Evaluation of epithelial functional and immune response to *Shigella flexneri* and its vaccine candidate infection*, in vitro*.

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Background: Shigellosis is a leading cause of diarrheal disease worldwide particularly in developing countries where it is estimates that 163 million cases with more than one million deaths occur annually. The pathogenesis of *S. flexneri* is based on the bacteria's ability to invade and replicate within the colonic epithelium, which results in severe intestinal inflammatory response and epithelial destruction. Vaccine development remains a high priority given the disease burden and increasing antibiotic resistance.
Aim: to determine 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. Indeed, infected cells showed the disruption of tight junction components at the cell-cell boundary. The effects on epithelial barrier function induced by the bacteria are not caused by epithelial cell death as determined by cell viability tests. *Shigella* infection of Caco2 cells induced the secretion of IL-8. Interestingly, heat-killed bacteria induced the strongest IL-8 production. The attenuated vaccine strain of *Shigella* (CVD1208S) elicited a pro-inflammatory response with no damage of the intestinal barrier integrity.
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.