Specific and cross-reactive immune response to oral Salmonella Typhi Ty21a and parenteral Vi capsular polysaccharide typhoid vaccines administered concomitantly

Pakkanen, Sari H.

2015-01-09

Pakkanen , S H , Kantele , J M , Savolainen , L E , Rombo , L & Kantele , A 2015 , ' Specific and cross-reactive immune response to oral Salmonella Typhi Ty21a and parenteral Vi capsular polysaccharide typhoid vaccines administered concomitantly ' , Vaccine , vol. 33 , no. 3 , pp. 451-458 . https://doi.org/10.1016/j.vaccine.2014.11.030

http://hdl.handle.net/10138/203411
https://doi.org/10.1016/j.vaccine.2014.11.030

Vaccine 33 (2015) 451–458

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Specific and cross-reactive immune response to oral *Salmonella* Typhi Ty21a and parenteral Vi capsular polysaccharide typhoid vaccines administered concomitantly

Sari H. Pakkanen[a,b], Jussi M. Kantele[c], Laura E. Savolainen[a], Lars Rombo[d,e], Anu Kantele[a,b,f,g,*]

[a] Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, POB 21, 00014 Helsinki, Finland
[b] Department of Medicine, Division of Infectious Diseases, Helsinki University Hospital POB 348, 00029 Helsinki, Finland
[c] Department of Medical Microbiology and Immunology, University of Turku, Kiinamyllynkatu 13, 20520 Turku, Finland
[d] Karolinska Institutet, Department of Medicine/Solna, Unit for Infectious Diseases, Stockholm, Sweden
[e] Centre for Clinical Research, Sörmland County Council, Eskilstuna, Sweden
[f] Department of Medicine, University of Helsinki, POB 20, 00014 Helsinki, Finland
[g] Aava Travel Clinic, Medical Centre Aava, Helsinki, Finland

ARTICLE INFO

Article history:
Received 30 June 2014
Received in revised form 5 November 2014
Accepted 17 November 2014
Available online 26 November 2014

Keywords:
Typhoid fever
Paratyphoid fever
Salmonella gastroenteritis
Salmonella Typhi
S. Paratyphi
Non-typhoid *Salmonella*
Typherix
Typhim Vi
Vivotif
Salmonella Typhi Ty21a
Vi polysaccharide
Antibody-secreting cell
Cross-reactive
ELISPOT
HomingClinicalTrials.gov NCT02121145

ABSTRACT

Background: Since protective efficacy of the current typhoid vaccines—oral whole-cell *Salmonella* Typhi Ty21a and parenteral Vi-capsular polysaccharide preparation—is not optimal, and no vaccines are available against paratyphoid or non-typhoidal *Salmonella* (NTS) serotypes, new approaches deserve to be explored. The immunological mechanisms elicited by the two typhoid vaccines are mainly targeted against different structures. We studied whether these vaccines would enhance *S.* Typhi-specific immune response and cross-reactivity against other *Salmonellae*, if administered concomitantly.

Materials and methods: Volunteers were immunized simultaneously with Ty21a and Vi vaccines (Ty21a + Vi group) or with either of the two singly (Ty21a and Vi groups). All volunteers were investigated for circulating specific and cross-reactive plasmablasts, identified by ELISPOT as IgA, IgG or IgM antibody-secreting cells (ASC) reactive with *S.* Typhi, *S.* Paratyphi A/B/C, or selected NTS serotypes (*S.* Enteritidis, *S.* Typhimurium).

Results: In the Ty21a + Vi group, no specific or cross-reactive plasmablasts were detected before vaccination. After vaccination, the number of *S.* Typhi-specific plasmablasts (878 ASC/10^6 PBMC, 95%CI 554–1201) proved higher than in the Ty21a (339 ASC/10^6 PBMC; $p < 0.001$) and Vi (149 ASC/10^6 PBMC; $p < 0.001$) groups. Likewise, cross-reactive responses in the Ty21a + Vi group were higher than in the Ty21a and Vi groups (Ty21a + Vi vs Ty21a: ASC against *S.* Paratyphi A/B, *S.* Enteritidis and *S.* Typhimurium $p < 0.05$, against *S.* Paratyphi C $p < 0.01$; Ty21a + Vi vs Vi: against *S.* Paratyphi C not significant, others $p < 0.0001$). A gut-directed homing profile was seen among O antigen-specific and a systemic one among Vi antigen-specific plasmablasts.

Conclusions: Concomitant administration of Ty21a and Vi vaccines is well tolerated and induces an additive immune response to the two vaccines. Thus it enhances the magnitude of both typhoid-specific plasmablast responses and those cross-reacting with paratyphoid and most important NTS serotypes. The data encourage concomitant use of Ty21 and Vi vaccines for those at risk.

Abbreviations: ASC, antibody-secreting cell; CLA, cutaneous lymphocyte antigen; HR, homing receptor; iNTS, invasive non-typhoid *Salmonella*; NTS, non-typhoid *Salmonella*; PBMC, peripheral blood mononuclear cell; PBS, phosphate-buffered saline.

* Corresponding author at: Division of Infectious Diseases, Department of Medicine, Helsinki University Central Hospital, POB 348, FIN-00029 HUS, Helsinki, Finland. Tel.: +358 50 309 7640; fax: +358 9 471 75900.
E-mail address: anu.kantele@hus.fi (A. Kantele).

http://dx.doi.org/10.1016/j.vaccine.2014.11.030

1. Introduction

Diseases caused by *Salmonella* species constitute a serious health problem worldwide. *Salmonella enterica* subspecies *enterica* serotype Typhi (*S.* Typhi) causes typhoid fever, while *S. enterica* subspecies *enterica* serotype Paratyphi A/B/C (*S.* Paratyphi A/B/C) are the etiological agents of paratyphoid fever. Together known as enteric fever, typhoid and paratyphoid mostly prevail in developing countries, where they pose a health risk also to visitors. Non-typhoidal *Salmonellae* (NTS) cause food-borne gastroenteritis and invasive non-typhoidal salmonellosis (iNTS); they are encountered both in the developing and developed world. Globally, the annual incidence of typhoid fever is estimated at 22 million [1], paratyphoid fever at 5.4 million [1], and NTS diseases at 94 million cases [2]. Because of increasing antimicrobial resistance, the need for preventive measures such as vaccination has become more and more urgent.

Currently, two types of vaccines—oral live attenuated *Salmonella* Typhi Ty21a (Vivotif®) and the parenteral capsular Vi polysaccharide preparations (Typherix® or Typhim Vi®)—are available against typhoid fever, while none are licensed against paratyphoid or NTS serotypes. Interestingly, Ty21a and, to a lesser extent, the Vi vaccine have been shown to elicit cross-reactive immune responses against paratyphoid serotypes [3–9] and the most common NTS *Salmonellae* [10,11]. The main underlying cause of cross-reactivity are the O-antigenic structures these strains share with *S.* Typhi: while *S.* Typhi expresses O-9,12, *S.* Paratyphi A and B carry the O-12, and many NTS *Salmonellae* express either O-9,12 (e.g. *S.* Enteritidis) or O-12 (e.g. *S.* Typhimurium). Specific and cross-reactive humoural immune responses can be explored at single-cell level with the help of plasmablasts appearing transiently in the circulation after vaccination [5,10,16–18]. Indeed, plasmablasts are of special interest when studying typhoid vaccines: they have been suggested as surrogate markers of protection against the disease [19]. Plasmablasts represent recently activated B cells trafficking, via lymphatics and blood, from the site of antigen encounter to their final effector sites [20]. Before homing to tissues, these cells can be caught from the circulation for a period of approximately one week, their magnitude peaking on day 7 [16,17]. The homing process is known to be tissue-specific: sc. homing receptors (HR) and chemokine receptors (CCR) on lymphocyte surface recognize their ligands in target tissues [20]. Analysis of HR and CCR on circulating plasmablasts can be used to explore the expected localization of the immune response [21–25]. The cells are guided by the HR $\alpha_4\beta_7$-integrin to the intestine [26], L-selectin mainly to systemic sites [27], and cutaneous lymphocyte antigen (CLA) to the skin [28].

Even if the two typhoid vaccines, Ty21a and Vi, have in field trials exhibited a similar protection rate of 60–70% against typhoid fever [29,30], their underlying protective mechanisms differ. Ty21a is a live Vi-negative whole-cell vaccine shown both to elicit humoral [19] and cell-mediated immune responses [19], while the Vi preparation consists of purified capsular Vi polysaccharide eliciting a humoural response mainly to the Vi antigen [18,19]. We hypothesized that, given at the same time, the benefits of both vaccines could be exploited. This is the first study to explore immune response to the two vaccines administered concomitantly.

2. Materials and methods

2.1. Study design

Three groups were immunized: one receiving both Ty21a and Vi vaccines simultaneously (Ty21a + Vi group), the other two either Ty21a (Ty21a group) or Vi vaccine (Vi group). Circulating specific and cross-reactive plasmablasts were identified by enzyme-linked immunospot assay (ELISPOT) as IgA, IgG and IgM antibody-secreting cells (ASC) with given specificity. The plasmablasts' homing potentials were characterized by combining immunomagnetic cell sorting with the ELISPOT. Results of the Ty21a and Vi groups have been reported earlier [9,11,18].

The study protocol was approved by the ethics committee of the Helsinki University Central Hospital and the Finnish Medicines Agency (EudraCT 2009-012949-33), and registered in the databases required (ClinicalTrials.gov NCT02121145). Written informed consent was obtained from all subjects. The investigation was conducted at the Travel clinic of the Medical Centre Aava, Helsinki University Central Hospital and the Haartman Institute, University of Helsinki.

2.2. Volunteers, vaccinations and samples

The Ty21a and Vi groups, each comprised 25 vaccinees, 17 females and 8 males, aged 22–62, mean age 32, as presented earlier [9,11,18]. The Ty21a + Vi group included twenty-four Finnish-born volunteers (11 females, 13 males, aged 22–29, mean 25 years) with no history of enteric fever or typhoid vaccination. They received both the oral *Salmonella* Typhi Ty21a vaccine (Vivotif®, Crucell AB, Leiden, The Netherlands, lot 3000620) and the parenteral Vi capsular polysaccharide vaccine (Typherix®, GlaxoSmithKline Biologicals s.a., Rixensart, Belgium, lot ATYPB096AF with endotoxin contents of 13.30EU) at the same time. The oral vaccine, containing at least 2×10^9 live bacteria/capsule, was administered as one capsule on days 0, 2, and 4, and the Vi vaccine as one 0.5 ml dose intramuscularly (25-mm needle) on day 0. Blood samples were drawn on days 0 and 7. The Ty21a and Vi groups were immunized 2010–2011 and the Ty21a + Vi group 2013. The ELISPOT assay was validated before the study, and proved to be highly repetitive (data not shown).

Combined use of the two vaccines was well tolerated: 7/24 vaccinees reported mild adverse effects (loose stools 2, one each: stomachache, nausea, flatulence, dizziness after injection, aphtha in the mouth) resembling those seen in the Ty21a (stomachache 1, tiredness 1) and Vi (one each: fever, loose stools, pain at the injection site, constipation) group.

2.3. Isolation of peripheral blood mononuclear cells (PBMC)

PBMC were separated using Ficoll-paque centrifugation of heparinized venous blood, as described before [16].

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

Because of limited numbers of PBMC, HR analyses could not be carried out for all volunteers. Four in the Ty21a + Vi group were subjected to an analysis of HR expressions on O-9,12-specific ASC and one volunteer to HR expressions on Vi antigen-specific ASC as described earlier [10,18,22]. Briefly, PBMCs (3.4×10^6 PBMC per HR) were incubated with monoclonal antibodies against $\alpha_4\beta_7$ (ACT-1, Millennium Pharmaceuticals, Cambridge, MA), L-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 and ELISPOT assay.

2.5. ELISPOT assay for specific and cross-reactive ASC

PBMCs and, for HR analyses, the receptor-positive and -negative cell populations, were assayed for ASC with ELISPOT as described earlier [10,16]. In brief, 96-well microtitre plates (Maxisorp, Nunc, Roskilde, Denmark) were coated with whole-cell bacteria (Table 1)

Table 1
Description of bacterial strains and specific and cross-reactive plasmablast responses. Data are provided on bacterial strains[a] used in the ELISPOT assay, the O and Vi antigens of each strain, proportion of responders, and numbers of antigen-specific ASC shown by volunteers in the Ty21a + Vi, Ty21a and Vi groups (arithmetic means of Ig(A + G + M)-ASC and 95% CI). Response to *S.* Typhi is calculated as the total of responses to the O-9,12 and Vi antigens. The data on the Ty21a or Vi groups have been retrieved from our previous reports [9,11,18].

Bacterial strain[a]	Strain	O antigens and Vi antigen	Plasmablast response					
			% Responding			Arithmetic mean (95%CI) ASC/10^6 PBMC		
			Ty21a + Vi	Ty21a	Vi	Ty21a + Vi	Ty21a	Vi
S. Typhi	Value calculated	9, 12, Vi	100	100[b]	100[b]	878 (554–1201)	339 (155–521)[b]	149 (81–217)[b]
S. Paratyphi A	RHS6716	1, 2, 12	92	88[c]	60[c]	228 (117–339)	111 (31–197)[c]	20 (2–37)[c]
S. Paratyphi B	RHS6744	1, 4, 5, 12	96	92[c]	72[c]	310 (149–471)	137 (39–236) [c]	22 (4–39)[c]
S. Paratyphi C	ATCC 13428	6, 7, Vi	63	36[c]	64[c]	104 (21–187)	4 (1–6)[c]	22 (8–35)[c]
S. Enteritidis	RHS634	1, 9, 12	100	100[d]	96[d]	555 (341–768)	364 (235–493)[d]	38 (19–55)[d]
S. Typhimurium	8965	1, 4, 5, 12	96	92[d]	76[d]	297 (153–442)	222 (105–339)[d]	22 (8–37)[d]
S. Hadar	RHS148	6, 8	29	24[d]	8[d]	5 (1–9)	1 (1–2)[d]	1 (1–1)[d]
Yersinia enterocolitica	RHI4823	–	8	0[b]	4[b]	1 (0–1)	0 (0–1)[b]	1 (1–2)[b]

[a] *S.* Paratyphi C strain was obtained from the American Type Culture Collection (ATCC, Manassas, VA), while the others were from the National Institute for Health and Welfare, Helsinki, Finland.
[b] Data retrieved from our previous report [18].
[c] Data retrieved from our previous report [9].
[d] Data retrieved from our previous report [11].

or Vi antigen, as described earlier [5,10,18]. To confirm the expression of the various O and Vi antigens on the coating strains, each strain had been analysed at the Finnish national reference laboratory (Gastroenterological Unit of the National Institute for Health and Welfare in Helsinki) according to their routines. Next, PBMCs were 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 (SouthernBiotech, 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 or by microscope was interpreted as a print of one ASC. A responder was defined as one having at least 3 ASC/10^6 PBMC; this limit was marked as LOD (limit of detection of the response).

2.6. Statistics

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

Statistical analyses were carried out with JMP software version 11.0.0 (SAS Institute Inc, Cary, NC, USA). The distributions of the ASC were verified with Shapiro–Wilk's test. Since not all proved normal even after log transformations, Wilcoxon signed-rank test was used for comparisons.

3. Results

3.1. S. Typhi-specific ASC responses

None of the vaccinees in the Ty21a + Vi group had circulating *S.* Typhi-specific ASC before vaccination. On day 7, such ASC were found in all volunteers: 636 (95%CI 394–879) ASC (IgA + IgG + IgM)/10^6 PBMC as an arithmetic mean of O-9,12-specific, and 250 (95%CI 52–449) Vi-specific ASC, totalling 878 (95%CI 554–1201) *S.* Typhi-specific ASC (Fig. 1 and Table 1). The respective figures for Ty1a and Vi groups have been reported earlier [18].

3.2. Cross-reactive ASC responses

None of the volunteers in the Ty21a + Vi group had circulating ASC cross-reactive with *Salmonellae* before vaccination. By day 7 most vaccinees had developed plasmablasts cross-reactive with serotypes sharing O or Vi antigen(s) with *S.* Typhi: 22/24 showed a response to *S.* Paratyphi A, 23/24 to *S.* Paratyphi B, 15/24 to *S.* Paratyphi C, 24/24 to *S.* Enteritidis and 23/24 to *S.* Typhimurium (Table 1 and Fig. 2). Minuscule amounts of plasmablasts against *S.* Hadar (O-6,7) were found in the blood of 7/24 vaccinees, and of 2/24 vaccinees against *Yersinia enterocolitica*, the negative control strain (Table 1 and Fig. 2B). Results for the Ty1a and Vi groups have been reported earlier [9,11]. In the Ty21a + Vi group, the responses to strains sharing two O antigens (O-9,12; *S.* Enteritidis) with *S.* Typhi proved higher than responses to strains sharing only one O antigen (O-12; *S.* Typhimurium) as shown in Table 2. We have previously reported a respective difference in the Ty21a and Vi groups [9,11]. As a rule, all those with a strong response to *S.* Typhi also showed strong cross-reactive responses and vice versa—a logical consequence in this study actually exploring the same cells for specific and cross-reactive capacity. The proportions of responders and arithmetic mean of typhoid-specific and cross-reactive ASC are presented in Table 1.

3.3. Comparison of ASC responses between Ty21 + Vi, Ty21a and Vi groups

The Ty21a + Vi group showed a higher *S.* Typhi-specific ASC response than the other two groups (Fig. 1). Likewise, the cross-reactive responses to *S.* Paratyphi A and B (Fig. 2A), *S.* Enteritidis and *S.* Typhimurium (Fig. 2B) proved stronger in the Ty21a + Vi group than the others (Fig. 2B). The response to *S.* Paratyphi C was found higher in the Ty21a + Vi than the Ty21a group ($p < 0.01$), and equal to that in the Vi group (Fig. 2A). Compared to the "gold standard"—the *S.* Typhi (total of O-9,12-and Vi)-specific ASC response in the Ty21a group—the Ty21a + Vi group exhibited equal cross-reactive response to *S.* Paratyphi A, B and to *S.* Typhimurium, and higher to *S.* Enteritidis (Fig. 3).

3.4. Expression of $\alpha_4\beta_7$, L-selectin and CLA on O-9,12-specific ASC

In the Ty21a + Vi group, the O-9,12 antigen-specific ASC showed a mucosal homing profile: almost all of these cells expressed the intestinal HR, $\alpha_4\beta_7$-integrin, while the peripheral lymph node HR, L-selectin, and the cutaneous HR, CLA were found less frequently. At the same time, the Vi-specific ASC exhibited a systemic homing profile: high proportion of L-selectin$^+$ and lower proportion of $\alpha_4\beta_7^+$ or CLA$^+$ cells (Fig. 4).

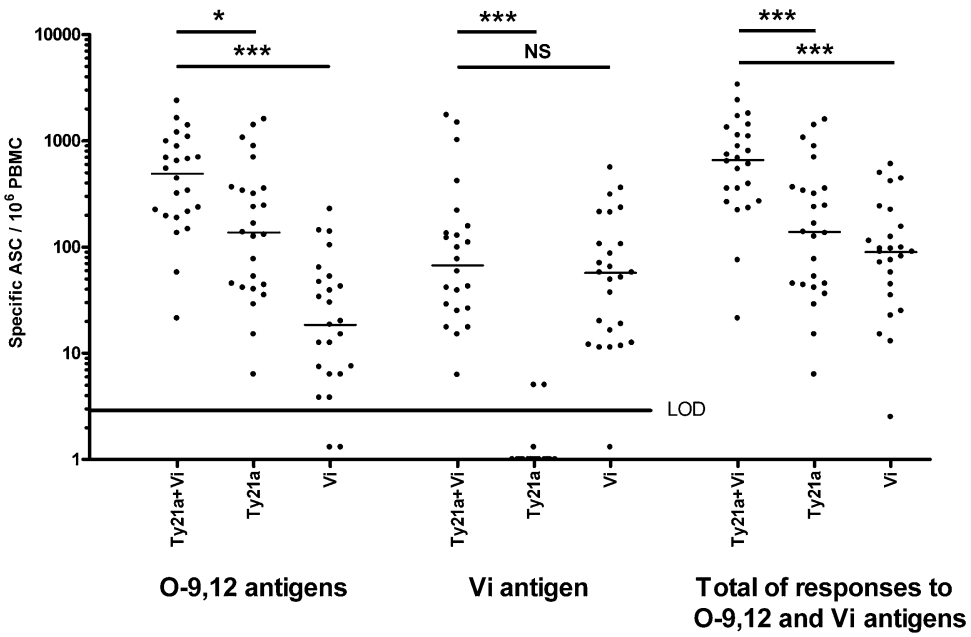

Fig. 1. Typhoid-specific plasmablasts in volunteers immunized concomitantly with the Ty21a and Vi vaccine or with either of the two singly. Data are presented as numbers of circulating ASC (/10^6 PBMC) specific to O-9,12 or Vi antigen and as the total of these responses. Dots represent results of individual vaccinees and lines the medians of Ig(A + G + M)-ASC on day 7 after vaccination. LOD = lower limit of detection of the response. No LOD values are given for the total response, since these figures represent a calculated value. Results of statistical comparisons (Wilcoxon signed-rank test) to Ty21a and Vi groups are provided: *** $p < 0.001$; ** $0.001 < p < 0.01$; * $0.01 < p < 0.05$; NS = not significant. Data on the Ty21a and Vi groups have been published earlier [18].

4. Discussion

The Ty21a and Vi vaccines are considered to exploit different immunological mechanisms when conferring protection against typhoid fever. As neither of them has an optimal protection rate, new approaches deserve to be explored. This is the first study to explore concomitant administration of the two vaccines. One of the major findings was that the plasmablast responses appear to be additive, suggesting that the two vaccines can well be given together. An enhanced plasmablast response was found not only against *S.* Typhi, but also against paratyphoid A and B strains, and selected NTS serotypes, all sharing O antigens with *S.* Typhi.

The levels of *S.* Typhi-specific plasmablasts are of special interest since, in earlier studies, increasing numbers of plasmablasts [16] have been found to correlate with increasing protective efficacy of typhoid vaccines in field trials [31,32]. Accordingly, plasmablast levels have been suggested as surrogate markers of protection [19]. Measuring plasmablasts at single-cell level offers a more sensitive approach to studying humoural immune response than determining serum antibodies [16,18,33].

In our plasmablast assay, whole bacteria were used for coating to cover all possible surface antigens. As shown previously, however, the responses appear to be mainly targeted against the LPS structures, since the differences in them have proved to strictly obey the expression of O antigens: a high response is seen to any *Salmonella* strain with both typhoidal O antigens (O-9,12), a moderate one to any strain with only one (O-12), and none/only a negligible one to all those with no typhoidal O antigens. The minuscule response to *Salmonella* Hadar (O-6,8) in this study was presumably due to the presence of minor antigens shared, such as some protein structures. A response profile correlating with O antigen expressions has been shown for both the Ty21a and the Vi vaccine [9,11]. For the Ty21a, a whole cell vaccine carrying the O-9,12 antigens, this is an expected finding, whereas for the Vi vaccine containing purified Vi capsular polysaccharide, the response appears to consist of plasmablasts part of which are Vi-specific and others targeted to the contaminating LPS [11,18]. In fact, in early studies reporting serum antibodies to LPS in Vi vaccinees, the levels have proved to correlate positively with the LPS content of the preparation: 5% of LPS caused seroconversion in 83% of vaccinees, and 0.2% of LPS in only 26% [34].

Concomitant administration of the two vaccines brought about an additive plasmablast response and thus an enhanced *S.* Typhi-specific response. While Ty21a is known to induce a strong O antigen-specific response and the Vi vaccine mostly a Vi-specific one [18], the enhancement proved to be produced by the

Table 2
Comparison of the responses to different pathogens in Ty21a + Vi group. The statistical comparison (Bonferroni corrected Wilcoxon signed rank test) was carried out between the responses to various strains. Significant differences are indicated with asterisks (*** $p < 0.001$; ** $0.001 < p < 0.01$; * $0.01 < p < 0.05$). NS = not significant. Response to *S.* Typhi is calculated as the total of responses to the O-9,12 and Vi antigens.

Bacterial strain	Comparison with plasmablast response to						
	S. Typhi	*S.* Paratyphi A	*S.* Paratyphi B	*S.* Paratyphi C	*S.* Enteritidis	*S.* Typhimurium	*S.* Hadar
S. Paratyphi A	***						
S. Paratyphi B	***	**					
S. Paratyphi C	***	NS	**				
S. Enteritidis	***	***	***	***			
S. Typhimurium	***	*	NS	***	***		
S. Hadar	***	***	***	*	***	***	
Yersinia enterocolitica	***	***	***	***	***	***	NS

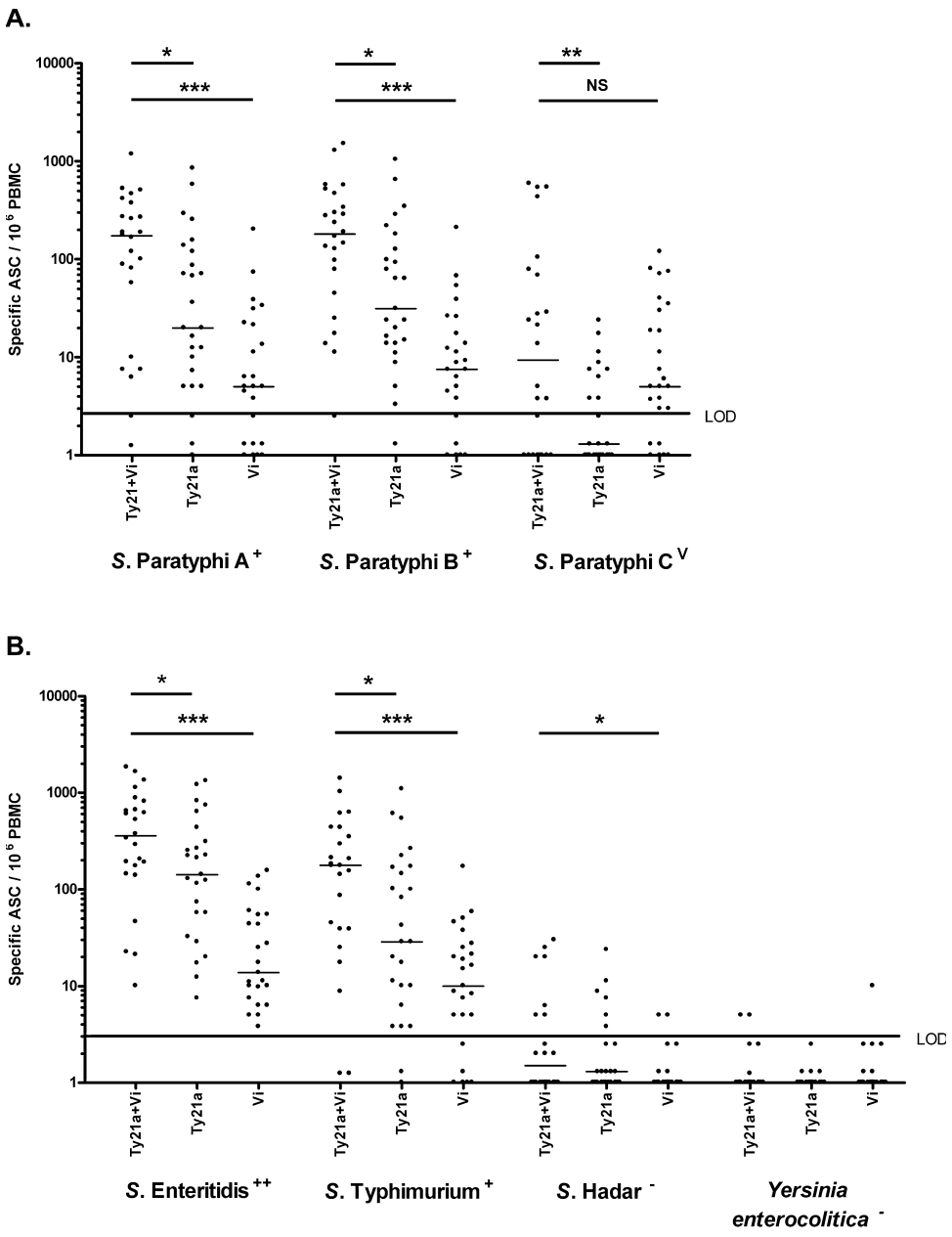

Fig. 2. Cross-reactive plasmablast in volunteers immunized concomitantly with the Ty21a and Vi vaccines vs. either of the two singly. Data are provided as the numbers of circulating ASC (IgA + G + M) cross-reactive with *S.* Paratyphi A/B and C in panel A and *S.* Enteritidis, *S.* Typhimurium, *S.* Hadar and the negative control *Yersinia enterocolitica* in panel B on day 7 after vaccination. LOD = lower limit of detection of the response. The short horizontal lines provide the medians of the responses. Results of statistical comparisons (Wilcoxon signed-rank test) to the Ty21a and Vi groups are provided: *** $p < 0.001$; ** $0.001 < p < 0.01$; * $0.01 < p < 0.05$; NS = not significant. + indicates strains with one O antigen (O-12) and V indicates Vi antigen in common with *S.* Typhi. Data on the Ty21a and Vi groups have been published earlier [9,11].

O antigen-specific response, for the Vi-specific one equalled that in the Vi group. This result is logical: Ty21a does not express the Vi antigen, nor does it contribute to the Vi-specific plasmablasts in the Ty21a + Vi group, while the Vi vaccine contributes both a Vi-specific and a low O-9,12-specific one [18]. The total *S.* Typhi-specific response in the Ty21a + Vi group was thus by and large the total of responses elicited by the two vaccines. Logically, the enhanced immune response observed should indicate higher protective efficacy, yet such a conclusion needs to be verified by field trials.

Data on concomitant use of any vaccines is scarce. Simultaneous administration has in some cases been shown to decrease the response to one of the individual preparations [35,36]. The response in the Ty21a + Vi group approximately equalled the total of responses in the Ty21a and Vi groups, suggesting that these vaccines not influence each other, but instead the responses be additive, thus encouraging their concomitant use.

We have previously reported that the Ty21a and Vi vaccines both elicit a cross-reactive response to *S.* Paratyphi A and B [5,9], Ty21a one greater in magnitude [9]. The cross-reactivity is attributed to the O-12 antigen shared between *S.* Typhi and these paratyphoid strains. The magnitude of the cross-reactive plasmablast response remains smaller than that to *S.* Typhi; accordingly, in a field trial in Chile, lower protective efficacy has been reported against *S.* Paratyphi B than against *S.* Typhi [37]. As to *S.* Paratyphi A, several immunolocigal studies have shown cross-reactivity [3–9], and two have suggested some degree of cross-protection [38,39]. The present study showed an enhanced

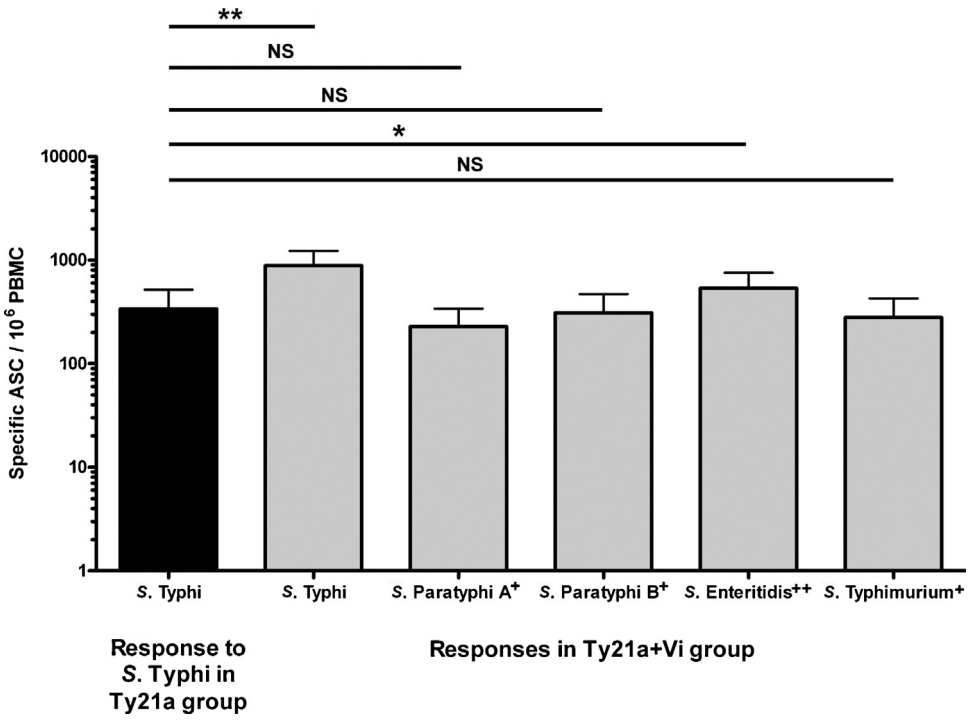

Fig. 3. Cross-reactive plasmablast responses in group Ty21a + Vi in comparison to "gold standard", *S.* Typhi-specific response elicited by Ty21a. Data are provided as numbers of circulating ASC specific to *S.* Typhi and cross-reactive to *S.* Paratyphi A and *S.* Paratyphi B, *S.* Enteritidis, *S.* Typhimurium in volunteers in the Ty21a + Vi group (grey bars), and as *S.* Typhi-specific ASC (total of O-9,12 and Vi-specific ASC) in the Ty21a group (black bar). Statistical comparisons (Wilcoxon signed-rank test) are indicated with asterisk (* $0.01 < p < 0.05$), NS = not significant. $^{+}$ Indicates strains with one O antigen (O-12) and $^{++}$ two O antigens (O-9 and O-12) in common with *S.* Typhi. Data on Ty21a group have been published earlier [18].

cross-reactive response in the Ty21a + Vi group against both *S.* Paratyphi A and B, suggesting that concomitant administration of the two vaccines may potentially enhance cross-protectivity against *S.* Paratyphi A and B, yet field trials are needed to confirm this. The significance of the cross-reactive response to *S.* Paratyphi C remains limited due to this pathogen's rarity as cause of disease.

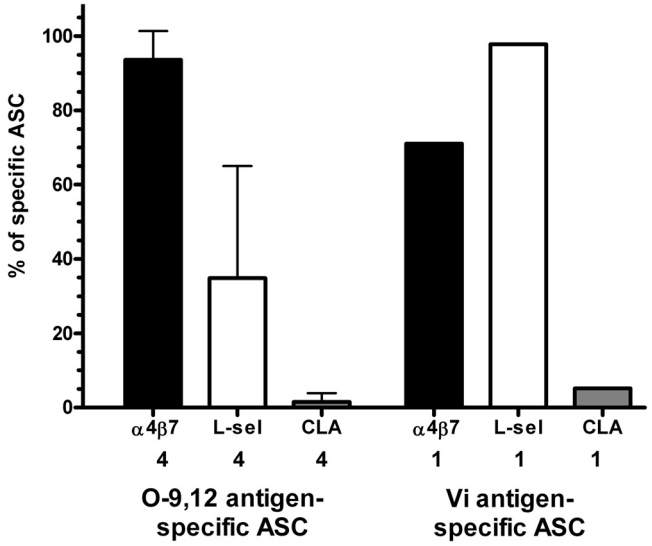

Fig. 4. Homing potentials of circulating O-9,12- or Vi-specific plasmablasts in Ty21a + Vi group. The bars indicate the arithmetic means + 95% CI of the proportions of $\alpha_4\beta_7^+$, L-selectin$^+$ or CLA$^+$ plasmablast (IgA + IgG + IgM) reactive with O-9,12 or Vi antigen. The number of volunteers for whom the data were pooled are indicated under the bars. Statistical comparisons were not performed due to the scarcity of tested volunteers.

The lack of vaccines against NTS and iNTS diseases has directed interest towards the cross-reactive potential of typhoid vaccines [10,11]. In the Ty21a and Vi vaccines such mechanisms are linked to O antigens shared between the NTS and *S.* Typhi strains. Although the potential cross-protection has not been explored in field trials, numerous animal experiments attest to the significance of O antigen-specific antibodies for protection [40–44]. Studies with *S.* Typhimurium-infected mice have shown that both systemic IgM and IgG and mucosal sIgA against O antigens prevent lethal infections [41–43]; intestinal secretory IgA (sIgA) also prevents local intestinal disease [42]. O antigen-specific sIgA promotes agglutination and clearance by blocking bacterial binding to epithelium receptors, capturing them in mucus, and assisting their removal by peristaltic and mucociliary activities [40]. It also affects the functionality of *Salmonella*, rendering it temporarily avirulent [45,46]. Serum IgG and IgM antibodies against O antigen have been demonstrated to generate complement-[43,47–49] and opsonization-mediated phagocytosis and killing of *Salmonella* [3,44]. Furthermore, systemic O antigen-specific IgM antibodies have proved more protective than the corresponding IgG antibodies in mice [43]. Likewise, studies with humans support the protective role of O antigen-specific antibodies: serum antibodies against O antigens have been suggested to serve a protective function in antibody-induced complement-mediated killing [48,49]; anti-*Salmonella* IgG and IgM antibody titres and bactericidal activity of serum against NTS have been reported to correlate negatively with number of NTS bacteraemia cases [48].

In the present study the two most common serotypes, *S.* Enteritidis and *S.* Typhimurium, were selected as representatives of NTS. Consistent with the number of common O antigens, the response to *S.* Enteritidis (O-9,12) has in our previous investigations proved as high as the response to *S.* Typhi, while that to *S.* Typhimurium (O-12) has been somewhat lower [10,11]. Now, after concomitant

administration of the two vaccines, the cross-reactive O antigen-specific responses were found higher than in either of the groups receiving only one vaccine. Indeed, in the Ty21a + Vi group, the magnitude of responses against strains with only one typhoid O antigen equalled the level of responses to strains with two O antigens in the Ty21a group. In the Ty21a + Vi group, the response to *S.* Typhi proved higher than that to *S.* Enteritidis, presumably because of the Vi response, yet both exceeded the responses to strains with only one typhoidal O antigen.

In addition to serotypes investigated here, the typhoidal O antigens are expressed by several other common NTS and iNTS types [12–15,50,51]. For example, *S.* Dublin, commonly isolated in iNTS in Africa [50] expresses both the typhoidal O antigens and the Vi antigen. *S.* Heidelberg, a serotype which has caused several outbreaks in US recently [51], expresses O-12 antigen. Hence, the clinical significance of the potential cross-protection would presumably cover more strains than the two most common ones explored here.

The number of *S.* Typhi -specific plasmablasts has been shown to correlate with protective efficacy against typhoid fever in field trials [16,31,32]. Three doses of Ty21a vaccine in enteric-coated capsules have provided a protective efficacy of 60–70% against *S.* Typhi in field trials [29,30]. Our study, using the same vaccination protocol in the Ty21a group, showed a mean of 339 (155–521) *S.* Typhi-specific plasmablasts/10^6 PBMC. If this response in the Ty21a group was taken as the "gold standard" against which other responses were compared, the Ty21a + Vi group had a higher response to *S.* Typhi and *S.* Enteritidis, and an equal one to *S.* Paratyphi A, *S.* Paratyphi B, and *S.* Typhimurium. Whether this implies enhanced protection against *S.* Typhi and *S.* Enteritidis, and equal protection against the others, needs to be explored in field trials. Importantly, however, while typhoid fever and paratyphoid fever share similar pathogenesis, NTS disease differs in this respect: instead of systemic manifestation, it is generally restricted to the intestinal region. The possible correlations between protection and plasmablast levels may also prove different.

The homing potential of plasmablasts in the Ty21a + Vi group was found to depend on their antigen-specificity: O-9,12 antigen-specific cells exhibited a mucosal and Vi-specific a systemic homing profile. This accords with earlier findings in the Ty21a and Vi groups [18] and the dogma that homing profiles of plasmablasts depend on the site of antigen encounter [21–25]. Most O-9,12-antigen-specific cells were elicited by the Ty21a vaccine in the gut and had a homing profile resembling that in the Ty21a group [5,10,18,23]. All Vi-specific cells were induced by the Vi vaccine and had a homing profile identical to that in the Vi group, consistent with the parenteral administration route [11,18]. These homing data accord with the discussion above, suggesting that concomitant administration of the two vaccines be immunologically additive, and allowing exploitation of the two vaccines' range of mechanisms. Evidently, strong response to all relevant antigens in both mucosal and systemic compartments of the immune system provides an optimal setting for protection.

5. To conclude

Our study shows that concomitant administration of the Ty21a and Vi vaccines is well tolerated and brings about an additive plasmablast response indicating that these vaccines can indeed be given together. An enhanced immune response is elicited not only to *S.* Typhi but also to paratyphoid and NTS serotypes. Despite the lack of studies exploring protective efficacy, it appears reasonable to presume a relation between immune response and clinical efficacy. As long as new vaccines against *S.* Typhi, *S.* Paratyphi and/or NTS are not available, we suggest that concomitant administration of Ty21a and Vi vaccines be encouraged for those at significant risk.

Funding

Funding source: The previously reported data on the Ty21a group and the Vi groups had been partly sponsored by the Finnish Governmental Subsidy for Health Science Research and by Crucell Ltd. The new data presented in this study (Ty21a + Vi group) was not supported by any external funding.

Role of the funding source: The funding sources had no involvement in study design, data collection, analysis, interpretation of data, conception of manuscript, or the decision to submit the article for publication.

Contributions

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

Conflict of interest statement

AK and LR have acted as members in advisory board meeting of Pfizer (AK), GlaxoSmithKline (AK, LR), and Novartis (AK, LR), and received honoraria for that. AK has acted as consultant to Crucell on vaccination immunology and received funding for a previous investigator initiated study. AK has received funding from Pfizer for an investigator-initiated study. AK and LR have 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. SHP, JMK and LES declare no conflicts of interest.

Acknowledgements

We thank the volunteers who participated in this study, and Professor Anja Siitonen for providing strains for our assays.

References

[1] Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. Bull World Health Organ 2004;82:346–53.
[2] Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, et al. International collaboration on enteric disease 'Burden of Illness' studies, the global burden of nontyphoidal *Salmonella gastroenteritis*. Clin Infect Dis 2010;50:882–9.
[3] Wahid R, Zafar SJ, McArthur MA, Pasetti MF, Levine MM, Sztein MB. Live oral *Salmonella enterica* serovar Typhi vaccines Ty21a and CVD 909 induce opsonophagocytic functional antibodies in humans that cross-react with *S.* Paratyphi A and *S* Paratyphi B. Clin Vaccine Immunol 2014;21:427–34.
[4] Wahid R, Simon R, Zafar SJ, Levine MM, Sztein MB. Live oral typhoid vaccine Ty21a induces cross-reactive humoral immune responses against *Salmonella enterica* serovar Paratyphi A and *S.* Paratyphi B in humans. Clin Vaccine Immunol 2012;19:825–34.
[5] Pakkanen SH, Kantele JM, Kantele A. Cross-reactive gut-directed immune response against *Salmonella enterica* serovar Paratyphi A and B in typhoid fever and after oral Ty21a typhoid vaccination. Vaccine 2012;30:6047–53.
[6] Tagliabue A, Villa L, De Magistris MT, Romano M, Silvestri S, Boraschi D, et al. IgA-driven T cell-mediated anti-bacterial immunity in man after live oral Ty 21a vaccine. J Immunol 1986;137:1504–10.
[7] Nisini R, Biselli R, Matricardi PM, Fattorossi A, D'Amelio R. Clinical and immunological response to typhoid vaccination with parenteral or oral vaccines in two groups of 30 recruits. Vaccine 1993;11:582–6.
[8] D'Amelio R, Tagliabue A, Nencioni L, Di Addario A, Villa L, Manganaro M, et al. Comparative analysis of immunological responses to oral (Ty21a) and parenteral (TAB) typhoid vaccines. Infect Immun 1988;56:2731–5.
[9] Pakkanen SH, Kantele JM, Kantele A. Cross-reactive immune response induced by the vi capsular polysaccharide typhoid vaccine against *Salmonella* Paratyphi strains. Scand J Immunol 2014;79:222–9.
[10] Kantele A, Pakkanen SH, Siitonen A, Karttunen R, Kantele JM. Live oral typhoid vaccine *Salmonella* Typhi Ty21a—a surrogate vaccine against non-typhoid *Salmonella*? Vaccine 2012;30:7238–45.

[11] Pakkanen SH, Kantele JM, Herzog C, Kantele A. Cross-reactive immune response elicited by parenteral Vi polysaccharide typhoid vaccine against non-typhoid *Salmonellae*. Vaccine 2014;32:544–51.
[12] Maclennan CA, Levine MM. Invasive nontyphoidal *Salmonella* disease in Africa: current status. Expert Rev Anti Infect Ther 2013;11:443–6.
[13] Center for Disease Control and Prevention. Surveillance for foodborne disease outbreaks—United States, 2009–2010. MMWR Morb Mortal Wkly Rep 2013;62:41–7.
[14] Feasey NA, Dougan G, Kingsley RA, Heyderman RS, Gordon MA. Invasive non-typhoidal *Salmonella* disease: an emerging and neglected tropical disease in Africa. Lancet 2012;379:2489–99.
[15] European Centre for Disease Prevention and Control. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011. EFSA J 2013;11:3129.
[16] Kantele A. Antibody-secreting cells in the evaluation of the immunogenicity of an oral vaccine. Vaccine 1990;8:321–6.
[17] Kantele A, Arvilommi H, Kantele JM, Rintala L, Makela PH. Comparison of the human immune response to live oral, killed oral or killed parenteral *Salmonella typhi* TY21A vaccines. Microb Pathog 1991;10:117–26.
[18] Kantele A, Pakkanen SH, Karttunen R, Kantele JM. Head-to-head comparison of humoral immune responses to Vi capsular polysaccharide and *Salmonella* Typhi Ty21a typhoid vaccines—a randomized trial. PLoS One 2013;8:e60583.
[19] Guzman CA, Borsutzky S, Griot-Wenk M, Metcalfe IC, Pearman J, Collioud A, et al. Vaccines against typhoid fever. Vaccine 2006;24:3804–11.
[20] Mora JR, von Andrian UH. Differentiation and homing of IgA-secreting cells. Mucosal Immunol 2008;1:96–109.
[21] Quiding-Järbrink M, Nordström I, Granström G, Kilander A, Jertborn M, Butcher EC, et al. Differential expression of tissue-specific adhesion molecules on human circulating antibody-forming cells after systemic, enteric, and nasal immunizations. A molecular basis for the compartmentalization of effector B cell responses. J Clin Invest 1997;99:1281–6.
[22] Kantele JM, Arvilommi H, Kontiainen S, Salmi M, Jalkanen S, Savilahti E, et al. Mucosally activated circulating human B cells in diarrhea express homing receptors directing them back to the gut. Gastroenterology 1996;110:1061–7.
[23] Kantele A, Kantele JM, Savilahti E, Westerholm M, Arvilommi H, Lazarovits A, et al. Homing potentials of circulating lymphocytes in humans depend on the site of activation: oral, but not parenteral, typhoid vaccination induces circulating antibody-secreting cells that all bear homing receptors directing them to the gut. J Immunol 1997;158:574–9.
[24] Kantele JM, Kantele A, Arvilommi H. Circulating immunoglobulin-secreting cells are heterogeneous in their expression of maturation markers and homing receptors. Clin Exp Immunol 1996;104:525–30.
[25] Kantele A, Arvilommi H, Iikkanen K, Savilahti E, Mäkelä HP, Herzog C, et al. Unique characteristics of the intestinal immune system as an inductive site after antigen reencounter. J Infect Dis 2005;191:312–7.
[26] Berlin C, Berg EL, Briskin MJ, Andrew DP, Kilshaw PJ, Holzmann B, et al. Alpha 4 beta 7 integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1. Cell 1993;74:185–95.
[27] Camerini D, James SP, Stamenkovic I, Seed B. Leu-8/TQ1 is the human equivalent of the Mel-14 lymph node homing receptor. Nature 1989;342:78–82.
[28] Picker LJ, Kishimoto TK, Smith CW, Warnock RA, Butcher EC. ELAM-1 is an adhesion molecule for skin-homing T cells. Nature 1991;349:796–9.
[29] Engels EA, Lau J. Vaccines for preventing typhoid fever. Cochrane Database Syst Rev 2000;2:CD001261.
[30] Fraser A, Paul M, Goldberg E, Acosta CJ, Leibovici L. Typhoid fever vaccines: systematic review and meta-analysis of randomised controlled trials. Vaccine 2007;25:7848–57.
[31] Levine MM, Ferreccio C, Black RE, Germanier R. Large-scale field trial of Ty21a live oral typhoid vaccine in enteric-coated capsule formulation. Lancet 1987;1:1049–52.
[32] Ferreccio C, Levine MM, Rodriguez H, Contreras R. Comparative efficacy of two, three, or four doses of TY21a live oral typhoid vaccine in enteric-coated capsules: a field trial in an endemic area. J Infect Dis 1989;159:766–9.
[33] Lundgren A, Kaim J, Jertborn M. Parallel analysis of mucosally derived B- and T-cell responses to an oral typhoid vaccine using simplified methods. Vaccine 2009;27:4529–36.
[34] Tacket CO, Ferreccio C, Robbins JB, Tsai C-M, Schulz D, et al. Safety and immunogenicity of two *Salmonella typhi* Vi capsular polysaccharide vaccines. J Infect Dis 1986;154:342–5.
[35] Gilchrist SA, Nanni A, Levine O. Benefits and effectiveness of administering pneumococcal polysaccharide vaccine with seasonal influenza vaccine: an approach for policymakers. Am J Public Health 2012;102:596–605.
[36] Kerzner B, Murray AV, Cheng E, Ifle R, Harvey PR, Tomlinson M, et al. Safety and immunogenicity profile of the concomitant administration of ZOSTAVAX and inactivated influenza vaccine in adults aged 50 and older. J Am Geriatr Soc 2007;55:1499–507.
[37] Levine MM, Ferreccio C, Black RE, Lagos R, San Martin O, Blackwelder WC. Ty21a live oral typhoid vaccine and prevention of paratyphoid fever caused by *Salmonella enterica* Serovar Paratyphi B. Clin Infect Dis 2007;45(Suppl 1):S24–8.
[38] Meltzer E, Sadik C, Schwartz E. Enteric fever in Israeli travelers: a nationwide study. J Travel Med 2005;12:275–81.
[39] Dong BQ, Yang J, Wang XY, Gong J, von Seidlein L, Wang ML, et al. Trends and disease burden of enteric fever in Guangxi province, China, 1994–2004. Bull World Health Organ 2010;88:689–96.
[40] Michetti P, Porta N, Mahan MJ, Slauch JM, Mekalanos JJ, Blum AL, et al. Monoclonal immunoglobulin A prevents adherence and invasion of polarized epithelial cell monolayers by *Salmonella typhimurium*. Gastroenterology 1994;107:915–23.
[41] Michetti P, Mahan MJ, Slauch JM, Mekalanos JJ, Neutra MR. Monoclonal secretory immunoglobulin A protects mice against oral challenge with the invasive pathogen *Salmonella* typhimurium. Infect Immun 1992;60:1786–92.
[42] Endt K, Stecher B, Chaffron S, Slack E, Tchitchek N, Benecke A, et al. The microbiota mediates pathogen clearance from the gut lumen after non-typhoidal *Salmonella* diarrhea. PLoS Pathog 2010;6:e1001097.
[43] Saxén H, Mäkelä O, Svenson SB. Isotype of protective anti-*Salmonella* antibodies in experimental mouse salmonellosis. Infect Immun 1984;44:633–6.
[44] Colwell DE, Michalek SM, Briles DE, Jirillo E, McGhee JR. Monoclonal antibodies to *Salmonella* lipopolysaccharide: anti-*O*-polysaccharide antibodies protect C3H mice against challenge with virulent *Salmonella* typhimurium. J Immunol 1984;133:950–7.
[45] Forbes SJ, Martinelli D, Hsieh C, Ault JG, Marko M, Mannella CA, et al. Association of a protective monoclonal IgA with the O antigen of *Salmonella enterica* serovar Typhimurium impacts type 3 secretion and outer membrane integrity. Infect Immun 2012;80:2454–63.
[46] Forbes SJ, Eschmann M, Mantis NJ. Inhibition of *Salmonella enterica* serovar *typhimurium* motility and entry into epithelial cells by a protective antilipopolysaccharide monoclonal immunoglobulin A antibody. Infect Immun 2008;76:4137–44.
[47] Boyd MA, Tennant SM, Saague VA, Simon R, Muhsen K, Ramachandran G, et al. Serum bactericidal assays to evaluate typhoidal and nontyphoidal *Salmonella* vaccines. Clin Vaccine Immunol 2014;21:712–21.
[48] MacLennan CA, Gondwe EN, Msefula CL, Kingsley RA, Thomson NR, White SA, et al. The neglected role of antibody in protection against bacteremia caused by nontyphoidal strains of *Salmonella* in African children. J Clin Invest 2008;118:1553–62.
[49] Trebicka E, Jacob S, Pirzai W, Hurley BP, Cherayil BJ. Role of antilipopolysaccharide antibodies in serum bactericidal activity against *Salmonella enterica* serovar Typhimurium in healthy adults and children in the United States. Clin Vaccine Immunol 2013;20:1491–8.
[50] Tennant SM, Diallo S, Levy H, Livio S, Sow SO, Tapia M, et al. Identification by PCR of non-typhoidal *Salmonella enterica* serovars associated with invasive infections among febrile patients in Mali. PLoS Negl Trop Dis 2010;4:e621.
[51] CDC—Center for Disease Control and Prevention. Multistate outbrakes of multidrug-resistant *Salmonella* Heidelberg infections linked linked to foster farms brand chicken. ⟨http://www.cdc.gov/salmonella/heidelberg-10-13/index.html?s_cid=cs_002⟩ (Accessed April 10, 2014).