Human milk oligosaccharides (HMOS) can potentially reduce allergy development

Authors: Eline Geervliet, Suzie Grigorjan, Susan Roelofs

In the previous two blogs of this series, we have discussed the development of a functional in vitro bronchial epithelial mucosal immune model. We also highlighted the current knowledge about HMOS in the context of immune development and asthma specifically. In this blog, we are going to evaluate the function of two specific HMOS, 2'FL and 3FL, in this in vitro model. We will study the crosstalk between bronchial epithelial cells (BECs), dendritic cells (DCs) and T cells, after exposure to house dust mite (HDM).

HDM exposure in a complete bronchial model vs. HDM exposure to DCs

The experimental set-up is elaborately discussed in the first blog of this series. Shortly: Calu-3 cells are cultured in air-liquid interface (ALI) set-up for two weeks to form a strong BEC layer. The BEC transwell is added to monocyte-derived DC culture and BECs are exposed to HDM for 24 hours. After 24 hours, the DCs are combined with T cells to allow DC-T cell interaction. As a control, a DC culture exposed to HDM, without BEC barrier is used. A schematic representation is shown in **Figure 1A**.

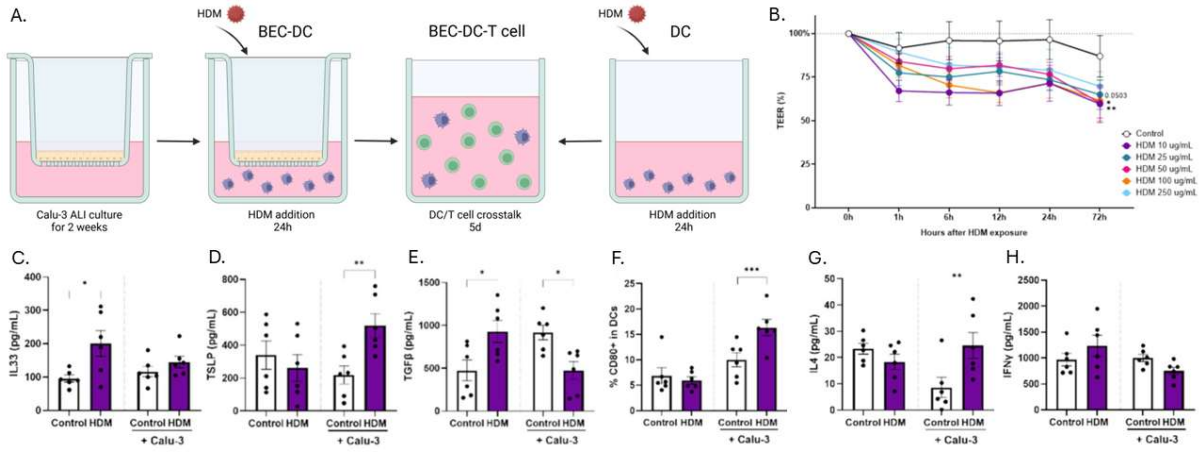

Figure 1. *(A) schematic overview of the set up; Calu-3 bronchial epithelial cells (BEC) were cultured for 14 days in ALI prior to coculture with monocyte-derived dendritic cells(moDCs). BEC-DCs and DCs alone were apically exposed to 10 µg/ml HDM for 24 h. After 24 h of exposure to HDM, primed DCs were collected for analysis and coculture with allogenic naïve Th cells for 5 days. (B) TEER values of mature BEC layers 0h, 1h, 6h, 12h, 24h, and 72h, after HDM exposure of different concentrations. (C-F) Upon 24 h of 10 µg/mL HDM exposure, supernatants and moDCs were collected to measure secreted levels of (C) IL-33, (D) TSLP, (E) TGFβ, and) the percentage of moDCs expressing the costimulatory markers. (G-H) After subsequent coculture with of primed DCs with naïve Th cells, supernatants and cells were collected to measure the expression of (G) IL4 and (H) IFNγ. Copied from [1].*

Exposure of HDM to a bronchial epithelial layer can disrupt the barrier function, even at concentrations as low as 10 µg/mL HDM. The significant decrease in TEER, as measured using the Locsense Artemis ST impedance spectrometer, is caused by proteolytic breakdown of tight junction proteins, see **Figure 1B** [1].

Next, the interaction between BECs and DCs during exposure of HDM was examined and compared to DCs exposed to HDM. Subsequently, the T cell response after co-culture with

either HDM-treated DC or HDM-treated BEC-DC as studied. In the absence of BECs, DCs exposed to HDM show increased levels of interleukin (IL)33 and transforming growth factor (TGF)β (**Figure 1C and E**). Furthermore, the expression of IL8 was decreased and the percentage of CD86 expressing DCs was increased (data not shown [1]). Even though HDM exposure made DCs produce signals usually linked to allergy (IL33, CD86), the presence of TGFβ and reduced IL8 suggest the DCs might actually be shifting towards a more "calm", regulatory state.

When BECs were exposed to HDM in the BEC-DC model, the DCs expressed higher levels of CD80, produced more thymic stromal lymphopoietin (TSLP) and less TGFβ (**Figure 1D-F**). This caused the DCs to change in a way that made them promote a type 2 (allergy-related) immune response. As a result, T cells exposed to these DCs released more IL4 (a type 2 cytokine) and less IFNγ (a type 1 cytokine) (**Figure 1G-H**), similar to what happens in asthma. The increase in TSLP and decrease in TGFβ seemed enough to push the immune response towards an allergic, type 2 pattern.

The immunological effects of 2'FL and 3FL in the bronchial epithelial immune model

After the observation that HDM only induced a type 2 driven immune response in presence of BECs, Zuurveld et al investigated the immunomodulatory effect of 2'FL and 3FL using the BEC-DC-T model [1]. The same experimental setup shown in **Figure 1A** was used, but this time, 2'FL or 3FL was added to the basolateral compartment at concentrations comparable to those found in the bloodstream of infants after breastfeeding.

Both 2'FL and 3FL reduced the HDM-triggered release of TSLP and IL-8 by DCs and possibly also BECs, as shown in **Figure 2A-B**. This decrease was linked to lower IL4 and IL4/IFNγ expression in

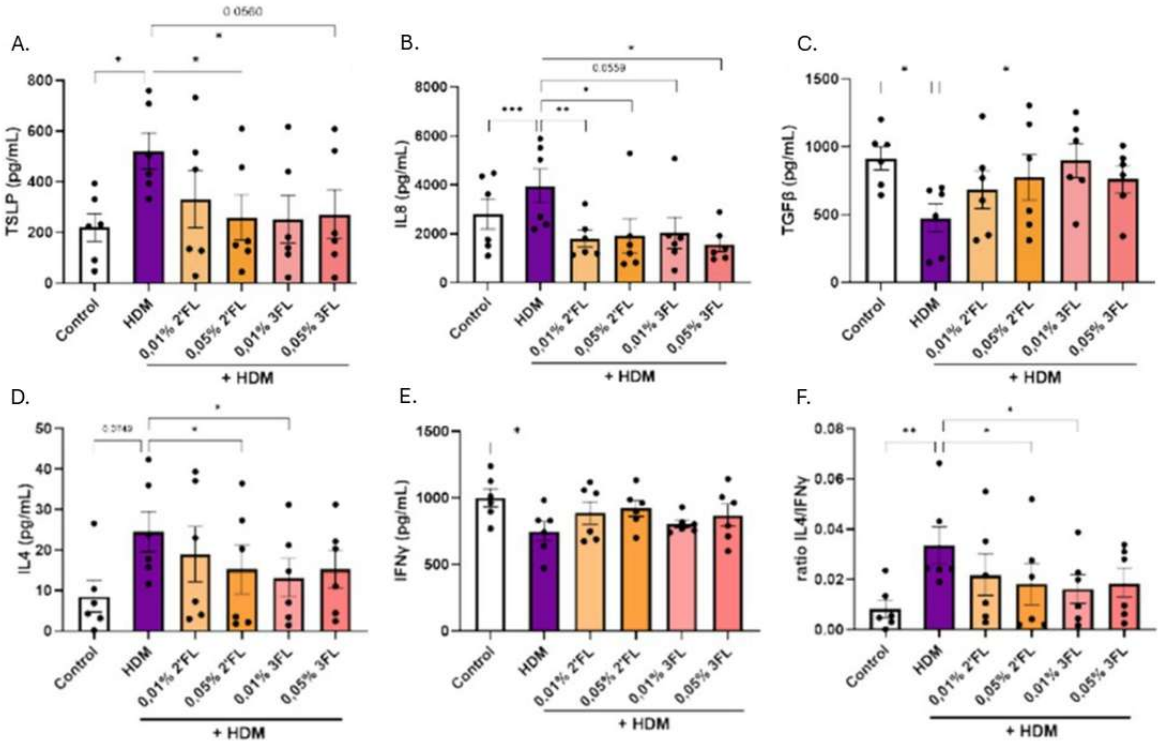

Figure 2. *Upon HMOS preincubation and HDM exposure, supernatants and moDCs were collected to measure secreted levels of (A) TSLP, (B) IL8, and (C) TGFβ. After subsequent coculture with of primed DCs with naïve Th cells, supernatants and cells were collected to measure secreted levels of (D) IL4, (E) IFNγ and (F) the ratio of type 2 IL4 and type 1 IFNγ secretion. Copied from [3]*

T cells, as depicted in **Figure 2D-F**. In short, these HMOS not only suppressed allergic-type activation in BEC-DC cultures but also prevented the T cells from developing a type 2 cytokine profile. This suggests HMOS can block HDM-induced TSLP release, altering DC function to reduce allergic reactions.

Notably, 0.01% 3FL restored TGFβ levels reduced by HDM exposure in BEC-DC cocultures (**Figure 2C**) and lowered IL-4 secretion while maintaining IFNγ expression in T cells. This suggests 3FL helps balance immune responses by enhancing regulatory TGFβ without affecting Treg development.

Conclusions

This study highlights the critical role of the bronchial epithelial barrier in shaping immune responses to HDM exposure. While HDM-exposed DCs alone showed a mixed, somewhat regulatory immune profile, the presence of BECs shifted the response toward a pro-allergic, type 2 pattern, characterized by increased TSLP production, higher CD80 expression, and reduced TGFβ levels. This, in turn, led to enhanced IL-4 secretion and diminished IFNγ expression in T cells, mirroring allergic airway inflammation such as asthma.

Importantly, the addition of HMOS 2'FL and 3FL effectively mitigated these HDM-induced allergic responses in the BEC-DC-T model. Both HMOS reduced TSLP and IL-8 secretion, and prevented the skewing of T cells towards a type 2 cytokine profile. Specifically, 0.01% 3FL restored TGFβ levels and maintained a balanced T cell response, reducing IL-4 while preserving IFNγ expression. These findings suggest that 2'FL and 3FL may hold therapeutic potential in modulating epithelial-immune interactions and preventing allergic airway inflammation.

Acknowledgement

This blog is based on the article from Zuurveld et al. None of this work nor data is produced by Locsense B.V. all rights reserved to M. Zuurveld *et al.*, "HMOS 2'FL and 3FL prevent house dust mite induced proinflammatory cytokine release in vitro and decrease specific IgE production in a murine allergic asthma model," *Front Nutr*, vol. 12, Feb. 2025, doi: 10.3389/fnut.2025.1491430.

References

[1] M. Zuurveld *et al.*, "HMOS 2'FL and 3FL prevent house dust mite induced proinflammatory cytokine release in vitro and decrease specific IgE production in a murine allergic asthma model," *Front Nutr*, vol. 12, Feb. 2025, doi: 10.3389/fnut.2025.1491430.