These findings suggest the involvement of EGC in Division of Infectious Diseases and Enteric glial cells (EGCs) regulate neuron function and increased risk of functional diarrhea has been reported in patients after C. difficile infection (CDI).

The goal of this study was to investigate the effects TcdA and TcdB on the S100B/RAGE/NFκB pathway in EGCs and their role in the pathogenesis of CDI.

### Methods

**a) In vitro**

- Cell viability (MTT)
- Morphology cell
- Gene expression of glial factors (GDNF, GFAP and S100B) and IL-6 and RAGE.
- Western Blot and ELISA for S100B (immunofluorescence for NFκB, RAGE and IL-6) and TcdA and TcdB-induced S100B, IL-6 and RAGE.
- Activation of NFκB (immunofluorescence for NFκB p65).

Investigating the mechanism involved in the TcdA and TcdB-induced S100B and IL-6 expression on EGCs:
- FPZ-ZM1 (RAGE antagonist)
- Gallelacatone (STAT3 inhibitor)
- LY 294002 (PI3K inhibitor)
- Antibiotic cocktail (vancomycin, gentamicin, metronidazole, clindamycin)

**b) In vivo**

- C57BL/6 mice 8 weeks old
- Swiss mice 8-9 weeks old
- Euthanasia

<table>
<thead>
<tr>
<th>Antibiotic cocktail</th>
<th>After 0.5, 2, 6, 12, 18, 24 or 48h</th>
<th>TcdA (10, 50 and 100 ng/mL) or TcdB (0.1, 1 and 10 ng/mL)</th>
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<tbody>
<tr>
<td>Toxin A (50 μg/ileal loop) or PBS alone</td>
<td>1h</td>
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**Results**

**Cell viability**

- **RAGE gene expression**
- **IL-6 gene expression**
- **S100B gene expression**

**S100B extracellular**

**S100B intracellular**

**GFAP gene expression**

**GDNF gene expression**

**Conclusion**

- TcdA and TcdB induce EGC morphology changes, loss of viability, and release of S100B, cause NFκB activation and alters expression of glial factors (GDNF and GFAP), RAGE and IL-6 in vitro and in vivo.

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