

Antibodies to the Vi capsule of *Salmonella* Typhi in the serum of typhoid patients and healthy control subjects from a typhoid endemic region

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Abstract

Background: There is very little published data on the antibody response to the Vi capsular polysaccharide (Vi-CPS) of *S. Typhi* during naturally acquired typhoid fever in an endemic area.

Methodology: An indirect ELISA, using tyraminated, purified Vi-CPS, was used to assay anti-Vi-CPS antibodies from typhoid fever cases and controls living in the Ho Chi Minh City and Mekong Delta region of Viet Nam.

Results: Antibody response to Vi-CPS is significantly higher in typhoid patients who have been ill for more than two weeks than those who are in the first two weeks of illness. The anti-Vi-CPS response is similar for adults and children. Anti-Vi-CPS antibodies can be detected in the sera of non-typhoid patients. The frequency with which this occurs increases with age, probably reflecting increased exposure to *S. Typhi*.

Conclusions: Anti-Vi_CPS is elicited in persons infected with *S. Typhi* but only after a prolonged duration of illness. Vaccine trials have shown anti-Vi-CPS antibodies to be protective; thus early treatment of typhoid patients, i.e. in the first two week of illness before the Vi-CPS response is elicited, may inhibit the development of this protective immune response.

Key Words: Vi capsule, *Salmonella* Typhi, typhoid fever, antibodies, Viet Nam, Vietnam

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Introduction

Salmonella enterica sub-species *enterica* serovar Typhi (*S. Typhi*) is the causative agent of human typhoid fever. Unlike most other serovars of *Salmonella enterica*, *S. Typhi* can express a carbohydrate capsule known commonly as the Vi-CPS antigen. The expression of this antigen is dependent upon environmental signals [1], is important for extra-cellular survival and protection against the oxidative burst of neutrophils [2], and reduces the TNF-alpha response of human macrophages after uptake. The current view is that Vi-CPS is involved in immune evasion during infection of the human host [3] and is therefore of critical importance during infection.

The Vi-CPS antibody response during infection is of interest because it is protective [4] and can be used for the identification of *S. Typhi* carriers [5, 6].

Material and Methods

Patients and serum samples

This study was performed at the Hospital for Tropical Diseases, Ho Chi Minh City, and The Dong Thap Provincial Hospital, Cao Lanh, Dong Thap, Viet Nam using serum samples collected from children (< 15 years) and adult patients with typhoid fever and also from hospital and community control subjects. These subjects were being recruited into either treatment or epidemiology studies of typhoid fever, which have been reported elsewhere [7-9]. Serum samples from typhoid patients and hospital controls were collected either before or within the first few days of treatment and processed within a few hours of collection. Sera from community controls were

processed within 8 hours of collection. Samples were stored at -20°C until assayed. Length of illness was taken as the time from onset of symptoms to when the sample was collected. Clinical details were recorded on a standard form. A diagnosis of typhoid fever was established by the isolation of *S. Typhi* from either bone marrow or blood.

Serum samples for the epidemiology risk factor study were collected from typhoid patients, hospital controls and community controls between May 1995 and August 1996. Hospital and community controls were recruited following the admission of a typhoid patient to the study, and were age (within five years) and sex matched with the typhoid patients. Following the recruitment of a typhoid patient, the next age-sex matched non-typhoid patient admitted to Cao Lanh Provincial Hospital was enrolled as the hospital control. Where possible patients presenting with fever were enrolled into the study, but in the absence of a suitable febrile patient a non-febrile patient was recruited. Blood cultures were performed if the patient was suspected of having typhoid fever. Diagnoses of hospital controls are given elsewhere [7]. Community controls were recruited within one month of the typhoid patient. These were recruited from the nearest household to the typhoid patient in which a suitably age-sex matched subject resided. None of the patients or controls enrolled into the study reported having been vaccinated against typhoid fever, and none of the community controls were febrile at enrolment into the study or reported having had a fever in the preceding four weeks. The median age (inter-quartile range (IQR), range) of the typhoid patients was 7 (5-14, 2-33) years compared to 6 (5-15, 2-39) years for the hospital controls and 8 (5-13.5, 3-39) years for the community controls.

Serum samples from the typhoid patients recruited to treatment trials (undertaken between 1993 and 1995) were used to compare the anti-Vi-CPS response of adults ($n = 97$) and children ($n = 63$) at different stages of the disease. The median (IQR, range) in age and duration of illness was 18 (11-26, 1-63) years and 12 (9-17, 3-33) days, respectively.

Enzyme-linked immuno-sorbent assays (ELISAs)

In-house indirect ELISAs were established to detect anti-Vi-CPS total immunoglobulin (Ig) and

IgG. All reagents used were purchased from Sigma unless stated otherwise. The wells of the plates were washed at least three times between each step with wash buffer (saline containing 0.05% (v/v) Tween 20). Immulon 1b flat bottomed 96 well microtitre plates (Dynex Technologies, Billingham, UK) were coated overnight at 4°C with $50\mu\text{l}$ of either tyraminated Vi-CPS ($1\ \mu\text{g/ml}$, a gift from Myron Levine, CVD, Baltimore, USA) in phosphate buffered saline (PBS, pH 7.4, Ag+) or PBS alone (Ag-). The plates were subsequently blocked for 1 hour at 37°C with $100\ \mu\text{l}$ PBS containing 1% bovine serum albumin (BSA). Sera were assayed at either a single dilution (1/500 for total Ig, 1/250 for IgG) or serially diluted (doubling-dilutions starting at a dilution of 1/50). Sera were diluted in PBS containing 0.1% BSA and 0.05% Tween 20 (0.1% BSA/PBST) and $50\ \mu\text{l}$ were applied to the appropriate wells and the plates incubated for 4 hours at room temperature. Bound antibodies were detected using heavy chain-specific goat antibodies directly conjugated to alkaline phosphatase (Sigma). These were diluted 1/5000 in Tris buffered saline containing 0.1% BSA and 0.05% Tween 20 (0.1% BSA/TBST). Fifty μl were added to each well and the plates were incubated overnight at 4°C . Fifty μl of p-nitrophenylphosphate ($1\ \text{mg/ml}$) were added to each well and the plates incubated at ambient temperature in the dark for 30 to 40 minutes. The absorbance at 405 nm was determined using an automated ELISA reader (BioRad, Hemel Hempstead, UK).

For sera assayed at a single dilution, antibody levels are expressed in optical density (OD) units. These were taken as the mean absorbance of three wells with antigen minus the OD of a single well without antigen. A series of standards ($n=5$) were included on each plate to which the ODs of the samples were adjusted. Inter-assay variation was monitored by the inclusion of high and low control sera on each plate. Blank wells containing serum buffer alone (0.1% BSA-PBST) were included on each plate to monitor background. For the titration assays, sera were assayed in triplicate (two wells Ag+, one well Ag-) and the titre was taken as the highest dilution giving a net OD (mean OD of Ag+ wells minus OD of Ag- well) of ≥ 0.2 . Serial dilutions ($n=6$) of a standard serum sample with a known titre were included on each plate and titre of the samples adjusted accordingly.

Blank wells with no sera were included to monitor background.

IgG sub-class ELISAs

IgG sub-class ELISAs were performed using the above protocol but with the following modifications. The sera were assayed at 1/100. Following incubation with the serum samples, 50 µl of biotinylated detecting antibody (0.5 µg/ml, G17-1 (IgG1) and G18-21 (IgG2), Pharmingen) were added to each well and the plates incubated overnight at 4°C. Fifty µl of avidin-alkaline phosphatase conjugate (dilute 1/4000 in 0.1% BSA/TBST) were added to each well and the plates incubated for one hour at room temperature.

Statistical methods

Serum antibody titres for the three groups of subjects were compared using paired t tests on log10 transformed data using SPSS for Windows v7.5.

Results

Typhoid patients vs control

The average antibody titres in the sera from typhoid patients were higher than those in the sera for the community and hospital controls ($p \leq 0.001$, Figure 1). For the control subjects (hospital and community) the frequency of detectable ($\geq 1/50$) serum IgG titres increased with age: 1/47 (2%) in the 2-5 year age group; 4/43 (9%) in the 6-10 year age group; 13/40 (33%) in those over 10 years. Overall the majority of patients with typhoid fever had anti-Vi-CPS IgG titres $<1/50$; however, most of the patients in this study were in the first week of illness.

Children and adults with typhoid fever

We were interested in looking at serum anti-Vi-CPS antibody levels in a larger group of typhoid patients with a longer history of illness to see if the antibody response was elicited later in the disease. Single serum samples were obtained from adults and children with non-severe typhoid fever. Anti-Vi-CPS total Ig and IgG levels were determined using a single dilution of the serum samples and were expressed in OD units. For cases in the first week of illness both children and adults had very low antibody levels (Figure 2a). For cases with a history of illness greater than fourteen days there was a slightly higher average antibody level in

adults compared with children but this did not reach statistical significance ($p > 0.05$) because half of the adults had a very low antibody titre.

Figure 1. A comparison of Vi-CPS antibody levels (anti-Vi_CPS IgG) in typhoid fever cases (TF), hospitalised cases (HC) and healthy community controls (CC). Titres of $\geq 1/50$ were detected in sera from 22/65 (34%) typhoid patients, 9/65 (14%) hospital controls and 10/65 (15%) hospital controls.

The dotted line represents the cut-off (titre of 1/50) used between raised (i.e. detectable) and undetectable levels of antibody.

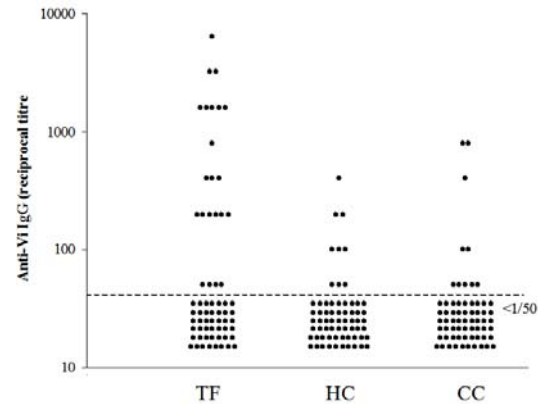
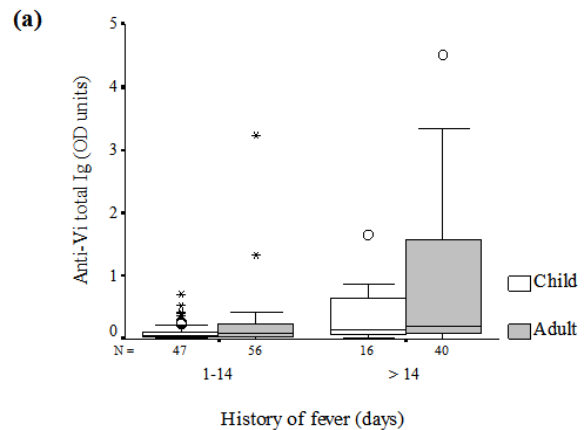
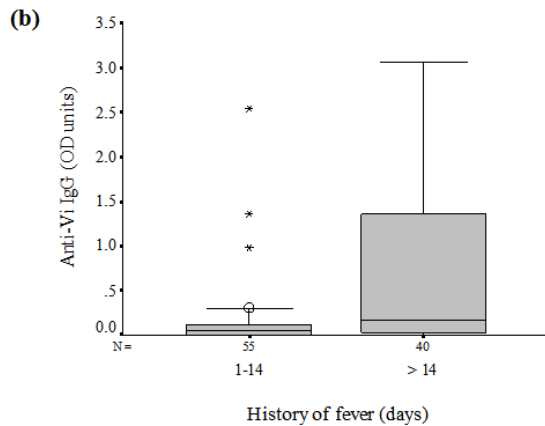


Figure 2. Boxplot of anti-Vi total Ig (a) and anti-Vi IgG (b) OD values in relation to age and duration of illness. Child ≤ 14 years, adult > 14 years.

Solid line = median, box = quartile, bars = range, O and * = outliers and extremes, respectively.





Discussion

Anti-Vi-CPS antibodies detected in serum of healthy subjects – increases with age

The data from this study show that anti-Vi-CPS antibodies can be elicited during a naturally acquired infection with *S. Typhi*, albeit late (after two weeks) in the disease and only in 35% (23/65) of cases. Our data argue against the belief that Vi-CPS antibodies are not raised during an acute infection with *S. Typhi* [10]. However, the methods used here were more sensitive than those used in previous studies, which lacked sensitivity and specificity [11]. Whether or not these antibodies are protective for typhoid fever has yet to be determined but the evidence from vaccine studies shows that immunisation with purified Vi-CPS can confer protection [4]. As the Vi-CPS antigen is a T independent antigen, the protection afforded by Vi-CPS vaccines is most likely to be antibody mediated. The same patients produced antibodies to LPS and flagella protein antigens, and so the lack of response to Vi-CPS is not caused by an inability to produce antibody [7], nor is it caused by the failure of *S. Typhi* to express Vi-CPS *in vivo*. In Viet Nam, as in Pakistan, over 99% of clinical isolates of *S. Typhi* express the Vi-CPS [12]. This raises the question as to whether the prompt diagnosis and treatment of an *S. Typhi* infection is conducive to the development of protective immunity, or whether patients successfully treated in hospital should be immunised before being discharged. Vi-CPS, however, is not the only antigen to which protective antibodies can be raised. A live attenuated *S. Typhi* vaccine, Ty21a, does not express the Vi-CPS antigen but is an effective vaccine, even giving some cross protection against *S. Paratyphi B* infection [13].

In this study, raised anti-Vi-CPS IgG titres were seen in 10/65 of the non-typhoid control subjects. This may be due to exposure to the antigen over time or it may indicate carriage of *S. Typhi*. Unfortunately we were unable to determine carrier status because the serum samples were processed some time after the sample collection, and stool culture on the subjects with raised titres was not possible. Although the numbers of sera tested in this present study were relatively small, our results are similar to the findings of others in typhoid endemic regions [14, 15]; there is an increase in Vi-CPS antibody levels with age.

By the end of the second week of illness, anti-Vi-CPS antibodies are produced by approximately one third of acute cases of typhoid fever. There is very little difference in the antibody response between adults and children but this may be masked by the two thirds of cases who did not produce an antibody response to Vi-CPSa antigen.

Acknowledgements

All studies were approved by the Scientific and Ethical Committee of the participating institutions and informed consent was obtained from all participants or, in the case of children, from their parent or guardian.

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