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Title: Mild pH alteration leads to intracellular retention of IL-8 by airway epithelial cells

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Body: Background: In children with severe neurodisability, chronic microaspiration can lead to recurrent lower respiratory tract infections and cause pulmonary fibrosis. Previous research on acid lung injury has focused on the effects of very low pH on airways, primarily through direct instillation of acid onto airway epithelial cells (AECs). This is not physiologically relevant to injury caused by chronic microaspiration where mild but persistent airway acidification occurs. Aim: To investigate the effects of mildly acidic conditions on inflammatory (IL-8) protein secretion by AECs Methods: A549 cells were cultured to 70% confluence in 96 well plates. DMEM media was adjusted to appropriate pH using 1M hydrochloric acid. Cells were exposed to media for 24h at pH6.5, pH6 and pH5.5. E.coli LPS, alone or with Brefeldin A, were used as positive controls for IL-8 production and intracellular protein retention. AEC IL-8 mRNA expression was measured by RT-PCR (Taqman). Extracellular (cell supernatants) and intracellular (whole cell lysate) protein concentration was measured by ELISA (R&D). Intracellular IL-8 expression was normalised to total protein concentration measured by BCA assay. Results: Increased IL-8 mRNA expression but not IL-8 protein secretion, was observed at pH6.5, pH6 and pH5.5 at 24h. Intracellular IL-8 protein increased as media acidity increased at pH6.5, pH6 and pH5.5. Conclusions: Mild acidification of A459 cells causes intracellular retention of IL-8 protein indicating that lowering pH of airways may disrupt intracellular protein trafficking. This may be due to activation of stress response pathways.