

Community-Based Safety, Immunogenicity, and Transmissibility Study of the *Shigella sonnei* WRSS1 Vaccine in Israeli Volunteers

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We describe the first community-based evaluation of *Shigella sonnei* strain WRSS1, a live, oral candidate vaccine attenuated by a 212-bp deletion in the *virG* (or *icsA*) plasmid virulence gene. Three single-dose regimens of WRSS1 (5×10^3 CFU, 2×10^4 CFU, and 4×10^5 CFU) were tested with cohorts of 15 adult volunteers. The vaccine was generally well tolerated at the 10^3 - and 10^4 -CFU doses. There were no fevers and there was one report of moderate diarrhea in 30 vaccinees; five additional vaccinees reported mild diarrhea. At the 10^5 -CFU dose, there were two reports of low-grade fevers and four reports of moderate diarrhea. The geometric means for immunoglobulin A (IgA) antibody-secreting cells (ASC) against lipopolysaccharide (LPS) were 30, 75, and 193 ASC per 10^6 peripheral blood mononuclear cells (PBMC) for the 10^3 -, 10^4 -, and 10^5 -CFU doses, respectively. The IgG means were 40, 46, and 135 ASC per 10^6 PBMC, respectively. The 10^4 -CFU dose of WRSS1 gave the best balance of safety and immunogenicity, since all vaccinees had a significant IgA ASC response and 73% had a response of more than 50 ASC. The anti-LPS seroconversion rate (threefold) for IgA was 60% and the IgG rate was 27% for the 10^4 -CFU cohort. Each vaccinee and a cohabitating household contact delivered daily perianal stool swabs for bacteriological culture. WRSS1 colonized vaccinees for a median of 5 days, and one individual excreted WRSS1 intermittently for 23 days. None of the 45 household contacts were colonized with WRSS1 after a cumulative 192 days of cohabitation with colonized vaccinees, suggesting that adventitious vaccine spread was not common in the community setting.

Shigella sonnei infection is an inconvenient and possibly serious health threat to travelers, expatriates, and soldiers who enter less-developed countries. For example, *S. sonnei* was responsible for 54% of all *Shigella* infections in a survey of almost 2000 clinical isolates collected over a 13-year period from Finnish travelers (calculated from data in reference 9). Of travelers suffering from shigellosis while they were visiting Africa, 43% were infected with *S. sonnei*, and 51% of the travelers with shigellosis in the Far East were infected with this species. For *Shigella*-positive Finnish travelers in Europe, South and Central America, the Soviet Union, and the Middle East the percentages of infection with *S. sonnei* isolates were somewhat higher, ranging from 60% to 67%. These and other data suggest that a vaccine protecting against *S. sonnei* would eliminate a majority of the shigellosis cases experienced by travelers, expatriates, and soldiers. For example, such a vaccine would have protected troops from almost 90% of shigellosis during Operation Desert Shield (10).

The predominance of *S. sonnei* as a cause of diarrhea in travelers is sometimes in stark contrast to the occurrence of this species among indigenous peoples. For example, *S. sonnei* was isolated from only 3% of native patients suffering from shigellosis in Sub-Saharan Africa, 5% of native patients in South Asia, and 15% of native patients in East Asia and the Pacific. This species did not predominate in native patients with shigellosis even in the Middle East (29% of isolates) or in Latin America (31% of isolates) (16). It is well known that the general level of environmental and personal hygiene affects the proportions of shigellosis that are attributable to *S. sonnei* and to *Shigella flexneri*. Improved hygiene reduces the ratio of *S. flexneri* (serogroup B) to *S. sonnei* (serogroup D) (B:D ratio) (2). Importantly, the B:D ratio can vary greatly within a relatively small geographical area. In southern Israel, for example, *S. sonnei* causes more than 70% of the shigellosis in the urban Jewish population of Beer-Sheva, while *S. flexneri* causes almost 70% of the shigellosis in the Muslim Bedouins living in the adjacent Negev desert towns and settlements (6). Since travelers from industrialized countries tend to lodge and dine in comparatively well-developed urban environments, they are exposed to *S. sonnei* more often than would be predicted from the overall B:D ratio for a less-developed country.

The United States Army Medical Research and Materiel Command has collaborated with the Center for Vaccine Development (CVD), University of Maryland School of Medicine, Baltimore, MD, and more recently with the Medical

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Corps, Israel Defense Force, and the Tel-Aviv Sourasky Medical Center in volunteer safety and immunogenicity trials of the *S. sonnei* WRSS1 candidate vaccine. WRSS1 was constructed at the Walter Reed Army Institute of Research as a $\Delta virG$ (or $\Delta icsA$) mutant of an *S. sonnei* strain that stably maintained the form I lipopolysaccharide (LPS) phenotype (8). The *VirG* gene product is a virulence determinant that activates the N-WASP-Arp2/3 complex and induces F-actin polymerization at the nongrowing poles of shigellae in the cytoplasm of infected epithelial cells (5). The resulting actin tail provides a motive force for intracellular and intercellular spread of the bacteria (1). Like wild-type *Shigella*, *virG* mutants invade gut-associated lymphoid follicles through M cells, inducing release of interleukin 1 β (IL-1 β) from macrophages in the underlying gut-associated lymphoid tissue (20). In concert with IL-1 β from infected macrophages, IL-8 released from infected epithelial cells elicits a localized infiltration of neutrophils into lymphoid follicles and into the surrounding epithelium. Unlike wild-type *Shigella*, however, *virG* mutants do not propagate beyond a limited number of epithelial cells surrounding the follicles, and IL-8-mediated inflammation is confined to the follicular area (21).

Extensive dose selection trials are necessary to demonstrate the safety of vaccines attenuated by *virG* mutation (4, 11). In the initial trials of WRSS1 at the CVD, single-dose regimens with doses ranging from 3×10^3 to 3×10^6 CFU were evaluated with a total of 27 vaccinees (15). Seven placebo controls were included in these trials for the purpose of double blinding. The only presumptive vaccine reactions that were characterized as severe were headaches reported by two subjects. All other reactions were characterized as mild; however, three subjects had transient fever (6 to 12 h), and three met the clinical definition of diarrhea (two or more liquid stools totaling more than 200 ml within 48 h). Twenty-two (82%) of the 27 vaccinees excreted WRSS1 on at least one day, and 52% were excreting the organism when antibiotic treatment commenced at the beginning of study day 7. WRSS1 proved to be remarkably immunogenic against homologous LPS because even the lowest dose elicited a geometric mean of 99 immunoglobulin A (IgA) antibody-secreting cells (ASC) per 10^6 peripheral blood mononuclear cells (PBMC). At the 5-log and 6-log doses, the immune responses against LPS rivaled those seen after clinical disease. Nonetheless, there was no clear dose relationship to either vaccine reactogenicity or excretion in the small cohorts of the first trial, and it was concluded that WRSS1 should be assessed in further volunteer trials (15).

The current trial was designed to evaluate the safety, immunogenicity, and intestinal persistence of WRSS1 in a community-based setting in Tel-Aviv, Israel. Our studies showed that 10^4 CFU is the optimal vaccine dose to test in phase 2 trials. A unique aspect of the study design was an initial evaluation of the potential for adventitious spread of WRSS1 to household contacts. The uniformly vaccine-negative fecal swabs cultured from household contacts suggested that adventitious spread of WRSS1 may not be a significant problem.

MATERIALS AND METHODS

Vaccine. WRSS1 was manufactured under current good manufacturing procedures at the Walter Reed Army Institute of Research Pilot Bioproduction Facility in Forest Glen, Md. The cryopreservative was phosphate-buffered saline

with 7.5% dextran T10, 2% sucrose, and 1% glycerol. The lyophilized product is stored at -80°C . Vials of WRSS1 lot 0451 have consistently yielded approximately 3×10^{10} CFU, with >90% form I colonies (smooth LPS phenotype), since manufacture in 1997.

Subjects. The study was conducted under an IND application to the United States Food and Drug Administration. Healthy male and female community volunteers who were 21 to 39 years old were recruited at the Tel Aviv Sourasky Medical Center Clinical Research Centre (CRC), Tel-Aviv, Israel. Subjects were enrolled as vaccinees if they shared an apartment or house with one other adult who agreed to enroll as a household contact. All vaccinees were HLA-B27 negative and had no children in the household. Informed consent from both vaccinees and contacts was obtained in accordance with the Tel-Aviv Sourasky Medical Center Institutional Review Board, the Israel Ministry of Health Institutional Review Board, and the Human Subjects Research Review Board, Office of the U.S. Army Surgeon General. As a part of the informed consent procedure, vaccinees and contacts were instructed to wash their hands thoroughly after they used the toilet, and vaccinees were also instructed not to prepare food for others until they were no longer excreting the vaccine.

Study design. Three groups containing 15 subjects each were formed, and these subjects were enrolled as outpatient vaccinees. For each vaccinee, a contact volunteer sharing the same living space and toilet facilities was enrolled. The study design was a dose-escalating, open-label trial starting with a single 10^3 -CFU dose for the first cohort, followed after a 4-week interval by a 10^4 -CFU dose for the second cohort and then after a 4-week interval by a 10^5 -CFU dose for the third cohort. Vaccinees who excreted WRSS1 for 20 days or longer were treated with a 3-day course of ciprofloxacin (500 mg per os twice a day).

Inoculation and clinical evaluation. Enrollment of volunteers, administration of the study agent, clinical evaluation, and sample collection took place at the CRC under full medical and nursing supervision. Fasting volunteers ingested 2 g of sodium bicarbonate buffer dissolved in 150 ml of water, followed 1 min later by 31 ml of water containing the assigned vaccine dose. During the first five study days and on days 7, 9, and 14, vaccinees reported to the CRC with a completed clinical evaluation form (CEF) that was reviewed by a study physician. At these times, blood pressure, pulse, and oral temperature were determined, and a physical examination was performed at the discretion of the physician. A temperature of $\geq 100^\circ\text{F}$, as measured by study personnel, was required for documentation of a fever. Contacts were required to complete a CEF that was delivered to the CRC by the cohabitating vaccinee. Contacts were not required to visit the CRC following recruitment and consent unless they were experiencing possible vaccine-related symptoms with clinical significance. Vaccinees and contacts were not required to submit any CEFs after study day 14 or after they had delivered at least three consecutive negative stools, but they were instructed to report any change in health status for up to 60 days. Mild diarrhea was defined as one or two liquid stools passed within a 24-h period, while moderate diarrhea was defined as three to five liquid stools within a 24-h period. The occurrence of soft stools was also noted but was not documented as diarrhea. Symptoms were classified as mild (not affecting normal activities and causing only slight discomfort), moderate (affecting, but not preventing, normal activities), and severe (preventing normal activities). A question about general perceptions of wellness was also included in the CEF. All vaccinees reported to the CRC on study days 30 (± 4 days) and 60 (± 7 days) for completion of a follow-up questionnaire concerning general health status and any joint pain or swelling.

Bacteriology. Vaccinees and contacts were provided with written instructions for collection of self-administered, postdefecation perianal swabs after the first bowel movement of each day for up to 28 days. These swabs were placed in Cary-Blair agar gel (Copan Italia, Brescia, Italy) and stored in a cool place (refrigerator recommended) until they were delivered to the CRC. The protocol required a minimum of three consecutive stool cultures that were negative for WRSS1 before a vaccinee was released from responsibility for delivery of daily stool samples. In practice, however, five or more consecutive WRSS1-negative swabs were usually cultured from each subject. All perianal swabs were transferred from the CRC to the Israel Defense Force Army Health Branch Research Unit in Tel-Aviv for culture. Briefly, a fecal swab was streaked for isolation on MacConkey agar, xylose lysine desoxycholate agar, and salmonella-shigella agar (Hylabs, Nes-Ziona, Israel) and incubated overnight at 37°C . Representative non-lactose-fermenting colonies (MacConkey agar and salmonella-shigella agar) or non-xylose-fermenting colonies (xylose lysine desoxycholate agar) were transferred to biochemical test media (Kligler iron agar, urea semisolid agar, Simmons citrate agar, and lysine iron agar), and isolates resembling *Shigella* were typed by slide agglutination in group D antiserum (Denka Seinken Co., Tokyo, Japan). The genotypes of all *S. sonnei* isolates from each volunteer were confirmed to be WRSS1 by PCR using a set of primers amplifying the entire *virG* gene and a second set of primers flanking the 212-bp deletion in WRSS1 (8).

TABLE 1. Clinical response to WRSS1

Symptom	No. of volunteers/total no. (%)	
	10 ³ and 10 ⁴ CFU ^a	10 ⁵ CFU
Any soft stool	17/30 (57)	10/15 (67)
Any liquid stool	6/30 (20)	4/15 (27)
Moderate diarrhea (>two liquid stools/24 h)	1/30 (3)	4/15 (27) ^b
Fever (≥100°F)	0/30 (0)	2/15 (13)
Total with moderate or severe gastrointestinal signs or symptoms ^c	5/30 (17)	5/15 (33)

^a More signs and symptoms were reported by volunteers who ingested the 10³-CFU dose than by volunteers who ingested the 10⁴-CFU dose, and the data for these two doses were combined for the safety analysis.

^b The frequency of diarrhea significantly increased with the 10⁵-CFU dose compared to the 10³- and 10⁴-CFU doses ($P = 0.036$).

^c Diarrhea, nausea, or abdominal pain.

Immunology. The enzyme-linked immunospot assay was used to enumerate IgA, IgG, and IgM ASC in peripheral blood on study days 0, 7, and 9 (19). Data were expressed as the number of ASC recognizing *S. sonnei* LPS per 10⁶ PBMC. A significant response was >3 standard deviations (SD) from the day 0 mean for all vaccinees (for IgA, 4.1 ASC; for IgG, 4.6 ASC). Circulating antibody recognizing *S. sonnei* LPS was assessed by an enzyme-linked immunosorbent assay (ELISA) using serum drawn on study days 0, 14, 30, and 60. Responses were expressed as endpoint titers derived from a linear regression analysis of eight doubling dilutions using an adjusted optical density of 0.3 (3). A threefold increase in the titer compared with the day 0 baseline value for each individual was considered seroconversion.

Statistical analysis. Associations between vaccine dose and signs or symptoms, excretion, and the immune response were assessed by Fisher's exact test. A multiple analysis of variance was used to compare the geometric means of ASC and ELISA titers for the three vaccine groups. Linear regression analysis was used to test the trend in the ASC response as a function of the vaccine dose.

RESULTS

Clinical response. The first cohort of 14 vaccinees received a dose of approximately 5×10^3 CFU of WRSS1. In order to bring the size of the first group to 15 subjects, an extra vaccinee (vaccinee 115) was recruited to the second group, and this subject received approximately 2×10^3 CFU. The most common complaint in the 10³-CFU cohort was abdominal pain; 10 subjects characterized this reaction as mild, and two subjects characterized it as moderate. Nausea was the next most frequent complaint, and four subjects characterized the reaction as mild. Two subjects characterized headaches as moderate. Three vaccinees reported one or two liquid stools that qualified as mild diarrhea, and one reported four liquid stools in the morning of study day 1 that qualified as moderate diarrhea (Table 1). The latter subject also reported severe nausea and vomiting during the morning of day 5. The second cohort of 15 vaccinees received a dose of approximately 2×10^4 CFU of WRSS1. Again, the most common complaint was abdominal pain; eight subjects characterized this reaction as mild, and two characterized it as moderate. Only three subjects reported nausea, and one volunteer characterized this reaction as moderate. Two subjects in this group reported a single liquid stool that qualified as mild diarrhea. Since there was no apparent dose-related trend in vaccine reactions seen in the first two groups, the safety summary in Table 1 combines the data for the 10³- and 10⁴-CFU groups.

The 3-log and 4-log doses of WRSS1 were well tolerated since only 1 of 30 volunteers (3%) who ingested one of these doses reported moderate diarrhea. Five additional vaccinees reported mild diarrhea; thus, a total of six vaccinees (23%) reported any liquid stool. However, 17 additional vaccinees reported a noticeable change in bowel habits, which was characterized as soft stools; thus, a cumulative total of 23 of 30 vaccinees (77%) had at least one soft or liquid stool. Nonetheless, only five of these vaccinees (17%) reported either moderate diarrhea or moderate to severe subjective symptoms, such as intestinal pain (cramps) or nausea, and only one vaccinee (3%) in the 3-log and 4-log dose cohorts reported feeling "bad" on the CEF general wellness question. None of these volunteers chose to self-medicate because of subjective symptoms.

The third cohort of 15 volunteers received approximately 4×10^5 CFU of WRSS1. Abdominal pain was reported by nine of these subjects; three of the subjects characterized the reaction as moderate, and one subject characterized the reaction as severe. Six subjects reported nausea; three characterized the reaction as moderate, and one subject also reported vomiting. Four subjects in this group reported moderate diarrhea with an average of four liquid stools for 2 days. Two of the subjects with diarrhea also had low-grade fevers of 100°F as measured by study personnel at the CRC. Six additional vaccinees reported a noticeable change in bowel habits that was characterized as soft stools; a cumulative total of 10 subjects (60%) had at least one soft or liquid stool. The severity and duration of signs and symptoms increased with the higher dose of vaccine, and it should be noted that four vaccinees (27%) reported feeling "bad" on their CEFs. Four vaccinees also chose to self-medicate because of headaches. No vaccine-related sequelae were identified in the 30- and 60-day follow-up questionnaires for any cohort.

Vaccine excretion. WRSS1 vaccinees submitted a total of 423 fecal swabs for bacteriological culture, which was an average of more than nine swabs per subject. For vaccinees, 137 (32%) of the fecal cultures were positive for WRSS1. A total of 390 fecal swabs were obtained from household contacts, which again was an average of almost nine swabs per contact. Since normal fecal flora was cultured from all swabs, we concluded that the perianal sampling technique was successful. Two contacts were positive for *Campylobacter jejuni*, and one was positive for *Shigella boydii*, but none of the swabs was positive for WRSS1 (Fig. 1). Thus, colonization of household contacts by adventitious spread of the vaccine was not detected by the methods used in our study.

WRSS1 was detected in fecal samples from nine vaccinees (60%) in the 10³-CFU group, 12 vaccinees (80%) in the 10⁴-CFU group, and 11 vaccinees (73%) in the 10⁵-CFU group. For Fig. 1, we assumed that the period of vaccine colonization ranged from the first day of vaccination until the last day of positive culture even though gaps of 1 to 5 days routinely occurred between positive samples. Thus, we extended fecal culture beyond the protocol standard of three consecutive negative samples to a study average of 5.6 negative samples after the last positive culture, and collection of samples from the contacts was extended concomitantly. In the 10³-CFU group, there were two vaccinees who excreted WRSS1 for an unusually long period; volunteer 113 consis-

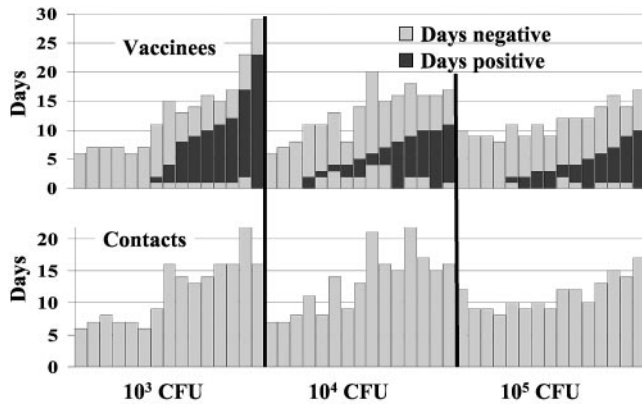


FIG. 1. Isolation of WRSS1 from stool cultures of vaccinees or household contacts. The number of days positive for WRSS1 includes all days from the first day of vaccine culture until the last day of vaccine culture regardless of the number of positive or negative cultures between these two events. Data for each vaccinee-contact pair are aligned vertically.

tently excreted for 17 days, and volunteer 115 consistently excreted for 10 days but was also positive on days 17 and 23. Even though they had delivered negative swabs for 3 or 4 days since the last positive swab, these volunteers were treated with ciprofloxacin beginning on study days 21 and 28, respectively, in order to guarantee that the vaccine had been eliminated. Excluding these two volunteers, WRSS1 was excreted for an average of 8 days (10^3 -CFU group), 5 days (10^4 -CFU group), or 4 days (10^5 -CFU group), suggesting that the number of organisms in the original dose of vaccine was not related to the duration of colonization. The average cumulative colonization period was 6 days, and the median was 5 days. All *S. sonnei* isolates from each excreting volunteer were confirmed to be WRSS1 by PCR.

Immune response. Vaccination with any dose of WRSS1 elicited vigorous IgA and IgG ASC responses at day 7 or day 9 against *S. sonnei* LPS in most volunteers. Almost all vacci-

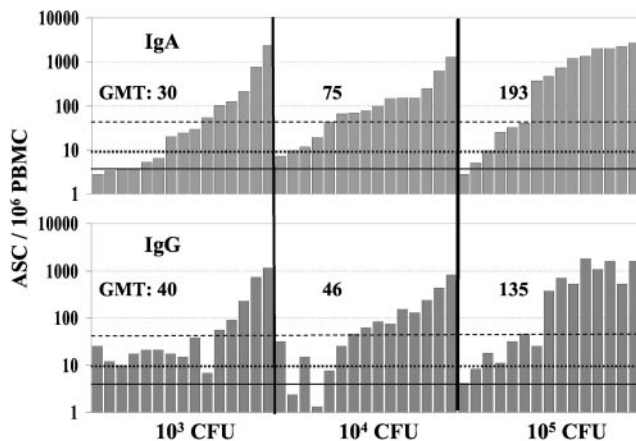


FIG. 2. Peak numbers of IgA and IgG ASC against *S. sonnei* LPS with the GMT for each dose of WRSS1. The solid line indicates 3 SD of the day 0 mean IgA or IgG ASC values for all volunteers; the dotted line indicates 10 ASC per 10^6 PBMC; and the dashed line indicates 50 ASC per 10^6 PBMC.

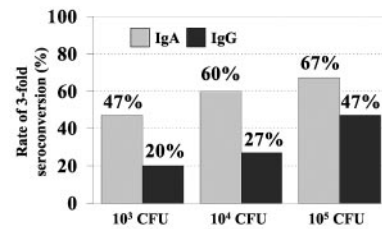


FIG. 3. Rates of threefold ELISA anti-LPS seroconversion at day 14 for each dose of WRSS1.

nees (91%) had significant IgA and IgG ASC responses that exceeded 3 SD of the day 0 mean (Fig. 2), but there was a trend toward a higher percentage of substantial ASC responses (≥ 10 ASC per 10^6 PBMC) for the 4-log (93%) and 5-log (87%) doses compared to the 3-log dose (60%). This trend was reflected in the increasing geometric mean number (GMN) of IgA and IgG ASC (Fig. 2) and in the increasing proportion of threefold or greater ELISA seroconversion against *S. sonnei* LPS (Fig. 3). The IgA ASC GMN for the cohort that received the 3-log dose (30 ASC per 10^6 PBMC) was not significantly different from the IgA ASC GMN for the 4-log cohort (75 ASC per 10^6 PBMC) or the 5-log group (193 ASC per 10^6 PBMC) ($P = 0.057$), but the trend toward a higher ASC response was significant ($P = 0.016$). The percentage of threefold serum IgA responses increased from 47% for the 3-log dose to 67% for the 5-log dose (Fig. 3), but this trend was not significant. The mean peak ELISA geometric mean titers (GMT) on day 14 were similar for all three cohorts. There was a strong positive correlation between excretion of WRSS1 and a substantial ASC response or seroconversion ($P > 0.01$), but there was no correlation between the occurrence of symptoms and the immune response. The IgM response was modest at all three doses of WRSS1, with GMN of 3.3 ASC for the 3-log dose, 2.8 ASC for the 4-log dose, and 6.0 ASC for the 5-log dose. Only one vaccinee who received the 3-log dose and three vaccinees who received the 5-log dose had IgM seroconversion, and no significant increases in the IgM ELISA GMT were recorded at any dose.

DISCUSSION

The 3-log and 4-log doses of WRSS1 were well tolerated, although vaccination was often associated with temporary changes in bowel habits and intestinal motility. These reactions were characterized as mild, with the exception of one report of moderate diarrhea for 1 day. Although self-reported observations in community-based trials are less rigorous than professional evaluation of vaccine reactions in clinic-based studies, the current data are consistent with the previously published results of a WRSS1 inpatient trial. In the latter study, one of six vaccinees who ingested a 3-log dose had mild diarrhea and a short-term fever, while none of four vaccinees had symptoms after ingestion of a 4-log dose (15). Thus, the cumulative rate of moderate diarrhea or fever was 5% in all inpatient and community-based studies of 3-log or 4-log doses of WRSS1. The moderate intestinal cramps accompanied by abdominal pain (13% of vaccinees) and headache (7% of vaccinees) reported after ingestion of a 3-log or 4-log dose of WRSS1 are

probably an unavoidable consequence of intestinal invasion because the innate response to shigellae is strongly biased toward acute inflammation (7, 20). However, these doses of WRSS1 appear to be sufficiently attenuated to warrant further clinical trials, although it should be expected that a few vaccinees will experience some painful cramps or headache and most vaccinees will experience a temporary change in bowel habits, usually characterized by soft stools but occasionally characterized by liquid.

After ingestion of a 5-log dose of WRSS1, fever occurred in 2 of 15 vaccinees in the current study and in 2 of 10 vaccinees in the previous study (cumulative rate, 16%). The occurrence of fever and/or moderate diarrhea in both inpatient and community-based trials with the 5-log dose of WRSS1 was 28%, which discouraged further volunteer trials with this dose. A recent clinical trial of the *S. flexneri* 2a CVD 1208 vaccine suggested that deletion of the *Shigella sen* and *set* enterotoxin genes ameliorates diarrhea and fever in vaccinees (14). Although *S. sonnei* does not carry the *sen* chromosomal enterotoxin gene (18), the *set* plasmid gene, encoding the ShET2 enterotoxin, is present in this species (17). We are now characterizing a *virG sen* mutant that may be a more attenuated second-generation *S. sonnei* vaccine.

Since *virG* attenuation does not alter the physiology of WRSS1, the vaccine would be expected to colonize volunteers as efficiently as wild-type *S. sonnei*. Indeed, 60% of volunteers who ingested a 3-log dose of WRSS1 excreted the vaccine, and it was detected in the stools of 80% and 73% the volunteers in cohorts that received 4-log and 5-log doses of WRSS1, respectively. One of the most important goals of the current community-based study was a preliminary assessment of the risk of adventitious spread of WRSS1 to household contacts. The rate of such spread could be as high as 25% if the contagion of excreted WRSS1 mimics the contagion of wild-type *S. sonnei* for household contacts (22). However, 32 contacts were exposed to housemates excreting WRSS1 for a cumulative total of 192 days (27 weeks), and none of their perianal swabs were positive for WRSS1. The apparent absence of adventitious vaccine spread may reflect relatively low levels of WRSS1 excretion. In the CVD study, WRSS1 was excreted at levels of 10^4 to 10^6 CFU per g of stool, depending on the dose of vaccine ingested (15), and these levels of excretion are 10- to 1000-fold lower than the level that typifies shigellosis (13). It is also likely that the normal standards of hygiene practiced by the vaccinees and contacts helped to prevent the adventitious spread of WRSS1 (12), especially since relatively few liquid stools were passed by vaccinees.

Although WRSS1 can elicit type 1 cell-mediated responses against proteinaceous invasion plasmid antigens (IpaB, IpaC, and IpaD) (15), antibody against LPS is the primary effector of protective immunity. The anti-LPS IgA and IgG responses elicited by WRSS1 in the current trial were generally similar to the responses seen in the previous phase 1 trial of this vaccine (15). When the IgA ASC data from the two studies are combined, the GMN totals are 46 ASC (68% with ≥ 10 ASC) for the 3-log dose, 73 ASC (95% with ≥ 10 ASC) for the 4-log dose, and 223 ASC (84% with ≥ 10 ASC) for the 5-log dose. Likewise, the percentages of threefold serum IgA responses for all phase 1 trials of WRSS1 were 55% (3-log dose), 58% (4-log dose), and 70% (5-log dose).

A small challenge trial of the *S. flexneri* 2a SC602 $\Delta virG$ candidate vaccine suggested that a level of ≥ 50 total anti-LPS IgA and IgG ASC per 10^6 PBMC is associated with complete protection against diarrhea, dysentery, or fever (4). In the current studies, 11 of 15 (73%) volunteers who received the 4-log dose of WRSS1 met this criterion of immunity (Fig. 2). Of note, all SC602 vaccinees were protected from fever and dysentery even if they had < 50 ASC, and the mild diarrhea suffered by volunteers with the lower immune responses was insignificant compared to the severe symptoms suffered by nonimmunized control volunteers (4). Since a 4-log dose of WRSS1 elicited significant anti-LPS IgA responses in all volunteers, we are optimistic that this vaccine could protect most, if not all, vaccinees after experimental challenge with *S. sonnei*. An efficacy trial of the 4-log dose of WRSS1 is planned as the next phase in development of *S. sonnei* candidate vaccines.

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