Cross-reactive immune response elicited by parenteral Vi polysaccharide typhoid vaccine against non-typhoid *Salmonellae*

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**A R T I C L E   I N F O**

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**A B S T R A C T**

Background: Despite 155 000 deaths and over 90 million cases – and the current emergence of antimicrobial resistance – no vaccines are available against non-typhoid *Salmonellae* (NTS). We recently presented immunological arguments for using the oral *Salmonella* Typhi Ty21a as surrogate vaccine against NTS strains: Ty21a elicits intestinal antibodies against typhoidal O-9,12 antigen, and numerous NTS strains share one or both of these structures with *S. Typhi*. The Vi polysaccharide vaccine can, presumably because of contaminating typhoidal lipopolysaccharide, also elicit a humoral response to O-9,12, although a lower one in magnitude than the Ty21a. In this study, the Vi vaccine was explored for cross-reactive immune response to various NTS strains, and compared to that elicited by the Ty21a vaccine.

Materials and methods: Volunteers immunized with the Vi polysaccharide (Typhirix®; n = 25) were investigated for circulating plasmablasts secreting antibodies reactive with six NTS serotypes. The results were compared to those for 25 age- and gender-matched volunteers vaccinated with Ty21a (Vivotif®), as partly presented in our previous study. The cross-reactive plasmablasts elicited by the Vi vaccine were also analyzed for homing receptor expressions.

Results: 49 out of 50 vaccinees showed a cross-reactive plasmablast response against *S. Enteritidis* sharing both O-9 and O-12 antigens with *S. Typhi* (mean: 95% CI 37: 19–55 and 363: 234–493 plasmablasts/106 PBMC in the Vi and the Ty21a group, respectively). The response against strains only sharing O-12 was weaker (22: 8–38 and 222: 105–338 against *S. Typhimurium*). Strains without typhoidal O-antigens generated no significant reactivity. The cross-reactive plasmablasts elicited by the Vi vaccine had systemic homing properties.

Conclusions: The Vi vaccine elicited an immune response cross-reactive with several NTS strains. This response was lower than that in Ty21a-vaccinated volunteers. The clinical significance of these responses deserves further research with respect to both gastrointestinal and invasive NTS (iNTS) disease.

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1. Introduction

There are no vaccines available against non-typhoid *Salmonellae* (NTS). Recently, it has been shown that the oral *Salmonella* Typhi Ty21a vaccine elicits a cross-reactive immune response against NTS strains [1] expressing one (O-12) or both (O-9,12) of the typhoidal O-antigens. These NTS strains include the two most common ones, *Salmonella enterica* subspecies *enterica* serotype Enteritidis (*S. Enteritidis*) and *S. Typhimurium*. We have lately shown that an immune response to typhoidal O-antigens is produced both by the Ty21a vaccine and the Vi capsular polysaccharide typhoid preparation [2], the latter presumably because of the trace amount of LPS remaining even after purification. This opens up the interesting possibility of also the Vi vaccine eliciting an immune response cross-reactive with NTS strains sharing O-antigens of *S. Typhi*.

*S. enterica* subspecies *enterica* includes typhoidal serotypes (Typhi and Paratyphi) causing enteric fever (typhoid and paratyphoid), and approximately 2500 NTS serotypes causing mainly gastroenteritis, but also invasive NTS (iNTS) disease [3]. NTS annually accounts for more than 90 million cases of gastroenteritis...
and 155,000 deaths worldwide [4]. iNTS is rapidly emerging in Africa, affecting especially infants and those who are malnourished or infected with HIV or malaria [5–10]. Most NTS diseases are caused by S. Enteritidis and S. Typhimurium [3,7,8,10,11]. S. Enteritidis shares its O-antigen profile (O-9,12) and S. Typhimurium one O-antigen (O-12) with S. Typhi. It is noteworthy that typhoidal O-antigens are also expressed by several other common NTS and iNTS isolates [3,10–12].

NTS gastroenteritis is generally self-limited, not requiring any treatment, whereas iNTS may manifest as a fatal disease, and antimicrobials are frequently needed. In Africa, even with accurate antibiotic treatment, a case fatality rate of 22–25% has been reported for adults and children [8], and 47% for those with HIV [5]. The emerging antimicrobial resistance among NTS strains complicates the problem further [13,14]. Vaccines are urgently needed. As long as a targeted NTS vaccine is not available, the situation calls for more research into the cross-protective capacity of current typhoid vaccines.

The cross-reactivity of an immune response can be studied at a single-cell level by investigating antigen specificity of the plasmablasts appearing in the human peripheral blood after vaccination [15–18]. These cells migrate to local lymph nodes and then, via the lymphatics and blood, to sites of expected antigen encounter, after which they can be found in the circulation for approximately one week [15–17,19,20]. Homing receptors (HR) and chemokine receptors of these plasmablasts provide information of the expected localization of the immune response [15,17,19,21]. The α4β7-integrin has been recognized as the HR guiding the cells to home to the intestine [22], while 1-selectin mainly mediates more systemic homing [23], and cutaneous lymphocyte antigen (CLA) homing to skin tissue [24].

Numerous studies have characterized S. Typhi-specific plasmablasts in the circulation after typhoid vaccination either with the whole cell vaccine [17,25] or the current Vi capsular polysaccharide preparation [2]. Also plasmablasts cross-reactive with either S. Paratyphi [26] or various NTS [1] strains have been explored after oral Ty21a vaccination. The present study is the first to explore plasmablasts cross-reactive with various NTS strains in volunteers immunized with the Vi vaccine.

2. Materials and methods

2.1. Study design

Volunteers given the Vi or the Ty21a vaccine were compared for circulating plasmablasts cross-reactive with six different NTS strains. These cells were identified by enzyme-linked immunospot assay (ELISPOT) as antigen-specific antibody-secreting cells (ASC). In a subgroup of the Vi group, the homing potentials of the cross-reactive plasmablasts were characterized by combining immunomagnetic cell sorting with the ELISPOT. Levels of specific antibodies were determined by ELISA in the serum and ALS (antibodies in lymphocyte supernatants) samples.

The study protocol was approved both by the ethics committee of the Helsinki University Central Hospital and the Finnish Medicines Agency, and entered in the registry of Current Controlled Trials Ltd. c/o BioMed Central (International Standard Randomized Controlled Trial Number ISRCTN68125331). Written informed consent was obtained from all study subjects.

2.2. Volunteers, vaccinations and samples

Fifty age- and gender-matched healthy Finnish-born volunteers with no history of enteric fever or typhoid vaccination were randomized in two groups (both groups comprised 17 females, 8 males, aged 22–62, mean age 32). Twenty-five received a parenteral Vi capsular polysaccharide vaccine (Typherin®, GlaxoSmithKline Biologicals s.a., Rixensart, Belgium, lots ATYPBO848C and ATYPBO966AF with endotoxin contents of 27.00 µg and 13.30 µg, respectively), and 25 the oral Salmonella Typhi Ty21a vaccine (Vivotif®, Crucell AB, Leiden, The Netherlands, lot 3001777). The Vi vaccine was administered as one 0.5 ml dose intramuscularly with a 25 mm needle on day 0. The oral vaccine, containing at least 2 × 10^9 live bacteria/capsule, was administered one capsule on each of days 0, 2, and 4. As previously reported, both vaccines proved well-tolerated [2].

ASC appear in the circulation on day 2–3, peaking on day 7 after both mucosal [15,16,21,25] and parenteral [2,25] immunizations. Accordingly, blood samples for ELISPOT and ALS were drawn before and 7 days after immunization. Serum samples were collected before and 28 days after vaccination.

The NTS-specific responses of the Ty21a group’s 22 volunteers have been incorporated in our recently published data on 35 volunteers (no age- and gender-matched controls were included) [1]. To allow comparison between typhoid- and NTS-specific immune responses, data on responses to S. Typhi in both the Ty21a and the Vi groups were retrieved from our recent report on typhoid-specific responses in these groups [2].

2.3. Isolation of peripheral blood mononuclear cells (PBMC)

PBMC were separated using Ficoll-Paque centrifugation of fresh heparinized venous blood, as described previously [16].

2.4. Separation of HR-negative and -positive cell populations

The expressions of HR on S. Enteritidis-specific ASC were explored in seven Vi-vaccinated volunteers, as described earlier [1]. In brief, PBMC (3.4 × 10^6 PBMC per HR) were incubated with monoclonal antibodies against α4β7 (ACT-1, Millennium Pharmaceuticals, Cambridge, MA), 1-selectin (Leu 8, Becton Dickinson, Erenbodegem-Aalst, Belgium), or CLA (HECA-452, a gift from Dr. Sirpa Jalkanen, Finland). Next, the cells were incubated with Dynal® M-450 magnetic beads coated with sheep anti-mouse IgG (Dynabeads, Dynal Biotech, Oslo), followed by magnetic separation.

2.5. ELISPOT assay of specific ASC

The PBMC – and for HR analyses, the receptor-positive and -negative cell populations – were assayed for ASC using ELISPOT, as described earlier [1,2,16]. The bacterial strains used for coating in the ELISPOT assay are shown in Table 1 along with the O-antigens they express. Preparation of the strains for coating has been described previously [1]. 96-Well microtiter plates (Maxisorp, Nunc, Roskilde, Denmark) were coated with the antigen preparations. The cells were then incubated in the wells, and the antibodies secreted were detected with alkaline phosphatase-conjugated goat anti-human IgA (Sigma–Aldrich), IgG (Sigma–Aldrich) and IgM (SouthernBiotek, Birmingham, England). The substrate (5-bromo-4-chloro-3-indolyl phosphate p-toluidine salt; Sigma–Aldrich) was added in melted agarose. Each spot enumerated with an AID ELISPOT reader was interpreted as a print of one ASC. A response was defined as at least 3 ASC/10^6 PBMC and marked as LOD (limit of detection of the response) in the figures.

2.6. ALS cultures

PBMC were cultured in RPMI 1640 as described previously [1]. Supernatants were collected after three days, and stored at −70 °C until assayed.
Table 1
Description of bacterial strains, plasmablast responses to each strain and results of statistical comparisons. The bacterial strains* used in the ELISPOT assay, the O-antigens of each strain, the number of plasmablasts (ASC/10^6 PBMC) specific to each strain in 25 volunteers vaccinated one week earlier with the Vi polysaccharide vaccine or the oral Salmonella Typhi Ty21a vaccine (means and 95% confidence intervals), magnitude of the response to each NTS serotype in percentages of the S. Typhi-specific response, and statistical comparison (Wilcoxon’s signed rank test with Bonferroni correction) between the responses to various strains and (Wilcoxon’s signed rank test) comparison between vaccine groups. The light grey in the background indicates results of the comparison in Ty21a-vaccinated volunteers (Wilcoxon’s signed rank test with Bonferroni correction); dark grey indicates results of the comparison in Vi-vaccinated volunteers, and white indicates comparison between Ty21a vs. Vi vaccine groups. Significant differences are indicated with asterisks (**P < 0.01; ***P < 0.001; NS, not significant).

<table>
<thead>
<tr>
<th>Antigens, their origin, and magnitude of response</th>
<th>Comparison with light grey: Ty21a-vaccinated volunteers</th>
<th>Comparison with dark grey: Vi-vaccinated volunteers</th>
<th>Comparison with white: Ty21a group vs. Vi group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial strain* Strain: O-antigens and Vi-antigen</td>
<td>Vi vaccine</td>
<td>Ty21a vaccine</td>
<td>Vi vaccine</td>
</tr>
<tr>
<td>S. Typhi</td>
<td>Value calculated</td>
<td>9, 12, Vi</td>
<td>149 (1–217)</td>
</tr>
<tr>
<td>S. Enteritidis</td>
<td>RHS634</td>
<td>1, 9, 12</td>
<td>38 (19–55)</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>8965</td>
<td>1, 4, 5, 12</td>
<td>22 (8–37)</td>
</tr>
<tr>
<td>S. Agona</td>
<td>RHS6160</td>
<td>4, 12</td>
<td>17 (3–30)</td>
</tr>
<tr>
<td>S. Stanley</td>
<td>RHS6766</td>
<td>4, 5, 12</td>
<td>16 (4–28)</td>
</tr>
<tr>
<td>S. Virchow</td>
<td>RHS6740</td>
<td>6, 7</td>
<td>2 (1–3)</td>
</tr>
<tr>
<td>S. Hadar</td>
<td>RHS148</td>
<td>6, 8</td>
<td>1 (1–1)</td>
</tr>
</tbody>
</table>

*All strains were obtained from the collection of the Gastrointestinal Infections Unit of the National Institute for Health and Welfare, Helsinki, Finland.

2.7. ELISA
Antibodies in serum and ALS samples were measured with ELISA, as described earlier [1]. Briefly, microtiter plates (Polysorp, Nunc) were coated with LPS of S. Enteritidis or S. Typhimurium (both from Sigma–Aldrich) and blocked. The samples were incubated in the wells overnight and horseradish peroxidase (HRP)-conjugated rabbit anti-human IgA, IgG and IgM antibodies (all from Dako) were used as secondary antibodies, and TMB peroxidase as substrate (3,3’,5,5’-tetramethylbenzidine and H_2O_2 in citric acid buffer; KPL, Gaithersburg, USA). A response was defined as at least two-fold titre increase from prevaccination level.

2.8. Statistics
The proportions of the receptor-positive ASC were calculated as follows: percentage of receptor-positive ASC = (100 × the number of ASC in receptor-positive cell population)/(the sum of the number of ASC in receptor-positive and receptor-negative cell populations).

Statistical analyses were carried out with JMP software version 9.0.0 (SAS Institute Inc., Cary, NC, USA). The distributions of the ASC and HR expressions were tested with Shapiro–Wilk’s test. Since not all distributions proved normal even after log transformations, Wilcoxon’s signed rank test with Bonferroni correction was used for comparisons between multiple groups, and Wilcoxon’s signed rank test for two groups.

3. Results

3.1. ASC response in Vi group
Before vaccination, no NTS-specific ASC were found in the circulation of 24/25 vaccinees in the Vi group. One volunteer had 118 ASC/10^6 PBMC to S. Stanley and 95 to S. Hadar (Fig. 1). Unfortunately, all antigens could not be measured for this vaccinee because of accidental loss of cells at the laboratory. It is noteworthy that the same volunteer had had a severe flu just a week ago. We have previously shown that infections can be associated with a polyclonal immune response [27].

A significant number of circulating S. Typhi-specific ASC has been reported earlier for all these vaccinees on day 7 [2]. In the present study 24 out of 25 showed a cross-reactive response to S. Enteritidis, 19/25 to S. Typhimurium and 16/25 to S. Agona, and 18/25 to S. Stanley, and 8/25 and 2/25 a minor response to S. Virchow and S. Hadar, respectively (Fig. 1 and Table 1). The responses were mostly IgA-dominated (Fig. 2), confirming previous reports that IgA [18,28] or IgA and IgM [2] predominate in responses elicited by parenteral polysaccharide vaccines.

3.2. ASC response in Ty21a group
Before vaccination, no ASC specific to S. Typhimurium, S. Agona, S. Stanley, or S. Virchow were found in the circulation of any of
Fig. 1. Plasmablast response to various non-typhoidal Salmonella strains on days 0 and 7 after immunization with Vi capsular polysaccharide vaccine. Numbers of circulating antigen-specific plasmablasts, identified as antibody-secreting cells (ASC) against S. Enteritidis, S. Typhimurium, S. Stanley, S. Agona, S. Virchow and S. Hadar in 25 volunteers immunized with the Vi vaccine. The lines represent the numbers of Ig(A+G+M) plasmablasts of individual vaccinees on days 0 and 7 after vaccination. LOD, lower limit of detection of the response. The upper indexes (+) indicate strains with (−) no O antigen, (+) one O-antigen (O-12) or (++) two O-antigens (O-9 and O-12) in common with S. Typhi.

Fig. 2. Immunoglobulin isotype distribution of NTS-specific cross-reactive plasmablasts in volunteers immunized with Vi vaccine. Immunoglobulin isotype distribution of antibodies secreted by plasmablasts reactive with S. Enteritidis, S. Typhimurium, S. Agona and S. Stanley (ASC/10^6 PBMC) in 25 volunteers immunized with the Vi polysaccharide vaccine. The dots represent results of individual vaccinees, and the lines the means of the number of plasmablasts secreting specific antibodies of the IgA, IgG or IgM isotype on day 7 after vaccination. The upper indexes (+) indicate strains with one O antigen (O-12) (+) or two O antigens (O-9 and O-12) (++) in common with S. Typhi.
the vaccinees. One volunteer had 5 ASC/10^6 PBMC reactive with S. Enteritidis, and another 40 ASC reactive with S. Hadar before vaccination, as reported earlier [1].

Seven days after vaccination, in addition to the response to S. Typhi [2], a significant number of S. Enteritidis-specific ASC was detected in all vaccinees. 23 out of 25 showed a response to S. Typhimurium, 22/25 to S. Agona and S. Stanley, 8/25 a minor response to S. Virchow, and 6/25 to S. Hadar (Fig. 3 and Table 1).

3.3. Comparison of ASC responses between Vi and Ty21a groups

All the cross-reactive responses to NTS proved lower for the Vi than the Ty21a group (Fig. 3). In both groups the highest cross-reactive responses were seen to S. Enteritidis sharing both O-antigens with S. Typhi, and responses were substantial also against strains only sharing one O-antigen, such as S. Typhimurium (Fig. 3).

3.4. Expression of α4β7, L-selectin and CLA on cross-reactive ASC in Vi group

In the Vi group, the majority of ASC cross-reactive with S. Enteritidis expressed the peripheral lymph node HR, L-selectin, whereas a lower proportion expressed the intestinal HR, α4β7-integrin, and only a minority the cutaneous HR, CLA (Fig. 4). In the Ty21a-vaccinated volunteers we have earlier reported [1] a mucosal homing profile with a high proportion of α4β7-expressing cells and lower proportion of L-selectin or CLA positive cells (Fig. 4).

3.5. Antibody responses in ALS and serum samples in Vi group

In the Vi group, the number of vaccinees responding with a twofold or higher rise in antibody titres in ALS and serum samples was smaller than the number of those responding in the ELISPOT assay (Table 2). For the Ty21a group respective results have been reported previously [1], and are now presented as a footnote to Table 2. The results of responses against S. Typhi, also reported earlier [2], are now disclosed in a footnote to Table 2.

4. Discussion

Non-typhoid Salmonella is a leading cause of foodborne illness, yet with no vaccines in clinical use. Recently, the Salmonella Typhi Ty21a vaccine has been suggested as a surrogate against NTS until a targeted product becomes available [1]. The present study is the first to compare the NTS-specific cross-reactive immune responses between the two currently employed preparations against typhoid fever: the parenteral Vi polysaccharide and the oral Ty21a vaccine.

The immunological basis for the cross-reactive response against a part of the NTS can presumably be ascribed to O-antigens shared between the vaccines and these NTS. Ty21a is a whole-cell vaccine incorporating the typhoidal O-9,12-antigens, whereas the Vi preparation contains these antigens only as a contaminating typhoid LPS remaining after purification [2]. Some of the NTS strains share both typhoidal O-antigens, while others only share the O-12 antigen or none at all. The cross-reactive plasmablast responses were almost exclusively seen against strains that have O-antigens in common with S. Typhi. Eight volunteers had a minor response to
Table 2

Antibody responses in serum and ALS cultures. Numbers of vaccinees responding in serum, ALS culture and ELISPOT assays to S. Enteritidis and S. Typhimurium. In the ELISA assays (serum and ALS), a responder was defined as an individual with at least two-fold increase in titre (in IgA, IgG and/or IgM isotype). In the ELISPOT assay, a responder was defined as having at least 3 ASC/10^6 PBMC on day 7. The samples for ELISPOT and ALS were collected on days 0 and 7, and serum samples on days 0 and 28 after the Vi polysaccharide vaccination. The number of vaccinees tested with each assay is indicated in the table.

<table>
<thead>
<tr>
<th>Salmonella enterica serotype</th>
<th>Responders in assay for</th>
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<tbody>
<tr>
<td></td>
<td>Serum antibodies/ELISA^1</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>25 vaccines</td>
<td>IgA</td>
<td>IgG</td>
<td>IgM</td>
<td>IgA/IgG/IgM</td>
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<tr>
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<td>7</td>
<td>6</td>
<td>7</td>
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<td>Typhimurium</td>
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<td>4</td>
<td>1</td>
<td>8 (32%)^a</td>
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<tr>
<td></td>
<td>ALS/ELISA^2</td>
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<tr>
<td></td>
<td>11 vaccines</td>
<td>IgA</td>
<td>IgG</td>
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<tr>
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<td>0</td>
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<tr>
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<tr>
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<td>17</td>
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<td>14</td>
<td>24 (96%)^2</td>
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<tr>
<td>Typhimurium</td>
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<td>14</td>
<td>8</td>
<td>10</td>
<td>19 (96%)</td>
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</table>

^1 Comparative results shown in our earlier study [1] with age- and gender-matched Ty21a-immunized volunteers were ^48%, ^85%, ^100% to S. Enteritidis, and ^20%, ^54%, and ^94% to S. Typhimurium.

^2 Proportions of responders to S. Typhi have been reported previously [2]: the percentage of responders with serum assay was 32%, with ALS 9%, and ELISPOT 96% in Vi-immunized volunteers, and 52%, 84%, and 100% in Ty21a-immunized volunteers, respectively. The ELISA assay was only based on S. Typhi IFS, not on the Vi-antigen.

NTS strains sharing no O-antigens; similar findings after Ty21a vaccination have previously been suggested to be due to some minor shared antigens, e.g. some protein structures [1]. Very low in magnitude, such responses have been considered to lack clinical significance.

The magnitude of all cross-reactive responses to NTS strains proved significantly lower in the VI than the Ty21a group. This result accords with our previous findings on immune responses to various typhoid antigens, where the O-antigen-specific responses were lower in the VI- than the Ty21a-vaccinated volunteers [2]. As for responses to NTS strains with only one typhoidal O-antigen, logically, the cross-reactive ones were significantly lower than those to S. Enteritidis or S. Typhi in both vaccination groups. Notably, all the VI-vaccinated volunteers with high responses to S. Typhi also had high cross-reactive responses. In 28% and 12% of cases, it exceeded an arbitrary limit of 50 cross-reactive ASC/10^6 PBMC against S. Enteritidis and S. Typhimurium, respectively. These data suggest that despite the generally low cross-reactive plasmablast responses to NTS in the VI group, in some volunteers the response reaches high levels as high as with the Ty21a, and may potentially have clinical relevance (see below).

In contrast to the intestinal homing profile of the Ty21a group’s response [1], the cross-reactive cells elicited by the parenteral Vi vaccine had a systemic homing profile (high proportion of L-selectin^+; lower of α4β7^+ and CLA^+). Indeed, homing profiles of plasmablasts depend on the site of antigen encounter: typhoid-specific plasmablasts exhibit an intestinal homing profile after oral vaccination [17] and in natural typhoid infection [2], while a parenterally administered whole cell Ty21a vaccine induces plasmablasts with a systemic homing profile [17]. Consistently, we have also shown an intestinal homing profile in NTS gastroenteritis [18,20].

A cross-reactive response could be identified in all three types of analysis, ELISPOT, ALS and serum, each showing a response to S. Enteritidis more frequently than to S. Typhimurium, in line with the number of O-antigens shared. In contrast to ELISPOT, however, a cross-reactive response in serum and ALS assays did not prove to be a frequent phenomenon; on the contrary, seroconversion was lacking in most volunteers. In the VI group, the seroconversion rate is known to depend on the amount of contaminating LPS in the vaccine preparation: Tacket et al. have shown that 5% and 0.2% LPS contamination in a VI vaccine preparation causes a seroconversion against S. Enteritidis in 83% and 26% of volunteers, respectively [29].

In accord with our previous report on Ty21a-vaccinated volunteers [1,2], also in the VI group, the ELISPOT illuminating responses at single-cell level proved more sensitive than measurements of antibody concentrations in serum and ALS by ELISA. While the ALS assay proved more sensitive than serum in assessing the immune response in the Ty21a group [1,2], in the VI group the reverse was found, consistent with a mainly intestinal response in the Ty21a and a mainly systemic one in the VI group. Indeed, the significance of any plasmablast response should never be estimated solely on the basis of its magnitude, but instead its targeting should be considered at the same time.

The protective efficacy of the O-antigen-specific antibodies against NTS deserves special interest, as cross-reactivity is based on these antigens. The efficacy of O-antigen-specific responses should be considered separately for systemic and mucosal antibodies.

The functional activity of the antibodies was not evaluated in the present study. MacLennan et al. have, however, shown a protective role for antibody-induced complement-mediated killing of NTS in African children with serum antibodies against O-antigens [9]. They found a relative absence of NTS bacteremia among children aged 1–4 months [9] and a peak after the maternal antibody levels have waned [30]. They have also shown a rise in anti-Salmonella IgG and IgM antibody titres, and bactericidal activity of serum against NTS with age, corresponding with a fall in NTS bacteremia cases [9], which suggests that serum Salmonella-specific antibodies may serve to protect against iNTS. Interestingly, in patients infected with HIV, an overproduction of S. Typhimurium O-antigen-specific IgG has been reported, and in contrast to studies in healthy children.
these antibodies have been found to correlate with impaired immunity against iNTS [31]. This dysregulation in HIV-infected persons has only been associated with antibodies of IgG isotype [31], not with IgM. Notably, the majority of cross-reactive serum antibodies in the present study belonged to IgA and IgM isotypes in Ty21a group and IgA in Vi group.

The potential protective efficacy of mucosal O-antigen-specific responses has been discussed in detail previously [1]. In brief, intestinal or mucosal secretory IgA have been found protective against S. Typhimurium O-antigens in numerous animal experiments [32–36]; these antibodies have been shown to block the invasion of S. Typhimurium [33]. In addition, introducing monoclonal O-antigen-specific antibodies in the intestinal tract has been proven to prevent invasive infection with S. Typhimurium [32]. Importantly, parenterally immunized mice did not mount detectable levels of O-antigen-specific mucosal antibodies and, in contrast to the mucosally immunized mice, none of them were protected against non-invasive wild type S. Typhimurium infection [36].

The clinical significance of a potential cross-protection conferred by any vaccine depends on the local occurrence of NTS strains carrying the O-9 or O-12 antigens. In Africa, most invasive NTS diseases are caused by S. Enteritidis (O-9,12) and S. Typhimurium (O-12) [37]. S. Enteritidis and S. Typhimurium represent 75% of the reported Salmonella cases in the EU [11], and 32% of all Salmonella isolates in USA (S. Enteritidis 17% and S. Typhimurium 15%) [3] where approximately half of all Salmonella strains carry typhoidal O-antigens, one quarter both O-antigens 9 and 12, and one quarter O-12 [3]. Hence, NTS strains with typhoidal O-antigens are common, and potential cross-protective efficacy deserves to be explored in the future.

5. Conclusions

The present study shows that the Vi vaccine elicits a mainly systemic humoral cross-reactive immune response to numerous NTS strains sharing O-antigens with Salmonella Typhi. The response was found lower than that in age- and gender-matched volunteers receiving the Ty21a vaccine known to elicit both intestinal and systemic responses. Efficacy studies are required, however, before any conclusions on clinical protective efficacy can be drawn. In the present situation, with NTS remaining a global health problem, antimicrobial resistance increasing, and vaccines for clinical use lacking, even a lower degree of cross-protective capacity in a preparation currently available should be welcomed and explored further.

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Contributions

AK, JK, CH and SP conceived and designed the experiments. SP carried them out, and AK, JK, and SP analyzed the data. AK contributed reagents, materials, and analysis equipment. AK, JK, CH and SP wrote the report.

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Conflict of interest statement: AK has participated as a member in advisory boards of Pfizer, GlaxoSmithKline, and Novartis, and received honoraria for that. She has acted as a consultant to Crucell on vaccination immunology, participated in international travel medicine meetings at the expense of Crucell and GlaxoSmithKline, and been reimbursed for giving lectures by Janssen, GSK, Baxter, and Pfizer. CH is a former employee of Crucell. SHP and JMK declare no conflicts of interest.

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