Development of an in vitro lung-immune model

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The respiratory system is constantly exposed to airborne pathogens, allergens, and pollutants, making the bronchial epithelium a critical first line of defense. Among these environmental triggers, house dust mites (HDMs) are a major source of allergens that can provoke strong immune responses, particularly in individuals with asthma and other allergic respiratory conditions. These lung conditions are closely linked to immune system dysfunction, where excessive inflammation or impaired immune responses contribute to disease progression [1]. An in vitro bronchial epithelial mucosal immune model is essential to study these cell-immune system interactions in a controlled environment. It enables researchers to investigate the cellular and molecular mechanisms of immune responses, assess the effects of environmental triggers like HDM allergens, and develop targeted therapies for respiratory diseases [2]. In this blog we showcase the development of an in vitro bronchial epithelial mucosal immune model using HDM as the allergenic trigger. This model has been developed by Zuurveld et al. [3].

Cell types

Allergic reactions happen through a series of events. Allergens penetrate the bronchial barrier and are taken up by antigen-presenting cells (APCs). These cells present the antigen to naïve T-helper (Th) cells which, in case of an allergic reaction, can differentiate to Th2 cells. The differentiated Th2 cells cause allergic reactions by secreting pro-inflammatory cytokines, recruiting immune cells to the lungs and activating B-cells into immunoglobulin E (IgE) producing plasma cells. So, several cell types are essential for construction of a representative bronchial epithelial immune model:

- Bronchial epithelial cells (BEC)
- Antigen-presenting cells (APC)
- T-helper cells (Th cells)

Since allergic sensitization, the development of an allergy, is linked to epithelial barrier dysfunction, a representative barrier is essential for a functional model. Therefore, **Calu-3 cells,** bronchial epithelial cells, cultured on a Transwell under air-liquid interface (ALI) conditions were used as model of the bronchial mucosal barrier. Unlike submerged cultures, ALI cultures better mimic physiological airway conditions by preserving ciliated characteristics and mucus production.

In the airways dendritic cells (DCs) are the APCs that bridge the innate and adaptive immunity by activating T-cells. Therefore, in this model **monocyte derived dendritic cells** were used as APCs.

Lastly, Th2 cells, which are crucial for allergic reactions by secreting cytokines (e.g., IL-4, IL-5, IL-13), recruiting eosinophils and activating B-cells, are derived from naïve T-cells. Therefore, **blood-derived naïve T-cells** are used in this model.

Workflow

Human lung adenocarcinoma Calu-3 cells were seeded apically on inserts of a 24-wells Transwell plate in liquid-liquid conditions. After 24h, all medium was removed and only 300 µl

basolateral medium was added to start air-liquid interface (ALI) culture. Medium was refreshed twice a week. Cells were cultured for 2 weeks until 100% confluency, differentiation of the cells was assessed by measuring trans-epithelial electrical resistance (TEER) using the Locsense Artemis (Locsense, The Netherlands).

Human peripheral blood mononuclear cells (PBMC) were isolated from buffy coats, that were obtained from healthy donors from the Dutch Blood Bank. Monocytes were isolated via negative selection using a QuadroMACS separator, LS columns and Monocyte Isolation kit II. Subsequently, these monocytes were differentiated to DCs using 100 ng/ml IL4 and 60 ng/ml GM-CSF. From the PBMC, naïve Th cells were isolated via a Naïve CD4+ T cell isolation kit according to manufacturer's instruction.

Confluent ALI-cultured Calu-3 cells were added to monocyte-derived DC suspension in a 24-well plate. Next, epithelial cells were apically exposed to 10 µg/ml HDM and incubated for 24h. DCs were collected for subsequent coculture with naïve Th cells and were transferred to a 48-well culture plate. Isolated naïve Th cells were added to the moDC suspension in the wells. Activation of naïve Th cells was aided by adding 5 ng/ml IL2 and 150 ng/ml anti-CD3 to the culture. The coculture was incubated for 5 days without medium refreshments. A schematic representation of the workflow is depicted in **Figure 1**.

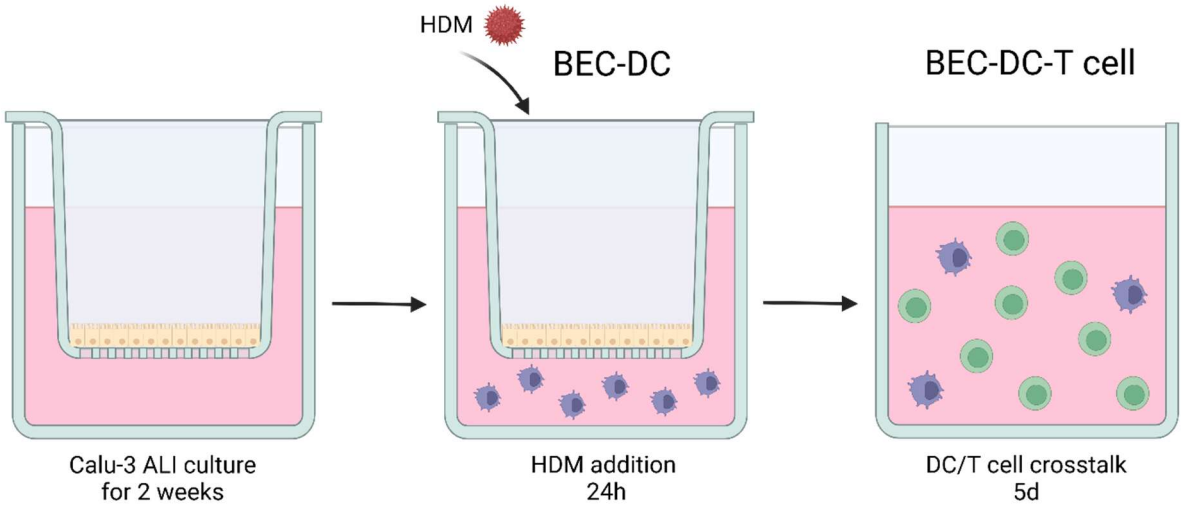

Figure 1. *A schematic overview of the coculture steps in this human in vitro bronchial mucosal immune model. Calu-3 bronchial epithelial cells (BEC) were cultured for 14 days in air-liquid interface (ALI) prior to coculture with monocyte-derived dendritic cells (moDCs). The co-culture was apically exposed to 10 µg/ml house dust mite (HDM) for 24 hours. After 24 hours of exposure to HDM, primed DCs were collected for analysis and coculture with allogenic naïve Th cells for 5 days.*

Acknowledgement

This blog is based on the article by 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] I. Gopallawa, R. Dehinwal, V. Bhatia, V. Gujar, and N. Chirmule, “A four-part guide to lung immunology: Invasion, inflammation, immunity, and intervention,” *Front Immunol*, vol. 14, p. 1119564, 2023, doi: 10.3389/FIMMU.2023.1119564.

[2] L. Wang, D. Hu, J. Xu, J. Hu, and Y. Wang, “Complex in vitro model: A transformative model in drug development and precision medicine,” *Clin Transl Sci*, vol. 17, no. 2, p. e13695, Feb. 2024, doi: 10.1111/CTS.13695.

[3] 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.