Immunological analysis of a putative virulence protein of *Haemophilus influenzae*

Kawasi Lett, Thomas Phillips, Arnold Smith and Miriam Golomb

*Haemophilus influenzae*, a gram negative bacterium, is part of the normal flora of the human upper respiratory tract; however, they are also known to be pathogenic. Nonencapsulated (nontypeable) *H. influenzae* (NTHi) are responsible for respiratory illnesses including bronchitis, otitis media, conjunctivitis, and pneumonia. Before widespread vaccination, encapsulated *H. influenzae* of serotype b (Hib) was a major cause of childhood meningitis. Although Hib meningitis has nearly disappeared from the developed world, invasive disease (meningitis and septicemia) has occasionally been associated with NTHi, which are vaccine-resistant. We are studying one such NTHi strain (R2866), isolated from a child with meningitis. R2866 has several genes that are absent in nonpathogenic *H. influenzae*, some of which may account for its increased virulence. A probable virulence gene, lav, is found in many pathogenic NTHi, including invasive strains, but not in commensal isolates. A phase-variable gene, lav has multiple copies of a GCAA repeat within the coding sequence; gain or loss of repeat sequences modifies the translational reading frame and turns expression on or off. A close homologue of lav exists in *Neisseria meningitidis*, the meningococcus, and it has been shown to have been acquired by horizontal passage from *H. influenzae*. Lav belongs to the AIDA-1/VirG/PerT family of autotransporters, bacterial virulence proteins, which facilitate their own secretion. Autotransporters consist of three distinct regions; an N-terminal signal peptide, an effector "passenger" domain, and a C-terminal beta-barrel domain. Subsequent to transport into the periplasm, the beta-domain forms a pore in the outer membrane through which the passenger domain is exported. Depending on the protein, the passenger can function as an adhesin, invasin, toxin, or IgA protease. Most passengers are cleaved at the bacterial surface via proteolytic enzymes and are either secreted into the medium or remain bound to the outer membrane by noncovalent forces. As a preliminary step in the investigation of Lav function, we have employed anti-peptide antibodies to visualize the cellular location, processing, and phase variation of Lav, by Western blotting, fluorescence microscopy and TEM. Unlike most autotransporters, the Lav passenger protein is not cleaved, but remains covalently bound at the surface.