
Circulating Epithelial Cell Cytokines Are Associated With Early-Onset Atopic Dermatitis

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Early onset of atopic dermatitis (AD) typically precedes the development of other atopic diseases, including food allergy [1] and asthma [2], thus suggesting a role for epithelial dysregulation in the emerging allergic phenotype. The pathogenesis of AD is not well understood, although it appears to result from complex interactions between epithelial and systemic immune networks, largely mediated through patterns of resulting cytokine cascades. Epithelial cell (EC) cytokines, including thymic stromal lymphopoietin (TSLP), interleukin (IL) 25, and IL-33, have emerged as potent inducers of inflammation at epithelial barrier sites. As all 3 cytokines share similar target cell populations and inducing stimuli, it is important to better understand these pathways, especially in infancy, when AD often first manifests.

While some of these cytokine pathways have been examined in older children with AD [3,4], to our knowledge, only 1 small longitudinal study has investigated levels of EC cytokines from birth over the course of infancy, where lower cord blood levels of IL-25, but not other EC cytokines, are associated with the onset of AD in infants at high-risk of AD due to maternal atopy [5].

This exploratory analysis was based on a population of infants at 4-6 months of age, either with moderate-to-severe eczema symptoms and a SCORAD [6] score of at least 15 or with no history of eczema symptoms. Parental written informed consent was provided, and approval was gained from the Human Research Ethics Committee at Princes Margaret Hospital, Perth (approval numbers 1635EP and 1782EP). At 1 year of age, the infants were again assessed for AD using the criteria of Hanifin and Rajka [7] and underwent skin prick

testing with hen's egg, cow's milk, wheat, tuna, peanut, cashew nut, grass pollen, perennial ryegrass, cat hair, and house dust mite. Sensitization was defined as a positive skin prick test result to at least 1 of the allergens.

Peripheral venous blood samples were collected at 4-6 months of age and centrifuged at 4000 rpm for 10 minutes; plasma was stored at -80°C until analysis. The Milliplex MAP Human TH17 Magnetic Bead Panel (Millipore) was used to measure IL-25 and IL-33. Quality controls were run on each plate. The plates were read in the Bioplex 200 system (Biorad). Values under or above the limits of detection were adjusted to the minimum and maximum value detected, respectively, according to previous methodology [8].

Acetone precipitation was performed to concentrate the TSLP for analysis. Four hundred microliters of sample was mixed with a volume 4 times that of the sample, vortexed, incubated for 2 hours at -20°C, and centrifuged at 14 000 rpm for 10 minutes; the supernatant was decanted, and the pellet was air-dried. The pellet was resuspended in 200 µL of enzyme-linked immunosorbent assay (ELISA) diluent. An acetone precipitation TSLP test was performed with spiked samples using standard TSLP to demonstrate that acetone precipitation did not modify TSLP ELISA results. The concentration of TSLP was determined using the human TSLP ELISA Ready-SET-Go! (Affymetrix eBioscience). When the TSLP concentration was not detected in a sample, the value of 4 pg/mL (half the limit of the detection kit) was allocated.

Since TSLP, IL-33, and IL-25 data were not normally distributed, they were reported as median (IQR) and analyzed using the Mann-Whitney test and the Kruskal-Wallis test with the Dunn-Bonferroni post hoc test. All analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp).

Plasma samples from 91 infants were analyzed. Fifteen (16.5%) infants had AD by 4-6 months of age (early AD), 20 (22.0%) infants developed AD between 6 months and 1 year of age (late AD), and 56 (61.5%) infants had no AD by 1 year of age. The characteristics of the participating infants are shown in Table 1 of the supplementary material.

Compared with infants who did not develop AD by 1 year of age, infants with early-onset disease (4-6 months) (n=15) had higher circulating levels of all EC cytokines. Median (IQR) levels were as follows: IL-33, 3121 (1167-6528) pg/mL ($P=.004$); IL-25, 430 (101-1128) ng/mL ($P=.011$); and TSLP, 28.8 (7.1-52.2) pg/mL ($P<.001$) (Figure and Supplementary Material, Table 2). Infants with early-onset AD also had higher circulating levels of IL-33 ($P=.004$) and TSLP ($P<.001$) than infants who developed AD between 6 months and 1 year (late-onset AD). We found no significant differences for any of the EC cytokine levels between those infants who developed AD between 6 months and 1 year (late-onset AD) and those infants who did not develop AD by 1 year of age (Figure and Supplementary Material, Table 2).

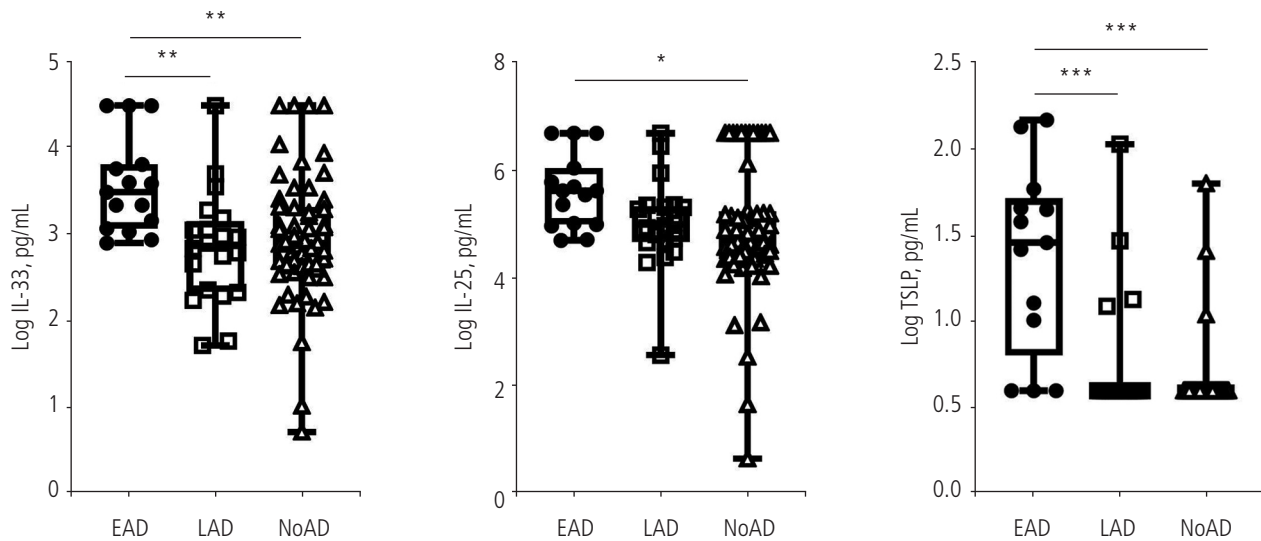


Figure. Concentration (pg/mL) (log₁₀-transformed) of epithelial cell-derived cytokines (IL-33, IL-25, and TSLP) in infants' plasma at 4-6 months of age. Comparison between infants who developed atopic dermatitis (AD) by 4-6 months of age (EAD) (n=15), infants who developed AD between 6 months and 1 year of age (LAD) (n=20), and those who did not (NoAD) (n=56). **P*<.05, ***P*<.005, ****P*=.001.

Clinical follow-up at 1 year of age revealed no differences in EC cytokine levels between infants (48 SPT-positive out of 82 [58.5%]) who were sensitized to at least 1 allergen at 1 year of age compared with those who were not sensitized (Supplementary Material, Table 3).

Here, we report for the first time that levels of TSLP, IL-33, and IL-25 are all elevated in infants who have early-onset AD prior to 6 months of age. This finding highlights possible mechanistic pathways involved in early life inflammation that are clinically expressed as AD. This is consistent with previous findings that higher levels of TSLP and IL-33 have been detected in older children with AD [4]. Our results extend these previous findings, showing that the cytokine patterns we studied are already emerging prior to 6 months of age.

Although AD has been associated with higher epidermal expression of IL-25 in lesional skin than in nonlesional skin [9], previous studies have not examined circulating levels. We demonstrate that plasma IL-25 levels at 4-6 months of age are also associated with early-onset AD. Our findings are consistent with the higher circulating IL-25 levels reported with asthma [10].

We acknowledge a certain degree of overlap in EC cytokine levels between those infants with and without AD in infancy and recommend future studies with longer term follow-up of clinical outcomes. We also acknowledge that these results need to be confirmed in a larger cohort.

Our findings improve our understanding of the relationship between EC cytokines in the emerging allergic phenotype. Elucidating how EC cytokines regulate target cell populations at different sites and stages of disease remains key and may reveal specific targets for future intervention. It also underscores the importance of epithelial events in initiating the allergic phenotype and the need to identify strategies to ameliorate this.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

Previous Presentations

- Child Health Symposium: 3-5 November 2020, Perth Children's Hospital, Perth, Australia. Oral presentation
- Combined Biologicals Sciences Meeting 2020: 27th November 2020, University of Western Australia, Perth, Australia. Poster.

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