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], suggesting a role of epithelial dysregulation in the emerging allergic phenotype. The pathogenesis of AD is not well understood but 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 these three 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 there is only one small longitudinal study investigating levels of EC-cytokines from birth over the course of infancy, where lower cord blood levels of IL-25, but not other EC-cytokines, were associated with the onset of AD during infancy 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 with a SCORAD [6] score of at least 15, or with no history of eczema symptoms. Parental written informed consent was provided, and approval 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

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assessed for AD using the criteria of Hanifin and Rajka [7] and were skin prick tested to 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 to at least one of the allergens.

Infant peripheral venous blood samples were collected at 4-6 months of age, centrifuged at 4000 rpm for 10 minutes and plasma was stored at -80°C until analysis. The Milliplex map human Th17 Magnetic Bead Panel (Millipore, Australia) was used to measure IL-25 and IL-33. Quality controls were run on each plate. The plates were read in Bioplex 200 system (Biorad, Australia). The values under or above the limits of detection were adjusted to the minimum and maximum value detected, respectively, according to previous methodology[8].

For the TSLP analysis, acetone precipitation was performed to concentrate the TSLP. 400µL of sample was mixed with a volume four times that of the sample, vortexed, incubated for 2 hours at -20°C, centrifuged at 14000 rpm for 10 minutes, the supernatant was decanted, and the pellet was air-dried. The pellet was resuspended in 200µL of enzyme-linked immunosorbent assay (ELISA) diluent. An acetone precipitation TSLP test was performed with spiked samples using standard TSLP to demonstrate that the acetone precipitation did not modify TSLP ELISA results. The concentration of TSLP was determined using the human TSLP ELISA Ready-SET-Go! (Affymetrixe Bioscience, USA). When the TSLP concentration was not detected in a sample, the value of 4 pg/mL, half of the limit of the kit detection was allocated.

TSLP, IL-33 and IL-25 data were not normally distributed, hence median, inter-quartile range and non-parametric Mann-Whitney U and Kruskal-Walliswith the Dunn-Bonferroni post-hocTests were applied for their analysis. All analyses were performed using the Statistical

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Package for the Social Science (SPSS) statistical software for Windows, version 25 (SPSS Inc., USA).

Plasma samples from 91 infants were analysed, 15 (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. Table 1 in supplementary material reports the characteristics of the participating infants.

Early onset by 4-6 months of age AD infants (n=15) had higher circulating levels of all EC-cytokines, IL-33 median=3121 (IQR 1167-6528) pg/mL, P=0.004;IL-25 median=430 (IQR 101-1128) ng/mL P=0.011; and TSLP median=28.8(IQR 7.1-52.2)(pg/mL)P=<0.001; compared with infants who did not develop AD by 1 year of age (Figure 1 and Supplementary Material, Table 2).Early onset AD infants also had higher circulating levels of IL-33 (P=0.004) and TSLP (P=<0.001), compared to infants who developed AD between 6 months and 1 year (late 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 AD) and those infants who did not develop AD by 1 year of age (Figure 1 and Supplementary Material, Table 2)..

Upon clinical assessment follow-up at 1 year of age, no differences in EC-cytokines levels were found between infants 48/82 (58.5%) who were sensitized to at least one allergen at 1 year of age compared to 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 six months of age. This finding highlights possible mechanistic pathways involved in early life inflammation, which are clinically expressed as AD. This is consistent with previous findings that higher levels of TSLP and IL-33 have been

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detected in older children with AD [4]. Our results have extended these previous findings to identify that these cytokine patterns are already emerging prior to six months of age.

Although AD has been associated with higher epidermal expression of IL-25 in lesional compared to non-lesional skin [9], previous studies have not examined circulating levels. Here 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 higher circulating IL-25 levels reported with asthma [10].

We acknowledge that there is some overlap of 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.

This study contributes further to 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 lead to focused future targets for 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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Author conflicts of interest

All authors have no conflict of interest associated with this manuscript.

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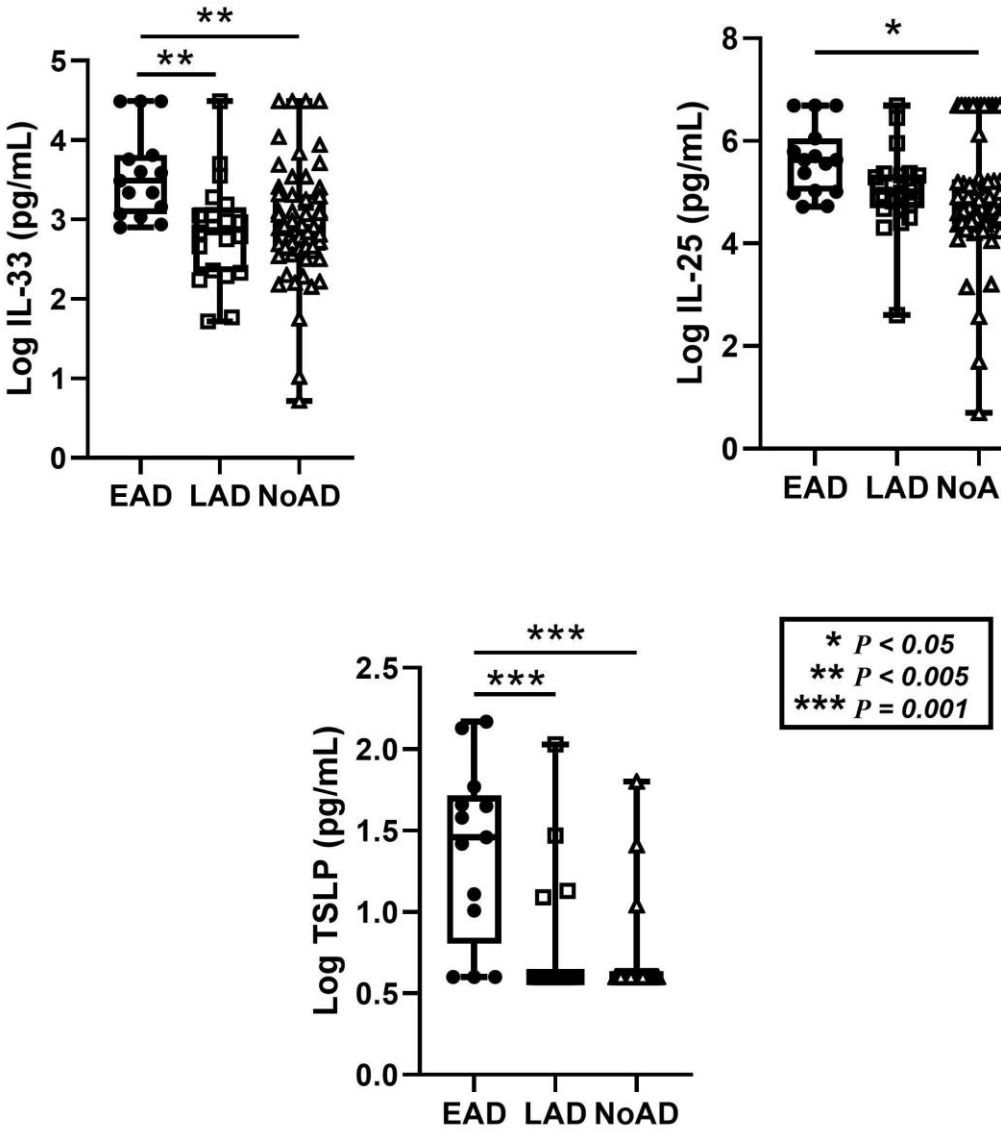
FIGURE LEGEND

Figure 1: Concentration (pg/mL) (log10 transformed) of EC-derived cytokines (IL-33, IL-25 and TSLP) in infants' plasma at 4-6 months of age comparing infants who developed atopic dermatitis (AD) by 4-6 months of age (EAD) (n=15), between 6 months and 1 year of age (LAD) (n=20) and those who did not (no AD) (n=56). * $P< 0.05$, ** $P< 0.005$, *** $P= 0.001$.



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