## Evaluation of the Role of the Bvg Intermediate Phase in Bordetella pertussis during Experimental Respiratory Infection.

The BygAS system of Bordetella pertussis was traditionally considered to mediate a transition between two phenotypic phases (Bvq(+)) and Bvq(-) in response to environmental signals. We characterized a third state, the intermediate (Bvg(i)) phase, which can be induced by introducing a 1-bp substitution into bvgS (the bvgS-I1 mutation) or by growing B. pertussis under conditions intermediate between those leading to the Bvg(+) and Bvg(-) phases. Like B. bronchiseptica, B. pertussis displays in its Bvg(i) phase a characteristic colony morphology and hemolytic activity and expresses a Bvg(i)-phase-specific polypeptide called BipA, whose synthesis is regulated by bvgAS at the transcriptional level. Based on our results, we hypothesize that the Bvg(i) phase of B. pertussis may be involved in facilitating transmission between hosts. Thus, a B. pertussis mutant carrying the bvgS-I1 mutation (GMT1i) persisted at wild-type levels only in the upper murine respiratory tract. Interestingly, a bipA deletion derivative of GMT1i displayed a reduced ability to colonize the nasal cavity of mice compared with GMT1i. However, in experimental mixed infections GMT1i expressing the Bvg(i) phase could establish an initial colonization in the nose and trachea of mice as efficiently as GMT1, but the wild-type strain outcompeted GMT1i at a later time point at all sites of the respiratory tract, suggesting that the Bvg(i) phase does not serve as a phenotypic phase specialized in colonization. Finally, even though B. pertussis expresses in vitro the Bvq(i) phase at the human nasal temperature, anti-BipA antibodies were undetectable in a large collection of sera from pertussis patients.

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