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Title: Influenza virus infection of human ciliated respiratory epithelial cells in culture

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Body: Introduction: Influenza is a major cause of paediatric respiratory disease initially infecting and replicating in the respiratory epithelium. Data is limited on the initial effects of influenza A virus on human ciliated respiratory epithelium. In this study we investigated the earliest stages of infection of ciliated epithelial cultures from healthy individuals. Methods Ciliated epithelial cells were cultured from nasal brush biopsies (n=7). Cells were infected with Influenza A/PR8 for up to 24h. Cilia were videoed using a high speed digital camera and the number of motile ciliated cells in each sample area was counted, half of which were used to determine the average CBF. Apical fluid was removed for evaluation of cytokine and chemokine response before and after infection. Cells were fixed and stained using anti-influenza HA antigen (Alexa594) and anti-β tubulin (FITC) antibodies. Confocal optical sectioning of stained slides was performed. Results Ciliary beat frequency was unaffected by influenza A infection over 24 hours, however, the number of motile ciliated cells (median (IQR)) was significantly decreased from 13 (6-14) to 5 (5-7) cells/4.2mm2 following infection. Influenza A infection caused a significant ($P<0.05$) down regulation of IL-6 secretion from 548pg/ml (418 - 1525) to 333 pg/ml (107 - 1304) and significant upregulation of MDC from 20545 pg/ml (9986 - 23181) to 22812 pg/ml (20342 - 30853) and IL-8 from 594 pg/ml (506 - 989) to 887 pg/ml (692 - 1190). Conclusion Ciliary beat frequency was unaffected by influenza A infection over 24 hours, however, a significant reduction in cells with beating cilia was observed.