IMMUNOLOGICAL STUDY OF TYPHOID FEVER IN MAN: HUMORAL IMMUNE RESPONSE

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INTRODUCTION

It has been generally accepted that cell mediated immunity plays an important role in the recovery from typhoid fever (Ushiba, 1965; Blanden *et al.*, 1966). Nevertheless the possible protective role of humoral antibodies have not been excluded (Ornellas *et al.*, 1970). Humoral antibodies are usually detected by the Widal test, the sensitivity of which is not yet satisfactory, since false negative was found in 24-34 % of patients (Stuart and Pullen, 1946; Chompoosang *et al.*, 1977). The other test is the indirect haemagglutination using either alkaline treated lipopolysaccharide or Vi antigen (Schubert *et al.*, 1959; Chernokhvostova *et al.*, 1969). The course of humoral immune response in patients with typhoid fever has been reported (Kumar *et al.*, 1974; Balakrishna *et al.*, 1977). The prominant immunoglobulin class of the antibody in patients with typhoid fever was predominantly IgM (Chernokhvostova *et al.*, 1969; Kumar *et al.*, 1974). Since indirect haemagglutination test has been shown to be more sensitive than the Widal test in experimental animal immunised with 0 somatic antigen (Carlsson *et al.*, 1972), studies were carried out to determine whether this finding is also applicable to typhoid fever in man. The study was designed also to cover the time course of the haemagglutinating antibodies response and the determination of the immunoglobulin class responsible for this antibody activity.

MATERIALS AND METHODS

Bacterial strains: *Salmonella typhi* 0901 and H901 obtained from the Faculty of Tropical Medicine, Mahidol University and the Department of Medical Science, Ministry of Public Health respectively were used throughout the study.

Subjects: The study was conducted in 45 patients with typhoid fever, whose ages ranged from 15 to 42 years. Diagnosis of these patients was done either by demonstration of positive blood culture (35 patients) or the presence of high 0 agglutinating antibody titer ($\geq$1:320) in a single serum specimen (10 patients). In addition 4 patients with paratyphoid A fever were studied. The controls included 26 healthy volunteers and 14 hospitalized patients 13 of whom had pyrexia of unknown origin (PUO) and the remaining one had scrub typhus.

Widal test: The classical tube method of Widal 0 and H agglutination was used (Bailey and Scott, 1974). The optical density of the bacterial suspension was adjusted to give 0.21 at 650 nm as measured by Coleman Junior II spectrophotometer which was equivalent to 9×10^8 CFU per ml. The antigen was found to give the same titer as the Widal 0 antigen from Difco (Batch no. 2842-56, control 623285) when tested against the same sera.

Indirect haemagglutination (IHA) test: The LPS used to sensitize sheep red blood cell (SRBC) was prepared from *S. typhi* 0901 according to the technique of Neoh and Rowley (1970). The LPS was rendered alkaline by treatment with NaOH according to the technique of Auzins (1968). The microtiter technique of indirect haemagglutination modified from the macrotechnique originally

described by Auzins (1968) was used. The immunoglobulin class of the IHA antibody was determined by the anti-immunoglobulin enhancement as described by Steel *et al.*, (1974) with minor alteration in that 2.5% SRBC suspension was used instead of 1% cell suspension. Preliminary testing showed that enhancement of IHA titer was optimum at the anti-immunoglobulin (Kallestad, Minnesota) dilution of 1:2000. This dilution was then chosen for use throughout the four week course of this study.

RESULTS

Result of serum antibody titers in 45 patients with typhoid fever and in the controls are presented in Table 1. The patients with typhoid fever had significant higher Widal 0 agglutinin, H agglutinin titers than those of the health individuals and febrile control. Twenty-three paired sera taken 7-14 days apart were available for testing. There was no statistically significant difference between the titers of the first and second samples.

Time course humoral responses from 72 specimens in 45 patients is shown in Fig. 1. In comparison with healthy control, there was a significant increase in the Widal 0 and H titers and IHA titers in the second week of illness and thereafter ($p < 0.001$). In addition,

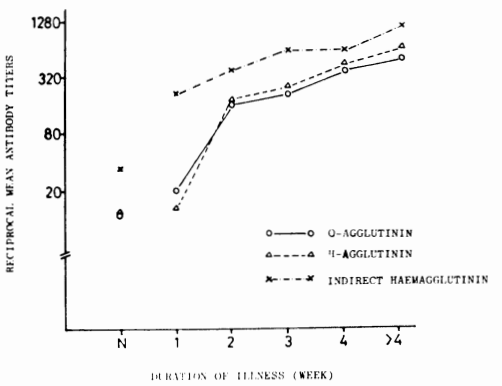

Fig. 1—Means of reciprocal 0 agglutinins, H agglutinins and indirect hemagglutinins in normal (N) and in patients with typhoid fever at various time of illness.

the IHA antibody titers were slightly higher than Widal 0 and H antibody titers in all stages of the tisease.

Analysis of variance of the antibody response at different time of illness revealed that there was a significant rise of Widal 0 and H antibody titers ($p < 0.05$), but not the IHA titer ($p > 0.05$). Further analysis by Duncan's new multiple range test showd that significant rise in titer was found only between sera the of first week and those of subsequent weeks.

Correlation between Widal 0 agglutinin and IHA titers in 72 specimens from 45 patients

Table 1

Reciprocal peak natibody titers in typhoid patient and control.

Subjects	No. tested	Geometric mean O titer		Mean H titer	Student t test
		O agglutinin	Haegmagglutinin		
Typhoid	45	340.34	565.80	320	1 vs 2 $p < 0.001$
Healthy control	26	10.72	31.62	11.48	
Fever control	14	10.47	25.12	11.75	1 vs 3 $p < 0.001$

Student 't' test 1 vs 2 = $p < 0.001$; 1 vs 3 = $p < 0.001$.

with typhoid fever showed very significant correlation (Fig. 2., $r = 0.66$; $p<10^{-7}$)

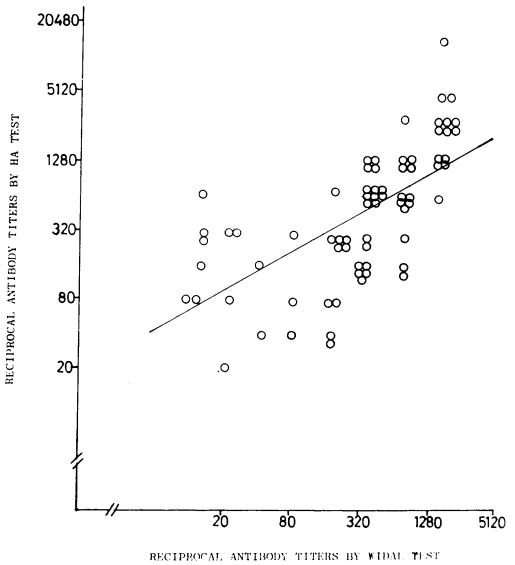

Fig. 2—Relationship between serum antibody titers measured by conventional Widal test and indirect hemagglutination test.

When comparison was made between the IHA and Widal agglutinating titers, it was found that there was significant difference as assessed by Wilcoxon matched-pairs signed-ranks test ($p < 0.01$) indicating that the antibody titer measured by IHA test was more sensitive than the Widal test.

The immunoglobulin class of IHA antibody was determined by the immunoglobulin enhancement test. Enhancement of four fold rise or more of haemagglutinating titers was considered significant. The immunoglobulin class in 45 patients with typhoid fever was found to belong to 3 main classes of IgG, IgM and IgA (Table 2). Strongest enhancement of IgG was found in most patients (Fig. 3). Distribution of immunoglobulins in relation to duration of illness is shown in Table 3. It was observed that during the first week of illness only IgG and IgM but not IgA were detectable. In subsequent weeks all three immunoglobulin classes were elevated.

Table 2

The percentage of immunoglobulin antibodies in sera of typhoid patients.

Immunoglobulin class	Percentage
IgG, IgM and IgA	38.89
IgG and IgM	48.61
IgG and IgA	4.16
IgM	2.77
IgG	5.56

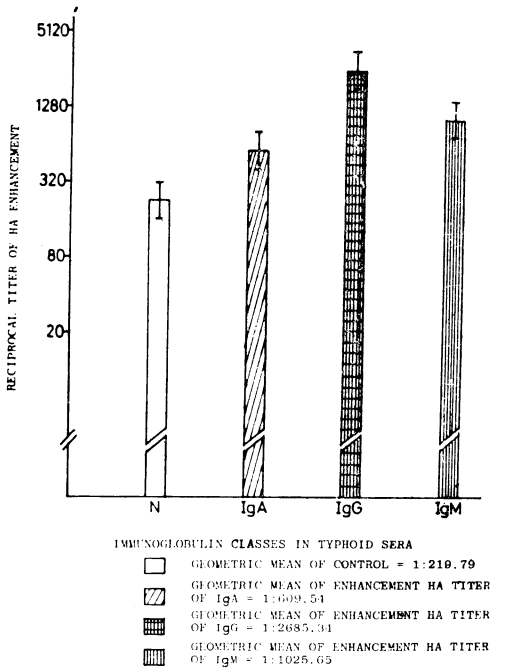

Fig. 3—Levels ($\pm$ 95% confidence limit) of hemagglutination enhancement of IgA, IgG and IgM patients with typhoid fever and control.

Natural IHA antibody against 0 antigen was demonstrated in all 22 healthy individuals but the titer was low (1:10-1:40). Immunoglobulin enhancement test in these sera showed that the antibody activity was associated chiefly with IgG and IgM.

Table 3

Relative distribution of immunoglobulin in sera of typhoid patients.

Duration of illness	Relative distribution of immunoglobulin antibody				
	IgA, IgG and IgM	IgG and IgM	IgG and IgA	IgG	IgM
1st week(2)*	-	100.0	-	-	-
2nd week(26)	34.6	50.0	7.7	3.9	3.9
3rd week(16)	18.7	56.3	-	18.8	6.3
4th week(24)	50.0	45.8	4.2	-	-
more than(4) 4 week	50.0	50.0	-	-	-

*Number of cases investigated.

DISCUSSION

The time course development of antibodies in patients with typhoid fever showed that the antibodies appeared on the first week of illness with the gradual increase in titer thereafter. This finding was similar to those reported by other investigators (Kumar *et al.*, 1974; Balakrishna *et al.*, 1977). Comparison between IHA and Widal test by Wilcoxon matched-pairs signed-ranks test showed that the IHA test was more sensitive than the Widal test. This contention was further substantiated by demonstration of lower antibody IHA titers in healthy unvaccinated persons. False negative Widal test in patients with typhoid fever has been reported in 34.17% of 360 cases (Stuart and Pullen, 1946), and in 24.2% of Thai patients with typhoid fever (Chompoosang *et al.*, 1977). In our studies, false negative Widal reaction was found only in 4 patients (8.9%) whose blood was taken during 12-19 days after the onset of clinical illness. The lower percentage of false negativity in our patients is not known. Since no detailed information was available with respect to the time course of clinical illness when sera were taken in the reports by Stuart and Pullen (1946) and by Chompoosang *et al.*, (1977) it was likely that some of the negative sera in these patients were taken in the early state of illness. The prior treatment with antibiotics was unlikely to affect significantly the subsequent Widal antibody response since raised Widal antibody titer was found in most patients. Such contention would be in corroboration with those of Robertson *et al.*, (1970) and Balakrishna *et al.*, (1977). False negative IHA was found in 3 patients indicating that specificity of Widal and IHA tests were similar.

Antibodies to somatic antigen of *S. typhi* were found in all three major immunoglobulin classes. The results were concordant with the report of Chernokhvostova *et al.*, (1969). However, our results showed that both IgG and IgM antibodies were produced in the majority of cases. This data contradicted that of Pinto and Dammaco (1964), who reported that the entirely IgM response were observed in patients with typhoid fever, as well as in healthy individuals receiving *Salmonella* vaccine (Chernokhvostova *et al.*, 1969). It was also shown by Kumar *et al.*, (1974), that antibody in typhoid fever was 2 mercaptoethanol (2 ME) sensitive and hence would presumably be IgM. However, they

reported that during the third week or more of illness, significant levels of anti-0 and anti-H antibodies resistant to 2 ME (IgG) developed which would indicate the development of other classes of antibodies besides IgM.

The strongest enhancement of haemagglutinating activity in the IgG class in most patients seems to be a paradox when taking into account that bacterial lipopolysaccharide antigen stimulates as a rule a prolonged and intensive synthesis of IgM antibody (Franklin 1964). It is possible that LPS antibody produced in patients is incomplete and its activity will be enhanced when anti-immunoglobulin is added. This type of incomplete (7S) antibody was demonstrated in typhoid carriers (Chernokhvostova *et al.*, 1969). In addition the natural agglutinating antibodies to the 0 antigen of *S. typhimurium* was shown by Robbins *et al.*, (1965) to belong to the IgG class when antiglobulin serum was added.

SUMMARY

Widal and indirect haemagglutination test were used to study the humoral immune response of 45 patients with typhoid fever. The Widal and IHA tests were positive in 41 (91.1%) and 42 (93.3%) of patients with typhoid fever after the second week of illness. Comparison between the Widal and IHA test showed that the latter was more sensitive as assessed by Wilcoxon matched-pairs signed-ranks test ($p < 0.01$). The anti-LPS antibody belongs to all three classes of immunoglobulins namely IgG, IgM and IgA with the confinement mostly to IgG and IgM. It was found that the level of haemagglutinating enhancement activity was strongest in IgG class. The low level of anti LPS-antibody was found in healthy persons with the pattern of immunoglobulin class of antibody similar to that in patients with typhoid fever.

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