

THE GRADUATE COLLEGE OF THE
UNIVERSITY OF OKLAHOMA HEALTH SCIENCES CENTER

ANNOUNCES THE FINAL EXAMINATION OF

Sarah Jane Bland

FOR THE DEFENSE OF THE DOCTOR OF PHILOSOPHY DEGREE GRADUATE
COLLEGE

Department of Microbiology and Immunology

Location: [http://ouhsc.zoom.us/j/92526133298?pwd=NINSeXByUmo
xSDZWdFVRdFJmZWVRGZz09](http://ouhsc.zoom.us/j/92526133298?pwd=NINSeXByUmo
xSDZWdFVRdFJmZWVRGZz09)

Password: 4jysl@Ns

Thursday, July 23, 2020, 10:00 am



*Characterization of factors influencing
cytotoxicity, structural stability, and receptor
binding of Clostridioides difficile toxin B*

COMMITTEE IN CHARGE: Jimmy Ballard,
PhD, Chair; Rodney Tweten, PhD; Mark Lang,
PhD; Ann West, PhD; David Dyer, PhD

ABSTRACT: *Clostridioides difficile* infection is a hospital and community acquired diarrheal disease that affects over half a million people and leads to nearly 30,000 deaths annually in the United States. Disease symptoms are mediated by the two major virulence factors of *C. difficile*, toxins A and B (TcdA and TcdB), with TcdB thought to be the primary virulence factor in humans. The hypervirulent toxin variant, TcdB2, exhibits differences in activity and receptor specificity when compared with TcdB1. A hypervariable region showing significant amino acid differences between TcdB1 and TcdB2, comprised of residues 1753-1852, is suggested to be vital for the intoxication process. Additional residue differences are observed in the TcdB2 binding region for the proposed physiological receptor.

The significance of the residue differences present in the hypervariable region and receptor binding regions of TcdB2 is not well understood. Interestingly, TcdB2 exhibits increased cytotoxicity when compared with TcdB1, despite an apparent lack of interaction with the primary receptor, Frizzled. TcdB2 instead interacts with CSPG4, a toxin receptor that is not highly expressed in the colonic epithelium. Recent reports have suggested CSPG4 expression varies depending upon the state of the microenvironment, and is influenced by inflammatory responses. Based on this data, we hypothesized that specific sequence variations in the hypervariable region of TcdB2 impact cellular interaction and intoxication, and that CSPG4 expression plays a role in the increased cytotoxic activity exhibited by TcdB2.

To address these hypotheses, we began by making targeted mutations in the hypervariable region, and discovered a particular mutant, TcdB2 $\Delta_{1769-1787}$, which showed a complete loss of cytotoxic activity and did not bind to cells. TcdB2 $\Delta_{1769-1787}$ also exhibited spontaneous auto-processing, indicating the mutant exists in a conformation that allows the active conformer of the autoprocessing domain to form. We showed the mutant TcdB2 $\Delta_{1769-1787}$ generated a protective immune response in a murine model of CDI when used as a vaccine candidate, suggesting the deletion mutant maintains important structural epitopes. Our studies further confirmed differences in receptor specificity between full-length TcdB1 and TcdB2, and support the idea that CSPG4 plays a larger role during intoxication than previously believed. Our data shows that cells made resistant to TcdB2 showed an almost complete loss of CSPG4 expression, while remaining susceptible to TcdB1, whereas cells overexpressing CSPG4 were found to be more susceptible to intoxication with TcdB2 than TcdB1. We report the novel finding that acute exposure to TcdB leads to increased expression of CSPG4, and toxin treated cells exhibit a higher a level of toxin binding than untreated cells. Importantly, we also show treatment of a colonic cell line with an inflammatory cytokine leads to increased CSPG4 expression. Collectively our findings indicate that the 1769-1787 region of the toxin is intimately important for cytotoxicity and maintaining structural stability in the toxin, and suggest CSPG4 plays an important role in TcdB2-mediated cell intoxication.