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Localized adherence and microcolony formation by nontypeable Haemophilus influenzae

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Haemophilus influenzae is a gram-negative bacterium that resides in the upper respiratory tract of most humans. Nontypeable strains of H. influenzae (NTHi) cause many upper respiratory infections, including otitis media, bronchitis, sinusitis, conjunctivitis, pneumonia, and complications of cystic fibrosis and COPD. We are investigating early stages of the process by which NTHi colonize the respiratory epithelium. When NTHi strain R2866 is allowed to adhere to a human lung cancer cell line (H292), binding increases in efficiency over a 4h period (a higher proportion of input bacteria bind per unit time). The autotransporter protein Lav, although not a primary adhesin, improves adherence approximately 2-fold in the 4h assay. Adherence is localized, with microcolonies of 50+ bacteria forming at discrete sites. In preliminary experiments, adherence sites appear to colocalize with structures formed by polymerized actin. We want to test the hypothesis that bacteria cause the actin structures to form and thereby facilitate their binding, and to investigate the role of Lav in facilitated binding. In particular, we wanted to mix diluted fluorescent red and green bacteria to see whether microcolonies grow clonally from one adherent bacterium, or are polyclonal in origin, suggesting recruitment to specific sites. Initially, we introduced GFP and RFP plasmids into our strains; however, expression of fluorescent proteins such as mCherry was relatively weak in NTHi. We therefore stained the surface of living bacteria with FITC and TRITC. Stained bacteria retained viability and the ability to adhere to H292. Confocal fluorescence microscopy showed colocalization of FITC-strained R2866 with polymerized actin structures at 30 minutes after infection. Even at 30 min after infection, before bacteria have replicated, clusters of bacteria are seen at sites of actin polymerization. Since the actin structures are rare in uninfected controls, this suggests that bacteria have induced them and that they in turn facilitate binding.