The Potential Role of Biofilm Phenotypes in Chronic Otitis Media with Effusion (COME)

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Abstract

Hypothesis: Middle ear pathogens (MEPs) originating from nasopharyngeal biofilms play a significant role in the pathogenesis of Chronic Otitis Media with Effusion (COME).

Objective: The role of bacterial infection in chronic suppurative otitis media and Acute Otitis Media (AOM) has been well established. There continues to be debate pertaining to the role of middle ear pathogens in COME. Work in our lab has previously demonstrated dense biofilms in the nasopharynx of patients with recurrent AOM. Through this study, we sought to elucidate the impact of biofilm phenotypes in COME.

Study Design: Adenoid specimens obtained from 7 COME patients were evaluated using Scanning Electron Microscopy (SEM). Obstructive Sleep Apnea (OSA) patients were used as controls. Biofilm density analysis was performed with Carnoy image analysis software. Polymerase Chain Reaction (PCR) analysis was performed on a subset of nasopharyngeal specimens obtained from COME patients and their matched middle ear fluids (MEFs).

Results: COME specimens had 31.80% mean biofilm density versus 0.04% in OSA controls. All MEFs demonstrated the presence of DNA from middle ear pathogens which included Staphylococcus pneumoniae, Moraxella catarrhalis, and Haemophilus influenzae. Any MEP identified in MEF was also identified in matched adenoid specimen.

Conclusion: These data suggest that MEFs from patients with COME contain MEPs and the source for these MEPs may be resistant adenoid mucosal biofilms.

Introduction

Otitis media with effusion (OME) is one of the most common causes for hearing loss in children. Patients with OME present with middle ear effusion behind an intact tympanic membrane, but do not show any signs or symptoms of acute infection. Presence of middle ear effusion leads to chronic OME.

Nasopharyngeal bacterial infection has been implicated as the most common factor in the pathogenesis of AOM and OME. The predominant bacteria that have been reported to play a role in the infections include Streptococcus pneumoniae, Haemophilus influenzae and Moraxella catarrhalis. The nasopharyngeal colonization by these bacterial pathogens has been shown to occur through the formation of biofilms.

Biofilms are bacterial colonies embedded within an extracellular matrix. Biofilms due to their resistance to antimicrobial and host defenses are considered a model for chronic and recalcitrant infections. Planktonic shedding of biofilms releases pathogens which ultimately cause infection.

Methods and Materials

Adenoid biopsies were obtained from COME and OSA patients. A total of 7 experimental (COME) and 7 control (OSA) specimens were obtained. In addition, middle ear effusions were also obtained from 3 of 7 COME patients.

Imaging
- All adenoid biopsies obtained after surgery were fixed in gluteraldehyde and prepared for SEM imaging.
- Imaging was done on all COME and OSA tissue specimens at various resolutions using a scanning electron microscope (JSM-6400, JEOL Ltd, Tokyo, Japan)

PCR
- Polymerase chain reaction (PCR) was performed on a subset of COME specimens – adenoid tissue and matched middle ear fluid.
- Specific primers for S. pneumoniae, M. catarrhalis, H. influenzae used.

Results: Nasopharyngeal mucosal biopsies from COME and OSA patients were analyzed using Scanning Electron Microscopy and biofilm density analysis was performed using Carnoy Image Analysis software. In addition, 3 out 7 COME nasopharyngeal specimens and their matched middle ear fluids were also analyzed using PCR. Patient group comprised of children 11 months to 15 years of age, both male and female.

SEM Image Analysis
- Discrete biofilm matrix structures with embedded bacteria at the mucosal surface were visible from the SEM images of all 7 COME specimens.
- For OSA specimens, SEM images showed a clear adenoid mucosal surface devoid of any discrete biofilm structure.
- COME specimens had 31.80% mean biofilm density with a standard deviation of 5.13% versus 0.04% mean biofilms density in OSA controls with a standard deviation of 0.11%

PCR Analysis
- All 3 Middle Ear Fluid samples demonstrated the presence of DNA from one or more middle ear pathogens.
- Any MEP identified in MEF was also identified in the corresponding adenoid COME specimen.

Conclusions

The ability of microorganisms to survive in biofilms despite significant antimicrobial pressure and the ability of these organisms to evade normal host immune response has significant clinical implications. Numerous investigators have proposed that the unique properties of biofilms perturb the host immune response. Nasopharyngeal mucosal biofilms from COME and OSA patients suffer from chronic and recalcitrant infections. Planktonic shedding of biofilms releases pathogens which ultimately cause infection.

Although bacterial biofilms have been documented in nasopharyngeal specimens of patients suffering from COME, there still continues to be controversy pertaining to this bacterial phenotype in the virulence mechanism.

To more definitely elucidate the impact of biofilm phenotypes in COME, this study sought to make a direct comparison of nasopharyngeal mucosal biofilms with age matched controls. This pilot study has documented the comparison in biofilm densities between COME and control specimens.

In addition to a distinct difference in biofilm coverage between the experimental and control specimens, PCR studies on a limited number of specimens have so far shown the presence of middle ear pathogens in the nasopharynx of specimens from COME patients. The data on middle ear effusions suggest that these middle ear fluids from COME patients contain middle ear pathogens, and the source for these pathogens may be nasopharyngeal mucosal biofilms.