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Functional analysis of *Shigella* needle tip protein IpaD reveals domains managing early virulence events

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Shigella is responsible for bacillary dysentery in human through host-mediated destruction of the colonic mucosa. *Shigella* invades the epithelium and induces an acute inflammation which accounts for the disease. As for numerous Gram-negative pathogenic bacteria, it uses a type III secretion system (T3SS) to inject effector proteins into eukaryotic cell which subvert cell signalling. This T3S apparatus spans, and protrudes beyond, the bacterial cell wall to form a molecular needle. To prevent early secretion of effectors, the needle is plugged by IpaB and IpaD. These proteins together with IpaC are also necessary to 'translocate' effectors within the host cell cytoplasm. Concretely, IpaD would insert IpaB and IpaC within the cell membrane to form a pore that allows the transit of effectors which remodel the actin cytoskeleton and promote *Shigella* internalization. This makes IpaD a "cornerstone" of the *Shigella* T3SS. Recently, we reported the localization of IpaD at the needle tip and an *in vitro* inhibition of the invasion process by polyclonal antibodies raised against its residues 131-332. In this work, we study the phenotype of twenty -10 amino acids- truncated IpaD variants and delineate two functional domains. First, the coiled-coil domain (131-174 and 272-332) seems to be important for the localization and the formation of a functional plug at the needle tip. Second, the central domain (175-271) is likely involved in the correct insertion of the pore within the cell membrane. Finally, we generated anti-IpaD monoclonal antibodies and we are currently (1) mapping their epitope as well as (2) investigating their ability to interfere with the entry process. In conclusion, our fine-tuned analysis of IpaD looks suitable to dissect early events governing the virulence of *Shigella* in an attempt to design a suitable subunit vaccine to protect against shigellosis. Indeed, IpaD is a remarkably conserved antigen amongst *Shigella* spp.

Keywords: Type III secretion system, tip proteins, epithelial cell invasion, protective antigen