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Localized adherence of *Haemophilus influenzae* to human lung cells in tissue culture

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The gram-negative coccobacillus *H. influenzae* is part of the respiratory mucosal flora of most healthy humans. Before the era of Hib vaccination, encapsulated *H. influenzae* of serotype b were the leading cause of childhood bacterial meningitis; at present, unencapsulated (nontypeable) strains of *H. influenzae* (NTHi) remain an important cause of respiratory infections such as otitis media, bronchitis, bacteremia, sinusitis, and pneumonia. Occasionally, NTHi strains (which are resistant to Hib vaccination) are isolated from Hib-vaccinated patients with meningitis or septicemia. During the past few years, much has been learned about how other pathogenic bacteria (particularly *E. coli*) colonize host tissue. Less is known about early steps in *H. influenzae* colonization, although a variety of non-pilus adhesins have been identified. We are studying the adherence of clinical NTHi strains to H292 (human lung carcinoma) cells in tissue culture, including the role of the autotransporter Lav, an outer membrane primary protein of unknown function which may play a role in adhesion or persistence. The NTHi being investigated have high molecular weight adhesins (either HMW or Hia). Quantitative adherence assays in which binding time was varied suggested that adherence is a multistep process. Bacteria exposed to H292 cells for 30-60 minutes bound less efficiently than those exposed for longer times (2-4h). The Lav protein did not contribute to short term binding but appeared to improve binding and internalization at 4h. NTHi labeled with a plasmid which expresses GFP (green fluorescent protein) were diluted and bound to H292 on cover slips. While adherence at 30 minutes was diffuse, adherence at 4h was highly localized, with microcolonies of 40-50 bacteria forming at discrete sites on cells. We are constructing a red fluorescent plasmid *for H. influenzae* to determine (by mixing red and green bacteria) whether microcolonies are the progeny of a single bacterial cell or arise by recruitment of multiple bacteria at the same prepared site. We are also staining H292 with fluorescently labeled phalloidin to determine whether microcolonies are associated with actin polymerization and cytoskeletal assemblies.