Abstract

Characterization of virulence mechanisms of *Bordetella Pertussis* using 2D and 3D in vitro airway test systems

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I. Abstract

The agent of whooping cough, B. pertussis, upon inhalation binds to the ciliated epithelial cells of the airway tract and produces several virulence factors to initiate pathogenicity. One of the most important virulence factors is adenylate cyclase toxin (CyaA). Previous studies have assessed the impact of CyaA on cell lines, however, the effects of CyaA on primary human airway epithelial cells (hAEC) are still unknown. In our investigation, we characterized the response of primary hAEC extracted from two different anatomic sites (nose and tracheo-bronchial region) upon intoxication with CyaA and its toxoid CyaA-AC-.To this end, cells were cultured either on monolayers or on 3D AEC-based tissue models with high in vitro/in vivo correlation, and comparative analyses were performed. In addition, we added to our investigation immortalized human bronchial epithelial cells (HBEC3-KT) to directly evaluate the differences in the response of primary human cells and a cell line, which has not been used before for such investigations. Furthermore, in the need of having in vitro test systems that +mimic with more precision the in vivo immune response of the human respiratory tract, we improved the primary airway tissue model by introducing monocyte-derived dendritic cells (MDDCs) into the system to evaluate its potential to allow MDDCs migration upon CyaA and CyaA-AC- intoxication. We were able to show that, compared to HBEC3-KT, primary hAEC were more sensitive to CyaA action. Moreover, in hAEC CyaA did affect cell viability, intracellular cAMP levels, dendritic cell migration, and compromises the innate immune functions, however, these effects were cell-type specific different Our findings reveal first insights into toxin-cell interaction using primary hAEC of different origins and confirm that the choice of respiratory epithelial cell type can significantly influence the outcome of research studies.

II. Image:

III. Reference:

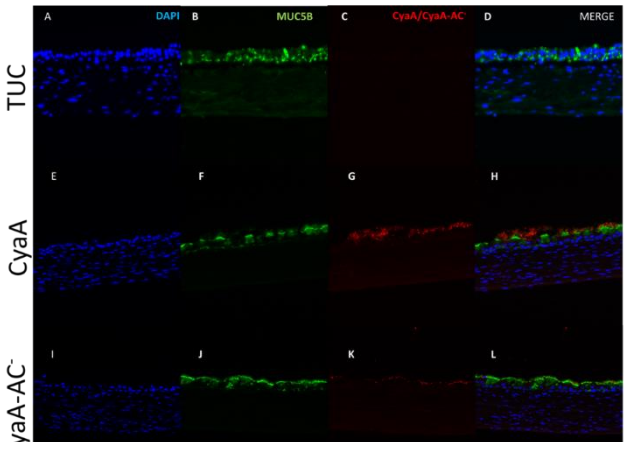


Figure 1. Fluorescently labeled CyaA and CyaA-AC- do not penetrate 3D airway mucosa models due to mucus layer. 3D HTEC-based models were treated with TUC buffer (A-D), 1.0 µg/ml Alexa Fluor 555-labeled CyaA (E-H) or CyaA-AC- (I-L) for 24 hours. Immunofluorescent images show mucus production and localization of CyaA and CyaA-AC-. MUC5B: mucin 5B, CK14 (green), DAPI: nuclear staining (blue), scale bar: 100 µm

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IV. Biography:

Maria Valeska Bianchi Martin was born in Caracas, Venezuela. At 22 years old she got her Cell biology degree at Universidad Central de Venezuela where she did her thesis in Tissue Engineering focus on Osteogenic Differentiation. She moved later on to Barcelona, Spain where she got her dual master degree in Biomedical Engineering at the Universitat de Barcelona and Universitat Politecnica de Catalunya. During her Master thesis she worked on human stem cells and thermo-responsible polymers. Since 2017 she is doing her PhD of Biomedicine at the University of Würzburg, Germany where she is currently working on primary human airway epithelial cells and *B. pertussis* Adelinate Cyclase Toxin at the Chair of Tissue Engineering and Regenerative Medicine of Würzburg

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