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Safety and tolerability of a live oral *Salmonella typhimurium* vaccine candidate in SIV-infected nonhuman primates

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ABSTRACT

Nontyphoidal *Salmonella* (NTS) serovars are a common cause of acute food-borne gastroenteritis worldwide and can cause invasive systemic disease in young infants, the elderly, and immunocompromised hosts, accompanied by high case fatality. Vaccination against invasive NTS disease is warranted where the disease incidence and mortality are high and multidrug resistance is prevalent, as in sub-Saharan Africa. Live-attenuated vaccines that mimic natural infection constitute one strategy to elicit protection. However, they must particularly be shown to be adequately attenuated for consideration of immunocompromised subjects. Accordingly, we examined the safety and tolerability of an oral live attenuated *Salmonella typhimurium* vaccine candidate, CVD 1921, in an established chronic simian immunodeficiency virus (SIV)-infected rhesus macaque model. We evaluated clinical parameters, histopathology, and measured differences in mucosal permeability to wild-type and vaccine strains. Compared to the wild-type *S. typhimurium* strain I77 in both SIV-infected and SIV-uninfected nonhuman primate hosts, this live-attenuated vaccine shows reduced shedding and systemic spread, exhibits limited pathological disease manifestations in the digestive tract, and induces low levels of cellular infiltration in tissues. Furthermore, wild-type *S. typhimurium* induces increased intestinal epithelial damage and permeability, with infiltration of neutrophils and macrophages in both SIV-infected and SIV-uninfected nonhuman primates compared to the vaccine strain. Based on shedding, systemic spread, and histopathology, the live-attenuated *S. typhimurium* strain CVD 1921 appears to be safe and well-tolerated in the nonhuman primate model, including chronically SIV-infected rhesus macaques.

1. Introduction

Nontyphoidal *Salmonella* (NTS) serovars are common causes of acute food-borne diseases worldwide. In immunocompetent individuals, the organism remains localized in the intestine, causing a self-limited gastroenteritis [1]. However, in immunocompromised individuals, infants, and the elderly, NTS pathogens can disseminate, resulting in potentially fatal bacteremia if untreated [2–4]. NTS bacteremia in sub-Saharan Africa has dramatically increased [5], emerging as a leading cause of hospital admission for adults, the majority of whom are HIV-infected [6–8]. With a high acute mortality rate of 47% [9], NTS and HIV coinfection poses a major challenge for HIV-infected African adults and children. Invasive NTS infections also constitute a significant health problem in sub-Saharan countries with low HIV prevalence [10,11]. The majority (~80–95%) of NTS from invasive disease in sub-Saharan Africa are *S. typhimurium* (or monophasic variants) or *S. enteritidis* [12].

Vaccination has been proposed as a strategy to control the spread and severity of NTS in Africa [12–14]. An effective live oral vaccine is licensed for use against typhoid fever [15], and

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recent studies in Malawi indicate the potentially protective role of antibodies against NTS in HIV-negative children [16,17] and HIV-infected adults [18]. A similar vaccine is feasible against NTS for which no licensed vaccines are available. Live-attenuated *S. typhimurium* vaccine strain CVD 1921, developed by the Center for Vaccine Development, was derived from a clinical isolate recovered from the blood of a 17-month-old toddler (HIV-status unknown) in Mali, West Africa and was attenuated by introducing deletions in *guaBA* (encodes guanine biosynthesis) and *clpP* (encodes the ClpPX protease that degrades the master flagellum regulator complex FlhD/FlhC) [14].

Here, we describe a proof-of-principle study that establishes the safety and tolerability of a live oral *S. Typhimurium* vaccine compared to wild-type *S. Typhimurium* infection in an SIV-infected rhesus macaque (RM) model. Although live *Salmonella* vaccines are not intended for HIV-infected individuals, demonstrating their safety in immunocompromised hosts is prudent given the high number of undetected HIV infections in the vaccine target population [19–21]. We evaluated clinical signs of disease including diarrhea and dehydration, and assessed the extent of shedding and systemic infection by detecting *Salmonella* organisms in tissues and feces. To determine the extent of mucosal barrier degradation, we measured lipopolysaccharide (LPS) and soluble CD14 (sCD14) plasma levels. Gastrointestinal damage was examined by histopathological analysis of intestinal tissue sections stained with hematoxylin and eosin (H&E) stain and immunohistochemistry.

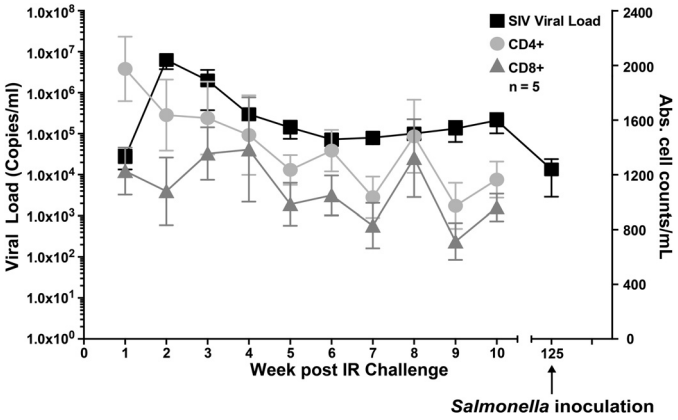

Fig. 1. Plasma SIV RNA levels, CD4, and CD8 cell counts after SIV infection. Five rhesus macaques were intrarectally (IR) challenged with SIVmac251. Mean plasma SIV RNA levels were measured by RT-PCR, and CD4 and CD8T cell counts were determined weekly for 10 weeks post-SIV infection. Plasma SIV RNA levels were determined immediately prior to *Salmonella* inoculation.

2. Materials and methods

Bacterial strains and culture conditions. *S. typhimurium* I77 (multi-locus sequence type ST19) is an invasive antibiotic-susceptible strain [22]. CVD 1921, a live-attenuated vaccine derived from strain I77 [14], harbors deletions in the *guaBA* and *clpP* loci. *Salmonella* strains were maintained on animal product-free Lennox media (Athena Environmental Sciences, Baltimore, MD, USA). For serum bactericidal assays, the invasive strain *S. typhimurium* D65 (ST313) was grown on animal product-free HS media at 37 °C. *guaBA* mutants were grown on media containing 0.005% guanine. The identity of *S. typhimurium* was confirmed by agglutination with antisera (Denka Seiken LTD, Japan, and Sifin Inst. Berlin, Germany) and/or O grouping and H typing PCRs [11] and differentiation between the wild-type strain and the vaccine strain was determined by growth on minimal medium [14,23] containing or lacking guanine, and PCR using primers that amplify the *guaBA* and *clpP* deletions.

Animals and experimental design. Animal use was approved by both the Vaccine Research Center Animal Care and Use Committee, and the holding facility's Institutional Animal Care and Use Committee (Bioqual Inc.) in accordance with the American Association for Accreditation of Laboratory Animal Care guidelines, all federal, state, and local regulations, and in compliance with *The Guide for the Care and use of Laboratory Animals*. Eleven adult Indian-origin *Salmonella*-free RMs (*Macacca mulatta*) were used, including six SIV-uninfected, and five with chronic SIVmac251 infection, confirmed by quantitative reverse transcription-polymerase chain reaction (RT-PCR) plasma viral load analysis with CD4 and CD8 cell counts [24] (Fig. 1). SIV-infected RMs did not exhibit overt SIV-related illness (e.g. SIV-related hepatitis, brain issues, giant cell pneumonia, etc.). These RMs were selected to reflect a chronic stage of SIV infection to simulate persistent HIV infections in humans that have not necessarily progressed to AIDS or whose illness has not been exacerbated by secondary infections.

RMs were inoculated orally with 5×10^9 Colony Forming Units (CFUs) of wild-type *S. typhimurium* I77 or with 10^{10} CFUs of the vaccine strain CVD 1921. Blood was collected on Days 0, 1, 4, 7, 10 and 12/13 to measure immune responses, and rectal swabs or fresh fecal samples were collected on Days 0–8, 10, and 12/13 to determine fecal shedding (Fig. 2). Following euthanasia on Day 12/13, sterile swabs and tissues were collected to assess the presence of bacteria and histopathology associated with vaccination and wild-type infection. Clinical observations for dehydration, diarrhea, demeanor, and food/water consumption were recorded daily. Health assessments of animals on Days 0, 1, 4, 7 and 10 included temperature, pulse, respiration, and weight.

Qualitative detection of S. typhimurium in tissues. Sterile tissue swabs of colon, small intestine, mesenteric lymph nodes, spleen, liver, and kidneys were collected, at the time of necropsy. Samples were inoculated onto selective and nonselective agar media and enrichment broths and incubated for 24–48 h at 37 °C. Specifically,

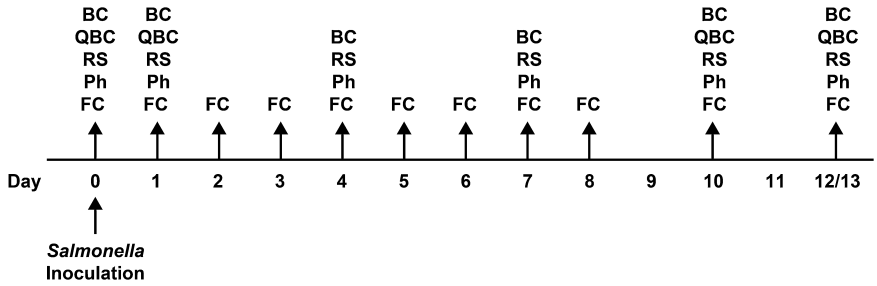

Fig. 2. Experimental timeline to evaluate the effect of *S. typhimurium* vaccine strain CVD 1921 versus wild-type *S. typhimurium* I77 in SIV-infected and uninfected macaques. At day 0, *S. typhimurium* was inoculated in four groups of rhesus macaques: (1) SIV+, wild-type; (2) SIV+, vaccine; (3) SIV-, wild-type; (4) SIV-, vaccine. $N = 3$ for each group except for SIV+ wild-type ($N = 2$). Blood collections (BC), cultures (QBC), rectal swabs (RS), physical exams (Ph), and fecal collections (FC) were performed at various timepoints as indicated.