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

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Shigella flexneri causes bacillary dysentery and is the major etiological agent of the endemic form of this disease, frequently affecting young children in developing countries.

It is responsible worldwide for an estimated 165 million episodes of shigellosis and 1.5 million deaths per year.
The bacterium is commonly found in water polluted with human feces. It is transmitted in contaminated food or water and through contact between people.

Upon infection, humans develop severe abdominal cramps, fever, and frequent passage of bloody stools.

Shigellosis is not only a significant cause of infant mortality in developing nations but maintains endemic levels of infection worldwide.
New treatments are needed for this highly infectious microbe because antibiotics are often inadequate and drug-resistant strains are on the rise.

Currently, no vaccines exist and the World Health Organization considers the development of a vaccine a priority.
The intestinal epithelium constitutes the largest and most important barrier against the external environment. It is equipped with multiple layers of innate defense systems and acts as a barrier against microbial invaders.

Despite the numerous host defenses, enteric pathogens are capable of circumventing the intestinal barrier function and can rather colonize within the epithelium.
Transcellular and paracellular fluxes are tightly controlled by membrane pumps, ion channels and tight junctions, adapting permeability to physiological needs.

Modulation of epithelial permeability properties is one of the common outcomes of bacterial infection of epithelial layers in vitro, and the in vivo correlates of these effects may induce or amplify diarrhea.
The aim of this study is to determine the intestinal mucosal biological effects triggered by wild-type *Shigella flexneri* and vaccine strain CVD 1208S.
Enterocyte-like cell line, Caco2, was infected with wild-type *Shigella flexneri* 2457T and vaccine strain CVD 1208S to evaluate initial host-pathogen interactions and the effect of exposure and colonization of these strains on mucosal barrier function.
Caco-2 cells (passages 23-30) were grown on transwells. Monolayers reached confluence in about one week with a baseline TEER between 750-1500 Ω.cm².

Cells were incubated with wild-type *S. flexneri* strain 2457T and mutant strains at different concentrations.

Changes in epithelial permeability were recorded and pro-inflammatory cytokine interleukin-8 (IL-8) was measured in culture supernatants.
CVD 1208S

- Live attenuated oral *Shigella* vaccine
- Deletions in genes regulating metabolic and virulence properties of wild type *S. flexneri* 2a strain 2457T
- Reconstructed from wt strain using animal-free media to comply with regulatory guidelines aimed to reduce the theoretical risk of transmissible BSE
S. flexneri effect on epithelial permeability is abolished by vaccine candidate CVD 1208S.
Changes in epithelial barrier permeability are independent of cell death.
*S. flexneri* causes disruption of tight-junctions and rearrangements of the cytoskeleton.
S. flexneri modulates Caco2 cells paracellular permeability

The vaccine candidate CVD 1208S does not elicit increased permeability.
S. flexneri induces an inflammatory response in infected Caco2 cells.

- Uninfected
- Conditioned media
- Heat Killed
- S. fl $10^7$
- 1208S $10^7$

IL-8
Inoculation of *Shigella* into human Caco2 cells caused severe mucosal damage, which was apparent as disruption of tight-junctions and loss of transepithelial resistance (TEER) in the absence of cell death.

Infection was associated with increased transport of dextran and BSA, indicating increased paracellular permeability.
*S. flexneri* induced a pro-inflammatory response in Caco2 cells. Data with heat killed bacteria showed that viable bacteria are not required to elicit an immune response.

Infection of Caco2 cells with an attenuated vaccine strain of *Shigella* (CVD 1208S) did not cause damage to the intestinal permeability barrier.
**CONCLUSIONS**

*S. flexneri* interferes with the intestinal epithelium barrier function by disrupting the role of components of tight junctions and inducing the release of pro-inflammatory immune molecules.

Preliminary data with CVD 1208S make this strain very attractive as a candidate vaccine.