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Bordetella Bsp22 forms a filamentous type III secretion system tip complex and is immunoprotective in vitro and in vivo

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Abstract

Type III secretion system (T3SS) tip complexes serve as adaptors that bridge the T3SS needle and the pore-forming translocation apparatus. In this report we demonstrate that Bsp22, the most abundantly secreted substrate of the Bordetella T3SS, self-polymerizes to form the Bordetella bronchiseptica tip complex. Bsp22 is required for both T3SS-mediated cytotoxicity against eukaryotic cells and haemoglobin release from erythrocytes. Bacterial two-hybrid analysis and protein pull-down assays demonstrated the ability of Bsp22 to associate with itself and to bind BopD, a component of the Bordetella translocation pore. Immunoblot and cross-linking analysis of secreted proteins or purified Bsp22 showed extensive multimerization which was shown by transmission electron microscopy to lead to the formation of variable length flexible filaments. Immunoelectron microscopy revealed Bsp22 filaments on the surface of bacterial cells. Given its required role in secretion and cell-surface exposure, we tested the protective effects of antibodies against Bsp22 in vitro and in vivo. Polyclonal antisera against Bsp22 fully protected epithelial cells from T3SS-dependent killing and immunization with Bsp22 protected mice against Bordetella infection. Of the approximately 30 genes which encode the Bordetella T3SS apparatus, bsp22 is the only one without characterized orthologues in other well-characterized T3SS loci. A maximum likelihood phylogenetic analysis indicated that Bsp22 defines a new subfamily of T3SS tip complex proteins. Given its immunogenic and immunoprotective properties and high degree of conservation among Bordetella species, Bsp22 and its homologues may prove useful for diagnostics and next-generation subunit vaccines.