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Systemic and Intestinal Immunities after Different Typhoid Vaccinations

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SUMMARY **The comparative studies of systemic and intestinal immunities to *S. typhi* were performed in 29 healthy volunteers during 2 years after receiving oral vaccination with attenuated *S. typhi* Ty21a in gelatin capsule, parenteral vaccination with acetone inactivated or heat inactivated-phenol preserved *S. typhi* Ty2. The methods used were immunobead ELISA for total secretory IgA and indirect ELISA for specific secretory IgA in the intestinal lavage fluid. The specific systemic IgG, IgM and anti-O, anti-H agglutinins were measured by indirect ELISA and Widal test respectively. The leukocyte migration inhibition test was used for the measurement of systemic cell-mediated immunity. The results indicate that the oral *S. typhi* Ty21a stimulated intestinal immunity better than both parenteral vaccines but evoked less systemic antibody response. The stimulation of systemic cell-mediated immunity by the live attenuated and acetone inactivated vaccine was comparable while stimulation by heat inactivated-phenol preserved vaccine was less pronounced.**

The same studies were performed in 26 healthy volunteers during 6 months following different doses of oral vaccination with *S. typhi* Ty21a in enteric – coated capsule. The results suggest that the stimulation of intestinal and systemic immunities by this vaccine is dosage dependent. Three doses of vaccine provide better stimulation than two doses and one dose, respectively.

Typhoid fever still remains a major public health problem in Thailand and other developing countries. Recent epidemiological surveillance data from the Ministry of Public Health of Thailand have shown that typhoid cases have not decreased in spite of regularized typhoid vaccination, improved hygiene, sanitation and diagnostic facilities (Epidemiological Surveillance Report, Ministry of Public Health, Thailand, 1974-1985). These data suggest that the parenteral heat inactivated-phenol preserved typhoid vaccine currently being administered is not very effective and that a more efficient typhoid vaccine should be given.

Existing vaccines for typhoid include oral attenuated *S. typhi* Ty21a, parenteral acetone inactivated *S. typhi* Ty2 and heat inactivated-phenol preserved *S. typhi* Ty2. Results of the first field trial of Ty21a in Alexandria, Egypt, showed that three doses of a liquid formulation of the oral vaccine provided 96% protection over three years without causing adverse reactions.[1] In another field trial in Santiago, Chile, three doses of Ty21a vaccine in an enteric-coated capsule formulation provided 65% protection over three years (M.M. Levine, personal communication). The heat inactivated vaccine (most widely used in developing countries because of low cost) and the acetone inactivated vaccine were evaluated in controlled field trials in other countries and found to be 40-90% effective.[2] Futhermore, the lack of simultaneous systemic and intestinal immunological response data after these vaccinations increases the difficulty in choosing the "ideal" vaccine for prophylaxis of typhoid fever.

The present paper reports the results of comparative studies of systemic and intestinal immunities done during two years following immunization with the 3 existing typhoid vaccines and six months after three different doses of the oral *S. typhi* Ty21a vaccination. The results indicate that routine dosage with the oral attenuated *S. typhi* Ty21a vaccine gave the best immunological stimulation and this immunological stimulation is dosage dependent.

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MATERIALS AND METHODS

Subjects

Fifty-five healthy male volunteers, aged between 19 and 21 years, were the subjects of this study. These volunteers had never had clinically apparent typhoid or paratyphoid fever, and had not received typhoid or paratyphoid vaccinations for at least 7 years prior to this study. They also had minimal or no anti-O or anti-H agglutinins and minimal or no specific cell-mediated immune response (CMIR) to *S. typhi* antigen by the leukocyte migration inhibition agarose test (LMIT).

The volunteers were divided randomly into 6 groups and vaccinated with following typhoid vaccines:

1. Oral attenuated *S. typhi* Ty21a vaccine in gelatin capsule (Vivotif®, kindly provided by Prof. Dr. R. Germanier, Swiss Serum and Vaccine Institute, Berne, Switzerland). Each vaccine capsule contained 2×10^7 viable microorganisms at the time of administration as confirmed by BHI agar pour plate method. Ten individuals were thus vaccinated and designated as the CO group. A single capsule was administered with $NaHCO_3$ on day 1,3 and 5 to each volunteer in this group. The day following ingestion of the 3rd vaccine capsule was considered to be the first day after vaccination. The 3 dosage-scheme was suggested for routine use by the manufacturer as the optimum dosage for protective immunological stimulation.

2. Parenteral acetone inactivated *S. typhi* Ty2 vaccine (kindly provided by Dr. I. Joó, Institute for Serobacteriological Production and Research, Budapest, Hungary). There were 1×10^9 microorganisms in 1.0 ml of this vaccine. Ten individuals were vaccinated and designated as the CA group. One ml of this vaccine was given subcutaneously only once to each volunteer. This dose was recommended for routine use in adults by the manufacturer. The day following the injection was considered to be the first day after vaccination.

3. Parenteral heat inactivated-phenol preserved *S. typhi* Ty2 vaccine (Pharmaceutical State Enterprise, Ministry of Public Health, Thailand). One ml of this vaccine contained 1×10^9 microorganisms. The nine volunteers were vaccinated and designated as the CH group. One subcutaneous injection of 0.5 ml of this vaccine was given to each volunteer. This dose was also recommended for routine use in adults by the manufacturer, since it can cause severe adverse reaction if 1.0 ml is given. The day following the injection was counted as the first day after vaccination.

The immunological studies in these first 3 groups were performed at the same time.

4. The remaining 26 volunteers were vaccinated with different doses of oral attenuated *S. typhi* Ty21a vaccine in enteric-coated capsules (kindly provided by Dr. E. Furer, Swiss Serum and Vaccine Institute, Berne, Switzerland). These 26 volunteers were divided randomly into another 3 groups and received 1, 2 (day 1,7) or 3 (day 1,4,7) vaccine capsules; groups were designated CO_1 (9 volunteers), CO_2 (9 volunteers) and CO_3 (8 volunteers), respectively. Each capsule contained more than 5×10^9 viable microorganisms as confirmed by our BHI agar pour plate method. The day following ingestion of the last vaccine capsule in each group was considered to be the first day after vaccination. The immunological studies of these last 3 groups were performed at the same time and about 1 year after the studies of the first 3 groups.

Preparation of antigen

The protein (Bp) antigen from *S. typhi* 0-901 was extracted with veronal buffer pH 8.4 and trichloroacetic acid exactly as described by Barber *et al.*[3,4] The protein was washed, resolubilized, lyophilized and stored at 4°C until used.

Sample collections

Blood and intestinal lavage specimens were collected at the same time from each volunteer before vaccination and at intervals of 1, 4, 12, 24, 36, 48 weeks, 1.5 and 2 years after vaccination in CO, CA and CH groups; and at intervals of 1, 4, 8, 12, 16, 20 and 24 weeks after vaccination in CO_1, CO_2, and CO_3 groups. The sera were aliquoted and stored at −20°C until used. The lymphocytes from the heparinized blood were processed and examined for cell-mediated immune responses within 1 hour after collection. Intestinal lavage specimens could be collected successfully from 9 vaccinees of the CO and CA groups, 7 vaccinees of the CH group and 8 vaccinees each of the CO_1, CO_2 and CO_3 groups. The method of collection followed Sack *et al.*[5] with slight modification as previously described.[6]

Study of cell-mediated immune responses (CMIR)

The leukocyte migration inhibition agarose technique (LMIT) as previously described[7] was used for detection of the systemic cell-mediated immune response to *S. typhi*. The optimum concentration of Bp antigen in the test was 125 μg/ml. The non-parametric Mann-Whitney U test was employed for the evaluation of the statistical significance of differences in this part of the study.

Study of humoral immune responses (HIR)

The humoral immune responses to *S. typhi* were measured from both sera and intestinal lavage fluids. Anti-O and anti-H agglutinating titers were determined in all sera of the volunteers

by the standard Widal test using antigen suspensions purchased from Gamma Diagnostic (Div. Gamma Biologicals, Houston, Texas).

The total intestinal secretory IgA (SIgA) level in each intestinal lavage specimen was determined by immunobead ELISA, using rabbit anti-human IgA covalently bonded to polyacrylamide beads (Bio-Rad Laboratories, Glattburg, Switzerland), as described by Sack and co-workers.[8] The results were expressed as milligrams of total SIgA in 1 ml of lavage fluid.

The systemic IgG and IgM specific to *S. typhi* in all vaccinee sera and the intestinal SIgA specific to *S. typhi* in all intestinal lavage specimens were determined by indirect ELISA according to the technique previously described.[6] The optimum concentration of Bp antigen used in all tests was 5 μg/ml.

The specific IgG and IgM in the sera were calculated in arbitrary antibody units (a.a.u.). The specific SIgA was calculated as a.a.u. per 1 mg of total SIgA. All these data were expressed in the results as fold rising of each value before vaccination.

RESULTS

Cell-mediated immune response

CO, CA and CH groups The results of LMIT to Bp antigen are shown in Table 1. By this assay, the LMI-index revealed that there was a good and comparable CMIR between CO and CA groups, whereas a poorer response was noted in the CH group. The CMIR could be detected in the CO group during the first week after vaccination, but disappeared before the 36th week.

The CMIR in the CA group started after the first week and disappeared before the 48th week. The CH group already had detetable specific CMIR before vaccination. Heat inactivated-phenol preserved typhoid vaccine boosted the primed CMIR poorly; a significant difference in the CMIR before and after vaccination in this group was noted at the 4th, 12th and 24th weeks post-vaccination. The peaks of CMIR in all groups were seen at the 12th week.

CO_1, CO_2 and CO_3 groups The results of CMIR are shown in Table 2. The LMI-index revealed that the response in the CO_3 group was the best, whereas, only minimal response and almost no response were observed in CO_2 and CO_1, respectively. The significant difference of LMI-index before and after vaccination in the CO_3 group was noted at 4 weeks through 24 weeks of the study and the peak of the response was seen at the 12th week.

Table 1 Leukocyte migration inhibition (LMI) index[a] in vaccinees who received different typhoid vaccines

Time	CO	CA	CH
0 week	0.80±0.04	0.84±0.04	0.74±0.06
1 week	0.67±0.04[c]	0.81±0.07	0.65±0.08
4 week	0.67±0.06[c]	0.57±0.07[b]	0.57±0.07[c]
12 week	0.57±0.06[b]	0.47±0.03[b]	0.56±0.05[c]
24 week	0.64±0.03[b]	0.66±0.06[b]	0.60±0.03[c]
36 week	0.73±0.06	0.69±0.06[c]	0.77±0.06
48 week	0.80±0.05	0.78±0.04	0.71±0.05
1.5 year	0.83±0.04	0.95±0.03[b]	0.86±0.07
2 year	0.77±0.03	0.76±0.05	0.79±0.03

CO = Oral attenuated *S. typhi* Ty21a.
CA = Parenteral acetone inactivated *S. typhi* Ty2.
CH = Parenteral heat inactivated-phenol preserved *S. typhi* Ty2.
[a]Mean ± SEM of 10, 10 and 9 vaccinees in CO, CA and CH groups, respectively.
[b]$p < 0.01$ versus control (time 0) by the Mann-Whitney U test.
[c]$p < 0.05$ versus control (time 0) by the Mann-Whitney U test.

Table 2 Leukocyte migration inhibition (LMI) index[a] in vaccinees who received different doses of *S. typhi* Ty21a orally in enteric coated capsules

Time	CO_1	CO_2	CO_3
0 week	0.94±0.05	0.80±0.04	0.83±0.05
1 week	0.85±0.05	0.78±0.05	0.85±0.06
4 week	0.91±0.07	0.72±0.06	0.66±0.03[c]
8 week	0.76±0.05[c]	0.67±0.04[c]	0.68±0.03[c]
12 week	0.86±0.05	0.68±0.03[b]	0.63±0.02[b]
16 week	0.78±0.03[b]	0.82±0.05	0.70±0.02[c]
20 week	0.85±0.05	0.77±0.05	0.68±0.03[c]
24 week	0.82±0.07	0.80±0.03	0.68±0.04[c]

CO_1 = One dose of vaccine.
CO_2 = Two doses of vaccine (day 1,7).
CO_3 = Three doses of vaccine (day 1,4,7).
[a]Mean ± SEM of 9, 9 and 8 vaccinees in CO_1, CO_2 and CO_3 groups, respectively.
[b]$p < 0.01$ versus control (time 0) by the Mann-Whitney U test.
[c]$p < 0.05$ versus control (time 0) by the Mann-Whitney U test.

The systemic humoral immune response

CO, CA and CH groups High titers of anti-O (1:640) and anti-H agglutinins (1 : 3,200) in the CH group were seen immediately in the first week and the peaks were at the 4th and 1st week after vaccination, respectively; this group showed the highest titer elevation of both agglutinins. Significant titers of anti-O agglutinin ($\geq$1:80 or GM $\geq$80) could be detected up to one and a half years after vaccination and more than 2 years for anti-H agglutinin ($\geq$1:100 or GM$\geq$ 100). The CA group showed an immediate but less pronounced elevation of the anti-O agglutinin titer associated with minimal and short-lived anti-H response. The CO group showed a minimal response of anti-O agglutinin and no anti-H agglutinin response (Tables 3 and 4).

The fold rising of specific IgM and IgG to Bp antigen are shown in Figures 1 and 2, respectively. The CH group provided the highest fold rising of these antibodies. The peak of Bp-specific IgM in all groups was in the first week and declined to pre-vaccination level at the 48th week. A significant rise of Bp-specific IgG was noted in the first week in the CH group and peaked in the 4th week. At the 48th week 33% (3/9) of vaccinees in the CH group still had more than twice the amount of this antibody than before vaccination but declined to pre-vaccination levels after 1 year. The CA and CO group had only minimal responses of Bp-specific IgG and this antibody also declined to pre-vaccination levels after 1 year.

CO_1, CO_2 and CO_3 groups No stimulation of anti-O and anti-H agglutinins were seen in all 3 groups.

The fold rising of Bp-specific IgM and IgG are shown in Figures 3 and 4. Minimum and comparable stimulation of these 2 antibodies was noted in all 3 groups.

The intestinal SIgA response

CO, CA and CH groups The SIgA response to Bp antigen is shown in Figure 5. Sixty-four percent (16/25) of vaccinees had detectable levels of this antibody before vaccination. After vaccination, the CO group showed the greatest increase of SIgA, while the other 2 groups showed only moderate to minimal average responses. The greatest amount of antibody was seen at the first week after vaccination. From 25 vaccinees on whom intestinal lavages were perfomed, all vaccinees (9/9) of the CO group, 44% (4/9) of the CA group and 71% (5/7) of the CH group had a specific SIgA response. The SIgA in CH group declined to pre-vaccination level after 1 year, while at 2 years after vaccination 78% (7/9) of the vaccinees in the CO group still had high elevation of this antibody and only 33% (3/9) of the vaccinees in the CA group had minimal rising.

CO_1, CO_2 and CO_3 groups The results of comparisons are shown in Figure 6. Before vaccination, 63 percent (15/24) of vaccinees had detec-

Table 3 The Widal anti-O agglutinin[a] in vaccinees who received different typhoid vaccines

Time	CO	CA	CH
0 week	21 ± 1.9	26 ± 7.6	23 ± 6.3
1 week	21 ± 1.9	70 ± 40.7	148 ± 61.4
4 weeks	23 ± 2.5	65 ± 56.5	235 ± 65.4
12 weeks	25 ± 2.9	43 ± 17.0	101 ± 27.4
24 weeks	26 ± 5.8	35 ± 17.3	80 ± 16.6
36 weeks	32 ± 5.5	40 ± 17.1	127 ± 32.1
48 weeks	30 ± 7.4	37 ± 13.6	137 ± 31.1
1.5 year	26 ± 7.6	28 ± 13.1	80 ± 16.6
2 years	23 ± 5.7	23 ± 5.7	54 ± 13.7

CO = Oral attenuated *S. typhi* Ty21a.
CA = Parenteral acetone inactivated *S. typhi* Ty2.
CH = Parenteral heat inactivated-phenol preserved *S. typhi* Ty2.
[a]Geometric mean titer ± SEM of 10, 10 and 9 vaccinees in CO, CA and CH groups respectively.

Table 4 The Widal anti-H agglutinin[a] in vaccinees who received different typhoid vaccines

Time	CO	CA	CH
0 week	54 ± 4.7	57 ± 14.2	73 ± 162.4
1 week	54 ± 4.7	100 ± 43.6	588 ± 393.1
4 weeks	57 ± 14.2	100 ± 43.6	272 ± 153.8
12 weeks	50 ± 0	50 ± 0	216 ± 73.3
24 weeks	50 ± 0	54 ± 4.7	159 ± 47.8
36 weeks	50 ± 0	54 ± 4.7	317 ± 150.0
48 weeks	50 ± 0	54 ± 4.7	294 ± 65.6
1.5 year	50 ± 0	54 ± 4.7	117 ± 34.6
2 years	50 ± 0	54 ± 4.7	136 ± 73.5

CO = Oral attenuated *S. typhi* Ty21a.
CA = Parenteral acetone inactivated *S. typhi* Ty2.
CH = Parenteral heat inactivated-phenol preserved *S. typhi* Ty2.
[a]Geometric mean titer ± SEM of 10, 10 and 9 vaccinees in CO, CA and CH groups respectively.

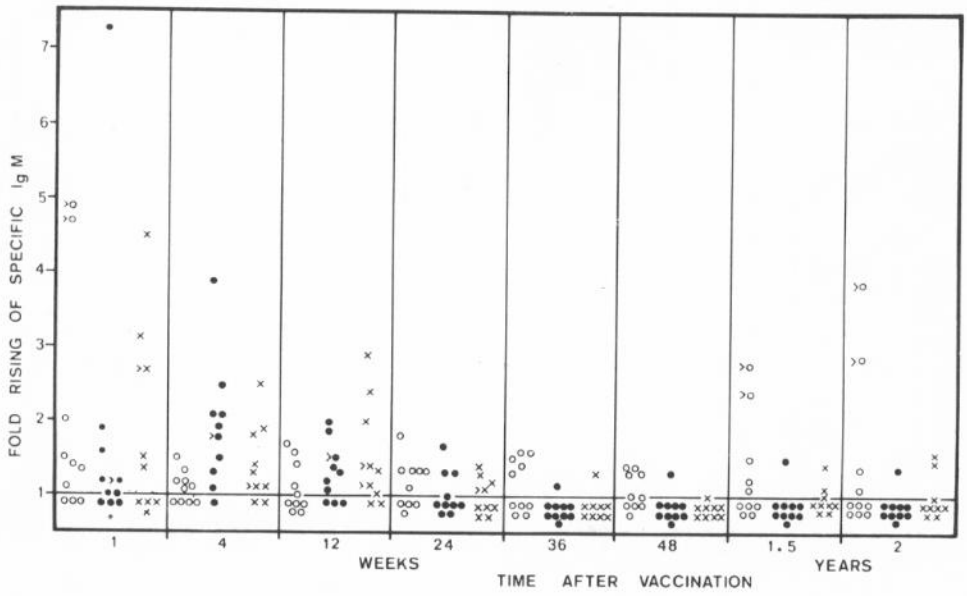

Fig. 1 The specific systemic IgM to Bp antigen in CO, CA and CH groups. Each amount of this antibody is shown as fold increase over the 1-fold value represented by the solid line, which is equal to the amount of this antibody before vaccination. o = CO; ● = CA; x = CH.

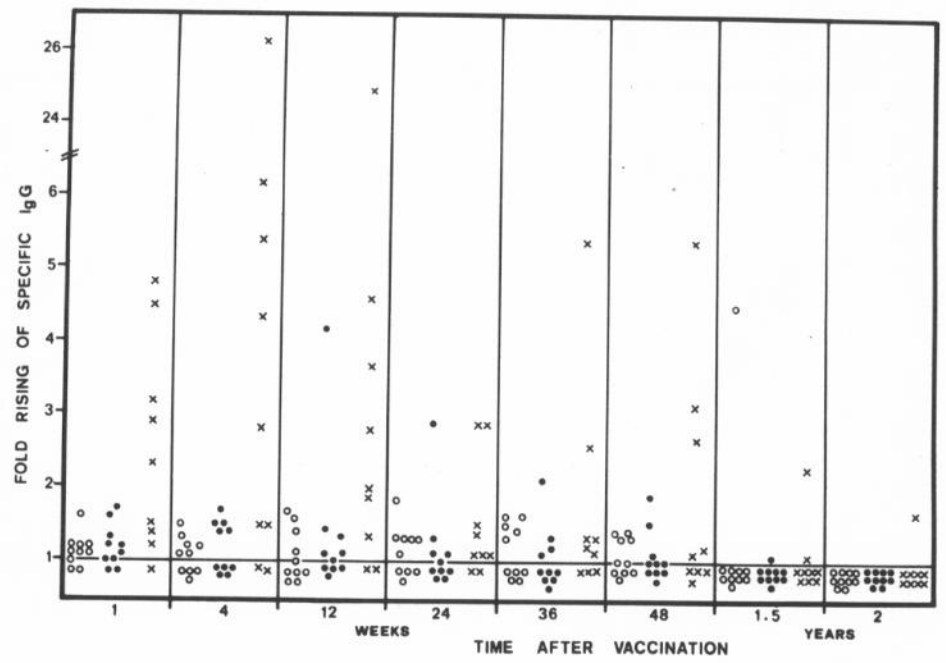

Fig. 2 The specific systemic IgG to Bp antigen in CO, CA and CH groups. Each amount of this antibody is shown as fold increase over the amount present before vaccination (solid line). o = CO; ● = CA; x = CH.

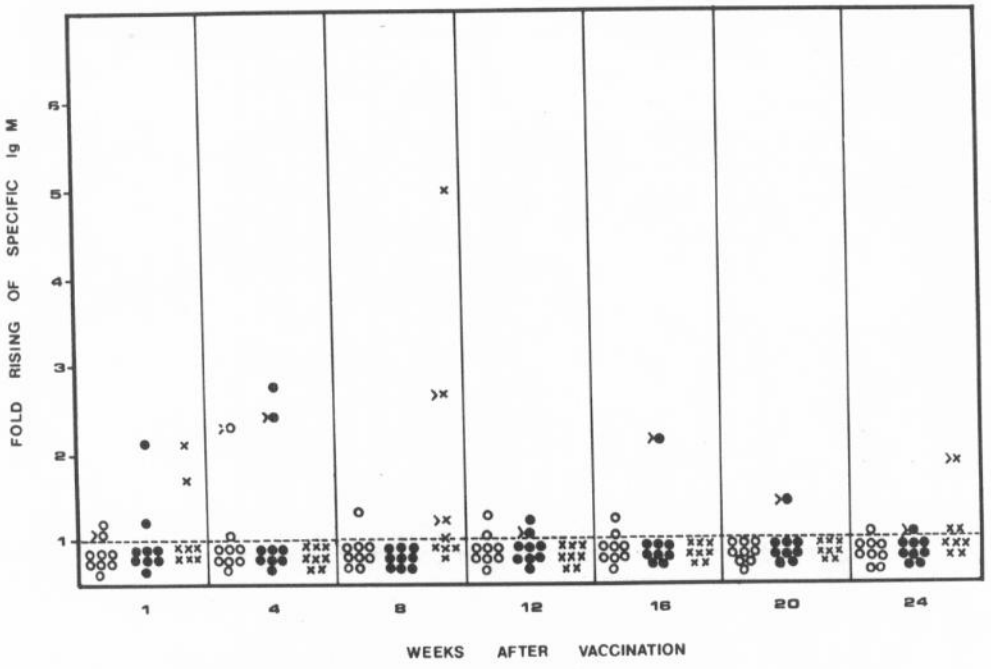

Fig. 3 The specific systemic IgM to Bp antigen in CO_1, CO_2 and CO_3 -groups is shown as fold increase over the amount of this antibody before vaccination, represented by the broken line. o = CO_1; ● = CO_2; x = CO_3.

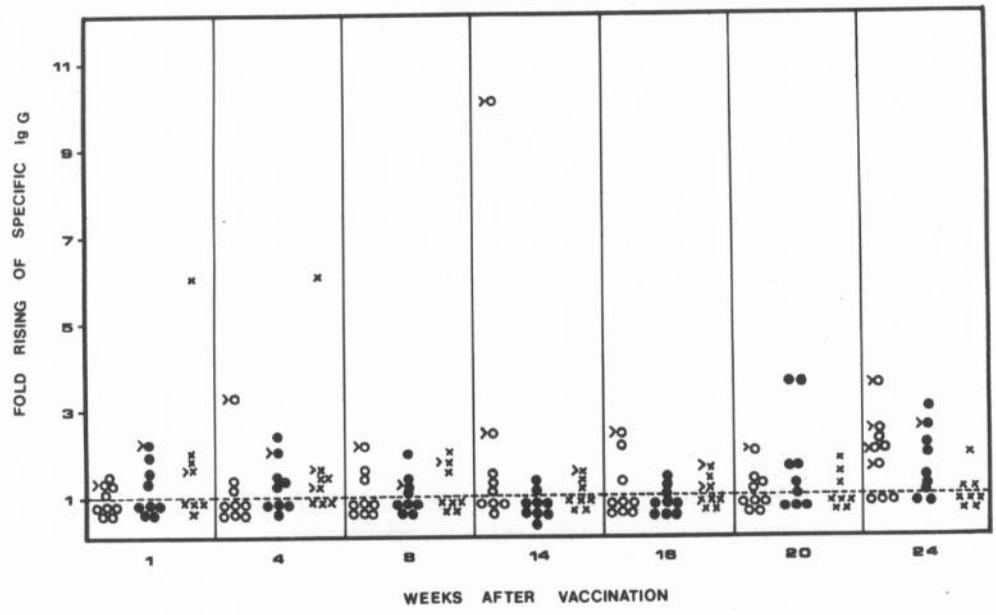

Fig. 4 The specific systemic IgG to Bp antigen in CO_1, CO_2 and CO_3 groups, shown as fold increase of the amount before vaccination (broken line). o = CO_1; ● = CO_2; x = CO_3.

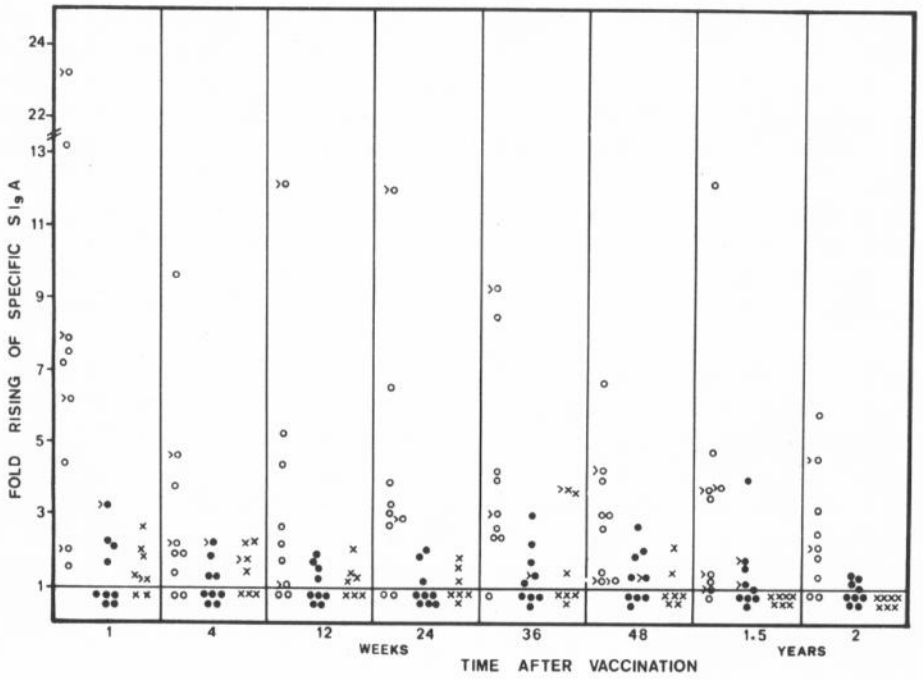

Fig. 5 The specific secretory IgA to Bp antigen in intestinal lavage fluid of the vaccinees in CO, CA and CH groups, shown as fold increase of the amount before vaccination (solid line). o = CO; ● = CA; x = CH.

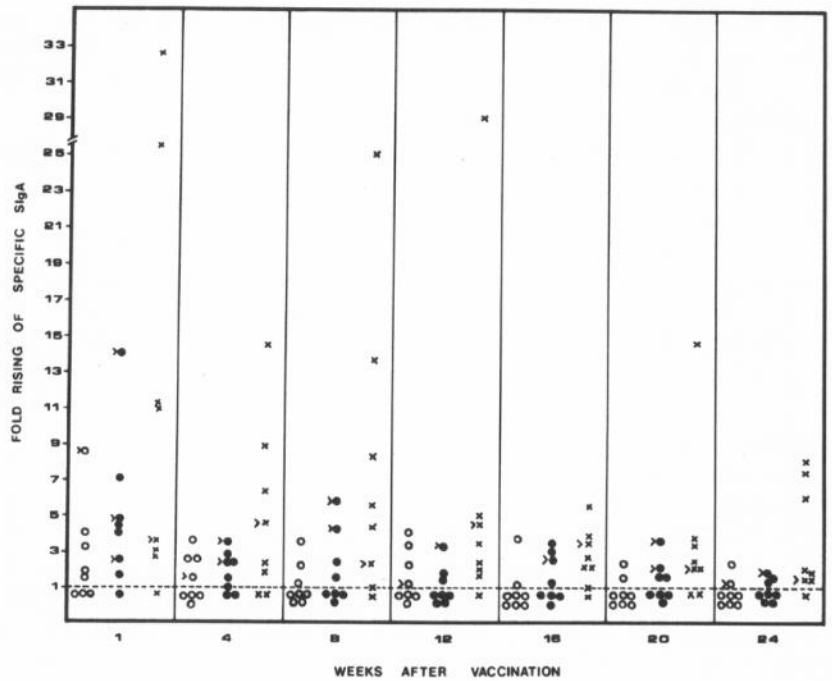

Fig. 6 The specific secretory IgA to Bp antigen in intestinal lavage fluid of the vaccinees in CO_1, CO_2 and CO_3 groups, shown as fold increase of the amount before vaccination (broken line). o = CO_1; ● = CO_2; x = CO_3.

table levels of Bp-specific SIgA as assayed from their intestinal lavages. The CO_3 group showed the greatest increase of Bp-specific SIgA after vaccination as compared to the other 2 groups. Eighty-eight percent (7/8) of the CO_3 and CO_2 group and 63% (5/8) of the CO_1 group had an increased SIgA response and the maximal response was seen at the first week after vaccination.

DISCUSSION

The efficacies of the existing vaccines for typhoid fever as reported from various field trails conducted in different typhoid fever endemic areas were 96% for oral attenuated *S. typhi* Ty21a,[1] 79-93% for parenteral acetone inactivated *S. typhi* Ty2 and 47 - 77% for parenteral heat inactivated-phenol preserved *S. typhi* Ty2.[2] A direct extrapolation of these separate trial results would suggest that the oral attenuated vaccine seems to be the best vaccine available for protection. However, a note of caution in the interpretation of such test results is indicated by a second field trial of oral Ty21a vaccine conducted in Chile, another endemic area of typhoid fever where the annual incidence rate of this infection was five times higher than in the first trial in Egypt.[9,10] In this trial, three doses of the practical enteric-coated capsule formulation of vaccine provided 65% protection during three years of surveillance (M.M. Levine, personal communication). It is not clear whether this discrepancy resulted from the change in formulation, fewer doses administered, or the much higher force of infection. However, the unsettled question of efficacy among these vaccines could be clarified feasibly by the comparative study of protective immunological response induced by the individual vaccines among groups of vaccinees who were matched statistically.

The exact mechanisms of protective immunity induced by these vaccines are not well characterized. However, they should follow the immunopathogenesis of typhoid fever, that is, the specific secretory IgA (SIgA), the first line of defense against the pathological microorganisms in the gut,[11-14] should play an important role in prevention. The specific anti-Vi antibody is beneficial in combating the microorganisms in the circulation, where they remain for a short while free from phagocytes.[15] The most important role of the immune system for eliminating these intracellular microorganisms and leading to recovery is specific cell-mediated immunity which is the co-function of antigen-specific T lymphocytes and monocytes macrophages.[16] Thus, the "ideal" vaccine should stimulate all three immune properties mentioned above.

The protective antigen(s) of *S. typhi* in human typhoid is/are not well elucidated. However, the protein antigen (Bp) of *S. enteritidis* and *S. typhimurium* has been shown to play an important function as a protective antigen in the mouse typhoid model.[17,18] In so far as humans comprise the sole natural host for *S. typhi*, and thus no animal model is available for experimental human typhoid, we can only postulate that the immune response in humans to this protein extracted from *S. typhi* plays the same role.

Our studies have shown that all 3 vaccines given in the routine dosages stimulate both systemic and intestinal immunity to Bp antigen in various degrees and patterns. The detectable levels of intestinal specific SIgA to Bp antigen in the vaccinees before vaccination indicates natural priming to *S. typhi*, since Thailand is known to be an endemic area of typhoid fever. The oral attenuated vaccine provided the best response of this specific SIgA in both magnitude and duration, while the other two vaccines provided minimal effect. This is to be expected as a result of direct stimulation of local gut lymphocytes by antigen in the oral vaccine.[12,14] Although Svennerholm *et al*[19] have shown that the parenteral route of vaccination could give rise to specific SIgA in intestinally-primed individuals, our results suggest that the magnitude and duration of the antibody response is much less than by the oral route.

The attenuated *S. typhi* Ty21a vaccine does not possess Vi antigen and the heat inactivated-phenol preserved vaccine possesses little of it, whereas, the acetone inactivated vaccine preserved most of the Vi antigen.[20-22] Based on this information, systemic anti-Vi antibody would not be elicited in the CO group, but should contribute to the immune response in the CA and CH groups, although determination of anti-Vi antibody was not included in the present study.

The systemic IgM and IgG to Bp antigen, anti-O (anti-lipopolysaccharide) and anti-H (anti-flagella) are not crucial in prevention and recovery from the disease.[16,23] Nevertheless, they are possibly indicators of the immunological response to *S. typhi*. The different responses among the CO, CA and CH groups in terms of such antibodies should indicate the unique properties of each vaccine since these antigens are present in all of them.[20-22] The patterns of IgM and IgG responses suggest that the vaccinees in the CH group, and probably in the other groups to the extent that they could be stimulated, were immunologically primed to *S. typhi*.

The specific CMIR, as measured by LMIT, revealed good and equivalent responses by the CO and CA groups and a relatively poorer response by the CH group. There is no clear evidence to explain why the oral attenuated vaccine, which has shown a poor stimulation of systemic antibodies, should provide a relatively good stimulation of cellular immunity. The possibility should be considered

that the antigenic determinants which induce humoral immunity may be different from those that induce cellular immunity.

These findings suggest that although the size of the inoculum is much less, the oral Ty21a vaccine given in routine dosage in gelatin capsule can stimulate the intestinal immunity and systemic cell-mediated immunity better, than the parenteral vaccines, but evoke less systemic antibody response.

When the oral Ty21a vaccine in enteric-coated capsules was given in different dosages to another 3 groups of volunteers, the results of both systemic and intestinal immunological studies revealed that the more vaccine received, the greater the immunity stimulated. Therefore, it can be concluded that immunological stimulation by oral *S.typhi* Ty21a vaccine is dosage dependent.

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