
Spore 1.5 1 Patch With 121 [EXCLUSIVE]

Fn was also found in the cytoplasm of IECs, suggesting uptake through receptor-mediated endocytosis 22, 23, which was corroborated by the stimulation of Ca²⁺-dependent endocytosis and the reduction in spore entry by incubation of the cells with the small GTPase inhibitor dynasore 15. Cytoskeleton rearrangement and, more specifically, the actin-driven endocytosis of particles has been previously reported in both, IEC and Caco-2 cells 24, 25, 26, 27, 28, 29. To better define the mechanism of spore uptake into Caco-2 cells, we utilized transmission electron microscopy (TEM) and confocal microscopy. Spores of strain R20291 were extracellularly seen bound between the apical and basal actin cytoskeleton and were found between the basal and apical endocytosis (Fig. 4e). The internalization of R20291 spores was confirmed by TEM by visualizing *C. difficile* spores (presumably extracellular) and/or endosomal-like membranes surrounding *C. difficile* spores and a membrane ruffle located at the interface of the spore and the apical membrane of Caco-2 cells (Fig. 4f and Si). Our data indicate that unroofed and enclosed spore-infected bacteria are able to enter polarized monolayers of epithelial cells, but the precise mechanism(s) and the ligand(s) that mediate spore internalization remain unknown. To investigate whether the spore *C. difficile* strain used in this study can bind to laminin/Vn, we carried out an ELISA experiment using laminin/Vn as substrate. As control, we assayed the presence of different proteins, including Fn, Vn, and Fibronectin (FN) (Supplementary Fig. 17a). *C. difficile* spores adhered to laminin/Vn and Fn in a concentration-dependent manner (Supplementary Fig. Importantly, the spore surface display of unglycosylated GD by the *gcdR* mutant was able to bind laminin/Vn and Fn, suggesting that the interaction between the spore surface with host Fn and laminin/Vn is mediated by GD without the need of polysaccharides *bgtCDA* (Supplementary Fig.

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