Assessing the Adhesion and Cytopathic Effects of *Bordetella Pertussis* in an In Vitro Human Airway Epithelial Tissue Model

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Abstract

Bordetella pertussis is a Gram-negative bacterium and the major pathogen responsible for whooping cough in humans. The recent resurgence of pertussis in the US calls for developing new treatments and vaccines that prevent bacterial colonization, transmission, and disease manifestation. The primary infection site for *B. pertussis* is the ciliated respiratory epithelium. Although the pathology of *B. pertussis* has been studied for decades, the mechanisms underlying the responses of individual cell types to infection remain unclear. Well-differentiated human tracheobronchial epithelial cultures, grown at the Air-Liquid Interface (ALI), closely resemble in vivo ciliated airway epithelium in both their structure and function and, therefore, have the potential to be developed as an advanced biological platform for studying the virulence mechanisms of *B. pertussis*. In this study, in vitro methods were developed for assessing *B. pertussis* adhesion and colonization in ciliated ALI cultures. The cytopathic effects of wild-type and non-virulent *bvg*-mutant *B. pertussis* were compared using functional assays that measure changes in cilia beating frequency, mucin production and epithelial integrity. While the *bvg*-mutant did not adhere, colonize and or produce ciliostasis in ALI cultures, as did wild-type B. pertussis, significant increases were observed in epithelial thickness and cytoplasmic vacuolization after exposure of ALI cultures to both the wild-type and *bvg*-mutant bacteria. Cytokine profiling further revealed infection-specific changes in chemokine secretion following infection with wild-type bacteria (i.e., IL-6, MCP-1/CCL2 and MIF) as compared with infection with *bvg*-mutant bacteria (i.e., MCD/CCL22). These results suggest that ciliostasis is a key early event during *B. pertussis* infection and is tightly associated with subsequent functional deficits in airway epithelial cells. The findings of this study provide mechanistic insight into the host-pathogen interaction of *B. pertussis* and support the potential application of the ALI airway model as a pre-clinical tool for evaluating the safety and efficacy of antimicrobial treatments for pertussis.

Introduction

Despite high vaccination coverage, large outbreaks of whooping cough have occurred in the US since 2004, with the number of pertussis cases increasing to over 48,000 in 2012. The pathogenic effects of *B. pertussis* depend on its ability to adhere, colonize and damage ciliated airway cells via various virulence factors. There are two types of pertussis vaccines, i.e., whole-cell vaccines and acellular vaccines, with the latter being more widely used in high income countries due to fewer side effects. Results from the baboon model of pertussis, however, indicate that the acellular vaccines currently licensed in the US, while preventing disease, fail to prevent pertussis colonization and transmission. This highlights the need for improved vaccines capable of not only inducing long-lasting immunity and preventing disease, but also to prevent colonization and transmission.

Well-differentiated human bronchial epithelial cultures, grown at the air-liquid interface, have many of the structural and functional features of in vivo ciliated airway epithelium. Thus, this in vitro model may provide a biological platform for understanding host-pathogen interactions in the human respiratory system (Fig. 1). In this study, we aim to use the ALI airway tissue cultures to investigate respiratory pathogen (such as *B. pertussis*) induced airway tissue responses, including changes in morphology, cilia beating frequency, inflammation and tissue repair functions, results of which may support its application as an alternative, advanced pre-clinical model for studying airway pathogens and potential for evaluating the efficacy of vaccines.

Materials and Methods

- ALI cultures were established from normal human bronchial epithelial cells (MatTek, Ashland, MA) using the PneumaCult™-ALI medium kit (STEMCELL™ Technologies, Canada). Cultures were grown on microporous membrane supports for 4 weeks to form a pseudostratified structure.
- *Bordetella pertussis* BP536, referred as wild-type (WT), and a Bordetella Virulence Gene regulatory locus deleted strain (BP536 Δ*bvgAS)* were provided by the Merkel lab. The bacteria were stored at −80°C and recovered by plating on Bordet-Gengou (BG) agar plates, supplemented with 1% proteose peptone (Becton-Dickinson, Franklin Lakes, NJ) and 15% defibrinated sheep blood, for 3 days at 37°C.
- Colonies of *B. pertussis* on a BG agar plate were resuspended and serially diluted in PBS. Twenty-five µL of bacterial suspension were pipetted onto the apical surface of ALI cultures at MOI 10, MOI 25 and MOI 50 and incubated for up to 10 days.
- Cell viability was measured using the LDH assay 5 or 10 days post inoculation (5 dpi and 10 dpi).
- Histological analysis of the infected ALI cultures was performed by staining tissue sections with H&E or labeling tissue sections for antibodies specifically recognizing *B. pertussis* antigen (Dxdiscovery, Reno, NV). Images were taken using 40 × magnification.
- Cilia beating frequency (CBF) was evaluated using the Sisson-Ammons Video Analysis software (SAVA, Ammons Engineer, Clio, MI).
- Changes in tissue permeability were evaluated by measuring the trans-epithelial electrical resistance (TEER) using an EVOM2 epithelial volt-ohmmeter (World Precision Instruments, Sarasota, FL).
- Mucin analysis was conducted by an Enzyme Linked Immunosorbent Assay (ELISA).
- Secretion of select cytokines into the basal medium was analyzed using a Bio-plex Pro Human Cytokine 40-plex assay kit (Bio-Rad, Hercules, CA).
- All data are presented as means ± standard deviation (SD). $^{*,\#}p<0.05$ was considered statistically significant compared to the corresponding PBS control using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test.

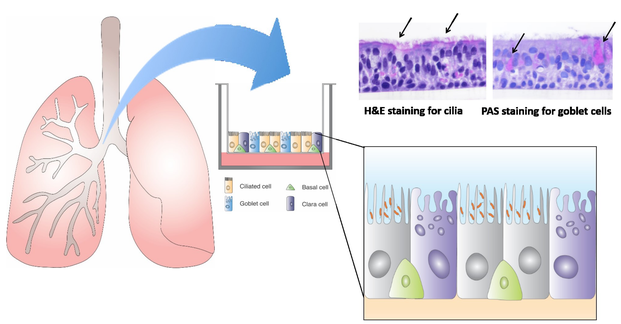
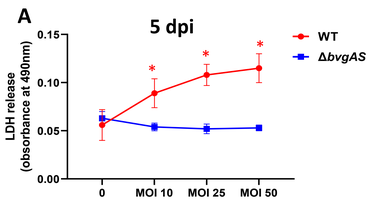


Figure 1. Hypothetical schematic representation of *B. pertussis* attachment to ciliated ALI airway tissue models.

Results and Discussion

B. pertussis induced cytotoxicity and morphological changes in the ALI cultures



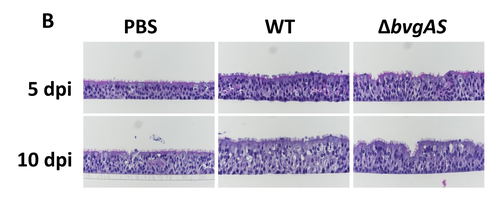


Figure 2. Wild-type *B. pertussis* induced a time-dependent cytotoxicity in the ALI cultures at MOI 50 (A). Significant increases in the epithelial thickness were observed after exposure of ALI cultures to both the wild-type and *bvg*-mutant bacteria at both time points (B and C). The wild-type bacteria adhered to ciliated cells in the ALI cultures (D). Note that intracellular *B. pertussis* antigens were found in the cultures infected with wild-type bacteria.

B. pertussis induced functional changes in the ALI cultures

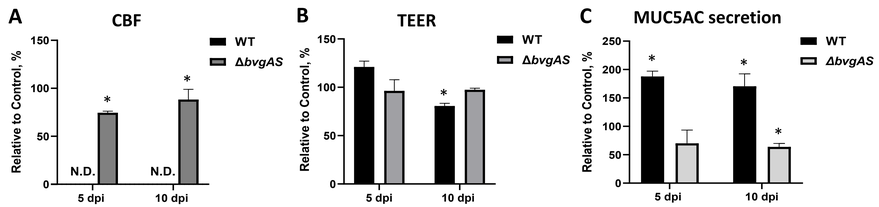


Figure 3. Functional changes in cultures infected with *B. pertussis* at MOI 50. Ciliostasis was observed only in cultures infected with the wild-type bacteria. N.D. : non-detectable (A). TEER was measured as an indicator for epithelial barrier function and was significantly decreased at 10 dpi by the wild-type bacteria (B). Disturbance of MUC5AC secretion following infection with the wild-type and *bvg*-mutant bacteria (C). Note that significant reduction in CBF and MUC5AC secretion were also observed in the *bvg*-mutant bacteria infected cultures.

Changes in key chemokines secretion following infection with *B. pertussis*

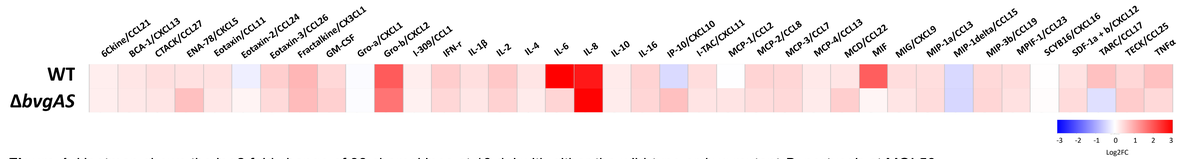


Figure 4. Heatmap shows the log2 fold change of 38 chemokines at 10 dpi with either the wild-type or *bvg*-mutant *B. pertussis* at MOI 50.

Conclusion

- In this study, we evaluated a comprehensive panel of endpoints in the ALI airway model relevant to many of the common pathophysiological features of respiratory diseases, including alterations in cilia function, epithelial integrity, mucin secretion, cytokine release, and tissue remodeling. Exposure of the ALI cultures to wild-type *B. pertussis* induced functional alterations (i.e., ciliostasis and the destruction of tight junctions) that are similar to those observed in the tracheobronchial epithelium of patients with pertussis. Cytokine profiling further revealed the modulation of key chemokines secretion might be tightly associated with *B. pertussis*-induced aberrant tissue remodeling (i.e., epithelial hyperplasia and cytoplasmic vacuolization) in ALI cultures.
- Findings from this project support the use of the in vitro human airway tissue model as a pre-clinical tool for evaluating the safety and efficacy of antimicrobial treatments for pertussis.