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Phase variation and host immunity against high molecular weight (HMW) adhesins shape population dynamics of nontypeable *Haemophilus influenzae* within human hosts

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HIGHLIGHTS

- A deterministic mathematical model of NTHi phase-variation and host immunity.
- Host immunity selects for an immune-evasive NTHi bacterial population.
- Large deletion events allow for maintenance of adherent NTHi bacterial cells.
- Adherent NTHi cells may be important for bacterial colonization and/or transmission.
- A robust immune response may increase NTHi transmission.

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ABSTRACT

Nontypeable *Haemophilus influenzae* (NTHi) is a bacterium that resides within the human pharynx. Because NTHi is human-restricted, its long-term survival is dependent upon its ability to successfully colonize new hosts. Adherence to host epithelium, mediated by bacterial adhesins, is one of the first steps in NTHi colonization. NTHi express several adhesins, including the high molecular weight (HMW) adhesins that mediate attachment to the respiratory epithelium where they interact with the host immune system to elicit a strong humoral response. *hmwA*, which encodes the HMW adhesin, undergoes phase variation mediated by 7-base pair tandem repeats located within its promoter region. Repeat number affects both *hmwA* transcription and HMW-adhesin production such that as the number of repeats increases, adhesin production decreases. Cells expressing large amounts of HMW adhesins may be critical for the establishment and maintenance of NTHi colonization, but they might also incur greater fitness costs when faced with an adhesin-specific antibody-mediated immune response. We hypothesized that the occurrence of large deletion events within the *hmwA* repeat region allows NTHi cells to maintain adherence in the presence of antibody-mediated immunity. To study this, we developed a mathematical model, incorporating *hmwA* phase variation and antibody-mediated immunity, to explore the trade-off between bacterial adherence and immune evasion. The model predicts that antibody levels and avidity, catastrophic loss rates, and population carrying capacity all significantly affected numbers of adherent NTHi cells within a host. These results suggest that the occurrence of large, yet rare, deletion events allows for stable maintenance of a small population of adherent cells in spite of HMW adhesin specific antibody-mediated immunity. These adherent subpopulations may be important for sustaining colonization and/or maintaining transmission.

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1. Introduction

Haemophilus influenzae is a Gram-negative coccobacillus that commonly resides within the human pharynx as a commensal and a potential pathogen. Non-encapsulated *H. influenzae* strains, which are commonly referred to as nontypeable *H. influenzae* (NTHi), are generally associated with localized infections of the respiratory tract such as pneumonia, sinusitis, and acute otitis media (AOM). AOM is a common childhood disease and in the United States approximately 83% of children have had at least one episode of AOM by the age of three and 45% have suffered three or more AOM episodes (Teele et al., 1989). In adults, NTHi strains are commonly associated with acute exacerbations in patients suffering from chronic obstructive pulmonary disease (COPD) (Garcha et al., 2012; Perotin et al., 2013; Sethi et al., 2002). Both acute exacerbations in COPD patients and AOM often result in antibiotic prescriptions (Lindenauer et al., 2006; Plasschaert et al., 2006). Thus, reducing incidence of NTHi-associated diseases can reduce a significant burden on the healthcare system, antibiotic usage and associated concerns regarding emerging antibiotic resistance.

NTHi strains are spread from person to person via infected respiratory droplets where they establish pharyngeal colonization, with prevalence ranging between 25% and 84% (Bou et al., 2000; Faden et al., 1995; Harabuchi et al., 1994). Since pathogenic NTHi arise from the community of NTHi strains colonizing healthy individuals, colonization marks one of the first steps of NTHi pathogenesis. Consequently, interventions that reduce, or prevent, colonization could potentially decrease the burden of NTHi disease.

Adhesin-mediated attachment to the host epithelium may play a critical role during the early stages of colonization, allowing newly transmitted NTHi cells to overcome host mucociliary clearance mechanisms. Thus, adhesins may confer a fitness advantage, increasing the probability of colonization following transmission. However, adhesins also tend to be antigenic, for example, NTHi colonization stimulates IgG, IgM, and IgA production that specifically targets surface localized adhesins (Barenkamp and Bodor 1990; Pichichero et al., 2010; Barenkamp 1986; Gnehm et al., 1985; Karasic et al., 1985). Thus, as the immunological environment changes, an adhesin that confers a fitness advantage during the early stages of colonization in a naïve host may become a liability when faced with an antibody-mediated immune response.

NTHi adherence to the host epithelium is mediated, in part, by the non-pilin high molecular weight (HMW) adhesins (St. Geme, 1993, 1994), which are present in approximately 40–75% of all NTHi isolates (Barenkamp and Leininger, 1992; Ecevit et al., 2004; Erwin et al., 2005; Erwin et al., 2008; St. Geme et al., 1998; van Schilfgaarde et al., 2000). Functional HMW adhesins are encoded by *hmwA* (Barenkamp and Leininger, 1992; Dawid et al., 1999), which displays extensive genetic diversity within and between isolates (Dawid et al., 2001; Giufre et al., 2006; Buscher et al., 2004). HMW adhesin amino acid diversity helps to define the tissue tropism of a particular strain (St. Geme et al., 1993; Buscher et al., 2004) and, importantly, generates antigenic diversity (Barenkamp and Bodor, 1990; van Schilfgaarde et al., 2000).

HMW-adhesin expression is phase variable. Tandem arrays of heptanucleotide simple sequence repeats (SSRs) located within the *hmwA* promoter region form the basis of HMW-adhesin phase variation (Barenkamp and Leininger, 1992; Dawid et al., 1999). During DNA replication, SSRs can be gained or lost by slipped-strand mispairing (Dawid et al., 1999) these changes are reversible and accumulate in a stochastic manner, independent of any external selective pressures. The number of repeats affects both *hmwA* transcription and translation—as repeat number increases, *hmwA*-transcript levels, and HMW-adhesin levels, decrease in a graded fashion (Dawid et al., 1999) (Fig. 1a). Ultimately this translates to population-level phenotypic diversity that may be

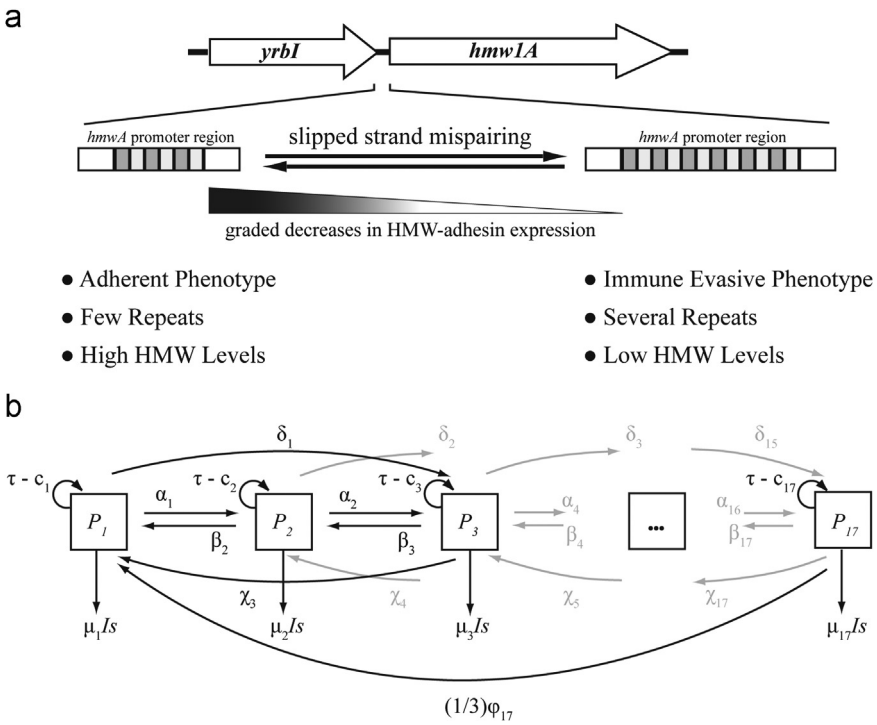

Fig. 1. (a) The HMW adhesin phase variation mechanism illustrated with NTHi 86028-NP *hmw1A*. Phase variation is mediated by the gain and loss of heptanucleotide repeats (shaded rectangles), located within the *hmw1A* promoter region between *yrbI* and *hmw1A* that are gained or lost during DNA replication by slipped-strand mispairing (Dawid et al., 1999). Differences in repeat number affect *hmwA* transcription, and HMW-adhesin levels, in a graded fashion such that fewer repeats are associated with increased *hmwA* transcription and HMW-adhesin production (Dawid et al., 1999). Phenotypically, cells can be characterized as "adherent" cells or "immune evasive" cells. (b) Schematic representation of the within-host NTHi population model. The total population was divided into 17 subpopulations, P_1 to P_{17}, designated by boxes. Each NTHi subpopulation is defined by the exact number of repeats located within the *hmwA* promoter. Arrows represent the rates at which NTHi cells transition between subpopulations or are cleared from the system; model equations, parameter names, and their baseline values are provided in the Appendix.