Abstract

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Titel of PhD thesis:

„Pathogenicity mechanisms of *Campylobacter jejuni* and *Campylobacter fetus*: characterization of pathogenicity factors and signaling in host cell invasion"

Campylobacter jejuni is the leading bacterial cause of food-borne illness worldwide and a major cause of Guillain-Barré paralysis. Invasion of host target cells has been reported as one of the primary reasons of tissue damage caused by this pathogen but molecular mechanisms are widely unclear. In the present study, I characterized the fibronectin-binding protein CadF as important pathogenicity factor expressed in all tested *C. jejuni* and *C. coli* strains. CadF is involved not only in adhesion but also required for maximal host cell invasion of *Campylobacter*. Additionally, the difference in molecular size and nucleotide sequence between CadF of *C. jejuni* and *C. coli,* described here, may potentially be applicable to discriminate these species in food and clinical specimens. Furthermore, detailed understanding of the signaling events induced by *C. jejuni* infection is presented in this study. It is shown that *C. jejuni* triggers membrane ruffling in the eukaryotic cell followed by invasion in a very specific manner first with its tip followed by the flagellar end. To pinpoint important signaling events involved in the *C. jejuni* invasion process, the role of small Rho family GTPases was examined for the first time. Using several molecular biological tools including specific GTPase-modifying toxins, inhibitors, siRNA and GTPase expression constructs it is shown that Rac1 and Cdc42, but not RhoA, are involved in *C. jejuni* invasion. In agreement with these observations, it was found that internalization of *C. jejuni* is accompanied by a time-dependent activation of both Rac1 and Cdc42. Furthermore, with use of ß1- and FAK-knockout cells, different expression constructs, siRNA and inhibitors it is shown that the integrins, EGFR, PDGFR, FAK, DOCK180, Vav-2, α-PIX and Tiam1 are critically involved in mediating *C. jejuni* invasion-promoting signals. It is proposed that activated integrins and PDGFR/EGFR interact during *C. jejuni* infection and trigger formation of various signaling complexes including FAK, DOCK180, Vav-2, α-PIX and Tiam1 leading to the activation of Rac1 and Cdc42 and stimulation of common downstream signaling pathways. This in turn causes actin rearrangements and efficient *C. jejuni* uptake. Moreover, evidence is presented that activation of Rac1 and Cdc42 involves the CadF protein and the flagellar apparatus. Thus, CadF appears to be a bi-functional protein enabling bacterial binding to host cells as well as stimulating integrin clustering, which subsequently can activate downstream factors triggering GTPase signaling in infected host cells. Collectively, results of this study suggest that *C. jejuni* invade host target cells by a unique mechanism and the activation of the integrins, FAK, Rac1 and Cdc42, but not RhoA plays a central role in this entry process.

Finally, the role of the surface array protein SapA and its phosphorylation in infection with *Campylobacter fetus* is here established. With use of SapA cloning, purification and *in vitro* tests as well as examination of SapA-non expressing strains, it is shown that Src-like PTKs mediate SapA phosphorylation and indicated that phosphorylated SapA plays significant role during *C. fetus* infection.