 (95% CI 1.1–2.0), Chi-square df 6.1; $P = 0.007$). Nine children were egg-allergic at 12 months and in only one of these nine children, OVA/IL-4-induced

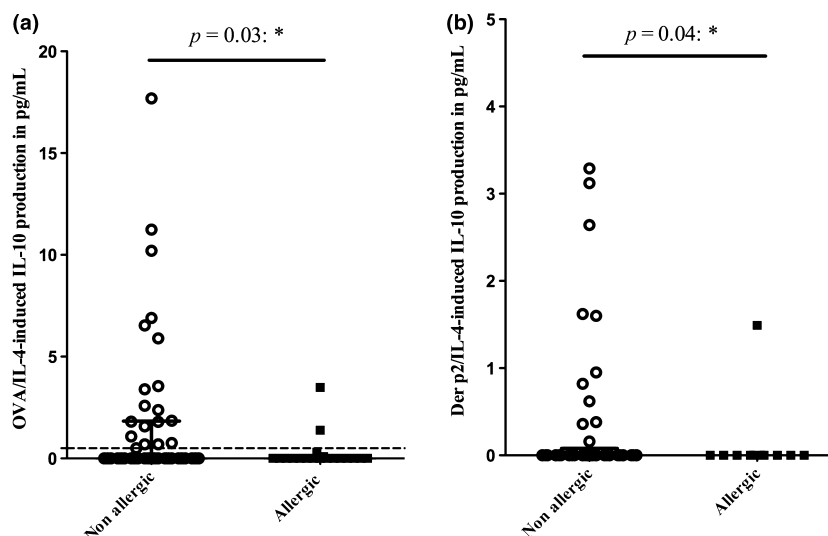


Fig. 1. Allergen + rIL-4-induced T-cell cytokine production. Cord blood mononuclear cells CBMC (1×10^6 /mL) ($n = 64$) were stimulated for 6 days with OVA ($1 \mu\text{g/mL}$; Sigma, St Louis, MO, USA) (a) or r Der p 2 ($1 \mu\text{g/mL}$) (b) in the presence of rIL-4 (5 ng/mL ; Shering-Plough, Kenilworth, NJ, USA). Supernatants were collected after 6 days, and the concentrations of IL-10 in the supernatants were detected by ELISA (Pharmingen). IL-10 in the supernatant of cells cultured without allergen was subtracted from the value in cultures with allergen. Cut-off value for RR calculation is indicated by a dotted line (a). Children were subdivided into two groups, depending on whether they developed clinical allergy before or at age 6 or not. * $P < 0.05$ by Mann-Whitney Test.

Correspondence:

Dominique MA Bullens, Pediatric Immunology, Department of Microbiology and Immunology, KU Leuven, Leuven, Belgium.

IL-10 at birth was above 0.5 pg/mL. Detectable r Der p 2/IL-4-induced cord blood IL-10 production similarly predicted the non-atopic outcome at age 6 (RR 1.4 (95% CI 1.1–1.8); Chi-square df 3.2; $P = 0.04$).

IL-10 production was similar in healthy children without ($n = 23$) and with ($n = 22$) maternal history of IgE-mediated disease (data not shown).

The setting of our study is thus different from the Utrecht study, as we analyzed blood at an earlier time point (at birth), and as we studied allergic sensitization associated with clinical allergy, rather than eczema. The results, however, point to the same conclusion on a defect in IL-10 production in allergy. Another difference is that we used allergens because we intended to measure specific T-cell responses. We cannot, however, rule out that we in fact measured TLR-4 responses. The OVA used to stimulate CBMC indeed contained substantial LPS levels. Moreover, Der p 2 by itself triggers TLR-4, potentially explaining why similar results could

be obtained by stimulating CBMC with this LPS-free allergen [4]. Our data thus add evidence to the observations by the Utrecht group [1], suggesting that IL-10 production at birth or very early in life contributes to induction and maintenance of (allergen-specific) tolerance. The mechanism of TLR-4-mediated protection against allergy remains hypothetical, but APC-induced IL-10 production upon TLR-4-triggering might favor the induction of iTreg cells. Deficits in Treg activity were associated by several groups with atopy development [5].

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References

- 1 Belderbos ME, Knol EF, Houben ML *et al*. Low neonatal Toll-like receptor 4-mediated interleukin-10 production is associated with subsequent atopic dermatitis. *Clin Exp Allergy* 2012; **42**: 66–75.
- 2 Bullens DMA, Kasran A, Dilissen E *et al*. In vivo maturation of T helper cells in relation to atopy. *J Allergy Clin Immunol* 2011; **128**:234–7.
- 3 Bullens DMA, Van den Keybus C, Dilissen E, Kasran A, Ceuppens JL. Allergen-specific T cells from birch pollen allergic patients and healthy controls differ in Th2 cytokine and in IL-10 production. *Clin Exp Allergy* 2004; **34**:879–87.
- 4 Trompette A, Divanovic S, Visintin A *et al*. Allergenicity resulting from functional mimicry of a Toll-like receptor complex protein. *Nature* 2009; **457**: 585–8.
- 5 Palomares O, Yaman G, Azkur AK, Akkoc T, Akdis M, Akdis CA. Role of Treg in immune regulation of allergic diseases. *Eur J Immunol* 2010; **40**: 1232–40.