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Regulation of bacterial trafficking in the nasopharynx

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Summary

Bacterial ‘colonisation’ of the nasopharynx by potential bacterial pathogens in early childhood is a normal, important series of events. They are part of a dynamic process in which the microbiota of the upper airways becomes established and evolves in early childhood. The potential pathogens ‘colonising’ the upper airways interact with resident, apparently non-pathogenic, bacteria; other potential pathogens including those from their own species; viruses and the hosts immune response. Environmental factors such as family dynamics, child care, antibiotic usage, smoking and indeed vaccines all have an impact. Understanding the significant of potential beneficial interactions as well as those factors that are detrimental to the host will be important in disease prevention in the future.

Keywords

Streptococcus pneumoniae, *Haemophilus influenzae*, Microbial populations; Bacterial interference; Bacterial competition; commensals

Introduction

Bacterial colonization of the nasopharynx (np) has long been recognized as a critical event in disease pathogenesis for both invasive diseases as well as respiratory tract infections. The predominant focus until recently has been on individual pathogens and the pathogenesis of disease following np acquisition. As examples, Faden and colleagues observed that early colonization of the np in infants with *Streptococcus pneumoniae* (SP) or nontypable *Haemophilus influenzae* (NTHi) was an important risk feature for early onset of middle ear disease[1] and Leach and colleagues reported that early colonization with NTHi and SP results in a 30 fold increase in risk for AOM in Aboriginal infants compared with colonization with *Moraxella catarrhalis* alone.[2] Revai and colleagues identified a significantly greater odds ratio for development of AOM during a viral upper respiratory tract infection for children colonized with an otopathogen compared with those who were not.[3] Furthermore, in invasive diseases due to *Neisseria meningitidis*, *Haemophilus influenzae* type B and *Streptococcus pneumoniae*, the specific pathogen is almost always present in the nasopharynx and in fact, sterilization of the nasopharynx to prevent further transmission has been incorporated into secondary prevention strategies for both meningococcal and HiB disease.

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The focus for disease prevention has been on risk factors for colonization with potential pathogens, such as age, gender, breast feeding, day care attendance, and exposure to smoke or the impact of selective pressure from antimicrobial treatment or conjugate vaccines on colonization with pathogenic strains as epidemiologic clues to identify strategies to prevent colonization and thereby prevent disease.[4,5] More recently, investigators have begun to view bacterial colonization with potential pathogens within the context of a microbial community and to explore the dynamics of colonization and the influences that result in persistence or clearance, disease or 'asymptomatic' carriage.[6] This review will explore and highlight our expanding understanding of the process of colonization and potential mechanisms that regulate the pathogen-commensal state with a focus on SP and NTHi. We will also speculate on how the results from '*in vitro*' and animal studies are relevant to clinical and epidemiologic observations primarily about otitis media, but potentially applicable to all bacterial respiratory tract infection.

Natural History of Colonization with Respiratory Pathogens in Children

Prospective studies in the first year of life in US infants report that initial colonization occurs during the first month and increases rapidly over the first six months.[1] *Moraxella catarrhalis* is both the earliest and the most prevalent among the respiratory pathogens during this period, achieving colonization rates of 55% in infants at six months and 72% by 12 months of age. SP and NTHi are found in 38% and 19% respectively at 6 months of life. Similar findings are reported in Aboriginal children with colonization beginning in the first weeks of life and increasing rapidly to greater than 80% for each of the three potential pathogens by 12 weeks of age.[2] In the Aboriginal child, all three potential pathogens are frequently present concurrently.

In cross sectional studies, pneumococcal colonization has peak prevalence between 2 and 3 years of age with an ~55% rate of carriage in Dutch children [7] and between 6 and 24 months in US studies.* Although SP and SA are recovered from different sites in the respiratory tract (np and oropharynx for SP and anterior nares or SA), the observation that *S. aureus* colonization is relatively low during the peak period of pneumococcal carriage and pneumococcal carriage is declining during adolescence, the peak age of *S. aureus* colonization raises questions about how these two pathogens interact but a definitive understanding has not as yet been established.

Risk factors that increase likelihood of colonization include household smoking, day care attendance, siblings and the presence of upper respiratory tract infection.[8] Animal studies support a 10–100 fold increase in recovery of NTHi in the presence of RSV[9] and SP during Influenza infection.[10] Prior antimicrobial therapy appears to have discordant effect on colonization, susceptible SP are reduced but NTHi and resistant SP are increased.

Studies of bacterial turnover suggest that one predominant strain of NTHi or SP is carried at a time yet children frequently are sequentially colonized with new strains.[11] Transmission in day care centers is common[12,13] as well as transfer to younger sibling in the household.[14]

Colonization with respiratory pathogens elicits an inflammatory response

Weiser and colleagues demonstrated that inoculation of SP into the nasopharynx in a mouse model results in signaling events that initiate an inflammatory response as well as promotes the opening of epithelial barriers at cellular junctions permitting cytokines and neutrophils to enter the lumen.[15] Within 24 hours, a mild rhinitis is present characterized histologically by the presence of neutrophils. Weiser describes the process as similar to the inflammatory response seen in the lungs during pneumococcal pneumonia. Such events are hypothesized

to be analogous to the mild rhinorrhea seen in humans following experimental nasopharyngeal challenge with SP. Despite the inflammatory process, pneumococci persist and take several weeks to clear apparently requiring monocytes and macrophages. Potentially this observation, inflammation following colonization with SP or NTHi, provides context as to why respiratory tract infections are more likely to arise shortly after bacterial colonization[16] and not from resident pathogens and supports a hypothesis that aspects of the inflammatory process may permit, or even promote, local or systemic invasion.

Colonization with respiratory pathogens elicits an immune response

Colonization with SP elicits local immune responses such that on rechallenge, an enhanced neutrophilic invasion that rapidly reduces the bacterial density within the np is observed.[15] The response appears related to either cellular or humoral defenses. These immune responses may partially explain the recognized age related decline in pneumococcal disease in children as nearly all children have been previously 'colonized' by age 3 [??Belongs here? Wroe P personal communication] Colonization is also recognized to induce protective systemic immunity, in some circumstances, as observed against *Neisseria meningitides* following colonization with *Neisseria lactamicus*.

Turnover of SP and NTHi strains within the np is common

Recent descriptions of competition between bacterial pathogens of the same species have demonstrated that pneumococci possess weapons such as bacteriocins that target other pneumococci.[17] In contrast, studies by Levin and colleagues demonstrate that nasal challenge in neonatal rats with an established resident strain permits a second strain to co-colonize when the resident and challenge strains are SP or NTHi, but not when the resident and challenge strains are *Staphylococcus aureas* (SA).[18] The clinical implication being that children can become colonized with more than one strain of SP or NTHi and that ongoing turnover of strains in the nasopharynx is observed. In contrast, once colonized with a resident strain of SA, neonatal rats are resistant to co-colonization with a new strain which may provide insight into why nasal colonization with methicillin resistant strains persists over months.

Competition among bacterial pathogens enhances the np inflammatory response

Competition between different species has also revealed that co-inoculation or sequential challenge results in increased inflammation and clearance or coexistence as the outcome appears to be strain specific.[19,18] In general, competition between SP and NTHi is immune mediated and associated with increased neutrophilic infiltration compared to colonization with either species alone. The observation that children colonized with more than one potential pathogen are more likely to develop AOM within a few days following onset of a viral respiratory tract infection suggests that the greater inflammation resulting from interspecies competition may contribute to impairment of mucosal defense mechanisms and development of disease.[3] The most severe outcome of this dense multispecies bacterial nasopharyngeal competition and resulting neutrophilic infiltration, tissue damage, increased mucus secretion, and decreased mucociliary clearance may be the persistent nasal discharge and concurrent chronic suppurative otitis media observed in Aboriginal children as hypothesized by Leach and colleagues.[20]

Do commensals 'regulate' bacterial virulence?

The evidence that interactions within the nasopharyngeal microbial community influence the outcome of colonization and its potential clinical relevance has been presented above. Future strategies for controlling the inflammatory response may lead to new approaches for preventing respiratory tract colonization from progressing to disease. The influence of microbial communities as a modifier for the development of disease following colonization remains poorly understood. Potentially the microbial population in concert, or specific key members, may dampen the immune or inflammatory response associated with colonization and potentially regulate the virulence of established otopathogens.

Studies of the microbial community using 16S rRNA has described differences in the commensal populations in children colonized with *S. pneumoniae* compared to those who are not colonized and children with and without OM.[21] The mean Shannon Diversity and Evenness, an ecological measure of species diversity in a community, were significantly lower in *S. pneumoniae* culture-positive than in culture-negative samples. Logistic regression analysis predicting presence of *S. pneumoniae* found the presence of several commensals to be protective against colonization. Logistic regression predicting the presence of OM revealed that the same commensals were also protective for OM. Alternatively, the presence of NTHi (a well recognized otitis pathogen) and unclassified Pasteurellaceae or *Actinomyces*, *Rothia*, *Neisseria* and *Veillonella* were associated with the presence of AOM. One hypothesis would be the presence of these commensals (themselves not AOM pathogens) increase the propensity of AOM pathogens to overcome host defenses and cause disease.

Studies of the quantitative microbiology of adenoids by Bernstein found Viridans streptococci to be the predominant normal flora in children who were not otitis prone and a significantly reduction in viridans Streptococci in adenoids from otitis-prone children.[22]. He hypothesized an alteration in environmental pH or competition for nutrients as potential mechanisms for the inhibition on NTHi by viridans streptococci. He further investigated the role of viridans group streptococci (*Streptococcus oralis*) in the prevention of colonization with NTHi, *Moraxella catarrhalis* or SP in an adenoid organ culture system.[23,24] The results indicate that some strains of *S. oralis* inhibit np colonization with potential pathogens.

A randomized trial of the role of re-colonization of otitis prone children with a mixture of five strains of streptococci reported a significant decrease in number of recurrences of acute otitis media in children treated with a nasal spray compared with those given placebo.[25] Differences in the persistence of secretory otitis were also seen in the treated group at the last study visit. The results suggest that the composition of the microbial community in the upper respiratory tract has a role in regulation of virulence as no differences in colonization with NTHi, SP or *Moraxella catarrhalis* were observed between control and treatment groups. In a second study, daily *Lactobacillus rhamnosus* GG, LC705, *Bifidobacterium breve* 99 and *Propionibacterium freudenreichii* JS administered as an oral capsule failed to demonstrate a reduction in AOM in otitis prone children compared to placebo.[26]

What are the potential implications of viewing bacterial virulence within the context of a microbial community?

The primary focus of antibiotic selection and vaccine impact has been on the role of such strategies on resistance of and/or colonization with respiratory tract pathogens. However, if as a growing body of evidence suggests, microbial communities regulate colonization and/or progression from colonization to disease, then a broader view of antimicrobial selection and

vaccine impact will be necessary. Antibiotic exposure results in clearance of susceptible pneumococci and accumulation of resistant pneumococci and conjugate vaccines prevent acquisition of vaccine type bacterial pathogens which results in increased colonization with non vaccine serotypes.[4,5] However studies to date have not addressed the larger picture that changes in key members and/or the overall diversity of the microbiome potentially facilitate or impair the body's natural defense and therefore impact the development of bacterial respiratory tract infection. Studies of the changes in respiratory tract flora and their implications will be important if we are to further translate the '*in vitro*' and animals studies to clinical relevance.

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Educational Aims

- To understand the dynamic nature of bacterial trafficking through the nasopharynx and upper airways
- To recognize the importance of this process in the development of both acute and chronic diseases of the respiratory system including the middle ear
- To understand that 'colonisation' is a dynamic event generating inflammatory and immunological responses which may produce upper airways symptoms including coryza
- To understand the complex interaction of host and environmental factors that influence the impact of potential pathogens taking up residence in the upper airway