THE HARTWELL FOUNDATION

2021 Individual Biomedical Research Award

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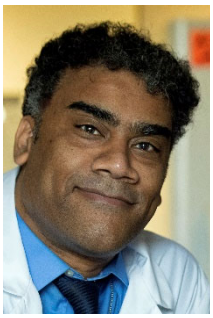

Engineering Protozoa to Prevent and Treat *C. difficile* Infection

Clostridioides difficile (*C. difficile*) infection (CDI) is a disturbingly common gastrointestinal disease in children. A toxin-producing bacteria called *C. difficile* causes symptoms that can range in severity from acute, self-limiting diarrhea to life-threatening colon damage. The virulence of the pathogen is the result of two major toxins, A and B, secreted by the bacteria and that disrupt the lining of the gut. Approximately 24,000 children in the United States suffer from a primary CDI infection every year, and the incidence is rising. Though the clinical course of the disease is typically milder in children than adults, for about 25% of affected children the recurrence of CDI causes significant disruption and contributes to clinically relevant complications that may include fever, dehydration, dangerously low blood pressure, kidney failure, weight loss, ruptured colon, bowel perforation, sepsis, and in rare cases death. There is no vaccine available for *C. difficile* prevention and approved treatment options are limited, including supportive care like aggressive rehydration, removal of adjunct antibiotic therapy, and initiation of effective anti-CDI measures. Children with medical comorbidities such as previous antibiotic use, immunosuppression, inflammatory bowel disease, and cystic fibrosis, among others, are at an increased risk of recurrent CDI. Preferred anti-CDI measures most often include evidence-based use of metronidazole and vancomycin, antibiotics that have limited activity against normal gut flora but potent activity against *C. difficile*. Fecal microbiota transplantation, probiotics, and a neutralizing antibody that blocks a toxin associated with CDI, have achieved variable success but depend on external conditions and the health of the patient. For a small subset of children, recurrent CDI is incurable. To address the need for a treatment option that will effectively reduce disease severity and decrease the incidence of relapse, I propose a drug delivery platform based upon a harmless unicellular protozoa that can survive as an intestinal parasite. Based upon my discovery that Entamoeba protozoa can be genetically engineered to secrete antibodies capable of neutralizing C. difficile toxin B, I propose to genetically engineer a harmless non-pathogenic form of the protozoa, which when introduced into the gut lumen of a C. difficile infected host will constitutively secrete human antibodies to neutralize the presence of both potent C. difficile toxins. I hypothesize that delivery of anti-toxin antibodies into the gut by this approach will offer effective protection against the debilitating recurrence of CDI and limit devastating complications. To engineer Entamoeba protozoa, I will use CRISPR/Cas9 technology to edit genomic DNA and express a known single-chain variable fragment that recognizes the desired toxin. The safety and efficacy of this drug delivery technology will be evaluated in vivo using an established antibiotic mouse model of CDI and amebic co-infection. If my approach to manage CDI is successful, clinical translation will improve health outcomes and the quality of life of the many children who endure this disease.