

# House dust mite induces M-CSF secretion in human airway epithelia

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An allergic reaction can result in airway remodelling and in an asthmatic condition, which is a life-threatening state. Thus, the identification of respiratory allergens is of great interest. House dust mite (HDM) is a common allergen that can elicit sensitization in susceptible individuals and upon further exposure an allergic reaction. The human airway epithelium (HAE) possesses an immunological function, exerted via a secretion of various mediators affecting the activation of immune cells. These include macrophage colony-stimulating factor (M-CSF), which plays a role in inflammatory macrophages proliferation, DCs trafficking and IgE production and has been shown to be present in greater abundance in both mice and humans following allergen exposure. Here, we report apical M-CSF secretion following HDM exposure using *in vitro* human nasal (MucilAir<sup>TM</sup>) and alveolar (AlveolAir<sup>TM</sup>) epithelium cultured at the air-liquid interface. The epithelia were reconstituted with primary cells isolated from either healthy or allergic donors.



#### Alveolar cells

Figure 1. Schematic representation of HDM exposure testing strategy in (A) human nasal epithelium (MucilAir<sup>TM</sup>) and (B) human alveolar epithelium (AlveolAir<sup>TM</sup>). (BioRender.com)

## 2. HDM has no effect on tissue integrity (TEER) and cytotoxicity (LDH)



Figure 2. Effect of 3-day apical exposure to HDM with or without wash after 30 minutes on (A) TEER and (B) LDH release in human nasal (MucilAir<sup>™</sup>) and alveolar (AlveolAir<sup>™</sup>) epithelium. MucilAir<sup>™</sup> model: Healthy (pool of 14 donors), Allergic 1 (donor 0965), Allergic 2 (donor 0133), Allergic 3 (donor 0419). AlveolAir<sup>TM</sup> model: AlveolAir (donor 822). The dotted line represents the treshold value of 100  $\Omega \times cm^2$  below which the tissue integrity is considered compromised. NT = non-treated, Veh = Vehicle (NaCl). Each series conducted in triplicates (n = 3). (A) Data represented as mean ± SEM of all series tested. (B) Data represented as mean ± SEM of 1 series for Allergic 3, which is representative of all series and donors. (GraphPad Prism)



Figure 4. Effect of 3-day apical exposure to HDM on apical M-CSF secretion in human alveolar epithelium (AlveolAir<sup>™</sup>). Measured by ELISA. The dotted line represents the limit of quantification based on the lowest amount of the standard (15.6 pg/ml). NT = non-treated, Veh = Vehicle (NaCl). Data represented as mean  $(n = 3) \pm SEM$ . Unpaired Student's t-test between Vehicle and HDM,

Figure 3. Apical M-CSF secretion in human nasal epithelium (MucilAir™) measured by ELISA. (A) Apical M-CSF secretion after 3 days of HDM apical exposure conducted in three series. (B) Dose-dependent M-CSF secretion after 3 days of HDM exposure. (C) Apical wash 30 minutes upon HDM exposure prevents M-CSF secretion measured on day 3. The dotted line represents the limit of quantification based on the lowest amount of the standard (15.6 pg/ml). NT = non-treated, Veh = Vehicle (NaCl). Data represented as mean (n = 3) ± SEM. Unpaired Student's t-test between Vehicle and HDM or HDM and HDM with or without wash, \*p<0.05, \*\*p<0.005. (GraphPad Prism)

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### 5. Constitutive apical expression of M-CSF receptor in human nasal epithelium (MucilAir<sup>™</sup>)



Figure 5. Immunofluorescence staining of M-CSF receptor (Alexa Fluor 546) and nuclei (DAPI) in human nasal epithelium. Apical and basal expression assesed by confocal microscopy. 3D view obtained from z-stack images ( $x = 160 \mu m$ ,  $y = 160 \mu m$ ,  $z = 40 \mu m$ ). (ZEISS ZEN 3.10)

#### 6. Conclusion

- Overall, HDM has no disruptive or cytotoxic effect on HAE. •
- HDM induces increase in M-CSF apical levels in both nasal (MucilAir<sup>™</sup>) and alveolar (AlveolAir<sup>™</sup>) • models.
- HDM-induced upregulation of M-CSF can be prevented by apical wash 30 minutes following HDM • exposure, demonstrating the ability of the model to test allergen-removing agents such as nasal sprays.
- MucilAir<sup>™</sup> constitutively express M-CSF receptor on the apical surface. •
- As next steps, inclusion of immune cells on MucilAir<sup>™</sup> and AlveolAir<sup>™</sup> as well as repeated exposures • may be considered.