Shigella flexneri causes severe impairment of mucosal barrier integrity by disrupting tight-junction mediated function *in vitro*.

M. Fiorentino, K. Lammers, T. Dyson, S. Vogel and A. Fasano.
Mucosal Biology Research Center, University of Maryland, School of Medicine, Health Science Facility II, Baltimore, MD 21201.

Background: Shigellosis, a major form of bacillary dysentery, is caused by infection with *Shigella* organisms. In poor countries, *Shigella*-caused dysentery is endemic and causes an estimated 163 million illness episodes annually and more than one million deaths. The pathogenesis of *S. flexneri* is based on the bacteria's ability to invade and replicate within the colonic epithelium, which results in severe inflammation and epithelial destruction.

Aim: to study the intestinal mucosal biological effects triggered by wild-type *Shigella flexneri.* and vaccine strain CVD 1208S.

Methods: To model the interactions of *Shigella* with human intestinal mucosa, we have studied *Shigella flexneri* infection in human colonic cell line Caco2 by monitoring its effect on intestinal permeability and intestinal epithelial immune response.

Results: Inoculation of *Shigella* into human Caco2 cells caused severe mucosal damage, which was apparent as a drastic reduction of the transepithelial electric resistance (TEER) to basal level in less than 24 hours from infection. This decrease in epithelial permeability can be accounted for a breakdown of the tight junction integrity, as shown by immunofluorescence staining of infected cells in which we observed the disruption of tight junction components at the cell-cell boundary. Cell viability tests following *Shigella* infection indicate that the effects on epithelial barrier function induced by the bacteria are not caused by epithelial cell death. Infection of Caco2 cells with an attenuated vaccine strain of *Shigella* (CVD1208S) did not cause damage to the intestinal permeability barrier.

Conclusions: Collectively, our experiments support a model in which *S. flexneri* can interfere with the intestinal epithelium barrier function by disrupting the role of components of tight junctions. In addition, the preliminary data with CVD 1208S make this strain very attractive as a candidate vaccine.