Humoral immunity following *Clostridioides difficile* infection and mechanisms of protection against disease

**COMMITTEE IN CHARGE:** Mark Lang, PhD, Chair; Jimmy Ballard, PhD; Lauren Zenewicz, PhD; Susan Kovats, PhD; Karla Rodgers, PhD.

**ABSTRACT:** The intracellularly active bacterial toxin, TcdB, is a major *Clostridioides difficile* virulence factor that contributes to inflammation and tissue damage during disease. Despite *C. difficile* causing an enteric disease, systemic TcdB-specific IgG is the best-known correlate of protection against infection-associated pathology. Immunization with an inactive TcdB fragment prevents *C. difficile* infection (CDI)-associated pathology. The protective immune response against inactive TcdB involves development of antigen-specific memory B cells and long-lived PC that encode TcdB-neutralizing antibodies.
Unlike the response to inactive TcdB, very little is known about the host humoral immune response to *C. difficile* and TcdB during primary and recurrent infection. In addition, whether the mechanism by which systemic IgG is delivered to the gut depends on specific receptor-mediated transport or is reflective of infection-induced damage to the gut remains unclear.

Herein, we used a murine model of recurrent *C. difficile* disease to demonstrate that an initial infection induces a serum IgM and mucosal IgA response against the toxin but a low serum IgG response, and this is associated with a lack of protection against disease during reinfection. Infection induced a partial expansion of the T follicular helper cell compartment, essential for B cell memory responses. Consistent with that, it failed to expand the memory B cell compartment. Furthermore, infection failed to stimulate a recall antibody response in pre-immunized mice although they were protected against associated disease.

We also tested the hypothesis that the neonatal Fc receptor (FcRn) is required for delivery of TcdB-neutralizing IgG to the gut and subsequent protection against *C. difficile*-associated pathology. FcRn-expressing mice and FcRn-deficient littermates were immunized subcutaneously with Alhydrogel adjuvant-adsorbed TcdB-based immunogen before challenge with live *C. difficile* spores. We show that FcRn was required for delivery of TcdB-specific IgG to the gut, and for vaccine-induced protection against *C. difficile* associated disease. The lack of FcRn expression had minimal effects on composition of the gut microbiome and did not affect susceptibility to *C. difficile* infection in non-immunized mice. Moreover, our data suggest that intraperitoneal injection of immune sera partially protect mice against disease and may bypass the requirement for FcRn.

These studies delineate the key humoral immune events that follow primary and recurrent *C. difficile* infection and provide a compelling inverse correlation between B cell memory and disease recurrence. They also elucidate a previously unappreciated mechanism by which immunization-induced systemic IgG protects the gut during enteric *C. difficile* infection. These findings may be beneficial for targeting of *C. difficile* -specific IgG to the gut.