Enteropathogenic *E. coli* and *S. flexneri* 2457T Adversely Affect Paracellular and Transcellular Permeability

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EPEC and *S. flexneri 2a* are causative agents of a disproportionately high number of fatalities in children under the age of five. Several vaccine strains have been developed and tested but none has been capable of generating a protective immune response in the absence of diarrhea. Because of the pressing need to develop new vaccines against EPEC and *S. flexneri*, we are characterizing the pathogenic effects caused by wild type and mutant strains of these pathogens in *in vitro* and *ex vivo* models. We employed polarized T84 cells, a crypt-like colonic cell line, and Caco-2 cells, which resemble small intestine enterocytes, to define the outcomes of infection with EPEC and *S. flexneri* 2a. Strains 1204 and 1208 are live, attenuated vaccine strains derived from *S. flexneri 2a* strain 2457T; strain 1204 is a guanine auxotroph and 1208 is a mutant of 1204 lacking functional ShET1 and ShET2 enterotoxins as well as the mucinase pic. Although T84 and Caco-2 cells respond differently to the pathogens, the loss of ShET1 has a major impact on transepithelial electrical resistance (TEER), paracellular permeability, and cytokine and chemokine release after a three hour apical infection. Strain CVD206 is a mutant of EPEC 2348/69 lacking the gene *eae* which encodes intimin, a necessary protein for the formation of attaching/effacing (A/E) lesions. A type 3 secretion system (T3SS) mutant of EPEC (*EscN*) was also tested in these models. Inactivation of the T3SS of EPEC has a detrimental effect on the ability of EPEC to decrease TEER and increase paracellular permeability in T84 and Caco-2 cells, although the effects of CVD206 vary between the two cell lines. In the *ex vivo* Ussing chamber model we exposed adult mouse tissue to bacterial supernatants containing secreted toxins and found that the strains that increased TEER (i.e. paracellular permeability) also increased short circuit current (i.e. transcellular secretory activity). Defining the proteins and toxins of *S. flexneri* and EPEC that are responsible for altering the function of tight junctions and paracellular permeability is essential to understanding the mechanisms responsible for causing diarrhea.