

ESTABLISHMENT OF A NON-HUMAN PRIMATE *CAMPYLOBACTER* DISEASE MODEL PRIOR TO THE PRE-CLINICAL EVALUATION OF *CAMPYLOBACTER* VACCINE FORMULATIONS

Islam D, Lewis MD, Bodhidatta L, Scott D, Baqar S and Mason CJ

Background: *Campylobacter jejuni* is a common cause of enteritis worldwide and a threat to international travelers and deployed U.S. forces. The clinical presentation of *Campylobacter* enteritis can range from a mild, watery diarrhea to a dysenteric-like disease with frank blood. Some strains are capable of eliciting a post-infectious polyneuropathy called Guillain Barre Syndrome (GBS). Challenge studies in humans are unethical due to the possibility of severe infection such as GBS. *Campylobacter* infection in non-human primates closely mimics the disease and immune response, seen in humans and it is believed that such a model would be more predictive of human outcomes. The *Macaca mulatta* are quite susceptible to *Campylobacter* infection, develop a human-like disease. Experimental infection in infant non-immune macaques elicits clinical signs of colitis with watery and occasionally bloody diarrhea. In this study, we attempted to determine the minimal dose of a pathogenic *Campylobacter jejuni* (CJ) 81-176 strain required to establish clinical signs and symptoms of disease in *M. mulatta* monkeys using an escalating dosage.

Methods: Monkeys were intragastrically challenged with CJ 81-176 to establish minimal dose required for infection ($\geq 80\%$ attack rate). Three groups of 10 monkeys were used sequentially starting with challenging one group with the smallest dose (10^7 ; group-1), followed by 10^9 (group-2) and the third group with 10^{11} (group-3). A control group of 5 monkeys was included. Fecal excretion of *C. jejuni* was monitored by obtaining fresh stool twice. Blood was drawn on challenge day (day 0) and thereafter on days 7, 14 and 28 for IgA and IgG titers (humoral immunity) and antibody secreting cell (ASC) assays with peripheral blood mononuclear cells (cell-mediated immunity). Fecal secretory-IgA was also measured. A number of clinical parameters (activity, appetite, stool consistency, mucous in stool, blood in stool, dehydration status, body weight, body temperature) were observed to measure clinical ranking of the monkeys. Clinical severity was determined bases on total clinical score.

Results: Eighty percent of the monkeys in group 2 and group 3 and only 30% of group 1 had mild diarrhea. Clinically all monkeys except one in group 1 was normal. All challenged monkeys shed *C. jejuni* 81-176 until day 13. *Campylobacter* antigen specific fecal s-IgA responses were observed in all three groups but the response was not dose-dependent. *Campylobacter* antigen specific ASC response is low in all challenged monkey groups. *Campylobacter* antigen specific plasma IgA response is dose-dependent, it is 80% in group 3 and 20% in group 1. In the control group, there was no shedding of *C. jejuni* and there was no plasma titer response, ASC response and fecal s-IgA response.

Conclusion: Elicited immune response (mainly plasma IgA) in three groups of rhesus monkeys was dose-dependent, indicating this monkey model can be used for pre-clinical evaluation of *Campylobacter* candidate vaccines. Although infectivity was low in those adult rhesus monkeys,

even the highest dose (10^{11}) was not able to make them clinically sick. In the future young naïve rhesus monkeys can be used for infectivity study and pre-clinical evaluation of *Campylobacter* vaccine formulations.

Vaccines for Enteric Diseases -- VED 2004 Conference. Montego Bay, Jamaica. 28-30 April 2004. (Poster)

FUNCTIONAL PIGLET MODEL FOR THE CLINICAL SYNDROME AND POSTMORTEM FINDINGS INDUCED BY STAPHYLOCOCCAL ENTEROTOXIN B

van Gessel YA, Mani S, Bi S, Hammamieh R, Shupp JW, Das R, Coleman GD and Jett M.

Staphylococcal enterotoxin (SE) B causes serious gastrointestinal illness, and intoxication with this exotoxin can lead to lethal toxic shock syndrome. In order to overcome significant shortcomings of current rodent and nonhuman primate models, we developed a piglet model of lethal SEB intoxication. Fourteen-day-old Yorkshire piglets were given intravenous SEB, observed clinically, and sacrificed at 4, 6, 24, 48, 72, or 96 hrs posttreatment. Clinical signs were biphasic with pyrexia, vomiting, and diarrhea within 4 hrs, followed by terminal hypotension and shock by 96 hrs. Mild lymphoid lesions were identified as early as 24 hrs, with severe lymphadenopathy, splenomegaly, and prominent Peyer's patches found by 72 hrs. Widespread edema-most prominent in the mesentery, between loops of spiral colon, and in retroperitoneal connective tissue-was found in animals at 72 hrs. Additional histologic changes included perivascular aggregates of large lymphocytes variably present in the lung and brain, circulating lymphoblasts, and lymphocytic portal hepatitis. Preliminary molecular investigation using gene array has uncovered several gene profile changes that may have implications in the pathophysiology leading to irreversible shock. Five genes were selected for further study, and all showed increased mRNA levels subsequent to SEB exposure. The use of this piglet model will continue to elucidate the pathogenesis of SEB intoxication and facilitate the testing of new therapeutic regimens that may better correlate with human lesions.

Exp Biol Med (Maywood). 2004; 229(10): 1061-71.

IDENTIFICATION AND CHARACTERIZATION OF ENTERIC PATHOGENS ISOLATED FROM CASES OF ACUTE DIARRHEA, ASYMPTOMATIC CONTROLS AND RETAIL FOOD IN A THAI VILLAGE

Bodhidatta L, McDaniel P, Mason CJ, Srijan A, Bangtrakulnonth A and Wongstitwilairung B

Background: The description of enteric pathogens isolated from environmental sources and from humans is an alternative approach to determine the transmission of diarrheal disease. This study microbiologically identifies and characterizes enteric pathogens isolated from children with and without diarrhea and uncooked food in a village in Thailand.