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Oral and Rectal Immunization of Adult Female Volunteers with a Recombinant Attenuated *Salmonella typhi* Vaccine Strain

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An attenuated strain of *Salmonella typhi* Δ*cya* Δ(*crp-cdt*) Δ*asd* expressing a gene encoding a hepatitis B virus core–pre-S protein was tested in female adult volunteers for its ability to elicit a systemic and a mucosal immune response. Specifically, our purpose was to evaluate the potential of such a vaccine strain to induce specific secretory immunoglobulin A (sIgA) at genital and rectal surfaces. Oral and rectal routes of immunization were compared: oral immunization induced seroconversion against the bacterial lipopolysaccharide (LPS) in six out of seven volunteers, while after rectal immunization only one out of six volunteers seroconverted against LPS. To our disappointment, the latter volunteer was also the only one who seroconverted against the carried antigen (pre-S1), demonstrating the poor ability of this live vaccine to induce an immune response against the carried antigen. Anti-LPS sIgA was found in both the vaginal and cervical secretions of a volunteer who presented a strong seroconversion after oral immunization (16-fold increase in anti-LPS IgG). Smaller amounts of anti-LPS sIgA were found in the rectal secretions of one orally and one rectally immunized volunteer and in the saliva of three orally and one rectally immunized woman. Our data show for the first time that it is possible to induce specific sIgA in the genital and rectal tracts of women by using an *S. typhi* vaccine strain.

Hepatitis B virus (HBV), human papillomavirus, candida, chlamydia, and human immunodeficiency virus all infect women through the genital tract and, for human immunodeficiency virus, the rectal mucosa. To provide specific protection against such pathogens, it would be of great interest to design mucosal vaccination strategies that could induce specific secretory immunoglobulin A (sIgA) in the secretions of the genital and rectal tracts. In addition, a systemic response that could prevent the systemic spread and infection by microorganisms that have escaped the first line of defense is also desirable. Live as well as inert mucosal vaccines can trigger both types of responses. For HBV, a safe and efficient plasma-derived or recombinant vaccine already exists, but its cost and the requirement of multiple injected doses prevent its use in mass vaccination programs. Oral, single-dose, and lower-cost vaccines are therefore desirable.

The uptake of antigens at mucosal inductive sites such as intestinal Peyer's patches results in the priming of antigen-specific B and T cells, which subsequently migrate from the site of induction and mature into effector cells. The migrating IgA-expressing B cells home preferentially to sites close to that of induction but also to distant mucosal sites where they differentiate into IgA-secreting plasma cells (32). The mucosal immune response at specific sites varies with the type of antigen, the route of administration, and the species studied (24, 26, 30, 33, 50). In particular, little is known about the mechanism whereby mucosal immune responses in the female genital tract are triggered. We have recently shown that attenuated *Salmonella typhimurium* recombinant strains induced specific IgA in vaginal secretions when mice were immunized by various mucosal routes, while specific IgAs were efficiently produced in rectal secretions only when rectal immunization was performed (8, 26, 43, 44). Attenuated *Salmonella* strains are one of the best-characterized mucosal vaccine carriers. Recombinant *Salmonella* strains that are attenuated yet invasive have been used as oral vaccine vectors to carry protective epitopes of several pathogens into the mucosa-associated lymphoid tissue, thus inducing mucosal and systemic immune responses against both the carrier and the foreign antigens (5, 7, 9, 34–36).

The currently licensed oral vaccine against typhoid fever *Salmonella typhi* Ty21a (21), administered as a three-dose regimen of enteric coated capsules (10^9 CFU per capsule), provided 67% efficacy over a 3-year period. However, because *S. typhi* Ty21a requires high and multiple doses in liquid formulation for higher efficacy and because its mutations are not yet all characterized (2, 3, 13, 19, 42), newly attenuated *Salmonella* strains have recently been developed and tested in humans. These include nutritional auxotrophs in which pathways for biosynthesis of aromatic compounds have been interrupted (Δ*aro* mutants). The Δ*aroA* Δ*purA* mutants of *S. typhi* have been tested in human volunteers (31) and were shown to elicit specific cell-mediated immune responses but weak humoral responses. Other *aro* mutants (*aroC* and *aroD*) were insufficiently attenuated and caused fever and bacteremia (46). The double mutant Δ*aroC* Δ*aroD* Ty2 (CVD 908) was safe and elicited IgG antibodies against lipopolysaccharide (LPS) in 80% of the immunized adult volunteers (25, 47). *S. typhi* mutants in which the adenylate cyclase (*cya*) and the cyclic AMP receptor (*crp*) genes were deleted were also generated. The products of these genes are required for the transcription of

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many genes and operons that control transport processes and the expression of fimbriae, flagella, and some outer membrane proteins. One mutant, χ3927 (Δ*cya* Δ*crp* Ty2), was tested and shown to be immunogenic, but some volunteers vaccinated with this strain developed fever and vaccine bacteremia (46). Therefore, a novel strain, χ4073, was constructed by deleting a third gene (*cdt*), which is responsible for the colonization of deep tissue (6, 8, 29). This strain was administered to volunteers, and it proved to be completely safe at doses up to 5×10^8 CFU and generated seroconversion in 80% of the volunteers (6, 9a). This attenuated strain was furthermore endowed with genetic information encoding protective epitopes from HBV.

This recombinant HBV-*S. typhi* strain, designated χ4632(pYA3167), was constructed with the parent strain χ4073 and an Asd (aspartate-β-semialdehyde dehydrogenase) balanced lethal vector-host system (20) to allow stable and high-level expression of hybrid HBV nucleocapsid (HBc) particles containing protective epitopes of the pre-S region of the viral surface antigen (36, 38). Such hybrid HBc–pre-S particles expressed in avirulent *S. typhimurium* were able to trigger high-titer pre-S specific antibody responses in mice after oral immunization (38–40). In this study, we investigated both the systemic and mucosal immune responses after oral or rectal administration of this recombinant *S. typhi* vaccine strain to female volunteers.

MATERIALS AND METHODS

Volunteers and study design. The clinical protocols used were approved by the local ethical committee. Healthy adult females, 18 to 35 years of age, using oral contraception and who had no history of gallbladder disease and no known allergies to antibiotics were recruited from the University of Lausanne community. The study was explained in detail, and written, informed consent was obtained. To confirm the good health of the volunteers, their medical histories were studied and physical examinations, a battery of clinical hematology and chemistry tests, and serologic tests for HBV surface antigen, *S. typhi*, and human immunodeficiency virus type 1 were performed.

After the first medical visit, 13 female volunteers enrolled in this study. The volunteers were divided into two study groups. Oral vaccinations were performed in the first group ($n = 7$), and rectal vaccinations were performed in the second group ($n = 6$).

The hormonal cycles of the female volunteers were synchronized by the volunteers' taking their contraceptive pills. The volunteers all took contraceptive pills with estroprogestative association, but the pills had different brand names. The period for which the contraceptive pills were taken was either shortened or extended beyond the normal 21-day dosage period so that all volunteers began a new 21-day container of pills on the same day (31 days before immunization). This allowed vaccinations and samplings to be performed on the same days for all volunteers without variations due to their hormonal cycles.

Oral and rectal vaccinations were performed three times with *S. typhi* χ4632(pYA3167), with doses of 3×10^8, 3×10^9, and 1×10^9 CFU, respectively. The first vaccination (day 1) was performed 10 days after the first day of menstruation, the second vaccination (day 14) was performed 2 weeks later, and the third vaccination (day 42) was performed 4 weeks after the second vaccination. Sampling was performed just before the first vaccination (day 1) and then once every 2 weeks (days 14, 28, 42, and 56). One further sampling was performed 30 weeks after the first vaccination for those volunteers who responded to the vaccine.

The volunteers were monitored as outpatients. For a 2-week period after each vaccination, they recorded their morning and evening body temperatures, the number and consistency of their stools, and any other symptoms. They were instructed to call in the hospital in cases of concern. The volunteers were also clinically examined on the days of vaccination and/or sampling, and they were not treated with antibiotics during the study period.

Vaccines. *S. typhi* χ4632(pYA3167) is a Ty2 recombinant *S. typhi* Δ*cya* Δ*crp-cdt* Δ*asd* strain carrying the Asd vector with the following HBV envelope sequences: HBc antigen amino acids 1 to 75, pre-S1 amino acids 27 to 53, HBc antigen amino acids 81 to 156, and pre-S2 amino acids 133 to 143 (HBc–pre-S) (8, 36, 38).

The inoculum was prepared from a stock culture of χ4632(pYA3167), maintained in 1% peptone and 5% glycerol at −70°C. Three hundred microliters of such stock was inoculated into 5 ml of Luria-Burrous broth and incubated at 37°C as a static overnight culture. On the day of vaccination, a 1:10 dilution with prewarmed Luria-Burrous broth was made and incubated at 37°C with gentle shaking (150 rpm) until the optical density (OD) at 600 nm reached 1.0. The bacteria were then pelleted at $3,500 \times g$ for 15 min, resuspended in 20 ml of chilled phosphate-buffered saline (PBS), and placed on ice until use. Dilutions of the inoculum were plated onto MacConkey–1% maltose agar to determine the titers, and slide agglutinations with Vi antigen and group D antisera (Difco) were employed to confirm the identities of the organisms on the day of vaccination.

Vaccination. For oral vaccination, the inoculum was administered with sodium bicarbonate. Sodium bicarbonate (2 g) was dissolved in 150 ml of distilled water, and volunteers drank 120 ml of the solution to neutralize gastric acid. One minute later, volunteers drank the vaccine (1 ml) suspended in the remaining 30 ml of bicarbonate solution. Volunteers had nothing to eat or drink for 90 min before and after vaccination.

For rectal vaccination, the vaccine (1 ml) was diluted to 5 ml with PBS and introduced into the rectum with a cannula, and the volunteers were asked to lie down for 30 min.

Sampling. On the days of sampling, blood, saliva, feces, and vaginal, cervical, and rectal secretions were obtained. A specialized wick (2 by 25 mm; Polytronic) (see reference 24) was used to obtain cervical, vaginal, rectal, and salivary secretions. For saliva, two wicks were placed under the tongue for 1 min. The other specimens were collected during a gynecological examination; a speculum was inserted, and one or two wicks were placed in the endocervical canal (cervical secretion) while two wicks were placed at each side of the posterior vaginal wall (vaginal secretion) for 1 min. During rectoscopy, a rectal wash was performed with 5 ml of PBS, and then a wick was placed against the side of the rectal mucosa approximately 2.5 cm from the anal opening and left in place for 1 min (rectal secretion). The rectal wash was centrifuged 5 min at $10,000 \times g$, and the pellet obtained (feces) was resuspended in 200 μl of PBS containing 50 mM EDTA and protease inhibitors (soybean trypsin inhibitor [0.1 mg/ml] and phenylmethylsulfonyl fluoride [0.35 mg/ml]), and after 10 min bovine serum albumin (BSA) was added (1-mg/ml final concentration). The absorbant wicks were centrifuged for 5 min at $10,000 \times g$ in small, 0.5-ml Eppendorf tubes pierced at the bottom with a needle and placed in large, 1.5-ml Eppendorf tubes without lids. After centrifugation, the samples were collected from the bottom of the large Eppendorf tubes and 200 μl of PBS containing protease inhibitors (pepstatin [10 μg/ml], leupeptin [10 μg/ml], antipain [10 μg/ml], and benzamidine [50 μg/ml]) was added over the wicks in the small Eppendorf tubes and, after centrifugation, pooled with the collected secretions. Vaginal and cervical samples were sonicated for 2 min in a bath sonicator. All samples were stored at −70°C.

Immunology. The levels of IgG, IgA, and IgM specific to *S. typhi* LPS and to pre-S1 in serum samples, were determined, while the levels of sIgA specific to *S. typhi* LPS and to pre-S1 as well as that of total sIgA in all secretions were determined by enzyme-linked immunosorbent assay (ELISA).

For total sIgA, microtiter plates were coated overnight at 4°C with a mouse monoclonal anti-human IgA (200 ng per well; catalog number 05-5200; Zymed) in borate buffer (0.15 M boric acid, 0.025 M $Na_2B_4O_7 \cdot 6H_2O$, 0.15 M NaCl [pH 8.2]). Free binding sites were blocked with 10 mg of BSA per ml–0.1% Tween–PBS (blocking buffer) for 30 min at 37°C. Duplicate samples were diluted in blocking buffer and incubated for 2 h at 37°C. Washes were performed with PBS-Tween between incubations. The plates were incubated at 37°C for 1 h with a peroxidase-conjugated sheep anti-human IgA secretory component (catalog number, W90011P; Biodesign) which was preincubated at a dilution of 1:500 in PBS-Tween containing 10% human serum for 2 h at 4°C to remove contaminating anti-human IgA. The plates were developed with *o*-phenylenediamine (Sigma Chemical Co., St. Louis, Mo.) and H_2O_2, and the OD at 492 nm was measured after 15 min. Purified human sIgA (5 mg/ml) (catalog number, 55905; Cappel) was used as standard, and the total amounts of sIgA were calculated.

For the anti-LPS ELISA, plates were coated with 100 ng of *S. typhi* LPS (Sigma) coupled to methylated BSA (48). For the anti-pre-S1 ELISA, plates were coated with 100 ng of recombinant pre-S purified from *Escherichia coli* (11) in carbonate buffer (pH 9.6). The antigen-specific ELISAs were performed as described above for sIgA determination. For measurement of specific IgG, IgA, and IgM, biotinylated rabbit anti-human IgG, anti-human IgA, and anti-human IgM, respectively (catalog numbers, E 482, E 484, and E 483, respectively; DAKO), diluted 1:1,000, were used as second antibodies, and this was followed by a 30-min incubation at 37°C with a peroxidase-conjugated streptavidin (catalog number, P 397; DAKO) diluted 1:5,000. End point dilutions of samples were carried out. For specific sIgAs, end point dilutions were determined with preimmune and postimmunization samples which were diluted in order to have comparable amounts of total sIgA. The specific sIgA amount for each sample is expressed as the reciprocal of the highest dilution that yielded an OD at 492 nm three times that of the preimmune sample. These reciprocal dilutions were expressed in units and normalized to the amount of total sIgA (in milligrams per milliliter) in each sample. The limits of detection of positive samples depended on their total sIgA contents. The minimal positive titer detectable in all cases was 20 U/ml.

RESULTS

Clinical responses to the vaccine. In general, the high doses of vaccine were well tolerated. None of the volunteers reported fever (>38°C) after vaccination with the exception of volunteer 13, who reported symptoms of a common cold (sore throat,

TABLE 1. Positive immunologic responses in serum after oral or rectal immunization of volunteers with the attenuated recombinant *S. typhi* vaccine strain

Antibody test and volunteer no.[a]	Positive titer (U)[b] at indicated week of sampling:				
	2	4	6	8	30
S. typhi LPS IgG					
3	25,600	12,800	12,800	12,800	4,000
5				100	
6	6,400	3,200	3,200	3,200	
7	200	200	400	200	
8	200				
10				3,200	
S. typhi LPS IgA					
3	3,200	800	400	400	100
4	100			100	
6	800	200	100	100	
8	200				
S. typhi LPS IgM					
3	3,200	1,600	800	400	200
6	6,400	3,200	1,600	800	
7	800				
8	200				
Pre-S1 IgG					
10				1,600	

[a] Volunteers 3 to 8 were immunized orally, while volunteer 10 was immunized rectally.

[b] The reciprocal of the highest dilution yielding an OD three times that of the preimmune serum.

TABLE 2. Immunological responses in serum before and after oral or rectal immunization of volunteers with the attenuated recombinant *S. typhi* vaccine strain

Volunteer no.[a]	Antibody titer (U) in serum sample[b]					
	S. typhi LPS IgG		*S. typhi* LPS IgA		*S. typhi* LPS IgM	
	Pre[c]	Post[d]	Pre	Post	Pre	Post
2	50	50	<100	<100	200	400
3	800	12,800[e]	<100	800[e]	200	3,200[e]
4	200	400	<25	100[e]	400	800
5	50	200[e]	<100	<100	200	400
6	400	3,200[e]	<100	400[e]	200	3,200[e]
7	100	400[e]	<100	<100	200	800[e]
8	200	800[e]	50	200[e]	100	200
10	100	800[e]	<100	<100	100	100
11	400	400	<100	<100	200	200
12	<100	<100	<100	<100	200	200
13	200	200	<100	<100	200	200
14	<100	<100	<100	<100	200	200
15	100	200	<100	<100	200	200

[a] Volunteers 2 to 8 were orally immunized, and volunteers 10 to 15 were rectally immunized.

[b] Titer reported as the reciprocal of the dilution yielding an OD of ≥0.1. Peak titers to IgG were at week 2 for all volunteers shown except volunteers 5 and 10, for which the peaks were at week 8.

[c] Preimmune.

[d] Postimmune.

[e] A fourfold increase in titer (considered significant).

coryza, and cough) during 2 weeks after the third immunization (done in December) together with fever during 4 days. All the rectally immunized volunteers reported normal soft or firm stools with the exception of volunteer 10, who reported thick liquid stool on days 3, 4, 7, and 11 and days 4, 5, 6, and 9 after the first and second immunizations, respectively, and opaque watery stool on day 4 and thick liquid stool on days 5 and 6 after the third immunization. The orally immunized volunteers all reported at some point thick liquid stool (volunteers 2 to 8) and/or abdominal pain (volunteers 2, 3, and 8). In particular, volunteer 3 reported abdominal pain on days 1, 2, and 3 after the three immunizations and thick liquid stool on days 4, 6, and 7 after the first and second immunizations and volunteer 6 reported thick liquid stool on days 2, 3, and 4 and days 2 and 3 after the first and second immunizations, respectively, and opaque watery stool on day 1 after the third immunization.

Immunogenicity of the vaccine. The immune responses of the volunteers after vaccination are shown in Tables 1 through 3. In the serum, the highest dilutions which yielded an OD three times those of the preimmune samples are indicated as positive responses (Table 1). Overall, anti-LPS-positive titers were found in six out of seven orally immunized volunteers but only in one out of six rectally immunized volunteers. This immune response was specific to *S. typhi* LPS, as no positive responses to *E. coli* LPS were found (data not shown). A single immunization induced seroconversion against LPS in five out of the seven orally immunized volunteers. The levels of anti-LPS IgA and IgM decreased rapidly after the first immunization, while those of the anti-LPS IgG were for the most part stable during the first 8 weeks. After 30 weeks, only the highest anti-LPS IgG titer (25,600 U) (that of volunteer 3) remained positive. An effect of the multiple immunizations was observed only for volunteers 5 (orally immunized) and 10 (rectally immunized), who seroconverted after the third immunization. The anti-LPS immune response in rectally immunized volunteer 10 was further confirmed by the finding of 25 anti-LPS IgA-secreting cells per 4×10^6 peripheral blood lymphocytes in a week-8 sample of PBL analyzed by the enzyme-linked immunospot assay method (10) (data not shown).

Surprisingly, seroconversion against the carried antigen (pre-S1) was observed only after the third rectal immunization of volunteer 10 and only for the IgG isotype. Pre-S1 was used to monitor the immune response against the carried antigen because it is the most immunogenic epitope of the hybrid HBc–pre-S particle. The internal insertion of pre-S1 within the immunodominant region of the HBc antigen abrogated most of the native HBc antigenicity and immunogenicity while resulting in an enhanced immunogenicity for pre-S1 (35, 41). Indeed, we did not find any anti-HBc IgG in our serum samples (data not shown).

The anti-LPS antibody titers in preimmune and postimmune (peak titer) serum samples have also been measured (Table 2). The preimmune anti-LPS IgG titers were more variable (50 to 800 U) than those of IgM (100 to 400 U) or IgA (<100 U). Interestingly, the volunteers who had the highest preimmune anti-LPS IgG titers (volunteers 3 and 6, with titers of 800 and 400 U, respectively) generated seroconversions of higher magnitudes, with 16- and 8-fold increases in IgG, respectively, after immunization. These findings corroborate observations that prior exposure to *Salmonella* can enhance subsequent vaccine responses in mice (1). A similar correlation (although not with serum titers) was also described in response to *S. typhi* Ty21a, in which intestinal immune responses were evident only in subjects with some evidence of intestinal immunologic priming (12). This observation was however disputed by Forrest (16), who observed that the maximal intestinal immune response occurred in subjects with few or no preexisting intestinal antibodies.

TABLE 3. Positive mucosal immune responses after oral or rectal immunization of volunteers with the attenuated recombinant *S. typhi* vaccine strain

Volunteer no. and sample	Sample week	Positive titer of LPS sIgA/total sIgA (U/mg)[a]
3		
Vaginal	2	46,000
Cervical	2	105,000
Saliva	6	1,500
6		
Saliva	6	7,200
Saliva	8	6,600
7		
Saliva	8	3,200
8		
Rectal	2	16,000
Rectal	4	8,000
Rectal	6	6,000
Rectal	8	8,000
Rectal wash	2	2,200
10		
Saliva	4	14,000
15		
Rectal	8	2,000
Rectal wash	8	2,000

[a] Reciprocal of the highest dilution yielding an OD three times that of the preimmune serum (in units) divided by the total sIgA content (in milligrams).

All secretions (saliva, feces, and vaginal, cervical, and rectal secretions) have been tested by ELISA for their specific (anti-LPS and anti-pre-S1) sIgA contents. We should point out that we have measured sIgA and not IgA in mucosal secretions and that therefore our data are not biased by possibly contaminating or transudating IgA from the serum. This is particularly important when dealing with genital secretions, in which case half of the antibodies transudate from the serum (20% monomeric IgA and 30% IgG [49]). Because the amount of total sIgA is highly variable between secretion types as well as between samples, end point dilutions of the preimmune and postimmune samples have been performed with similar amounts of total sIgA. The positive titers, expressed in units, were divided by the total amount of sIgA (in milligrams) so that the specific mucosal immune responses could be compared (Table 3).

None of the mucosal samples contained significant amounts of anti-pre-S1 sIgA, while 6 out of 13 volunteers (volunteers 3, 6 to 8, 10, and 15) had one or more samples which contained significant amounts of anti-LPS sIgA. With the exception of volunteer 15, these volunteers also had anti-LPS immunoglobulins in their sera (Table 1). The number of mucosal samples positive for anti-LPS sIgA was low (14 out of 364 analyzed). The titers of anti-LPS sIgA varied between 1,500 and 105,000 U/mg of total sIgA, with the highest values found in the vaginal and cervical secretions of volunteer 3 2 weeks after immunization. Vaginal washes had very low amounts of total sIgA (<2 μg/ml), thus preventing the detection of specific sIgA. Low amounts of anti-LPS sIgA (1,500 to 16,000 U/mg of total sIgA) were found in saliva and rectal secretions. Unexpectedly, no significant amounts of anti-LPS sIgA were found in feces, which has previously been believed to be an indicator of the intestinal response (4, 23, 24, 27).

DISCUSSION

In this human trial we have tested the ability of an attenuated *S. typhi* vaccine carrier strain, χ4632(pYA3167), to elicit mucosal responses in the genital and rectal tracts. Previous experiments with *S. typhi* strains have analyzed the presence of specific IgA (or, less frequently, sIgA) at mucosal sites and in jejunal fluid, saliva, bronchoalveolar lavage fluid, and breast milk (2, 3, 18, 22, 46). In this study, we have analyzed vaginal, cervical, and rectal secretions for the presence of specific sIgA after oral and rectal immunizations with an *S. typhi* vaccine strain.

Despite some reactogenicity, the new vaccine strain was well tolerated by the volunteers, with orally or rectally administered doses as high as 3×10^9 CFU. The volunteers who reported abnormal stools and/or abdominal pain were those who responded immunologically to the vaccine (except volunteer 2), thus suggesting that these symptoms might be indicators of the take of the vaccine strain.

This study shows that the χ4632 strain is immunogenic. A fourfold increase in anti-LPS IgG was elicited in 43% of our volunteers after a single oral dose (3×10^8 CFU); this compares well with the 18% reported for a single oral dose of *S. typhi* Ty21a. However, the three spaced doses used in our vaccination schedule did not greatly improve the overall immune response, as only one of the orally immunized volunteers (volunteer 5) responded after the third immunization. In contrast, three alternate daily oral doses of 10^{11} CFU of *S. typhi* Ty21a or of *S. typhi* Ty21a expressing *Vibrio cholerae* LPS elicit seroconversion in 62 (17, 28) and 100% of the vaccines, respectively (45).

Rectal immunization with χ4632(pYA3167) was not very successful compared with that performed with three doses (2×10^{11} CFU) of *S. typhi* Ty21a. The former induced anti-LPS IgG after three doses in only one out of six volunteers (17%), while the latter induced anti-LPS IgG in three out of seven volunteers (43% [19]). However, we should note that the only anti-LPS-responding volunteer that was rectally immunized was also the only one in the study to respond to the carried antigen (pre-S1). For unknown reasons, the seroconversion rate against pre-S1 was low, at least as determined by the ELISA method used. In contrast, eight of nine mice orally immunized with an *S. typhimurium* strain carrying the same HBc–pre-S1 expression plasmid had anti-pre-S1 IgG in their sera (36) and intraperitoneal injection of our vaccine strain χ4632(pYA3167) in mice also elicited serum anti-pre-S1 IgG (37).

Overall, anti-LPS mucosal responses were detected in four out of seven orally and two out of six rectally immunized volunteers. These mucosal responses appeared to correlate with the magnitude of the serum IgG responses with some exceptions (volunteer 15) as already observed for intestinal IgA responses to *S. typhi* Ty21a (14). We have not taken samples of jejunal fluid and have used saliva to compare our results with those obtained with the Ty21a vaccine.

Three doses (10^{11} CFU) of *S. typhi* Ty21a elicited specific anti-LPS IgA in 70% of jejunal fluid samples from a large group of vaccinees ($n = 81$). This rate of response reached 100% when smaller groups of vaccinees were considered (17, 18). In contrast, only 13.6% (3 of 22) to 20% (1 of 5) of the vaccinees had some specific anti-LPS IgA in their saliva samples 2 weeks after immunization (14, 15). With our strain, three out of seven (43%) orally immunized volunteers had anti-LPS sIgA in their saliva samples at week 6 and/or 8 after immunization. This suggests either that χ4632(pYA3167) is more efficient at eliciting mucosal responses or that the response in

saliva arises later than 2 weeks after immunization (6 to 8 weeks).

Anti-LPS sIgA was found in the rectal secretions of two volunteers immunized by different routes (orally or rectally), one at all weeks of sampling (volunteer 8) and the other at week 8 (volunteer 15). Apparently, the rectal route of immunization did not induce better responses in rectal secretions than did oral immunization in contrast to results from our experiments with mice (26). We should note that no anti-LPS sIgA was found in the feces of these volunteers, suggesting that the sIgA was produced locally. Also, this rectal response did not correlate with anti-LPS serum titers. In contrast, large amounts of anti-LPS sIgA were found in the cervical and vaginal secretions of a volunteer who had a high-level serum response. Attention was paid to ensure that only sIgA and not IgA levels were measured, and thus we believe that anti-LPS sIgA was produced locally in the genital tract.

In conclusion we have shown for the first time that it is possible to induce specific sIgA both in the genital tract and in the rectal mucosa with an attenuated *S. typhi* vaccine strain. Clearly, more trials using different *Salmonella* strains and/or on a larger scale are needed to make more valid correlations.

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