

## Specific immune responses in typhoid fever & after TAB vaccination

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**The specific immune responses to *Salmonella typhi* were investigated in 131 patients suffering from typhoid fever and 34 healthy individuals after TAB vaccination. A proportion of individuals failed to develop either specific humoral or cell mediated immune responses. About 5 per cent of the patients with natural infection and nearly 9 per cent of the vaccine recipients failed to develop both the responses. Frequent reinfection and carrier state, and lack of absolute protection following TAB vaccination could be due to the inability of a proportion of naturally infected and TAB vaccinated individuals to mount sufficient specific immune responses, due to the same mechanism.**

Typhoid fever which is an endemic infectious disease in many parts of India poses an important public health problem on account of its morbidity and mortality, development of carrier stage, reinfections and relapses that are associated with this disease.

Being a facultative intracellular pathogen, *Salmonella typhi* is believed to evoke cell-mediated immune (CMI) responses in the host. However, the immunological basis for complete recovery from typhoid fever is not clearly defined. The immunogenicity of killed salmonella organisms as vaccines is also debated<sup>1-3</sup>, though it is still the practice to administer the TAB vaccine containing the killed organisms.

The present study, therefore, has been conducted to evaluate the acquired immune mechanisms in individuals suffering from natural infection with *S. typhi* and after the administration of TAB vaccine.

### Material & Methods

**Subjects :** The subjects for the study were selected from the Institute of Tropical Medicine, Hyde-

rabad. Patients having continuous fever from 5 to 15 days and clinically diagnosed to be suffering from typhoid fever were hospitalised. Patients (134) of either sex who were willing to participate in the study were registered. Their age ranged from 5 to 30 yr. After a thorough clinical examination, 10 ml of venous blood was drawn into a heparinized syringe and the investigations were carried out.

**Investigations :** For confirming the diagnosis-antibody titres to 'H' and 'O' antigens of *S. typhi* were determined following the standard method for Widal test<sup>4</sup>. Antibody titres for 'O' antigen measuring 1:80 were considered as positive for Widal test. Two-fold increase in titres after 4 wk confirmed the diagnosis. Widal negative samples were subjected for antigen detection by counter immunoelectrophoresis (CIEP) following the method described by Gupta and Rao<sup>5</sup>. An acid extract of *S. typhi* 0901 strain (obtained from Haffkine Institute, Bombay, India) was used as antigen to raise antisera in rabbits. Positive test was read from the band of precipitate between the anodal and cathodal wells, after passing a current of 5 m Amps for 45 min per slide.

Cell mediated immune responses (CMI) was measured by T-cell percentage determined by rosette formation technique<sup>6</sup>. Lymphocyte transformation test and leukocyte migration inhibition tests (LMIT) were carried out using PHA as mitogen and *S. typhi* extract as antigen following the standard methods<sup>7,8</sup>. Incorporation of <sup>3</sup>H thymidine into mitogen stimulated lymphocytes was measured and the results expressed as a ratio of T/C = cpm in test culture/cpm in control culture. Antigen specific as well as mitogen induced leukocyte migration inhibition was expressed as migration index - MI = Area of migration in antigen/mitogen treated system/area of migration in control system. Heat killed extract of *S. typhi* 0901 strain (10 µl of extract equivalent to 1 µg protein) was used per assay system. A migration index of <0.8 was considered as positive response to mitogen or antigen. All patients were treated in the hospital appropriately. The duration of hospital stay, and presence or absence of complications were recorded. Patients were followed for a period of 8 wk after discharge from the hospital. In 45 subjects, a repeat blood sample was collected at the end of 8 wk and all investigations repeated. Blood cultures were not attempted as several patients had antibiotic therapy before coming to the hospital. The details of the drug therapy could not be obtained from all.

**The vaccination :** Thirty four healthy volunteers working in the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, aged between 20-30 yr, were given two doses, each 0.5 ml of TAB vaccine sc (obtained from the Institute of Preventive Medicine, Hyderabad) at an interval of one month. The volunteers did not suffer from typhoid fever prior to the study period and their Widal test was negative before immunization. A second blood sample was obtained 8 wk after the second dose. All immunological tests as mentioned for the typhoid patients were performed.

All the paired samples were analysed statistically using paired, 't' test. The other analysis was done using Student's 't' test while T/C (<sup>3</sup>H thymidine incorporation) ratio was tested using 'Kruskall-Wallis one way analysis of variance by ranks' because of the wide variation.

### Results

All the individuals investigated had serum albumin levels > 3.5 g/dl.

Widal test was positive in 111 of 134 patients during the pyrexial illness. In 23 individuals it was found to be negative. Four of the 34 vaccinated subjects had negative Widal test.

Circulating *S. typhi* antigens were detected by CIEP in 20 of the 23 Widal negative subjects. The three patients who were negative for Widal as well as antigen were treated as cases of pyrexia, other than typhoid.

**Cell mediated immunity :** Mean T cell percentage was 51.1 per cent during typhoid fever. Mean migration index in response to PHA was 0.87 while the ratio of <sup>3</sup>H thymidine incorporation by lymphocytes in culture was 58.5. Comparison of the parameters between Widal positive and Widal negative groups indicated a significantly lower T-cell number in the former, with a higher MI (though not significant statistically). After recovery from infection, there was a significant increase in T-cell percentage from 51.1 to 57.8 per cent ( $P < 0.001$ ) and <sup>3</sup>H thymidine incorporation from 58.5 to 91.6 ( $P < 0.001$ ). MI, however, did not show any significant change (Table I).

Individuals during natural infection as well as the vaccine recipients demonstrated very little incorporation of <sup>3</sup>H thymidine into DNA while the migration index was positive with mean values of 0.75 and 0.79 respectively (Table II). Also there were no significant differences between the Widal positive and Widal negative groups.

**Table I.** General cell mediated immune responses in typhoid fever

Group	No. of subjects	Percentage T cells	(Data are mean ± SE)	
			Lymphocyte response to PHA	
			Proliferative response T cells*	Migration index
During typhoid	131	51.1±1.34	58.5±7.88	0.87±0.03
Widal+ve	111	49.4±1.14	63.0±8.91	0.92±0.08
Widal-ve	20	53.2±2.20	54.7±12.14	0.83±0.06
8 wk after recovery**	45	57.8±1.79†	91.66±12.70†	0.93±0.55

\*blastogenic index; \*\*45 pairs of samples tested by paired 't' test; † $P < 0.001$ , compared to pooled initial value.

**Table II.** Specific cell mediated immune responses in typhoid fever and after TAB vaccination  
(Data are mean  $\pm$  SE)

Group	No. of subjects	Lymphocyte response to <i>S. typhi</i>	
		Proliferative response T/c	Leukocyte migration inhibition MI
Typhoid	131	1.08 $\pm$ 0.07	0.75 $\pm$ 0.03
Widal +ve	111	1.02 $\pm$ 0.083	0.77 $\pm$ 0.05
Widal -ve	20	1.10 $\pm$ 0.11	0.77 $\pm$ 0.05
TAB vaccinated individuals	34	0.95 $\pm$ 0.06	0.79 $\pm$ 0.04

MI, migration index

Though the mean value for MI was positive there was a wide range of values. Forty four subjects during typhoid fever and nine volunteers after TAB vaccination remained negative for the leukocyte migration inhibition assay (LMIT) in response to *S. typhi* antigen. Of the 20 patients with typhoid fever who had negative Widal, 14 had LMIT positive

while 6 remained LMIT negative. The cell mediated immune responses of LMIT positive patients in relation to their Widal status is given in Table III. The mean T cell percentage of LMIT positive, Widal positive patients was 48.1. The MI with PHA as well as with *S. typhi* antigen was higher in the LMIT positive, Widal negative group compared to the Widal positive group. However, the differences were not statistically significant. The proliferative responses were comparable between the two groups.

LMIT negative patients showed lower T-cell percentage and higher values for MI in Widal positive group compared to the Widal negative group, though the differences were not statistically significant (Table IV). Of the 4 subjects who remained negative for Widal test after vaccination, three were negative for LMIT also, forming 9 per cent of the vaccinated subjects.

The duration of fever and presence or absence of complications were not different between Widal positive, LMIT positive, both positive or both negative groups. The mean duration of fever was 5-7 days after hospitalisation and most patients had an uneventful course.

**Table III.** Cell mediated immunity of patients with LMIT positivity to *S. typhi* antigen  
(Data are mean $\pm$ SE)

Group	Percentage T cells	Lymphocyte responses			
		PHA		<i>S. typhi</i> antigen	
		T/c	MI	T/c	MI
Widal +ve (73)	48.1 $\pm$ 1.86	54.4 $\pm$ 9.34	0.76 $\pm$ 0.030	1.03 $\pm$ 0.112	0.60 $\pm$ 0.023
Widal -ve (14)	52.5 $\pm$ 3.34	56.8 $\pm$ 19.34	0.80 $\pm$ 0.061	1.21 $\pm$ 0.169	0.65 $\pm$ 0.032

Figures in parentheses indicate number of subjects. MI, migration index; LMIT, leukocyte migration inhibition test

**Table IV.** Cell mediated immunity of patients with LMIT negativity to *S. typhi* antigen  
(Data are mean  $\pm$  SE)

Group	Percentage T cells	Lymphocyte responses			
		PHA		<i>S. typhi</i> antigen	
		T/c	MI	T/c	MI
Widal +ve (38)	50.2 $\pm$ 1.92	72.5 $\pm$ 18.870	1.08 $\pm$ 0.06	1.05 $\pm$ 0.134	0.97 $\pm$ 0.038
Widal -ve (6)	53.9 $\pm$ 3.34	52.71 $\pm$ 17.334	0.89 $\pm$ 0.188	0.97 $\pm$ 0.119	0.91 $\pm$ 0.061

MI, migration index. Figures in parentheses indicate number of subjects.

### Discussion

*S. typhi* is a Gram negative facultative intracellular pathogen that multiplies in the phagocytic cells of the reticuloendothelial system. It has been well established that macrophages as cellular components of the innate immune system play an important role in clearing the bacterial load during acute infection<sup>9</sup>. However, the role of humoral and cell mediated immune mechanisms in strengthening these innate cellular responses in offering protection against infection caused by *S. typhi* is not clear.

Data from experimental studies investigating the protective immune mechanisms in mice infected with *S. typhimurium* are controversial. Several investigators have reported that specific cell-mediated immune responses were protective to infection with *S. typhimurium*<sup>10,11</sup>. Kumar *et al*<sup>12</sup> and Sarma *et al*<sup>13</sup> reported the role of cell mediated immunity in human typhoid infection. Other studies suggested that acquired humoral mechanisms also play a significant role in the control of salmonella infections<sup>14</sup>. The importance of both T and B cell systems in mounting protective immunity to salmonella infections has also been indicated<sup>15,16</sup>. In the present study, we observed a significant improvement in the general cell mediated immune status following recovery suggesting an initial immunosuppression during the acute phase of infection, an observation similar to that of Hamza *et al*<sup>17</sup> and Rajagopalan *et al*<sup>18</sup>.

Eighty five per cent subjects with natural infection and 96 per cent vaccinees developed adequate titres of antibodies. Specific cell mediated immune responses were similar between the Widal positive and Widal negative groups. Lymphocyte proliferative response was negligible or absent (at the antigen concentration tested) and might be due to the inability of the bacterial lipopolysaccharide to stimulate the T or B cells *in vitro*, an observation similar to the one reported by Geha and Merler<sup>19</sup>. Positive LMIT in two-thirds of the patients and vaccinated subjects indicates successful sensitization of T cells by the *S. typhi* antigens. However, a sizeable proportion of the individuals failed to develop one or both of the acquired immune responses. Nearly 15 per cent of subjects remained persistently negative for Widal though there was positive evidence for *S. typhi* infection detected by CIEP. Twelve per cent of the

vaccinated individuals failed to acquire satisfactory titres of antibodies.

One-third of the patients as well as the vaccinated volunteers failed to develop positive LMIT response at the antigen dose tested, indicating failure of T cell sensitization. Interestingly 5 per cent of patients and 9 per cent of vaccinated subjects were found to be negative for Widal as well as LMIT. These observations indicate that a proportion of individuals may have failed to acquire one or both of the specific immune responses following exposure to natural infection or killed bacterial vaccine.

The uneventful recovery of all patients despite their varied immunological profile could be due to the function of macrophages and the adequate chemotherapeutic support.

Though clinical recovery from acute infection is not significantly affected, inadequacy of specific immune responses could contribute to lack of memory leading to re-infection. Rajagopalan *et al*<sup>18</sup> observed lowered cell mediated immune responses to be associated with complications during typhoid fever. It may be speculated that absence of specific cell-mediated immune mechanisms might interfere with the total bacterial clearance by macrophages and lead to carrier stage. Follow up studies are essential to establish these points. Lack of memory induction following TAB vaccination probably explains the absence of absolute protection offered by this vaccine and supports the suggestion made by Rajagopalan *et al*<sup>3</sup> to modify the TAB vaccine to induce specific cellular immune responses.

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