**Unencapsulated Haemophilus influenzae - What Kind of Pathogen?**

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*Haemophilus influenzae* are small, gram-negative, rod-shaped bacteria. Because of their special growth requirements, they do not grow on usual blood agar media, but flourish on the mucosal membranes of the human respiratory tract where they adhere to the epithelial cells by fimbriae (a potential vaccine component). Nasopharyngeal carriage of *Haemophilus influenzae* is very common, and in healthy carriers the bacteria are usually unencapsulated. The outer membrane of *Haemophilus influenzae* contains lipopolysaccharide (of so called R form, without O antigen) and major outer membrane proteins. The lipopolysaccharide is a virulence determinant. An extracellular enzyme, IgA protease, is another potential virulence determinant. The outer membrane of *Haemophilus influenzae* is a rather ineffective barrier towards antibiotics, and thus the major determinants of antibacterial resistance in *Haemophilus influenzae* are plasmid-coded enzymes that inactivate the antibiotic, and changes in the target molecules.

*Haemophilus influenzae* is a common human parasite and pathogen which colonizes the mucosa of the upper respiratory tract and causes disease by local spread or invasion. An important distinguishing feature between *Haemophilus influenzae* isolates is whether or not they are encapsulated. The disease spectrum caused by encapsulated *Haemophilus influenzae* is very different from that caused by unencapsulated strains. The pathogenic mechanisms and factors governing the course of diseases are likewise very different. In the majority of studies on the bacteriology, antigenicity, and pathogenicity of *Haemophilus influenzae*, the focus has been on encapsulated *Haemophilus influenzae*, and primarily on the most common pathogen, type b. By contrast, the *Haemophilus influenzae* strains found in maxillary sinusitis are almost exclusively unencapsulated. The purpose of this paper is to examine the properties and capabilities of this bacterium as a background to understanding how disease is caused and how we might be able to intervene in the course of disease. The relative paucity of information of these aspects still is a handicap to rational approaches.

*Haemophilus influenzae* as a Mucosal Colonizer

*Haemophilus influenzae* are traditionally described as small, slender, gram-negative rods with special nutritional requirements. These data are useful for diagnostic purposes, but not very helpful for understanding the bacteria as a cause of disease. The growth requirements suggest, however, that the bacteria would best survive in a natural host, and this is borne out by observation. Thus colonization of the respiratory tract is essential for the survival of the bacteria and its spread from person to person. In fact, colonization rates may be so high that 50% of a population may be found to be colonized at any time (1); the bacteria are thus very successful parasites.

Colonization of mucous membranes is believed to require adhesion of the organisms to the epithelium. The adherence can easily be seen in electron micrographs, and can also be mimicked in vitro with epithelial cells. In such assays, *Haemophilus influenzae* has been shown to adhere very well (2). In vitro, 100 or more bacterial cells can bind to each buccal epithelial cell (3). Large differences can be found between individual strains; nonencapsulated strains adhere better than encapsulated ones. Adhesion is mediated by fimbriae (long, thin, hair-like appendages extending out from the bacterial surface, which can be seen by electron microscopy) (4, 5). Fimbrial variation appears to be common in *Haemophilus influenzae*. While most strains are fimbriated and adhere well when first isolated from the nasopharynx, these properties are soon lost during cultivation in vitro (4). This sort of variation has been suggested to occur also during infection (6): bacteria which colonize the nasopharynx need to be fimbriated, whereas in tissues the loss of fimbriae might be advantageous (this would pertain to encapsulated *Haemophilus influenzae*).
The receptor for the *Haemophilus influenzae* fimbriae on red blood cells was recently identified (7). Epithelial cell adhesion of and colonization by *Haemophilus influenzae* seem to be enhanced by a viral infection (8, 9). In an experimental infection system, concomitant influenza A virus infection potentiates the disease (10). On the other hand, antibiotics in even subinhibitory concentrations may decrease the adhesion of and colonization by *Haemophilus influenzae* (11). Adhesion can be prevented, at least in in vitro assays, by employing measures which interfere with the binding of fimbriae to their receptor. This can be effected by soluble receptor-like molecules, which compete with the epithelial cell receptor, or by antibodies to the fimbriae. Human milk contains material that inhibits adherence of viral infection (8, 9). In an experimental infection with the site of colonization.

### *Haemophilus influenzae* as a Pathogen

As a gram-negative organism, *Haemophilus influenzae* has a morphologically typical outer membrane. Classically the outer membrane is a barrier that selectively allows the passage of only certain types of molecules into or out of the bacterial cell (13). The resistance of enteric bacteria to complement-mediated killing or to many antibiotics is due to a large extent to this barrier function. On the basis of this function, the outer membrane of *Haemophilus influenzae* is remarkably less efficient, as it allows the killing action of serum complement in the presence of antibodies. In encapsulated *Haemophilus influenzae* strains, the capsule may prevent binding of antibodies to noncapsular outer membrane components, and thus laboratory measurement of bactericidal antibodies gives a good indication of their potentially protective activity (14). By contrast, the unencapsulated forms do not have the protection afforded by the capsule and are generally sensitive to antibodies normally present in human serum (15). This sensitivity would, under usual circumstances, prevent the survival of unencapsulated *Haemophilus influenzae* strains in the bloodstream, and, indeed, such strains are rarely found as a cause of bacteraemic infections. The capsule furthermore protects encapsulated bacteria from phagocytosis, and, by inference, the nonencapsulated forms are susceptible to this mechanism of host defense, too. The net result is that the infection usually remains localized on the mucosal membranes without further spread or penetration to the tissues. This is, of course, the clinical picture seen in sinusitis, otitis media, and chronic bronchitis, the typical forms of disease produced by nonencapsulated *Haemophilus influenzae* strains.

The outer membrane of *Haemophilus influenzae* contains a set of major proteins (OMPs), some of which function as pores that allow the passage of small hydrophilic molecules through the membrane and are therefore essential for the survival of the bacteria, while the function of the others is not known. So far we do not know anything that would allow correlation of the OMPs with pathogenic mechanisms of the bacteria. The OMPs of different strains of *Haemophilus influenzae* can vary quite extensively, both in molecular size and antigenic properties. This variation is especially marked among nonencapsulated *Haemophilus influenzae* strains, whereas encapsulated type b strains form a more homogeneous group. OMP-variation forms a basis for serotyping (16); however, even if some serotype were to be found associated with disease (which is not yet the case with nonencapsulated *Haemophilus influenzae*), it does not imply that any particular OMP would be a virulence factor since the typing merely identifies a group of strains with similar properties.

Lipopolysaccharide (LPS) is a typical major component of the outer membrane of gram-negative organisms. It constitutes part of the endotoxin which is responsible for gram-negative shock and can also have local toxic effects. Thus the cessation of ciliary movement on respiratory endothelial cells and the sloughing off of ciliated cells seen when such tissues are experimentally infected with *Haemophilus influenzae* are probably due to endotoxin (17, 18). Whereas lipopolysaccharides of many other bacterial groups, e.g. the enteric bacteria, also carry polysaccharide side chains with distinct antigenic properties (the O antigen), the LPS of *Haemophilus influenzae* is devoid of these and resembles the short core-LPS molecules of rough enterobacterial mutants (19). Thus *Haemophilus influenzae* does not have the protection afforded by O antigen and is therefore sensitive to antibodies to both outer membrane proteins and LPS. The short LPS molecules (sometimes called LOS, for lipo-oligo-saccharide, to emphasize the shortness) can vary structurally and antigenically. This variation can also be exploited for serotyping. The different variants of LPS have been associated with differences in virulence of encapsulated *Haemophilus influenzae*; however, the mechanisms of their action are not clear.