The Type IVB Pili of *Salmonella enterica* Serovar Typhi Bind to the Cystic Fibrosis Transmembrane Conductance Regulator

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*Salmonella enterica* serovar Typhi expresses type IVB pili. We show that the prePilS protein (the soluble precursor form of the structural pilin) interacts with a 15-mer peptide representing the first extracellular domain of the cystic fibrosis transmembrane conductance regulator (CFTR), a recognized human epithelial cell receptor for serovar Typhi (G. B. Pier et al., Nature 393:79-82, 1998). This indicates that after mediating bacterial self-association (C. Morris et al., Infect. Immun. 71:1141-1146, 2003), the pili then act to attach the bacterial clumps to CFTR in the membrane of gut epithelial cells. These sequential type IVB pilus-mediated events cannot be performed by (for example) *S. enterica* serovar Typhimurium, which may explain why only serovar Typhi causes epidemics of enteric fever in humans.

The type IVB pilus operon of *Salmonella enterica* serovar Typhi contains a pilS gene encoding the structural pilin (1, 5). A pilS mutant of serovar Typhi was much reduced in adhesion to and invasion of human epithelial gastrointestinal cells in vitro, and soluble purified prePilS protein (retaining the signal sequence normally cleaved when the protein is excreted to form insoluble pili based on polymerized PilS) inhibited bacterial invasion (5). While the pili mediate bacterial self-association (3), these data did not explain why purified prepilin should inhibit serovar Typhi entry to human intestinal epithelial cells. This rather suggested that the prepilin might interact with an epithelial cell receptor.

It is known that the first extracellular domain of the cystic fibrosis transmembrane conductance regulator (CFTR) is a serovar Typhi receptor domain (4). To determine if soluble prePilS protein could interact with this domain of CFTR, bacteria of serovar Typhi strain J341 (Ty2 Vi−) (5), grown in Luria broth for 14 to 16 h at 30°C to reach optical densities at 600 nm of 0.5 to 0.7, were resuspended in Eagle’s basal medium, which also contained CFTR peptides, prePilS protein, or both. Then the bacteria were centrifuged for 10 min at 3,500 g. After incubation, cells were washed, treated with gentamicin, and concentrations of prePilS and CFTR 15-mer as independent variables showed that the prePilS-CFTR peptide interactions of both Fig. 1A and B were significant, with *P* < 0.001.

These data indicate that the prePilS protein and the CFTR 15-mer interact, and this in turn suggests that the PilS protein of the type IVB pili binds to CFTR in the INT407 cell membrane. It was not possible to use purified PilS protein in the test described above, because PilS is insoluble, precipitating in the affinity matrix upon thrombin release from a GST conjugate (data not shown). Because the signal sequence of prePilS will be removed upon PilS secretion by serovar Typhi, a phys-
biologically relevant interaction with CFTR would involve the PilS portion of prePilS.

Serovar Typhi bacteria use the type IVB pili for bacterial self-association (3). The data described here suggest that such aggregates may use the pili also to bind to the human intestinal cell membrane. The next step would logically be bacterial invasion of the intestinal mucosa, perhaps facilitated by association of bacterial lipopolysaccharide to pili-recruited CFTR (2). Because expression of the type IVB pili is confined to serovar Typhi and a few human-invasive strains of S. enterica serovars Paratyphi C and Dublin (our unpublished observations), expression of the type IVB pili may be important in the mediation of enteric fever in humans.

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REFERENCES


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