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Randomized, placebo-controlled, double-blind trial of the *Na*-ASP-2 Hookworm Vaccine in unexposed adults

Jeffrey M. Bethony[a,*,1], Gary Simon[b,1], David J. Diemert[a,c], David Parenti[b], Aimee Desrosiers[b], Suzanne Schuck[b], Ricardo Fujiwara[d], Helton Santiago[a], Peter J. Hotez[a,c]

[a] *Department of Microbiology, Immunology and Tropical Medicine, George Washington University Health Center, Washington, DC, United States*
[b] *Department of Medicine, George Washington University Health Center, Washington, DC, United States*
[c] *Albert B. Sabin Vaccine Institute, Washington, DC, United States*
[d] *Instituto René Rachou, Fundação Oswaldo Cruz, Belo Horizonte, Minas Gerais, Brazil*

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Summary *Necator americanus Ancylostoma* Secreted Protein-2 (*Na*-ASP-2) is a leading larval-stage hookworm vaccine candidate. Recombinant *Na*-ASP-2 was expressed in *Pichia pastoris* and formulated with Alhydrogel®. In a phase 1 trial, 36 healthy adults without history of hookworm infection were enrolled into 1 of 3 dose cohorts ($n = 12$ per cohort) and randomized to receive intramuscular injections of either *Na*-ASP-2 or saline placebo. Nine participants in the first, second and third cohorts were assigned to receive 10, 50 and 100 μg of *Na*-ASP-2, respectively, on study days 0, 56 and 112, while 3 participants in each cohort received placebo. The most frequent adverse events were mild-to-moderate injection site reactions; in 8 participants these were delayed and occurred up to 10 days after immunization. No serious adverse events occurred. Anti-*Na*-ASP-2 IgG endpoint titers as determined by ELISA increased from baseline in all vaccine groups and peaked 14 days after the third injection, with geometric mean titers of 1:7066, 1:7611 and 1:11,593 for the 10, 50 and 100 μg doses, respectively, compared to <1:100 for saline controls ($p < 0.001$). Antibody titers remained significantly elevated in all vaccine groups until the end of the study, approximately 8 months after the third vaccination. *In vitro* stimulation of PBMCs collected from participants with *Na*-ASP-2 resulted in robust proliferative responses in those who received vaccine,

* Corresponding author at: Ross Hall 727, George Washington University Medical Center, 2300 Eye Street NW, Washington, DC 20037, United States. Tel.: +1 202 994 3535; fax: +1 202 994 2913.
E-mail address: mtmjmb@gwumc.edu (J.M. Bethony).
[1] These authors contributed equally to this work.

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which increased with successive immunizations and remained high in the 50 and 100 μg dose groups through the end of the study. This first trial of a human hookworm vaccine demonstrates that the *Na*-ASP-2 vaccine is well-tolerated and induces a prolonged immune response in adults not exposed to hookworm, justifying further testing of this vaccine in an endemic area.

An estimated 740 million people are infected with the hookworms *Necator americanus* and *Ancylostoma duodenale*, mostly in rural areas of the tropics [1]. Although mortality due to infection is rare, the global burden of hookworm disease is high, with an estimated annual loss of 22 million disability-adjusted life years [2]. Hookworm infection is primarily acquired after skin contact with infective larvae (L3) in fecally contaminated soil. Following penetration of the host's skin, larvae enter the vasculature and pass through the heart to the lungs, where they become trapped in pulmonary capillaries. After migrating into the alveolae the larvae ascend the bronchial tree and are swallowed into the gastrointestinal tract where they develop into adult worms. As adult worms, they attach to intestinal microvilli, mate, and feed on host blood and mucosa [3,4]. Chronic infection with hookworm, often lasting between 5 and 7 years, results in long-term pathological consequences to the host. The intestinal blood loss caused by hookworm infection over years can result in iron-deficiency anemia, which is the major clinical manifestation of infection [3]. In children and adults living in resource-poor countries, where iron stores are often lower than those in developed countries due to dietary deficiencies, there is a well-established relationship between the intensity of hookworm infection, intestinal blood loss, and anemia [5—7].

While treatment with the benzimidazole class of anthelmintic drugs is highly effective against established hookworm infection [8], sustained chemotherapy programs have proven difficult to implement, especially in developing countries where there is often rapid reversion (e.g., within 12 months) to high levels of transmission after mass treatment. Therefore, development of a vaccine against hookworm has become a public health priority in the tropical and subtropical regions of the world [9]. Since it is by far the most prevalent hookworm worldwide, *N. americanus* is the primary target of hookworm vaccine development.

The development of a human hookworm vaccine is considered feasible due to past production of a safe and efficacious vaccine against the canine hookworm (*Anclyostoma caninum*), which was commercially available in the 1970s [10]. Since the canine vaccine consisted of irradiated L3, development of a hookworm vaccine for humans has focused on identifying antigens produced by the invading L3 and expressing them as recombinant proteins. As such, *Ancylostoma* Secreted Protein-2 from *N. americanus* (*Na*-ASP-2) was selected as the lead human hookworm vaccine candidate. *Na*-ASP-2 is a 21.3 kDa excretory/secretory (ES) product and is the most abundant ES product released by *N. americanus* L3 upon entry into the host [11,12]. Encouraging preclinical results showed that vaccination of canines and hamsters with recombinant ASP-2 reduced adult worm burden, fecundity (as measured by fecal egg counts), and *in vitro* migration of larvae through tissue [13—15]. Studies of populations living in hookworm-endemic areas also showed that anti-ASP-2 antibodies were associated with a reduced risk of acquiring heavy hookworm infection in areas of high transmission [14]. Based on this evidence, recombinant *Na*-ASP-2 was manufactured and tested in a phase 1 trial in healthy, hookworm-naïve adults, the results of which are reported herein.

Materials and methods

Vaccine preparation

The *Na*-asp-2 gene was cloned from an L3 cDNA library, amplified by polymerase chain reaction (PCR) using specific *Na*-asp-2 primers, and then transferred into the expression vector pPICZaA. A positive pPICZaA-*Na*-asp-2 clone was further characterized and transformed into *Pichia pastoris* for expression [16]. Clinical lots of vaccine were produced at the Walter Reed Army Institute of Research Pilot Bioproduction Facility (Silver Spring, Maryland, USA) according to current Good Manufacturing Practice. The 21.3 kDa recombinant protein was expressed, purified using a series of chromatography steps followed by diafiltration in phosphate-buffered saline (PBS), and then adsorbed to Alhydrogel® (Biosector, Denmark).

The *Na*-ASP-2 Hookworm Vaccine was supplied as an off-white suspension in vials containing 500 μg of *Na*-ASP-2 and 3000 μg of Alhydrogel® in 1.0 mL PBS, without stabilizers or preservatives. Potency studies of vaccine stored at 2—8 °C were conducted in mice every 6 months and confirmed that the lot used in the trial was stable and fully potent throughout the course of the study.

Prior to injection, the vaccine was diluted under aseptic conditions with Alhydrogel® diluent (3000 μg/mL) to reach the appropriate concentration of *Na*-ASP-2 while maintaining a constant concentration of Alhydrogel® (3000 μg/mL) such that each 0.5 mL dose of vaccine contained 1500 μg Alhydrogel®. The placebo was sterile, preservative-free saline solution (0.9% NaCl, Abbott Laboratories, Abbott Park, Illinois, USA). Individual doses of vaccine or placebo were supplied to the clinic in syringes that were masked with a label to obscure the contents and ensure that study personnel administering the vaccine remained blinded.

Study design

A randomized, double-blind, placebo-controlled dose-escalating phase 1 clinical trial in healthy adult volunteers was conducted to evaluate the safety, reactogenicity, and immunogenicity of the *Na*-ASP-2 vaccine formulated on Alhydrogel®. This study was performed under an Investigational New Drug application (BB-IND-12166) to the U.S. Food and Drug Administration and was conducted at the George Washington University Medical Center. The protocol, amendments to the protocol, informed consent