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A study of the efficacy of typhoid vaccine in inducing humoral and cell-mediated immune responses in human volunteers

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SUMMARY

The nature of protective immunity against typhoid fever in man is not at present well understood. Work on animal models and earlier studies from this laboratory indicate an important protective role for cellular immunity. The present work attempts to study the efficacy of the conventional typhoid vaccine in inducing specific cellular and humoral immune responses. The study on fifty-eight new army recruits and thirty-one civilian volunteers showed adequate humoral responses after vaccination. However, vaccination failed to induce a significant cellular immune response. In addition, a transient suppression of cellular immunity was observed in the immediate post-vaccination period in ten subjects who possessed natural cellular immunity before vaccination. These findings indicate the need for improving the typhoid vaccine so that it will induce cellular immunity as well as a humoral response. It also points to the necessity for obtaining detailed knowledge of the post-vaccination anergy as it could be important in timing public health programmes.

INTRODUCTION

The mechanism of acquired resistance against enteric fever in man is not at present well understood. In the animal model of typhoid, which includes extensive studies in mice and rats, the existence of both types of immune response, cellular and humoral, is well established. The former seems to play a major role in recovery from primary infection, as well as in resistance to reinfection (Ushiba, 1965; Blanden, Mackaness & Collins, 1966). Recent studies in man also indicate a possible protective role for the cell-mediated immune response (CMIR) in typhoid fever (Kumar *et al.*, 1974; Sarma *et al.*, 1977).

The currently available killed-typhoid vaccine induces a good humoral response but fails to protect all vaccinated individuals. It has been suggested that the lack of complete protection with killed vaccine may be due to its inability to induce any significant degree of cellular resistance (Ushiba *et al.*, 1959; Mackaness, Blanden & Collins, 1966; Collins, 1970; Cooper & Fahey, 1970). The present study was undertaken to investigate whether or not killed-typhoid vaccine would induce CMIR.

MATERIALS AND METHODS

Two different groups of the population were studied: group 1 consisted of healthy volunteers who were the new recruits to the Rajputana Rifles, Defence Services, Delhi Cantt, and group 2 consisted of healthy civilians from amongst the residents and staff of the All-India Institute of Medical Sciences, New Delhi. None of the subjects selected for either group had a history of typhoid vaccination nor had they suffered from typhoid fever during the preceding 5 years.

All subjects were given typhoid (TAB) vaccination and were tested for humoral responses as well as CMIR to antigens of *Salmonella typhi*. The CMIR was tested by the leucocyte migration test (LMT) using heat-killed and ultrasonically lysed *S. typhi* as antigen, whilst the humoral antibodies to H and O antigens of *S. typhi* were determined by the standard Widal test. In the majority of subjects, the immune responses were tested before, and 3 weeks after, the last dose of TAB vaccine. In some

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subjects in group 1 the tests were repeated again 8 weeks after the last dose of the vaccine. Those volunteers of group 2 who showed a positive LMT before vaccination were eliminated from the study. There were some subjects in group 1 who were investigated for immune responses only in the post-vaccination period. In them, the tests were performed either 3 or 8 weeks after the last dose of vaccine.

Dosage and route of administration of TAB vaccine The TAB vaccine used in this study was obtained from the Central Research Institute, Kasuali, India. It contained 10^8 heat-killed phenol-preserved *S. typhi* (strain Ty–2) and 5×10^7 each of *S. paratyphi* (A) and (B) per ml.

Volunteers in group 1 were given two injections of TAB vaccine, 0·5 ml and 1·0 ml respectively, subcutaneously at an interval of 10 days. Group 2 subjects received only a single dose of the vaccine administered intradermally in a dose of 0·1 ml.

Leucocyte migration test (LMT). The technique of Federlin *et al.* (1971) was used with minor modifications. The capillary tubes (Gelman–Hawksley, UK) were loosely packed with leucocytes separated from defibrinated blood utilizing 2% gelatin in phosphate-buffered saline. The cells were allowed to migrate in perspex chambers for 16 to 20 hr in minimum essential medium containing 10% foetal calf serum (Difco, USA) with or without antigen. The area of migration of cells was determined by projection and planimetry, and the migration index calculated by dividing the migration area in the presence of antigen with the migration area in the absence of antigen. As the normal range of the migration index was found to be 0·76 to 1·23, an index of less than 0·76 was considered as positive migration inhibition.

A VW strain of *S. typhi* isolated from the blood of a patient suffering from typhoid fever was used as the source of antigen in LMT. A smooth colony of the strain was subcultured on a nutrient agar slope and incubated at 37°C overnight. The growth was harvested in sterile physiological saline, washed twice in sterile saline, and the wet weight of the organisms was determined. The bacteria were then suspended in a suitable amount of sterile distilled water and ultrasonically lysed at 20 kHz for 15 min. The lysed suspension was heated at 56°C for 1 hr. After checking the sterility, the optimum antigen dose was determined. This was done by incubating an increasing dose of this antigen with freshly prepared leucocyte suspensions at 37°C overnight and checking the viability of the leucocytes by the trypan blue exclusion test. The highest non-toxic dose of antigen giving a positive and negative leucocyte migration inhibition in known positive and negative controls respectively was used in the test. For the present work the strength used was 3 mg (wet weight) of organisms per ml of fluid to fill the migration chamber.

Antibody titration. The sera were inactivated at 56°C for 30 min and serially diluted two-fold in 0·5 ml normal saline in two rows of test tubes. Equal volumes of H and O agglutinable suspensions of *S. typhi* (Central Research Institute, Kasuali) were added to separate rows of serially diluted serum. The dilutions used ranged from 1:20 to 1:640. The readings were taken after overnight incubation at 37°C in a water bath. The reciprocal of the highest dilution giving a positive result was taken as the titre. The end titre was not determined in samples that gave a positive result up to 1:640 dilution. For each group, the mean titre was calculated in natural logarithms. For calculation of mean titres the negative sera were assumed to be positive at a 1:10 dilution.

RESULTS

Table 1 summarizes the number of subjects studied before, and at different intervals after, TAB vaccination. In group 1 there were forty-three subjects who were studied both before as well as after the TAB vaccination. The remaining fifteen group 1 subjects were studied only in the post-vaccination period.

In group 2 of thirty-one subjects, there were six who could not be studied further after the initial observation. The rest of the twenty-five subjects were investigated before as well as 3 weeks after the TAB vaccination.

TABLE 1. Subjects studied in the present work

Subjects	Before TAB	After TAB			Total
		3 weeks	8 weeks	Not followed	
Group 1 (army recruits)	33	33	n.s.	0	33
	10	10	10	0	10
	n.s.	7	n.s.	0	7
	n.s.	n.s.	8	0	8
Group 2 (civilians)	31	25	n.s.	6	31

n.s. = Not studied; 0 = nil.

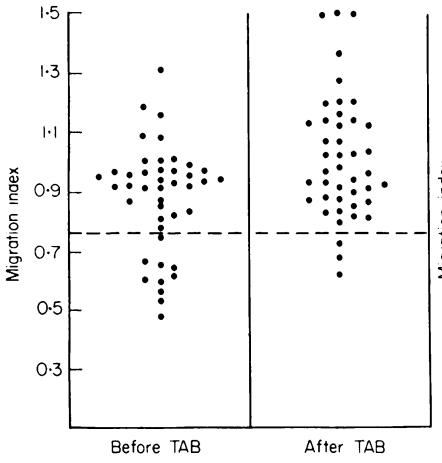

FIG. 1.

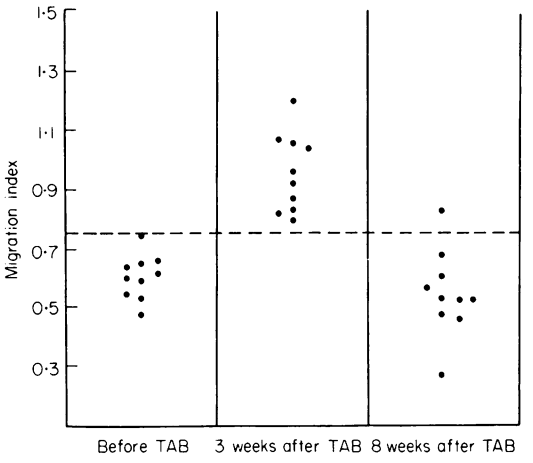

FIG. 2.

FIG. 1. LMT before and 3 weeks after a course of TAB (army recruits). $\chi^2 = 2{\cdot}95$, $P > 0{\cdot}05$.

FIG. 2. LMT at different intervals in ten subjects who were positive before TAB vaccination (army recruits).

Leucocyte migration test

In forty-three group 2 subjects, studied both before as well as after vaccination, thirty-three were LMT-negative and ten were LMT-positive before vaccination. 3 weeks after the vaccination, three out of thirty-three pre-vaccination LMT-negative subjects became LMT-positive while the other thirty remained LMT-negative (Fig. 1). All ten pre-vaccination LMT-positive subjects showed a negative LMT 3 weeks after the vaccination. However, nine of these ten subjects reverted to the LMT-positive state when retested 8 weeks after vaccination (Fig. 2). Of the remaining fifteen subjects who were studied only in the post-vaccination period, five out of seven at 3 weeks after vaccination and seven out of eight at 8 weeks after vaccination were LMT-negative. In group 2 of thirty-one civilians there were six subjects who were LMT-positive in the pre-vaccination period, but they could not be studied in the post-vaccination phase. Of the other twenty-five pre-vaccination LMT-negative subjects only three became LMT-positive 3 weeks after the vaccination (Fig. 3).

The results of the LMT are summarized in Table 2.

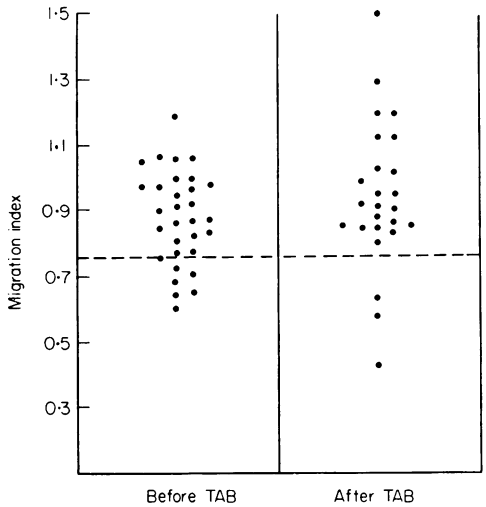

FIG. 3. LMT before and 3 weeks after a course of TAB (civilians). Pre-vaccination LMT-positive subjects have not been studied after TAB. $\chi^2 = 0{\cdot}56$, $P > 0{\cdot}05$.

TABLE 2. Summary of the results of LMT in subjects investigated in this study*

Before TAB (Number in each class in brackets)	After TAB			
	3 weeks		8 weeks	
	LMT-positive	LMT-negative	LMT-positive	LMT-negative
Group 1 (army recruits)				
LMT-positive (10)	0	10	9	1
LMT-negative (33)	3	30	n.s.	n.s.
Not investigated (7)	2	5	n.s.	n.s.
(8)	n.s.	n.s.	1	7
Group 2 (civilians)				
LMT-positive (6)	n.s.	n.s.	n.s.	n.s.
LMT-negative (25)	3	22	n.s.	n.s.

n.s. = Not studied.

* Figures indicate the number of subjects.

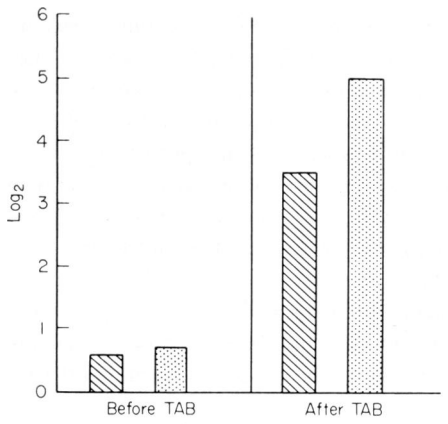

FIG. 4.

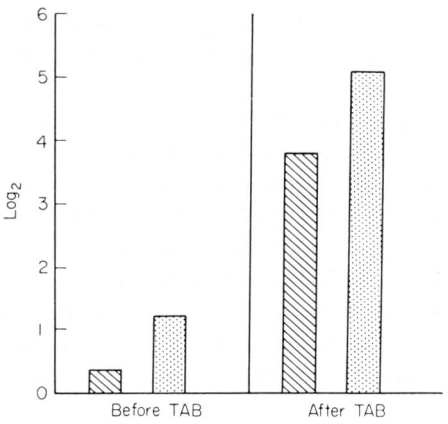

FIG. 5.

FIG. 4. Mean antibody titres before and 3 weeks after a course of TAB (army recruits). Mean O titre (▨), the rise of O titre is significant ($P<0{\cdot}001$). Mean H titre (▩), the rise of H titre is significant ($P<0{\cdot}001$).

FIG. 5. Mean antibody titres before and 3 weeks after a course of TAB (civilians). Mean O titre (▨), the rise of O titre is significant ($P<0{\cdot}001$). Mean H titre (▩), the rise of H titre is significant ($P<0{\cdot}001$).

Antibody response

The titres of O and H antibodies were significantly raised 3 weeks after TAB vaccination in both the groups. Group 1 subjects had mean initial titres of 0·69 and 0·74 for O and H agglutinins respectively before vaccination. 3 weeks after vaccination, the mean titres were 3·53 and 5·09 respectively ($P<0{\cdot}001$) (Fig. 4). In group 2 subjects the pre-vaccination mean titre was 0·36 for O and 1·2 for H. The post-vaccination mean titres were 3·88 and 5·08 for O and H agglutinins respectively ($P<0{\cdot}001$) (Fig. 5).

DISCUSSION

The main objective of this work was to investigate whether TAB vaccine would induce CMIR in addition to a humoral response. It was noted that only three out of thirty-three (9·09%) group 1 and three out of

twenty-five (12·0%) group 2 subjects who were LMT-negative before vaccination converted to the LMT-positive state 3 weeks after vaccination. It appears that TAB vaccine has almost uniformly failed to induce CMIR. Small doses of antigen and intracutaneous administration are known to be two conditions which preferentially favour the induction of CMIR (Report of WHO Scientific Group, 1969). For this reason, group 2 subjects were given 0·1 ml of the vaccine by the intracutaneous route and their responses compared with those of group 1 who received the more conventional larger dose by the subcutaneous route. Both schemes of immunization were equally poor with regard to the induction of CMIR. That the failure of either schedule to induce CMIR was not due to the low potency of the vaccine is clear as both the groups had the expected humoral response. These results confirm the preliminary observations reported earlier from this laboratory (Kumar *et al.*, 1974).

It could be argued that 3 weeks was not long enough for TAB vaccine to induce a CMIR. This possibility was examined by testing vaccination. There was no difference in the number of subjects giving a positive reaction at 3 or 8 weeks.

An interesting observation made in this study was the effect of TAB vaccination in subjects who showed as LMT-positive before vaccination. Among the forty-three army recruits, there were ten such individuals. When the LMT was repeated on these individuals 3 weeks after vaccination, all gave a negative reaction. When tested again 8 weeks after vaccination, nine had reverted to a LMT-positive state. This suggests that TAB vaccination can induce a transient state of unresponsiveness in subjects who are already sensitized to *S. typhi* antigens. This temporary suppression was restricted to CMIR, as the antibody response in these ten subjects was not significantly different from that of the other members of the same group at this period. Suppression of the humoral and cellular immuner esponses can be induced by antigens as well as antibodies (Heller & Siskind, 1973). A marked non-specific and transient reduction in skin reactivity was demonstrated when guinea-pigs previously immunized with bovine gamma-globulin, tuberculin and human serum albumin were given the same antigen intravenously or intraperitoneally (Kantor, 1975). Similarly, in persons who showed a strong positive mantoux reaction, the skin test induced a significant suppression of *in vitro* lymphocyte–blast transformation with purified protein derivative and PHA (Thestrup-Pederson, 1974). When normal subjects were skin tested twice with tuberculin at an interval of 2 days, those with a strongly positive reaction in the first skin test gave significantly smaller induration in the second test (Thestrup-Pederson, 1975). These observations suggest that the transient suppression following TAB vaccination is not an isolated finding. In view of this, the widespread practice of giving TAB vaccine during an epidemic both to close contacts of typhoid cases and to travellers just before going to endemic areas may not be good practice and perhaps should be avoided.

The development of humoral antibodies following TAB vaccination has been well documented (Olitzki, 1972). However, there is no uniform agreement with regard to the route of administration for maximum antibody production. Clasener & Beunders (1967) indicated that 0·1 ml of the vaccine administered intradermally produced antibody titres equal to those which followed subcutaneous administration of 0·5 ml of the vaccine. Others have reported contradictory findings (Van Gelder & Fischer, 1941; Morgan, Favorite & Horneff, 1943). The results of the present study indicate that one dose of 0·5 ml and one of 1·0 ml given subcutaneously at 10 days interval (group 1) only induced as high an antibody response as a single dose of 0·1 ml administered intradermally (group 2). Not only the final titres achieved, but also the rise in titres from the pre-vaccination levels were similar in the two groups. The antibody response was slightly better in group 2. Therefore it is suggested that intracutaneous route is as good as the more conventional subcutaneous route for the administration of TAB vaccine.

Further attempts at improving the efficacy of TAB vaccine should be directed to modifications that would induce a CMIR in addition to humoral antibodies.

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