RESEARCH NOTES

FALSE-POSITIVE WIDAL TEST IN NONTYPHOID SALMONELLA INFECTIONS

Despite its many well-known limitations, in many parts of the tropical world great reliance is often placed on the results of the Widal test performed on a single, acute-phase serum specimen in the diagnosis of typhoid fever. Unfortunately, these Widal test results are often used as a primary diagnostic indicator in place of more pertinent clinical, epidemiological and bacteriological investigations. Although many limitations of the Widal test (e.g., background titres in an endemic area, effect of TAB vaccination, anamnestic responses, antibiotic therapy) have been extensively documented, the problem of false-positives has not received sufficient attention. Such false positives have been reported in patients with chronic active hepatitis (Protell *et al.*, 1971. *Lancet, 2* : 330) and probably occur in other immunological disorders. Additionally, false positives may also arise as a result of infection with nontyphoid salmonella strains which share common antigens (Reynolds *et al.*, 1970. *J. A. M. A, 214*: 2192).

We report here four cases of nontyphoid salmonella infections where Widal test titres to *S. typhi* H and O antigens are significantly elevated. *S. typhimurium* and *S. blockley* was isolated from two patients and *S. enteritidis* from the other two cases (Table 1). Patient 3 also had *Staphylococcus aureus* isolated from the blood. Except for patient 4 (for the O antigen of *S. paratyphi* B) the highest antibody titres in all cases were detected against the H and O antigens of *S. typhi* (Table 1).

These false positive Widal test results to *S. typhi* antigens should be considered primarily in the light of antigen sharing among salmonella strains. In terms of its (O:H) antigens, *S. typhi* is (9, 12, Vi : d-), *S. typhimurium* is (1, 4, 5, 12 : i-1, 2,), *S. enteritidis* is (1, 9, 12 : g, m) and *S. blockley* is (6, 8 : k-1, 5) (Edwards and Ewing, 1962. Identification of Enterobacteriaceae, Burgess Publishing Co., Minneapolis). Thus, the sharing of O antigen 12 between *S. typhi, S. typhimurium* and *S. enteritidis* would account for the cross-reactions detected with the O antigen of *S. typhi* used in the Widal test. Similarly, the non-sharing with *S. blockley* is appropriately reflected in the absence of cross-reactivity to the O antigen of *S. typhi*. There is, however, no sharing of H antigens between these organisms. In relation to this, it has been suggested that high Widal titres in an endemic area may be due to an anamnestic response and that the anti-H response, in particular, is anamnestically responsive and easily stimulated by other, nontyphoidal stimuli (Wicks *et al.*, 1974. *S. Afr. Med. J., 48* : 1368). It is thus likely that the high levels of anti-H antibodies detected in these cases is a result of an anamnestic response to nontyphoid organisms.

The present report reemphasizes that caution needs to be exercised when utilizing Widal test results as the sole diagnostic test in typhoid fever. This would be especially relevant in areas where infections caused by nontyphoid salmonellae are a significant

Table 1

Widal test* titres in four cases of nontyphoid Salmonella infection.

Patient	Organism isolated in blood (b) and/or stool (s)	Antigen suspension	Reciprocal Widal test titres to	
			H antigen	O antigen
1	*S. typhimurium*(b)	*S. typhi*	640	⩾2560
		S. paratyphi A	40	< 40
		S. paratyphi B	80	160
2	*S. enteritidis*(b)	*S. typhi*	⩾2560	⩾2560
		S. paratyphi A	40	160
		S. paratyphi B	80	640
3	*S. blockley*(s)	*S. typhi*	⩾2560	<40
	Staph. aureus(b)	*S. paratyphi* A	640	<40
		S. paratyphi B	160	40
4	*S. enteritidis*(b)	*S. typhi*	⩾2560	⩾2560
		S. paratyphi A	80	320
		S. paratyphi B	80	⩾2560

* Widal test was performed according to standard procedures described previously (Pang & Puthucheary, 1983. *J. Clin. Pathol., 36* : 471). Antigen suspensions were from Wellcome Reagents, Kent, England.

component in the total spectrum of salmonella infections. For example, in a Malaysian study over a 10-year period, infections with *S. typhimurium*, *S. stanley*, *S. haifa*, *S. derby* and *S. agona* (all of which share some antigens with *S. typhi*) accounted for about 20% of all reported human salmonella isolations (Jegathesan, 1984. *J. Hyg., 92*: 359). Because of its low diagnostic specificity, the Widal test should be considered as the least accurate of the various diagnostic criteria available and every effort should be made to obtain bacteriological confirmation of disease.

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LYMPHOCYTE SUBSETS IN TWO CASES OF ACUTE MALARIA OF PREGNANCY AND IN CASES OF UNCOMPLICATED FALCIPARUM MALARIA IN WESTERN THAILAND

Pregnant women, particularly the primiparia, are affected to an extraordinary degree by falciparum malaria (subject reviewed by Desowitz, 1987. *Ann. Trop. Med. Parasit., 81* : 599). They are more likely to contract malaria; to have a more severe anemia; to abort spontaneously; and to die of the infection. The underlying mechanism(s) responsible for the pregnancy-related enhanced virulence is poorly understood. It has been suggested that immune unresponsiveness/dampening induced by the increased cortisol level during pregnancy may contribute to the pathogenesis by redirecting T and B cell traffic (Vleugels; 1984. *Cortisol and malaria immunity in human pregnancy.* Doctoral thesis, Catholic University of Nijmegen). Alterations in T:B cell and T helper:T suppressor cell ratios have been reported as occurring in acute malaria and it has been hypothesized that these alterations reflect an immunodepressive component to the mechanism of pathogenesis (Strickland *et al.*, 1979. *Z. Tropenmed. Parasit., 30*: 35 : Stach *et al.*, 1986. *Clin. Immunol. Immunopathol., 38*: 129 : Theander *et al.*, 1986. *Scand. J. Immunol., 24*: 73). Similar analysis of lymphocytes has not been done on women suffering from acute malaria of pregnancy and this present study was undertaken to (a) determine the reliability of labelling and preserving lymphocytes, under field conditions, for later processing by flow cytometry and (b) to begin characterization of lymphocyte types and subsets.

During the three-week period of this study at the Thongpaphum District Hospital, Kanchanaburi Province, two pregnant women were admitted with the diagnosis of acute malaria. Both cases proved to be fatal despite the administration of what would seem to have been an appropriate chemotherapeutic regimen and adjunctive physiologic support. Case 219 was a 22-year-old woman in her third trimester of her third pregnancy. She was semi-comatose at admission and aborted a dead fetus 24 hours later. She died on the third day of hospitalization. Case 231 was a 20-year-old primigravida in the first trimester. She too died on the third day after admission. Both women had a *P. falciparum* parasitemia, at admission, of approximately 10%. Also examined, were seven cases of non-acute uncomplicated falciparum malaria and one case of acute malaria in a 5-year-old child with hepatomegaly and marked jaundice.

Venous blood was collected into EDTA tubes. The lymphocytes were labelled with a panel of FITC or RD1-conjugated monoclonal antibodies and controls (Coulter Clone, Coulter Immunology, Hialeah, Florida, USA) according to the directions given by the manufacturer. Two technical modifications were made; 150 µl samples were used instead of 100 µl, and after the final wash the labelled lymphocytes were suspended in a solution of PBS-fixative (1: 5). The samples were then stored at 5 °C in the PBS-fixative for approximately one month after which

Table

Flow cytometry analyses of lymphocyte subsets in cases of acute malaria of pregnancy and in severe complicated and uncomplicated falciparum malarias.

	Case (age/sex)	Total T cells (CD2)	Total B cells (CD20)	T4 cells (CD4)	T8 cells (CD8)	T4 : T8
NORMAL ADULT VALUES	Coulter	83+5	10+5	50−65	25−35	1.8
	Rainut *et al.*	74.2	3.2	46	15.6	3.1
ACUTE MALARIA OF PREGNANCY	219 (22 yr F)	80	6	31	22	1.4
	231 (20 yr F)	85	9	36	21	1.7
ACUTE COMPLICATED MALARIA OF CHILDHOOD	5 (5 yr F)	63	12	31	18	1.7
UNCOMPLICATED FALCIPARUM MALARIA	1(45 yr M)	69	2	35	20	1.8
	2 (28 yr M)	55	9	17	15	1.1
	3 (12 yr M)	69	4	22	18	1.2
	4 (20 yr M)	72	5	46	15	3.1
	6 (7 yr F)	65	5	30	19	1.6
	8 (54 yr M)	47	5	10	5	2.0
	9 (20 yr M)	55	8	25	17	1.5

time they were brought to Hawaii and subsequently analyzed by flow cytometry (FACS). Under these conditions the cells appeared to be well-preserved, retained their monoclonal antibody markers, and were judged to yield acceptable results by flow cytometry. The technique thus lends itself to the study of lymphocyte characterization under semi-field conditions.

The results are shown in the Table from which it will be noted that there are differences between the normal values for adults as reported in several publications. The values given by Rainut *et al.*, (1987. *Human Immunol., 18* : 331) have, for the sake of expediency, been considered as representing the lower limit of normalcy while the values given by Coulter in their instruction sheet as an upper limit. Thus, normal values would be 83 ± 5 to $74\pm12\%$ for total T cells; 10 ± 5 to $3\pm1.3\%$ for total B cells; 65 to $46\pm13\%$ for T helper/inducer cells; and 35 to $15\pm4\%$ for T suppressor/cytotoxic cells.

An unexpected finding was the more marked lymphocyte alterations amongst the cases of uncomplicated malaria than in the severe malarias of pregnancy. Total T cells and T helper/inducer cells were reduced in 6 of 7 of those cases. However, T suppressor/cytotoxic cell numbers were reduced in only one of those cases. In the single case of severe malaria of childhood (Case 5) there were no significant abnormalities in lymphocyte percentages other than a modest

reduction in the T helper/inducer cells similar in magnitude to that observed for the two cases of severe malaria of pregnancy. The reason for the uncomplicated cases having greater changes in T cell values than the severe cases is not known. The samples from the uncomplicated cases were obtained at a later time after the onset of symptoms than the samples obtained from the severe cases. Thus, the alterations may be a consequence of continuing disease rather than a contributory cause of it.

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