Salmonella typhi induces increased mucosal permeability and elicits a strong pro-inflammatory response ameliorated by vaccine candidates *in vitro*

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INTRODUCTION

Typhoid fever is a common worldwide illness, transmitted by the ingestion of food or water contaminated with the feces of an infected person, which contain the bacterium *Salmonella typhi*.

The World Health Organization estimates that 16 million cases of typhoid fever occur annually, resulting in ~600,000 deaths.

In common with other enteropathogens, *Salmonella typhi* has developed means of breaching the mucosal epithelial barrier by usurping signaling mechanisms within host cells.

Infection is initiated in the intestinal tract, and severe disease causes widespread destruction of the intestinal mucosa.

Given the adverse consequences of *S. typhi* infection there is a critical need to understand the mechanisms of the disease process and the host immune response for the development of efficacious and cost effective vaccines.

THE INTESTINAL BARRIER

The intestinal epithelium constitutes the largest and most important barrier against the external environment.

It acts as a fence to prevent the passage of harmful intraluminal entities (foreign antigens, microorganisms and their toxins) and as a selective filter, allowing the translocation of essential dietary nutrients, electrolytes, and water from the intestinal lumen into the circulation.

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.

AIM

The aim of this study is to determine the effects of wild-type *Salmonella typhi* and vaccine strains, CVD 908-*htrA* and CVD 909, on the intestinal barrier function and immune response.

Methods

Enterocyte-like cell line, Caco2, was infected with wild-type *Salmonella typhi* and vaccine strains (CVD 908-*htrA* and CVD 909) to evaluate initial host-pathogen interactions and the effect of exposure and colonization of these strains on mucosal barrier function.

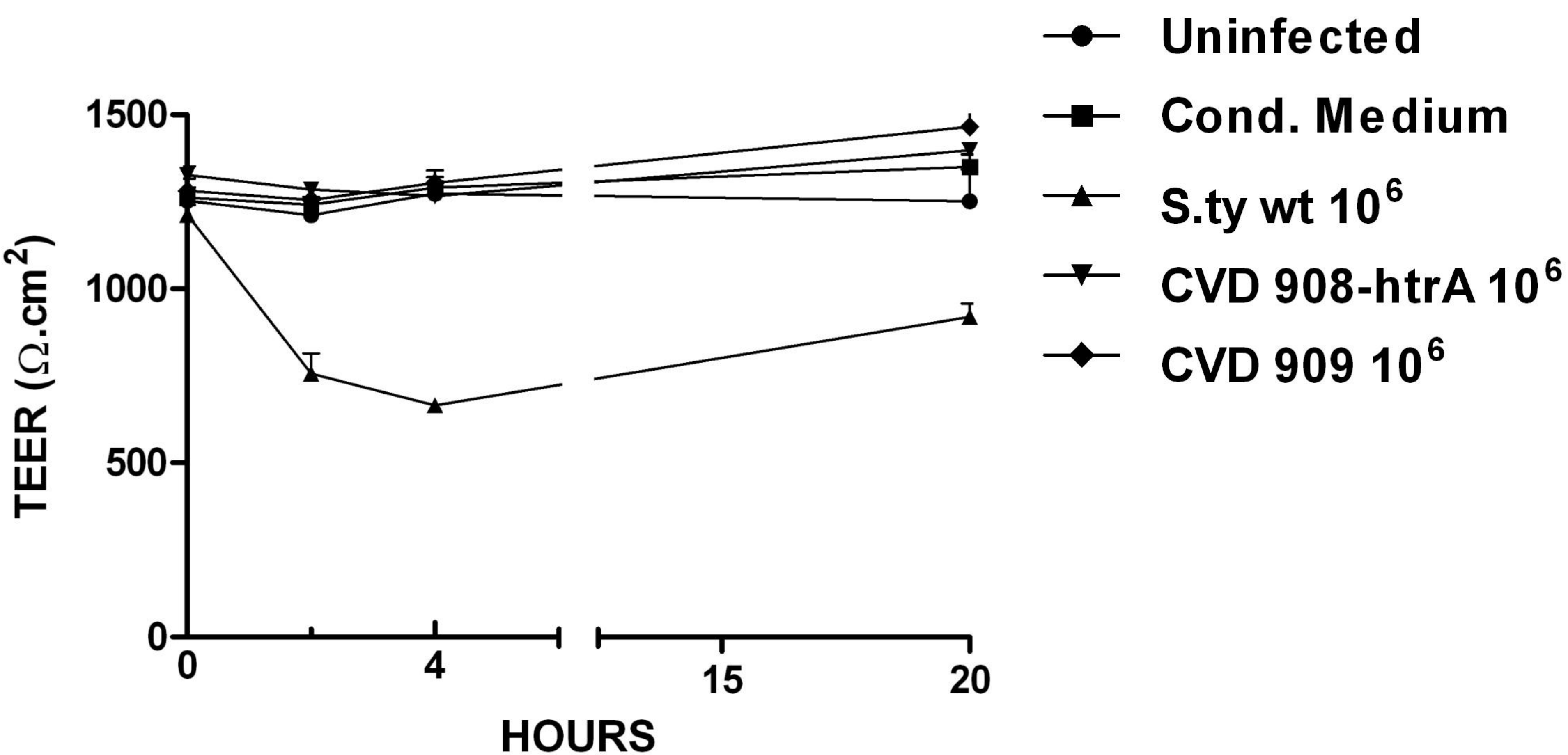
Caco-2 cells (passages 23-30) were grown on transwells. Monolayers reached confluence in about one week with a baseline TEER between 750-1500 $\Omega.cm^2$. Cells were incubated with wild-type *S. typhi* and vaccine candidate strains at different titers.

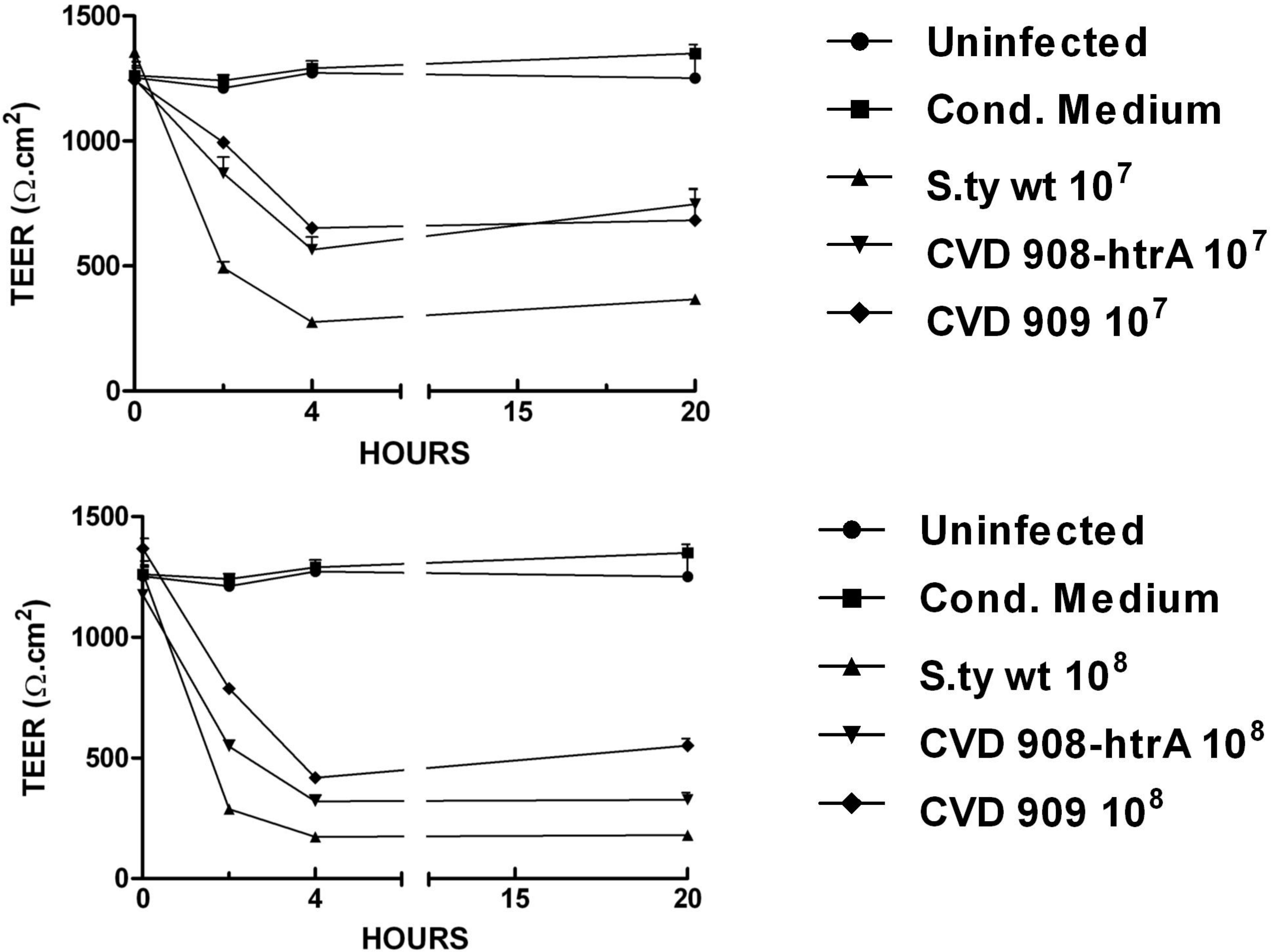
Changes in epithelial permeability were recorded and pro-inflammatory cytokines interleukin-8 (IL-8), interleukin-6 (IL-6) and TNF-α were measured in culture supernatants.

CVD 908-htrA/CVD 909

- Live oral typhoid vaccines
- Deletions in the genes of the pathway for the biosynthesis of aromatic metabolites
- Can't proliferate within mammalian cells
- CVD908-htrA bears a mutation in the *htrA* gene; when the gene is deleted the mutant becomes less virulent
- CVD909 constitutively expresses the Vi polysaccaride capsular antigen

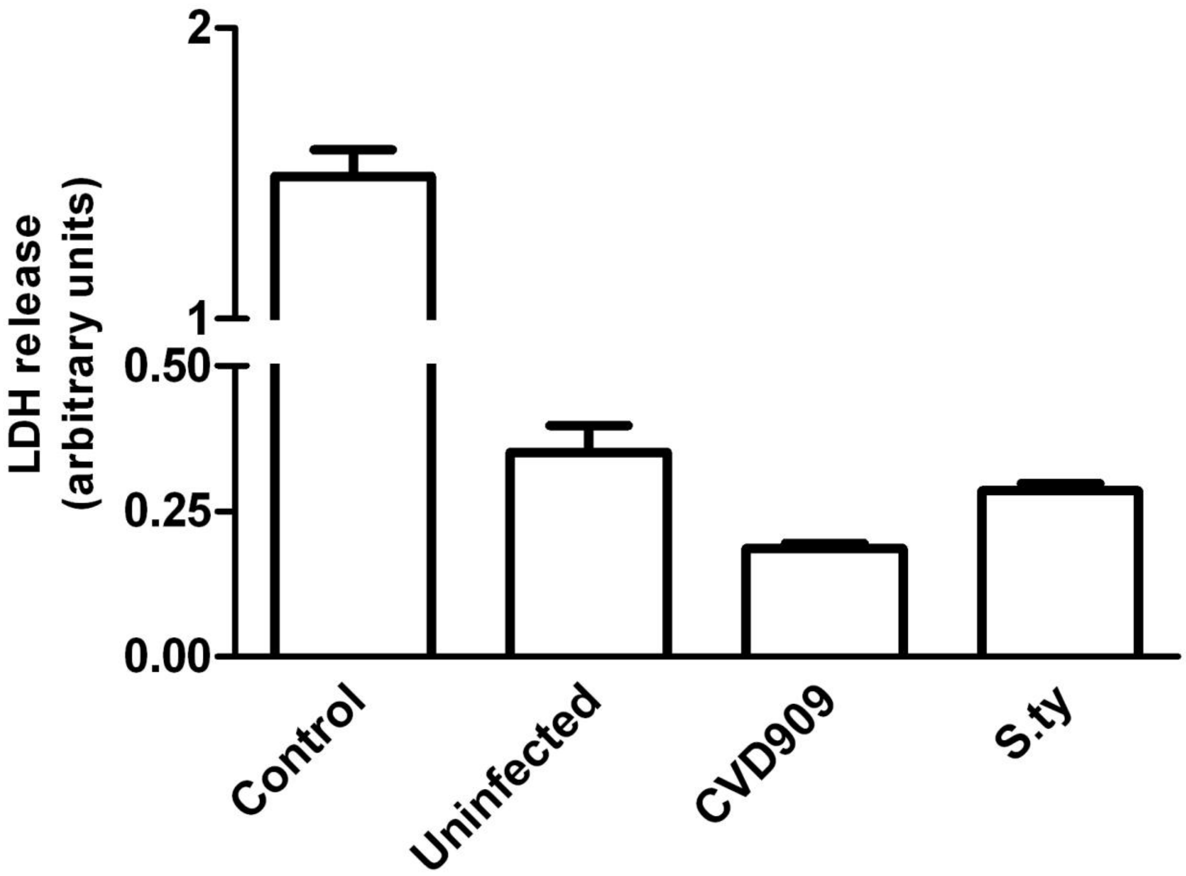
S. typhi dose-dependent increase of Caco2 monolayer permeability is ameliorated by vaccine candidates.





1500
1000
500
0
TEER (Ω.cm²)
0
4
15
20
HOURS
Uninfected
Cond. Medium
S.ty wt 10⁷
CVD 908-htrA 10⁷
CVD 909 10⁷
1500
1000
500
0
TEER (Ω.cm²)
0
4
15
20
HOURS
Uninfected
Cond. Medium
S.ty wt 10⁸
CVD 908-htrA 10⁸
CVD 909 10⁸

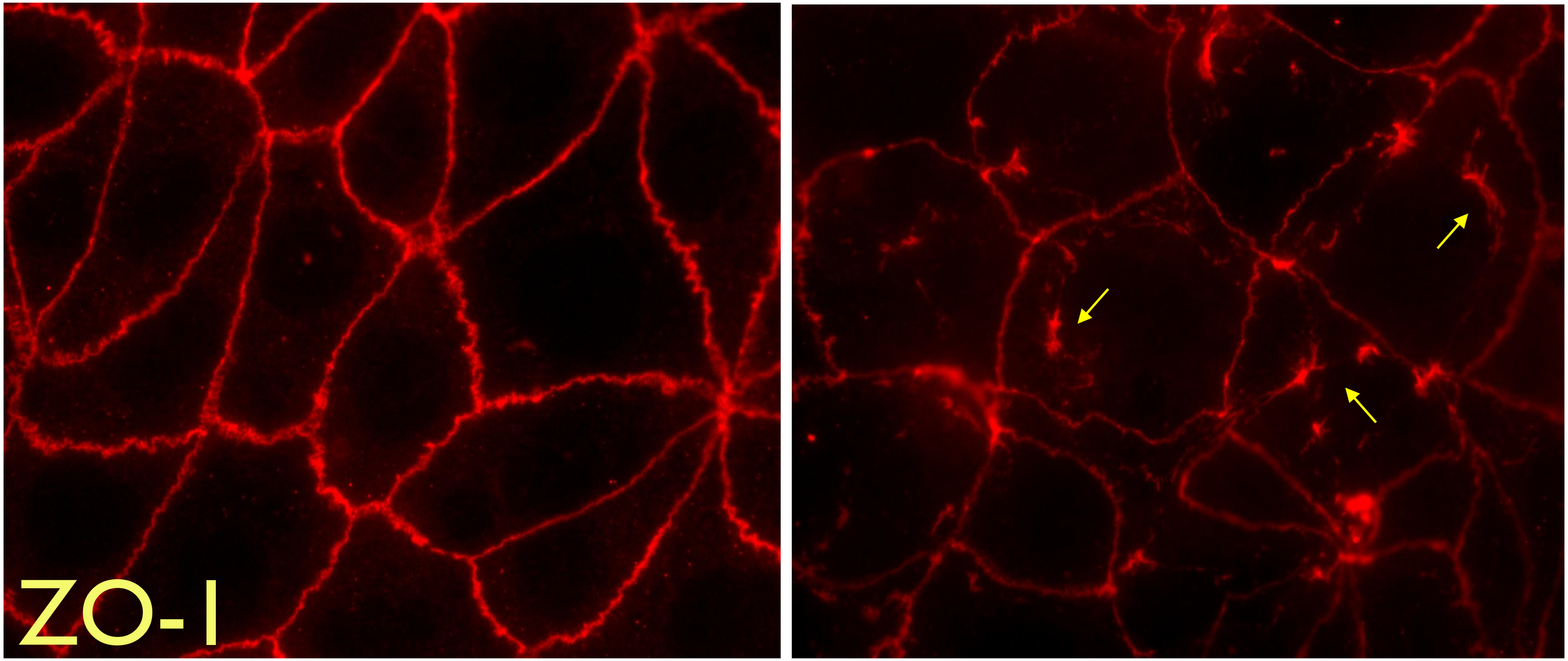
S.typhi-induced epithelial barrier function alterations are independent of cell death

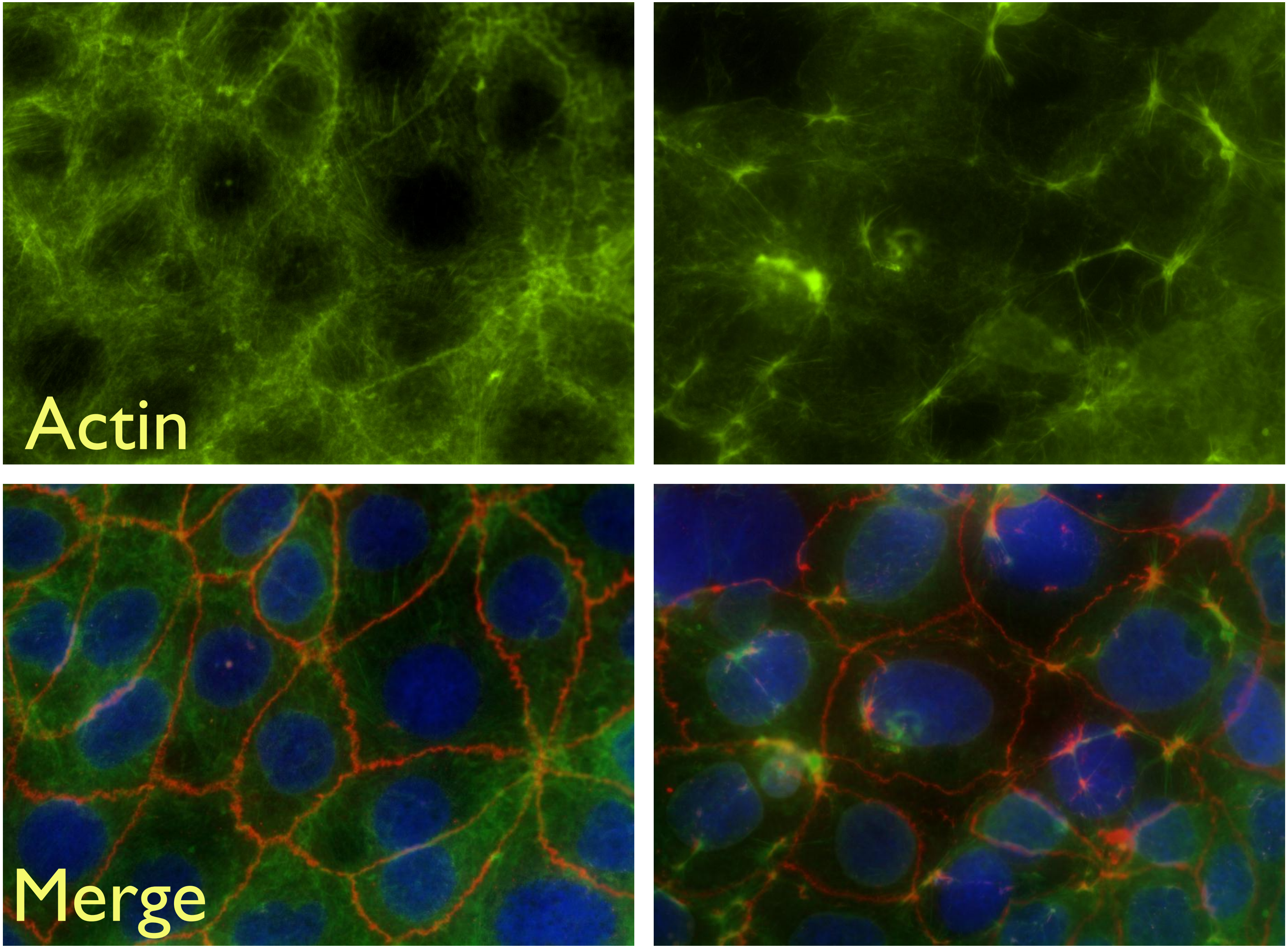


Changes in epithelial permeability are accounted for modulation of tight-junction barrier function

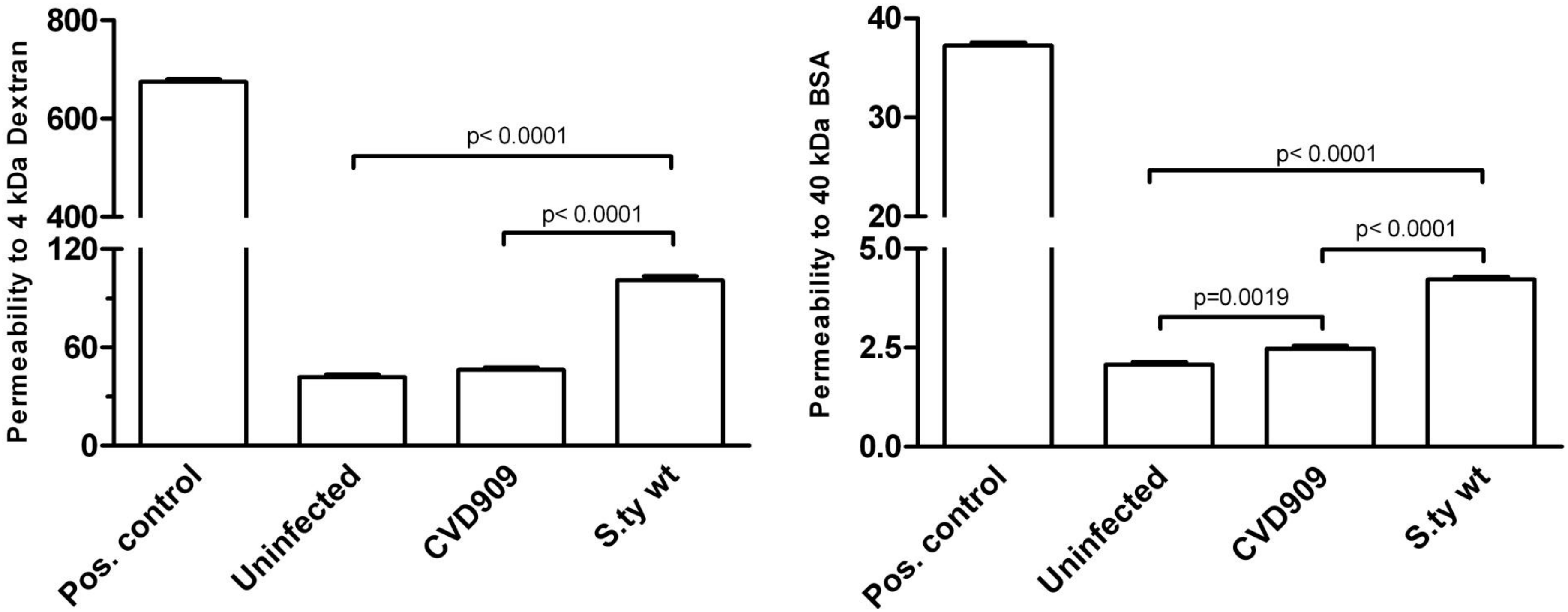
Control

Infected



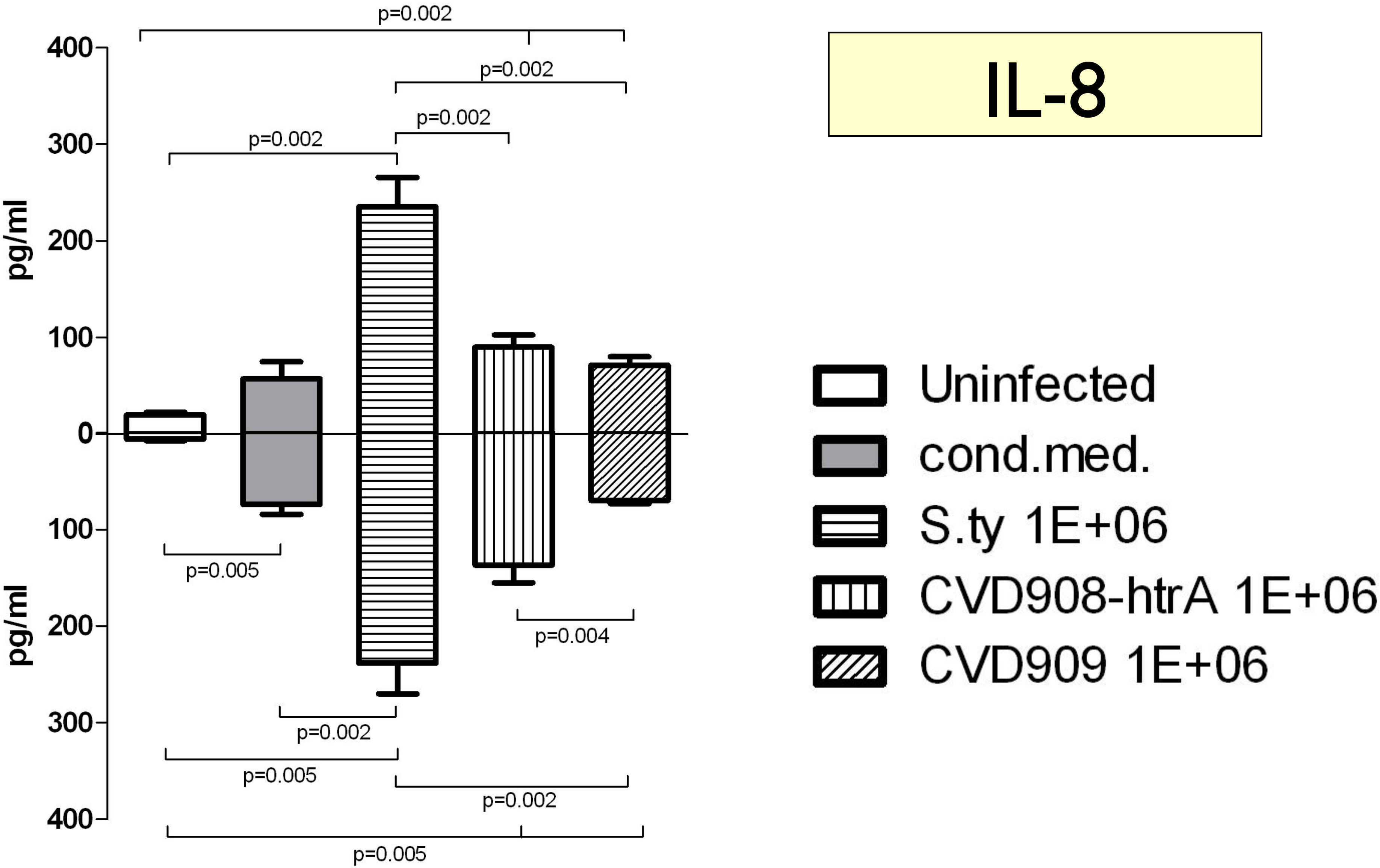

Actin
Merge

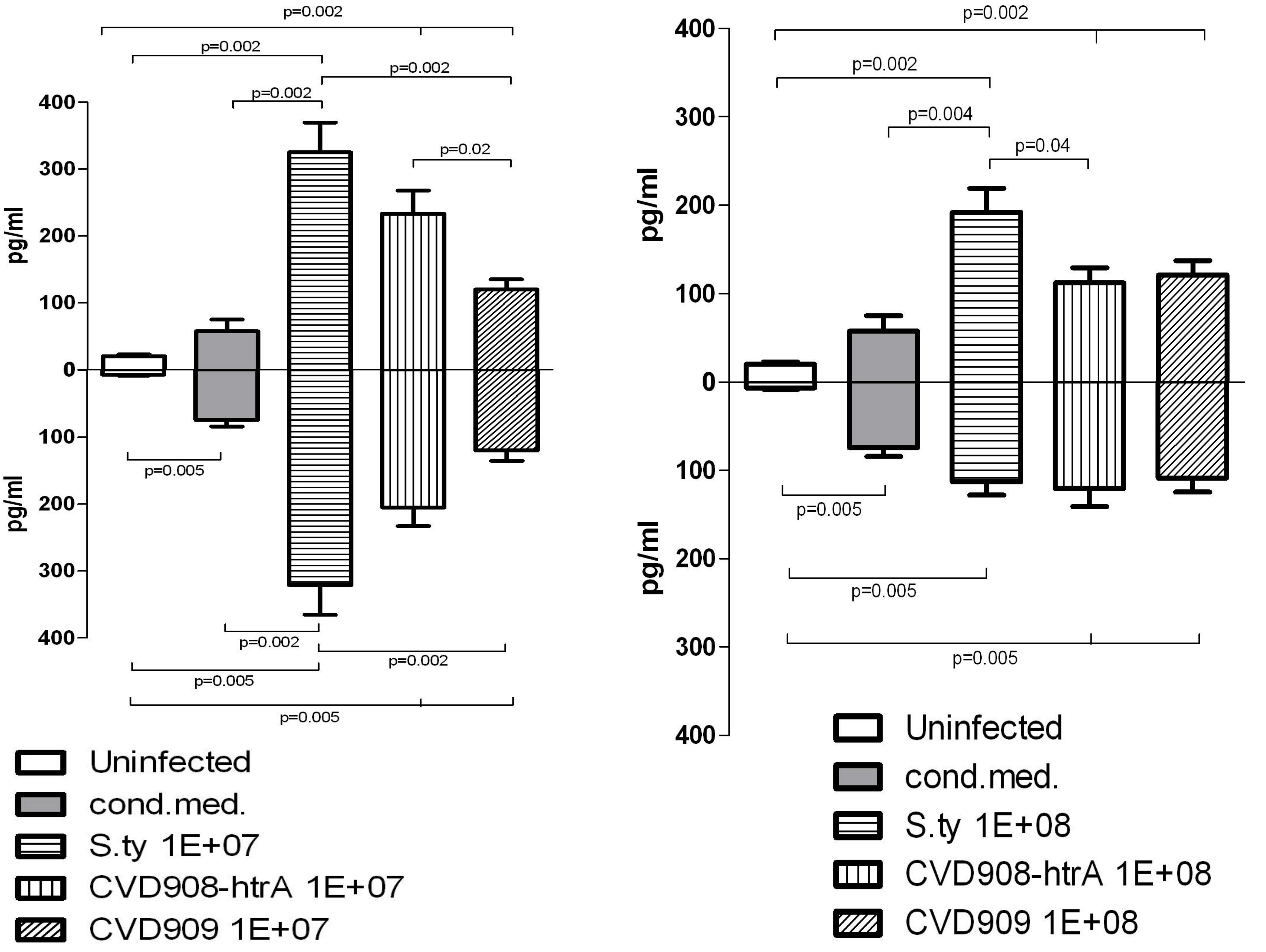
S. typhi increases the paracellular flux in Caco2 cell monolayers



The vaccine candidate CVD 909 does not elicit increased permeability

S. typhi triggers the release of pro-inflammatory cytokines




p=0.002
p=0.002
p=0.002
p=0.002
p=0.02
400
300
200
100
0
100
200
300
400
pg/ml
pg/ml
p=0.005
p=0.002
p=0.002
p=0.005
p=0.005
Uninfected
cond.med.
S.ty 1E+07
CVD908-htrA 1E+07
CVD909 1E+07
p=0.002
p=0.002
p=0.004
p=0.04
400
300
200
100
0
100
200
300
400
pg/ml
pg/ml
p=0.005
p=0.005
p=0.005
Uninfected
cond.med.
S.ty 1E+08
CVD908-htrA 1E+08
CVD909 1E+08

SUMMARY

Caco2 cells infected with wild-type *Salmonella enterica serovar typhi* exhibited a rapid decrease in transepithelial electrical resistance (TEER), marked changes in tight junction proteins organization and an increase in the paracellular flux of dextran and BSA as early as 4h post-infection.

Cell viability tests showed that barrier function disruption was not associated with cell death caused by bacterial infection.

S. typhi induced an immune response in Caco2 cell monolayer with the release of the pro-inflammatory cytokines IL-8. IL-6 and TNF-α.

Infection of Caco2 cells with the new oral vaccine strains, CVD 908-*htrA* and CVD 909, showed that both strains, but in particular CVD909, elicit a weaker immune response with minimal disruption of the barrier integrity, and hence offers reason for optimism for vaccine development.

CONCLUSIONS

Salmonella typhi causes specific and transient intestinal mucosal epithelial events, including changes in TEER and cytokine production that seem to be ameliorated by the engineering of specific vaccine candidates.