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Detection of Antibodies against *Salmonella typhi* Outer Membrane Protein (OMP) Preparation in Typhoid Fever Patients

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SUMMARY **An indirect ELISA was used to detect antibodies against outer membrane protein preparations (OMPs) from *Salmonella typhi*. Sera from patients with a definitive diagnosis of typhoid fever (TF) gave a mean absorbance reading, at 414 nm, of 1.52 ± 0.23 as compared to 0.30 ± 0.11 for sera from healthy individuals. This gave a positive to negative ratio of absorbance readings of approximately 5.1. Suspected TF patients (no isolation of *S. typhi*), with positive and negative Widal titers had mean absorbance readings of 1.282 ±0.46 and 0.25 ±0.19, respectively. Sera from patients with leptospirosis, rickettsial typhus, dengue fever, and other infections gave mean absorbances of 0.20 ± 0.08, 0.24 ± 0.08, 0.27 ± 0.08, and 0.31 ± 0.16, respectively. The sensitivity, specificity, positive and negative predictive values were 100%, 94%, 80% and 100%, respectively. The antibody response detected in the definitive TF cases was predominantly IgG in nature and no cross-reactivity was seen with OMP preparations extracted from *E. coli*. Variable reactivity was noted with OMP preparations obtained from other *Salmonella* spp. Three major OMPs are presented in the antigen preparation and strong binding of positive sera was detected to all three bands.**

The serological diagnosis of typhoid fever still relies largely upon the Widal test, which detects the presence of antibodies to the O (lipopolysaccharide) and H (flagellin) antigens derived from *Salmonella typhi*. This test, however, is difficult to interpret in endemic areas, due to the high background titers among the population.[1,2] As a result, newer methods have been developed which are based on detection of antibodies to the protein antigens of *S. typhi*.[3] For example, it has been suggested that outer membrane proteins (OMPs), which are abundant and exposed on the surface of the cell, might be of diagnostic value. OMPs have been shown to induce protective immunity to *S. typhi* and *S. typhimurium* in experimental models,[4-6] and there appears to be a significant increase in antibody titers to OMPs in acute-phase and convalescent-phase sera as compared with normal sera, and these antibody levels appear to be higher than antibody titers to O and H antigens.[7] An indirect ELISA has been previously developed for detection of serum antibodies against OMPs, with results that allowed discrimination between groups of TF-positive and TF-negative subjects.[8,9] Further evaluation of this test, especially in endemic areas, is both necessary and desirable in the quest for better serological tools in the diagnosis of TF. We report here the results of a study to evaluate the indirect ELISA in the detection of antibodies against *S. typhi* OMP in TF patients from two endemic areas, Mexico and Malaysia.

MATERIALS AND METHODS

Strains

In addition to *E. coli* HB101,[11] two strains of *S. tyhi* (*S. typhi* 100, a Malaysian isolate, and *S. typhi* IMSS-1, a Mexican reference strain,[4,10] *S. paratyphi* A, *S. paratyphi* B, *S.*

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typhimurium, S. anatum, S. aberdeen, and *S. worthington* were used in the study.

Sera

Several groups of human sera were used in the study. Group 1 consisted of 41 sera collected from patients with a definitive diagnosis of typhoid fever (TF) as indicated by positive Widal test titers and isolation of *S. typhi* from the blood and/or stool. Sera were collected at various times ranging from 1 week to 1 month after onset of disease. This group consisted of 38 Malaysian samples, and three control serum samples from hemoculture-confirmed Mexican TF patients. Group 2 consisted of eleven sera from suspected cases of TF with positive Widal titers, but negative blood cultures. Group 3 comprised of 48 sera from suspected TF cases, but with negative Widal titers, and negative blood cultures. The method of doing the Widal test, as well as the titers considered to be significant, have been reported previously.[2] Group 4 consisted of 28 serum samples from healthy blood donors. Group 5 is made up of sera collected from patients with other febrile illnesses common in the region, including dengue fever (9 samples), leptospirosis (17 samples) and rickettsial typhus (19 samples). Diagnosis of these illnesses were made on the basis of standard serological tests or isolation of the causative organism. Group 6 consisted of 30 Mexican patients with other infections, where the organism was identified by isolation, including bacteremia by : *S. enteritidis, S. paratyphi* B, *Salmonella* spp., *Proteus* spp., *Shigella flexnerii, E. coli, Pseudomonas aeruginosa, Staphylococcus epidermidis, Staphylococcus* coagulase positive, *Staphylococcus* coagulase negative, *Enterococcus* group D, *Streptococcus* group B, *Acinetobacter calcoaceticus;* septicemia by *Candida albicans;* and six serum samples from patients involved in a diarrhoeal outbreak by *Salmonella* group B.

Preparation of OMPs

Seven hour cultures of bacterial cells in nutrient broth were centrifuged at 1,400×g at 4°C for 10 minutes. The bacterial cell pellet was then resuspended in phosphate-buffered saline (PBS, pH 7.4), and sonicated at a setting of 20kHz or 20,000 cycles/sec (Vibra Cell sonicator, Sonic & Material Co., Danbury, Connecticut, USA). Sonicated cells were centrifuged at 1,400×g at 4°C for 10 minutes, and the obtained supernatant was centrifuged at 100,000 ×g at 4°C for 30 minutes and the pellet resuspended in 20 ml of PBS, pH 7.4 containing 2% Triton X-100 and incubated at 37°C for 20 minutes. The centrifugation step was repeated and the pellet resuspended in 1 ml of PBS, pH 7.4, and stored at -20°C until use. Protein concentration of the OMP preparation was determined by the Bradford method, [12] and lipopolysaccharide (LPS) content by 2-keto-3-deoxyoctonate (KDO) determination.[13]

ELISA for the detection of antibodies specific to *S. typhi* OMPs

Microtiter plates (96-well, flat-bottom, Nunc Plate, Intermed, Denmark) were coated overnight at room temperature with 100 µl OMP preparation at a concentration of 2.5 µg/ml protein. Plates were washed 3 times on the next day, with PBS containing 0.1% Tween-20 (PBS-Tween). Blocking was then carried out by the addition of 150 µl PBS, pH 7.4, containing 2% BSA, 0.1% Tween-20, and incubating for one hour at room temperature followed by three washes with PBS-Tween. Primary antibody (human sera) was then added (100 µl/well of a 1 in 3;125 dilution) and the plates were incubated for 1 hour at 37°C. After 4 washes with PBS-Tween, 100 µl of anti-whole human serum-horseradish peroxidase-conjugate (KPL Co., Gaithersburg, MD, USA) diluted 1:1000, was added to each well and the plates were incubated for one hour at 37°C. Plates were then washed 5 times with PBS-Tween before the addition of 100 µl of the substrate (2,2' -azino-bis(3-ethylbenzthiazoline-6-sulfonic acid, ABTS, Sigma Chemical Co., St Louis, MO, USA) at the concentration of 0.5 mg/ml. The colour was allowed to develop for 45 minutes at room temperature and the absorbance at 414 nm was measured by using a Titertek Multiskan MCC-340 ELISA reader (Flow Laboratories, Australia). Where indicated, horseradish peroxidase-conjugated rabbit anti-human IgG, IgM or IgA (Dakopatts A/S, Glostrup, Denmark) were also used instead of enzyme-labeled anti-whole human serum in the ELISA.

The cut-off absorbance value, between TF patients and controls, was the mid-point between the mean absorbance of the positive TF sera (Group 1) and the mean absorbance of the healthy blood donors (Group 4). The gold standard was defined as febrile patients with a clinical setting corresponding to TF and both a positive blood and/or stool culture and Widal test (Group 1).

Polyacrylamide gel electrophoresis (PAGE) and immunoblotting

SDS-PAGE analysis of OMP was performed in 12% polyacrylamide gels, containing 0.01% sodium dodecyl sulphate (SDS) and 8 M urea. OMP containing 10 µg of protein in PBS, pH 7.4, 1% SDS and 1% 2-mercaptoethanol, was boiled for 5 minutes prior to loading on the gel. Elecrophoresis was performed using an electrode buffer containing 0.025 M Tris (pH 8.3), 0.192M glycine, 0.1% SDS for 3 hours at 170-250 V. Gels were then stained with 0.1% Coomassie blue, followed by destaining with 40% methanol and 7% acetic acid. Molecular weight markers ranging from 29 to 205 kDa were used (Sigma Chemical Co., St Louis, MO, USA). Separated polypeptides

were transferred to nitrocellulose membranes by a semi-dry method in a SemiPhor TE70 system (Hoefer Scientific Instruments, San Francisco, CA, USA) for 3 hours at 134 mA. After that, membranes (10 × 14 cm) were blocked with 100 ml TNT (10 mM Tris, 150 mM NaCl, 0.05% Tween-20) containing 3% BSA (TNT-BSA) for 1.5 hours at room temperature. Fifty milliliters of the primary antibody (pooled sera from group 1 patients diluted 1 in 400) was then added, and incubated overnight at room temperature with gentle shaking. Membranes were washed 3 times with 100 ml TNT-BSA followed by the addition of 50 ml biotinylated goat anti-whole human serum (1 in 1,000 dilution, Clontech Laboratories Inc. Palo Alto, CA, USA) and incubated for 2.5 hours at room temperature. After 3 washes with TNT-BSA, streptavidin-alkaline phosphatase conjugate (1 in 1,000 dilution, Immunoselect, Life Technologies Inc., Gaithersburg, MD, USA) was added and the incubation was carried out for 10-15 minutes at room temperature. Membranes were then washed twice with 100 ml 0.1M Tris (pH 9.5), 0.15 M NaCl for 10 minutes and twice more with 100 ml 0.1M Tris (pH 9.5), 0.1 M NaCl, 50 mM $MgCl_2$ for 10 minutes. The substrate (NBT-BCIP, nitroblue tetrazolium Cl-5-bromo-4-chloro-3-indolylphosphate p-toluidine salt, Immunoselect, Life Technologies Inc., Gaithersburg, MD, USA) was then added at a concentration of 132 µl of NBT, 102 µl of BCIP in 40 ml of 0.1M Tris (pH 9.5), 0.1M NaCl, 50mM $MgCl_2$ and the colour development was allowed to proceed for 10-30 minutes. The reaction was stopped by the addition of 100 ml of 20 mM Tris (pH 7.5), 0.5M EDTA.

Inhibition ELISA.

The capability of either OMP preparations or LPS (Sigma Chemical Co., St Louis, Mo., USA), to inhibit the binding of anti-OMP antibodies to solid-phase bound OMP preparations (5 µg/ml), was assayed by an inhibition ELISA method. Human sera were diluted 1:625 or 1:125, when testing binding inhibition to OMP preparations or to LPS, respectively. These serum dilutions were five-fold concentrated with respect to the ELISA titers. Free antigen (OMP preparations or LPS), was present in two-fold serial dilutions, ranging from 25 to 0.39 µg/ml. The serum-antigen mixtures were incubated at room temperature for 90 minutes, washed, and 100 µl of diluted 1:1,000 anti-human immunoglobulin horseradish peroxidase-conjugated immunoglobulins (Dako Corporation, USA) were added per well, incubated for 90 minutes at room temperature, and the plates were added with 100 µl per well of peroxidase substrate solution : 38 mM $HOC(COOH)(CH_2COOH)_2$, 83 mM Na_2HPO_4, pH 5.5; 1 mg/ml ortho-phenylenediamine (Sigma Chem Co., USA), 0.4 µl/ml 30% H_2O_2. The antigen concentration that inhibited by 50% the binding of the respective human antibodies, to solid phase-bound OMP preparations was determined.

RESULTS

SDS-PAGE analysis of the OMP preparation indicated the presence of three major OMPs, OmpC, OmpF and OmpA with apparent molecular weights of 38.5, 37 and 34 kDa, respectively, and several minor polypeptides ranging from 18 to 103 kDa (Fig. 1).

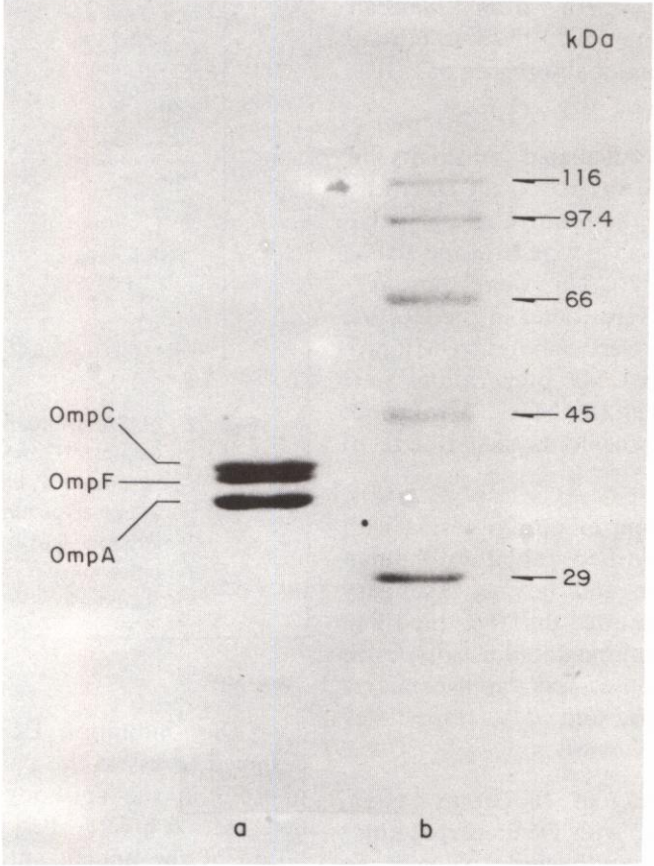

Fig. 1 SDS-PAGE profile of an OMP preparation from *S. typhi* grown in nutrient broth (lane a). The bands corresponding to OmpC, OmpF, and OmpA are indicated. Lane b contains the molecular weight markers.

ELISA testing (Fig. 2 and Table 1) of various sera with OMP preparation-coated plates showed significantly stronger binding of sera from confirmed cases of TF (group 1) as compared to sera from healthy blood donors (Group 4), with mean absorbance (at 414 nm) of 1.52 ± 0.23 and 0.30 ± 0.11, respectively, and giving a positive:negative absorbance ratio of 5.1. No differences were observed in the binding of sera from Mexican or Malaysian patients. Suspected TF patients with positive (Group 2) and negative (Group 3) Widal titers, but negative blood cultures, had a mean absorbance of 1.28 ± 0.46 and 0.25 ± 0.19, respectively. Sera from patients with other fevers common in the region (Group 5), namely leptospirosis, rickettsial typhus, and dengue fever, showed no significant binding to OMP preparations. Sera from Mexican patients with other infections (Group 6), had a mean absorbance of 0.31 ± 0.16.

The calculated sensitivity in the ELISA was 100%, the specificity 94%, and the positive and negative predictive values were 80% and 100%, respectively. No significant differences were found in the ELISA, nor in the electrophoretic OMP profile, when OMP preparations were from either the Mexican reference strain or the Malaysian isolate of *S. typhi*.

Testing of Group 1 sera with affinity purified, rabbit anti-human IgG, IgM, and IgA as secondary antibody showed that the proportion of the immunoglobulin isotypes in the response was approximately 60%, 20%, and 12%, respectively (data not shown).

Testing of 16 Group 1 sera, in ELISA, with OMP preparations from various bacteria, showed no cross-reactivity with an OMP preparation from *E. coli*, although variable reactivity with other *Salmonella* spp. was observed (Table 2).

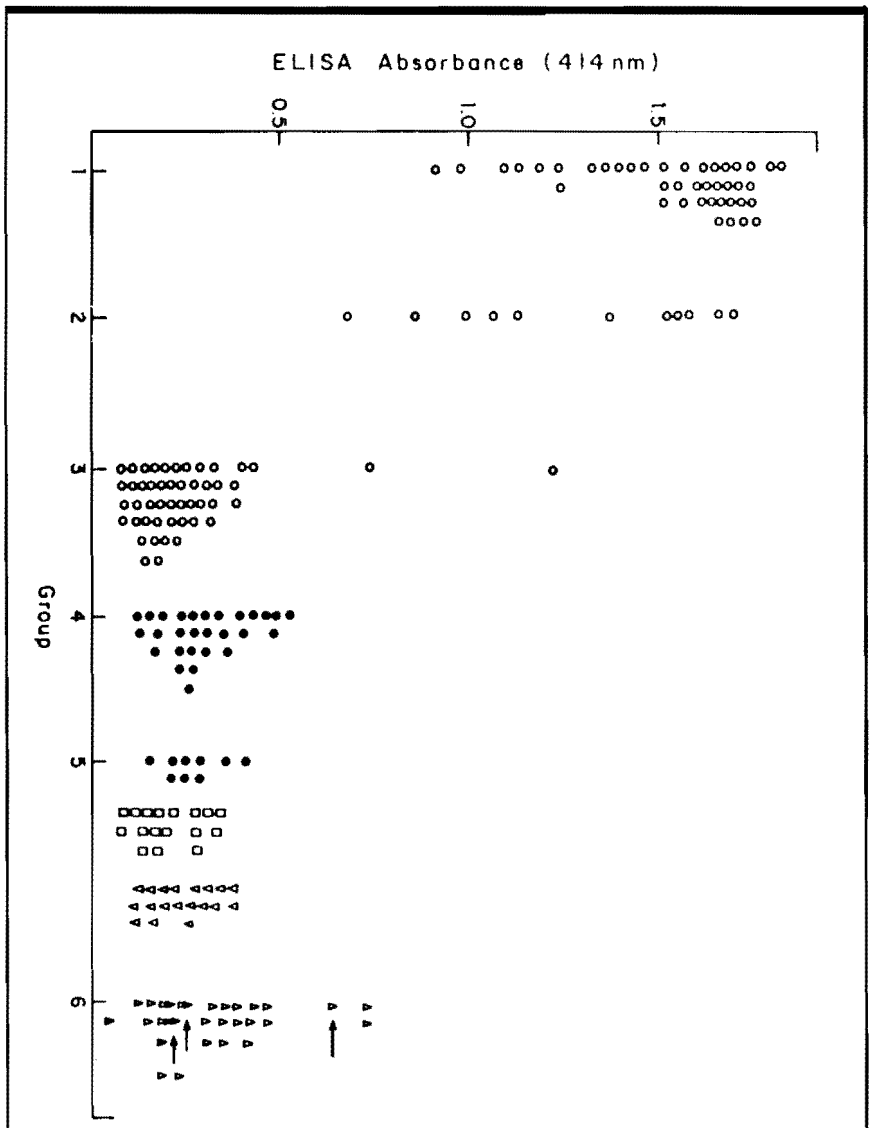

Fig. 2 ELISA absorbance readings corresponding to various groups of sera. Group 1:TF, blood and/or stool culture and Widal positive; Group 2: suspected TF, blood culture negative, Widal positive; Group 3: suspected TF, blood culture and Widal negative; Group 4: healthy blood donors; Group 5: leptospirosis, rickettsial typhus, and dengue fever; Group 6: other infections (bacteremias by *S. enteritidis, S paratyphi* B, and *Salmonella* spp., are indicated by an arrow in descending order).

The inhibition-ELISA was designed to assess the participation of LPS in the antibody response detected. A greater than 50% inhibition of the human antibody response, to solid phase-bound OMP preparation, was observed with 9 μg/ml at most of soluble OMP preparations; in contrast, soluble LPS did not inhibit 50% even with 25 μg/ml (Fig. 3).

Immunoblotting results with the *S. typhi* OMP preparation showed that strong antibody binding was detected to all three major OMP bands. Also, we detected a significant reaction against a 53 kDa polypeptide (Fig. 4).

Table 1 ELISA testing of various sera with OMP-coated plates.

Group	Source of sera	Absorbance at 414 nm (Mean ± S.D)
1	Typhoid fever (TF) (confirmed)	1.52 ± 0.23
2	Suspected TF (positive Widal)	1.28 ± 0.46
3	Suspected TF (negative Widal)	0.25 ± 0.19
4	Healthy donors	0.28 ± 0.10
5	Other fevers:	
	Leptospirosis	0.20 ± 0.08
	Typhus	0.24 ± 0.08
	Dengue	0.27 ± 0.08
6	Other infections	0.31 ± 0.16

Table 2 Reactivity of sera from definitive TF cases with OMPs from different sources.

OMPs source	Positive/Tested*	Percentage
S. typhi	16/16	100
E. coli	0/16	0
S. paratyphi A	2/16	12.5
S. paratyphi B	8/16	50
S. typhimurium	9/16	56
S. anatum	4/16	25
S. aberdeen	0/16	0
S. worthington	0/16	0

*14 Malaysian and 2 Mexican sera from Group 1 were tested. Control sera (Group 4) gave a baseline response.

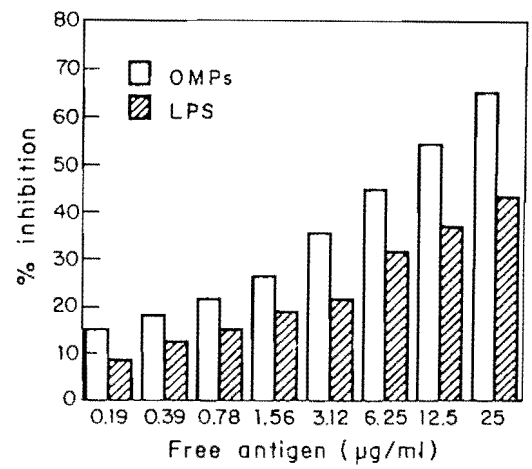

Fig. 3 Inhibition-ELISA. Inhibition of antibody binding from typhoid fever (TF) patient sera to solid phase-bound *S. typhi* OMP preparations, by increasing concentrations (0.19-25μg/ml) of free antigen. TF patient sera were diluted either 1:625 or 1:125, when used together with an OMP preparation or LPS as free antigen, respectively.

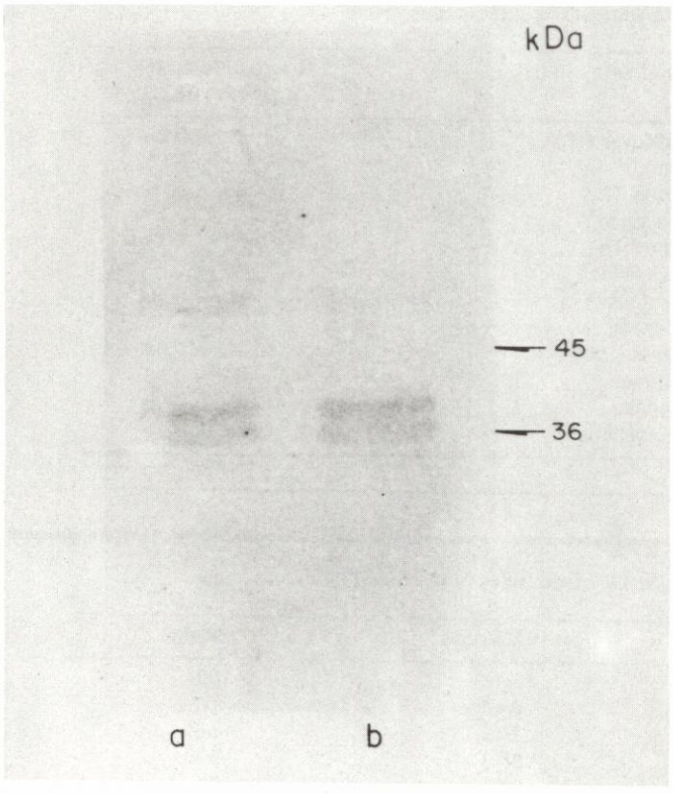

Fig. 4 Immunoblot of OMP preparations from *S. typhi* detected with sera from two TF patients (lanes a and b). Sera were diluted 1:400, and biotinylated goat anti-whole human serum was diluted 1:1,000. Molecular weight markers are indicated.

DISCUSSION

In the serological diagnosis of TF there is clearly a need to develop a single specific, sensitive, rapid and inexpensive test to replace the Widal test which, despite its many limitations, is still widely used especially in developing countries.[14] In particular, there has been an increasing interest in the role played by outer membrane proteins (OMPs) as diagnostic antigens and as candidate antigens for vaccines.[4-9,14] The study described herein clearly shows that antibodies to OMP preparations are present in TF patient sera from two distant endemic areas. The OMP preparation used contained three major bands, that of OmpC, OmpF, and OmpA, and the SDS-PAGE profile of these bands are in agreement with those published previously.[15]

The ELISA showed high specificity and sensitivity, with values of 100% and 94%, respectively. The mean absorbance values from typhoid sera was approximately 5.1 times higher than the mean absorbance of sera from typhoid-negative groups, which included sera from healthy blood donors and from patients with other febrile illnesses common in the region. Suspected TF cases, with positive Widal titers, rendered a lower mean absorbance value than TF-confirmed cases and higher dispersion in the absorbance values. Suspected TF cases, with negative Widal titers, produced a mean absorbance value lower than that with TF-confirmed or TF-suspected, Widal positive sera; but higher than that with TF-negative sera.

The test was positive in TF patients (Group 1) ranging from one week to one month of illness, and did not detect significant binding in normal sera from endemic areas. The response consisted mainly of IgG class antibodies (60%) with a minor IgM (20%) and IgA (12%) component. Individuals with suspected TF, and positive Widal titers, might not have rendered a positive blood culture due to antibiotic treatment, or improper specimen collection. Alternatively, if some of them are TF-negative, they could be showing an anamnestic response. Some individuals with suspected TF, and negative Widal titers, gave a positive response in the TF-ELISA. Thus, the TF-ELISA might be detecting positive cases that are negative by Widal. This question warrants further investigation.

The TF-ELISA correlates very well with a positive blood/stool culture. In this respect it offers an advantage to the Widal test, where significant titers of anti-O and anti-H antibodies in normal sera often pose interpretation problems.[1,2] In addition, the initial study to develop this test[8,9] showed that it also has the capability of differentiating patients with other gram-negative infections, such as those caused by enterotoxigenic *E. coli* (ETEC), *C. jejuni,* and *Salmonella* group B.[8,9] Consistent with this is the finding that ELISA testing of sera from TF cases with OMP preparations from *E. coli* showed no cross-reactivity. Variable reactivity was, however, noted with OMP preparations extracted from other *Salmonella* spp.

There also appears to be reduced dispersion in absorbance values[8,9] in contrast with other ELISA's.[16] Our data confirm the results of earlier studies[7-9] and also suggest no apparent quantitative or qualitative differences in the reactivity of

sera from Mexican or Malaysian patients with the OMP preparations.

The immunoblotting results with TF patient sera showed a significant reaction against OmpC, OmpF, and to a lesser extent against a 53 kDa band, and OmpA. Reaction to OmpC and OmpF is in agreement with that detected by Ortiz *et al.* [17] A 53 kDa band has been observed previously to be overexpressed under iron starvation, [9] and might correspond to a 50-52 kDa protein(s) reported recently. [18,19]

The present study also emphasizes the recent interest in the OMPs as important antigens in TF. [14] In relation to the wider use of OMPs in diagnostics there is a need for standardized methodology and more extensive field testing of OMP preparations as diagnostic antigens. It has been noted, for example, that various OMPs of different molecular weights are being detected in different parts of the world, [14] and that technical differences in OMP preparation (e.g. heat treatment, contamination with flagellin, etc) could account for such differences. It is not clear at present whether these various OMPs are similar or even identical; although the same results were obtained herein with OMP preparation from either Mexican or Malaysian strain of *S. typhi.* It should be noted that the OMP preparations used in the present study contains approximately 30% (w/w) LPS. In related to this, it has been proposed that OMP-LPS complexes are involved in binding to antibodies from typhoid sera. [6,8] Other reserach groups have previously shown that OMPs of 50 kDa or 52 kDa are potentially useful as diagnostic antigens. [18,19]

The fact that TF patient sera showed strong reactivity to OmpC also supports the suggestion of Mora *et al,* [20] that this porin is expressed at significant levels in the human body during infection, and that it is exposed on the surface of the cell. [21] Further evaluation and field testing of OMP-based assays, including exchange of reagents, sera, and test antigens between laboratories may eventually allow its more widespread use in areas endemic for typhoid fever.

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REFERENCES

1. Levine MM, Grados O, Gilman RH, Woodward WE, *et al.* Diagnostic value of the Widal test in areas endemic for typhoid fever. Am J Trop Med Hyg 1978; 27 : 795-800.
2. Pang T, Puthucheary SD. Significance and value of the Widal test in the diagnosis of typhoid fever in an endemic area. J Clin Pathol 1983; 36 : 471-5.
3. Tsang RSW, Chau PY. Laboratory diagnosis of typhoid fever. In : Pang T, Koh CL, Puthucheary SD, eds. Typhoid Fever : Strategies for the 90's. Singapore & London : World Scientific Publishing 1992 : 188-94.
4. Isibasi A, Ortiz V, Vargas M, Paniagua J, Gonzalez C, Moreno J, Kumate J. Protection against *Salmonella typhi* infection in mice after immunization with outer membrane proteins isolated from *Salmonella typhi* 9, 12, d Vi, Infect Immun 1988; 56 : 2953-9.
5. Matsui K, Arai T. An antigen to induce serovar-specific cellular immunity for experimental salmonellosis. In : Pang T, Koh CL, Puthucheary SD, eds. Typhoid Fever : Strategies for the 90's. Singapore & London : World Scientific Publishing 1992 : 176-81.
6. Udhayakumar V, Muthukkarupan VR. Protective immunity induced by outer membrane proteins of *Salmonella typhimurium* in mice. Infect Immun 1987; 55 : 816-21.
7. Calderon I, Lobos SR, Rojas HA, Palomino C, Rodriguez LH, Mora GC. Antibodies to porin antigens of *Salmonella typhi* induced during typhoid infection in humans. Infect Immun 1986; 52 : 209-12.
8. Verdugo-Rodriguez A, Santana FJ, Puete JL, Calva E, *et al. Salmonella typhi* outer membrane proteins in the diagnosis of typhoid fever. In : Pang T, Koh CL, Puthucheary SD, eds. Typhoid Fever : Strategies for the 90's. Singapore & London : World Scientific Publishing 1992 : 216-20.
9. Verdugo-Rodriguez A, Lopez-Vidal Y, Puente JL, Ruiz-Palacios GM, Calva E. Early diagnosis of typhoid fever by ELISA with *Salmonella typhi* outer membrane protein preparations. Submitted for publication.
10. Puente JL, Flores V, Fernandez M, Fuchs Y, Calva E. Isolation of an OmpC-like outer membrane protein gene from *Salmonella typhi.* Gene 1989; 83 : 197-206.
11. Bolivar F, Backman K. Plasmids of *Escherichia coli* as cloning vectors. Methods Enzymol 1979; 68 : 245-67.
12. Bradford MM. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72 : 248-54.
13. Karkhanis YD, Zeltner JY, Jackson JJ, Carlo DJ. A new and improved microassay to determine 2-keto-3-deoxyoctonate in lipopolysaccharide of Gram-negative bacteria. Anal Biochem 1978; 85 : 595-601.
14. Pang T, Calva E, Punjabi N, Rowley D. Report from an international symposium on typhoid fever. Asian Pac J Allergy Immunol 1992; 10 : 73-7.
15. Puente JL, Verdugo-Rodriguez A, Calva E. Expression of *Salmonella typhi* and *Escherichia coli* OmpC is influenced differently by medium osmolarity; dependence of *Escherichia coli* OmpR. Mol Microbiol 1991; 5 : 1205-10.
16. Appasakij HH, Banchuin N, Sarasombath S, Rungpitarangsi B, *et al.* Enzyme-linked immunosorbent assay for detection of *Salmonella typhi* protein antigen. J Clin Microbiol 1987; 25 : 273-7.
17. Ortiz V, Isibasi A, Garcia-Ortigoza E, Kumate J. Immunoblot detection of class-specific humoral immune response to outer membrane proteins isolated from *Salmonella typhi* in humans with

typhoid fever. J Clin Microbiol 1989; 27 : 1640-5.

18. Ismail A, Hai OK, Kader ZA. Demonstration of an antigenic protein specific for *Salmonella typhi*. Bochem Biophys Res Commun 1991; 181 : 301-5.

19. Sukosol T, Sarasombath S, Mongkolsuk S, Chairayoj S, Ekpo P. Molecular cloning of a 52kDa specific protein antigen of *Salmonella typhi*. In : Pang T, Koh CL, Puthucheary SD, eds. Typhoid Fever : Strategies for the 90's. Singapore & London : World Scientific Publishing 1992 : 43-51.

20. Mora GC, Barnier RA, Toro CS, Asenjo CA. Regulation of *Salmonella typhi* porins. In : Pang T, Koh CL, Puthucheary SD, eds. Typhoid Fever : Strategies for the 90's. Singapore & London : World Scientific Publishing 1992 : 64.

21. Puente JL, Bobadilla M, Arias C, Calva E. Genetic variation of the *Salmonella typhi* OmpC gene; a study on OmpC topology. In : Pang T, Koh CL, Puthucheary SD, eds. Typhoid Fever : Strategies for the 90's. Singapore & London : World Scientific Publishing 1992 : 59-63.