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Negative Pressure-induced Secretion of Inflammatory Mediators by Cultured Middle Ear Epithelial Cells: Relevance to Eustachian Tube Dysfunction and Otitis Media with Effusion

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1 Introduction

Otitis media (OM) is a common disease of the pediatric age group considered to be multifactorial in etiology. While most cases of symptomatic OM with acute onset resolve within one month of presentation, a significant percentage persists for months to years as OM with effusion (OME), and many children present with OME evidenced by middle ear (ME) mucosal inflammation and effusion without recent signs and symptoms. OME is a persistent inflammation that most often fails to respond to conventional medical therapies. Recent studies conducted by us show that *hydrops ex vacuo* is a valid explanation for the development and persistence of OME under certain conditions. Disrupting Eustachian tube (ET) function in animals causes middle ear (ME) pressure dysregulation reflected as underpressures, which in turn causes increased permeability of the mucosal vasculature and results in ME effusion. However, the mechanisms responsible for transducing the biological signals associated with the underpressure that result in ME mucosal inflammation have not been well studied. We hypothesize that transduction of the signal associated with middle ear underpressure initiates and sustains an inflammatory process that contributes to persistence of OME and to adverse changes in ME physiology. Our laboratory is using animal and cell culture model systems to further explore the effects of underpressure on the cells and tissues of the middle ear. Here we present our recent findings with middle ear mucosal epithelial cells in culture. The purpose for this study was to determine whether negative air pressure, relative to atmospheric pressure, induces middle ear mucosal epithelial cells to release inflammatory mediators.

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2 Materials and Methods

A human middle ear epithelial cell line (hMEEC-1, a generous gift from the House Institute, Los Angeles, CA) was used to set up cultures in transwell plates. Cells were seeded onto inserts forming the upper chambers and grown to confluence, then brought to the air/liquid interface for 24h. The inserts were divided into treatment and control groups and exposed to a physiologically relevant negative pressure (-250 daPa*) for an additional 24h. Negative controls were maintained at atmospheric pressure for 24h, and positive controls were exposed to interleukin-1beta, a well-established inflammatory agent. Cell secretions were collected individually from the basal side, in the medium, and apical side, in saline washes, of the cell layer and analyzed using a 27-plex human inflammatory cytokine panel (Bio-Rad Labs, Hercules, CA). *decaPascal = mm H2O, measured with a manometer

3 Results

Negative pressure alone induced secretion of pro-inflammatory mediators, notably IL-8 and RANTES, important chemokines for macrophage recruitment, and VEGF, an angiogenic factor. The pattern of increased secretion of key inflammatory mediators shared similarities with that induced by IL-1β, an early initiator of the inflammatory response. There were notable differences in the polarity of these secretions, basal vs apical, with an

overall tendency for secretions to be higher from the basal (or mucosal) side of the epithelial layer.

4 Conclusion

Exposure of middle ear epithelial cells to negative pressure, comparable to that induced by Eustachian tube dysfunction in patients with OME, triggered the release of key inflammatory mediators. The polarity of the secretions may also indicate potential mechanisms of actions of individual cytokines. Translating these results to the analogous in vivo condition of middle ear negative pressures, this response could contribute to persistent inflammation in middle ear mucosa.

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