

Short Communication

Thymic stromal lymphopoietin is present in human breast milk

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Thymic stromal lymphopoietin (TSLP) has emerged as a potential key mediator of both the initiation and maintenance of Th2 responsiveness and thereby the allergic inflammatory cascade. Given the possible role of TSLP in initiating Th2-mediated allergic disease more detailed investigation of TSLP expression and responsiveness in early life is warranted. Therefore, the presence of TSLP in breast milk was evaluated. Defatted human breast milk collected at 3–5 days and 11–26 days postpartum ($n = 22$ /group) and commercially available ready-made baby formula ($n = 3$) were analysed for TSLP with a specific ELISA. TSLP was detectable in breast milk but not in commercial ready-made formulas. Levels in breast milk were significantly higher ($p < 0.001$) at 3–5 days than at 11–26 days postpartum. The potential role of breast milk TSLP in the development of immune function within the neonatal gastrointestinal tract and the development of allergy in childhood remain to be determined.

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Pivotal to the development of allergic disease is the establishment of Th2 reactivity to allergens which supports the production of specific IgE and eosinophilia, and thereby the manifestation of allergic diseases such as eczema, hayfever and asthma. The early immunological mechanisms that predispose to Th2 responses are controversial, particularly whether allergen specific reactivity is evident at birth (1–3). Recently, it has been demonstrated that the bulk of allergen-dependent reactivity at birth reflects an initial burst of short-lived cellular immunity in the absence of conventional T cell memory (1); that emergence of Th2-biased allergen-specific responses from 6 months of age correlates better with sensitization than reactivity at birth measured in cord blood (2); and that allergen-specific IgE detectable at birth does not correspond to allergen-specific IgE detectable in the infant's blood at 6 months of age (3). Despite this,

identification of the factors contributing to the development of Th2 responsiveness to allergens in early infancy is fundamental to understanding the early life origins of allergic disease.

Thymic stromal lymphopoietin (TSLP) has emerged as a potential key mediator of both the initiation and maintenance of Th2 responsiveness and the allergic inflammatory cascade. TSLP is an IL-7-like cytokine produced predominantly by epithelial cells, including those of the skin and airways (4–7). Production of TSLP by epithelial cells is driven by exposure to microbial products (e.g. double stranded RNA), and by the combined action of pro-inflammatory (e.g. TNF α) and Th2 cytokines (4, 5). Expression of TSLP is increased in asthmatic airways (6) and TSLP is highly expressed by keratinocytes in lesional but not in non-lesional skin from subjects with atopic dermatitis nor in lesional skin from subjects with other skin inflammatory diseases such as nickel

induced contact dermatitis or cutaneous lupus erythematosus (7). Animal models provide extensive evidence of a role for TSLP in initiation of the allergic inflammatory cascade.

In humans, exposure to TSLP enhances the survival and maturation of Langerhans cells and CD11c+ myeloid dendritic cells, and leads to increased expression of MHC Class II and co-stimulatory molecules as well as increased TARC (thymus and activation-regulated chemokine; CCL17) and MDC (macrophage-derived chemokine; CCL22) production by these cell types. In turn, TSLP-treated Langerhans cells or CD11c+ myeloid dendritic cells stimulate T cells to produce IL-4, IL-5, IL-13 and TNF α whilst down-regulating IFN γ and IL-10, and enhance their proliferation (7, 8). The TSLP receptor, expressed predominantly by monocytes and myeloid dendritic cells, is a heterodimer of IL-7R α and TSLP-R and interaction with TSLP initiates phosphorylation of signal transducer and activator of transcription (STAT) 3 and STAT5 (9).

To ascertain if TSLP might contribute to the development of Th2 responsiveness to allergens in early infancy we investigated whether human breast milk contained TSLP. Unmatched samples of breast milk were collected from lactating women at 3–5 days ($n = 22$) and 11–26 days ($n = 22$) postpartum with approval of the local research ethics committee and the informed, written consent of the women. The sample was generally collected by expression from the non-feeding breast after completion of a feed. All samples analysed in this study were from different women and the atopic status of the mothers was unknown. Samples were stored at -80°C until analysis. Three different brands of ready-made commercially available formula designated suitable for infants from birth to 6 months of age were purchased. Human milk and formula were defatted by centrifugation ($13,000 \times g$, 10 min, 4°C). TSLP levels in aqueous milk were measured with a specific ELISA (Quantikine, R&D Systems, Abingdon, UK) according to the manufacturer's instructions. All samples were analysed on the same ELISA plate. Statistical analysis was conducted using Wilcoxon Rank Test (SPSS Version 13).

All human milk samples contained detectable levels of TSLP whereas commercial formulas did not. Levels of TSLP were significantly lower ($p < 0.001$) in milk collected 11–26 days postpartum than in milk collected 3–5 days postpartum. Lower concentrations of analytes in breast milk vs. colostrum frequently are described (10, 11), but analysis of changes in

TSLP levels at different times postpartum in matched samples would provide definitive information about this relationship. However, reductions in the TSLP concentration in breast milk would be offset by temporal increases in the volume of breast milk consumed. As TSLP has been described to be produced predominantly by epithelial cells (4–6) it is likely that mammary epithelial cells are the source of breast milk TSLP. Whether breast milk TSLP serves to benefit the mother, baby or both remains to be determined. Given the concentration of TSLP in breast milk and the volume of milk consumed by the baby it is likely that TSLP levels achieved within the gastrointestinal tract would be functional. However, it would be worthwhile considering whether TSLP can survive passage through the gastrointestinal tract and exposure to gastric and pancreatic fluids. Secretory IgA and lactoferrin, both abundant in breast milk, can be detected in the faeces of breast fed infants (10), but soluble CD14 (sCD14), also abundant in breast milk (11), is not detectable in stools of breast fed infants and is likely to remain intact in the upper gastrointestinal tract where it could exert a biological effect but be destroyed in the lower gastrointestinal tract (12).

Variation in TSLP activity in breast milk might underlie some of the controversy surrounding breast feeding and the risk of the development of allergy by the child. Maternal allergy or maternal TSLP genetic polymorphisms might be associated with differences in the abundance of TSLP in breast milk. Unfortunately, we did not have information about the atopic status of the women who provided samples for this study but are now collecting a larger number of samples from women of known atopic status to explore these possibilities. Additionally, the targets for TSLP activity in the gastrointestinal tract might account for variations in biological effects of ingested TSLP. Dendritic cells, monocytes/macrophages and mast cells are responsive to TSLP (5, 7–9, 13). Ascertaining the expression of the relevant receptors and functional effects of TSLP cells within the neonatal gut, although technically challenging should improve our understanding of the potential effects of TSLP in normal and pathological immunological responses in the gastrointestinal tract in early childhood. Understanding the interplay between TSLP and innate immune responsiveness in the neonatal gut during the transition from the germ free to the germ laden environment at birth also is critical in terms of the 'hygiene hypothesis'. It has been suggested that TSLP and TGF β_1 , which is abundant in

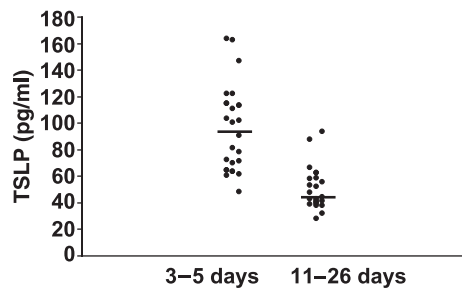


Fig. 1. Thymic stromal lymphopoietin (TSLP) levels in human breast milk at 3–5 days and 11–26 days postpartum ($n = 22/\text{group}$, $p < 0.001$). The median is shown as a bar.

breast milk, induce a tolerogenic DC phenotype within the gut (14). TSLP responsiveness by the human newborn has been demonstrated but requires further characterization (15). Given the emerging importance of TSLP in initiating (and maintaining) the allergic inflammatory cascade (5, 7, 8) more detailed investigation of TSLP expression and responsiveness in early life is warranted.

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