ANTIBODY RESPONSES TO HEAT-KILLED, PHENOL PRESERVED PARENTERAL TYPHOID VACCINE

WANPEN CHAICUMPA, YONGYUT WECHSATHANARAK,
SURANG TANTIVANICH and WISAI PLUEKSAWAN*

Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, Bangkok. *Special Prison Hospital, Department of Correction, Ministry of Interior, Thailand.

INTRODUCTION

Parenteral typhoid vaccines have been produced and used but no prospective evaluation for their efficacy was performed until 1960 when control field trials were conducted in Yugoslavia, Guyana, Poland and also in USSR under the auspices of the World Health Organization (Yugoslav Typhoid Commission, 1964; Hejfec *et al.*, 1966, 1969). Two of the most effective typhoid vaccines used in these trials are the acetone-killed and heat-killed vaccines. The results which involved large numbers of immunized individuals indicated that both vaccines provided significant immunity and that the acetone-killed vaccine was better than the heat-killed one.

It was observed that typhoid vaccine induced good humoral immune response but failed to protect all the vaccinated individuals. It was suggested that the lack of complete protection with killed vaccine may be due to its inability to induce any significant degree of cell-mediated immune response (Mackaness *et al.*, 1966; Collins, 1970).

In control field trials, there was a correlation between the level of H antibodies and degree of protection. In fact, in one trial with an H antigen-free vaccine, no protection was observed. Thus, H antigen may have a role in protection. However, it is possible that H antigen is only a marker for a heat-labile, not yet detected "protective antigen" (WHO, 1979). The importance of the study concerning the efficacy of the vaccine is dependent on the antibody response. The good vaccine should have high antigenicity and high avidity. Serological test in man revealed that the H agglutinins are most reliable indicator of the effectiveness of typhoid vaccine (Cvejetanovic and Vemura, 1965). The only direct test for protective immunity in man is the challenge study of vaccinated and non-vaccinated volunteer which is apparently difficult to perform. However, it was found that both anti-O and anti-H antibody responses following typhoid vaccination in the field coincided with the decreasing rate of the disease (Beneson, 1964). This could mean that anti-O or anti-H antibody is the factor which indicates whether protective immunity occurs or not.

The heat-killed, phenol preserved parenteral typhoid vaccine has been widely used for public health programmes in many countries including Thailand for many decades. In Thailand, the vaccine is prepared and distributed by the Government Pharmaceutical Organization, Ministry of Public Health. However, no assessment for efficacy of this type of vaccine has been performed. An experimental study was undertaken, which was accomplished by vaccinating human volunteers and observing the magnitude and duration of humoral immune responses elicited by the vaccine.

MATERIALS AND METHODS

The subjects were selected from healthy prisoners in the Bangkok Special Prison. They were clinically examined by the doctor and those with no history of typhoid vaccination or typhoid fever during the past 2 years were included in the study. The volunteers were informed about the study. They were divided into two groups. Each individual of the vaccinated group (49 volunteers) was injected subcutaneously with 0.5 ml of the heat-killed phenolized typhoid vaccine, (The Government Pharmaceutical Organization, Thailand). Each volunteer in the control group (37 persons) received subcutaneous injection of 0.5 ml steriled distilled water. Serum samples were collected from each individual of the two groups prior to injection, 1 week, 1 month, 3, 6, 9, and 12 months after the injections respectively.

Levels of specific antibodies to *Salmonella typhi* antigens in serum specimens were assessed by classical Widal test (Bailey and Scott, 1974) and enzyme-linked immunosorbent assay (ELISA). Salmonella somatic group D (typhoid O) and salmonella flagella group D (typhoid H) of Gamma Laboratory were used as O and H antigens in Widal test throughout the study. Febrile antigen positive control pool of sera (polyvalent salmonella and brucella) and febrile antigen negative control pool of sera were used for quality control of the Widal agglutination assays.

Lipopolysaccharide (LPS) was prepared from *Salmonella typhi* strain 0901 according to the method previously described (Sangpetchsong and Chaicumpa, 1982). The antigen was diluted to 50 µg/ml of carbonate buffer pH 9.6, and 50 µl of this preparation was used to coat the well of flat-bottom microtiter plastic plate. After the antigen plate was kept at 37°C overnight and thoroughly washed with physiological saline-tween solution, 100 µl of diluted serum sample was added to the wells. Three rows of serially diluted serum sample were prepared for detecting IgM, IgG and IgA antibody. After 30 minutes incubation, each well was washed three times with physiological saline-tween 20. Aliquots (100 µl) of 1 : 1000 peroxidase labelled anti-human IgA, IgG and IgM (Pasteur Institute) were added to appropriate wells, respectively. The plate was left at room temperature for 30 minutes then each well was washed thoroughly with PBS-tween. Freshly prepared ortho-tolidine solution 100 µl was added, mixed, and the ELISA end point was read at 30 minutes theerafter.

RESULTS

Reciprocal Widal anti-O antibody titres of vaccinated and control groups are shown in Fig. 1. It was found that the anti-O antibody peaked within 7 days after the typhoid vaccination. The mean titre at this time point was 1 : 52 which was significantly higher than the mean titre found before vaccination and the mean titre of the control individuals taken at the corresponding period ($p<0.01$). At the first and third months the mean titres of the vaccinated group were 1 : 42.4 and

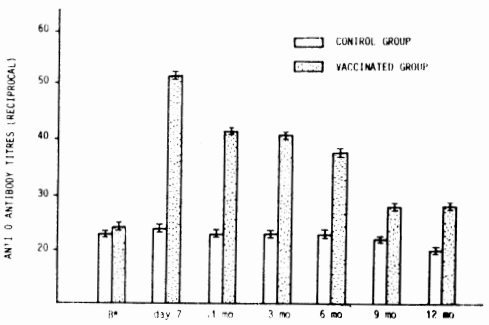

Fig. 1—Reciprocal mean ±S.E. of Widal anti-O antibody titres of typhoid vaccinated subjects and controls.
B* = Before injection with vaccine or distilled water.
mo = month(s)

1 : 40.8 respectively which were not significantly different from the titre at day 7. At the sixth month, the titre of the antibody decreased to 1 : 38.2. However, this was not different from the titres at the first and third months though significantly lower than the titre at day 7 and higher than the titre before vaccina tion. The mean titres detected at 9 months and 12 months after the vaccination were 1 : 27.7 and 1 : 28.2. These were similar to the titre before vaccination.

Widal anti-H antibody mean titres of the vaccinated and control groups are shown in Fig. 2. The mean titre at 7 days after the vaccination was 1 : 413.3 which was much higher than the titres before vaccination and those of the controls taken at the corresponding time point.

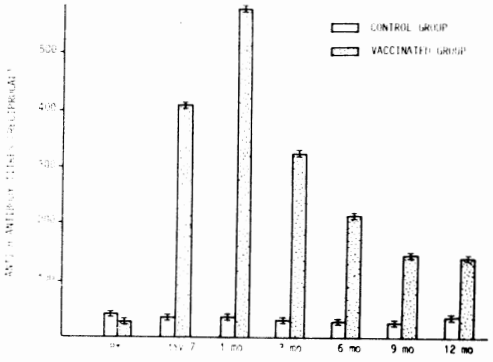

Fig. 2—Reciprocal mean ± S.E. of anti-H antibody titres of typhoid vaccinated subjects and controls.
B* = Before injection with vaccine or distilled water.
mo = month(s)

The anti-H antibody peaked at one month (mean titre = 1 : 583.4). Thereafter, the levels declined and by 12 months the mean titre was 1 : 146.6. However, this level was still 5 fold higher than those before vaccination.

Anti-O and anti-H titres of the control groups remained unchanged throughout the course of the study ($p > 0.01$).

Anti-LPS antibody response after the typhoid vaccination was found in all three classes of the immunoglobulins namely IgM, IgG and IgA. The data on the reciprocal mean ELISA titres of sera from vaccinated and control individuals are shown in Table 1. All of the antibody classes showed significant rise at 7 days after the vaccination. IgM and IgG levels were higher than the levels in the sera of control individuals collected at the corresponding times up to one year (longer time intervals were not studied). IgA antibodies were maintained at the levels higher than in the controls up to 6 months.

No change in ELISA titres was observed among the serum samples of the control individuals throughout the course of the study ($p > 0.01$).

DISCUSSION

The study revealed that antibodies to *Salmonella typhi* antigens were detected in the volunteers even before vaccination which implied that they had experienced certain degree of subclinical typhoid infections.

The anti-O antibody level assessed by the Widal test reached its peak at one week after the vaccination and showed a plateau until 6 months. Then declined to the same level as before vaccination or to those of the control individuals. However, the levels of specific IgM and IgG to LPS as detected by ELISA were significantly higher than in the controls up to 12 months, of which longer time intervals were not studied. The results thus indicate that the Widal test is less sensitive in detecting antibody to tyhpoid O somatic antigen than ELISA. These findings indicate that the Widal O-agglutination test detects only antibody molecules which have

Table 1

Mean ± S.E. of reciprocal anti-LPS IgM, IgG, IgA titres as detected by ELISA in sera of typhoid vaccinated subjects and controls.

Group	Pre-vaccination	Post vaccination					
	1st day*	7th day	1 mo	3 mo	6 mo	9 mo	12 mo
IgM							
Control	707 ± 0.38	778 ± 0.44	783 ± 0.37	684 ± 0.32	762 ± 0.40	744 ± 0.40	773 ± 0.49
Vaccinated	987 ± 0.48	1,994 ± 0.41	2,409 ± 0.52	2,486 ± 0.51	2,457 ± 0.56	2,216 ± 0.65	1,600 ± 0.72
IgG							
Control	639 ± 0.34	738 ± 0.37	676 ± 0.35	559 ± 0.31	546 ± 0.31	520 ± 0.36	509 ± 0.34
Vaccinated	753 ± 0.43	2,334 ± 0.56	2,124 ± 0.54	2,192 ± 0.56	1,766 ± 0.66	1,883 ± 0.54	1,703 ± 0.56
IgA							
Control	68 ± 0.31	70 ± 0.34	82 ± 0.35	84 ± 0.37	69 ± 0.34	59 ± 0.31	70 ± 0.43
Vaccinated	73 ± 0.45	146 ± 0.53	150 ± 0.50	120 ± 0.47	128 ± 0.63	98 ± 0.54	97 ± 0.61

* Before injection with vaccine or distilled water.

full capacity in cross-linking the epitopes on different typhoid bacilli and these epitopes are randomly distributed on the bacterial surface. ELISA, on the other hand, detects any antibody molecule (or even antibody fragments) which were able to bind to the epitopes with or without cross-linking.

In recovery from typhoid infection, cell-mediated immunity was found to have a major role. However, in the early phase of pathogenesis caused by the typhoid bacilli, specific antibodies may be play an important factor in protecting against the disease. This protective effect may be achieved through the agglutinating activity of the intestinally located antibodies. Thus, the pathogenic bacilli which survived the host's hostile factors in the upper part of the gastrointestinal tract and arrived at the small intestines would be agglutinated by antibodies in the intestinal lumen or within the mucus gel and subsequently being removed by forward propulsion to the large intestine where bacterial antagonism would lead to killing of the pathogens.

Intestinal antibodies may be from two sources i.e. serum derived and locally produced (Pierce and Koster, 1981). Locally produced antibodies are predominantly of the IgA class and to the lesser extent the IgM. Serum derived specific antibodies are mainly IgG which can passively leak from the circulation to the intestine. From our study, IgG specific antibodies as detected by ELISA sustained in the sera of the vaccinated volunteers at high levels for longer than 12 months after the vaccination. These may leak into the intestines and function therein. However, the levels of O-agglutinating antibodies as detected by Widal test lasted only for 6 months. It can be assumed that if the prevention of invasion by *S. typhi* is to be due to agglutination of the bacilli by O-antibodies, the protective period of the vaccine (based on serum antibodies) is 6 months. However, it is not known whether the subagglutinating doses of the specific antibodies (those detected by ELISA after 6 months) would be able to confer any protection by any other means. It is also reasonable to assume that after the parenteral vaccination, certain portion of the inoculated antigen would be selectively or preferentially arrived at the intestinal lymphoid tissues and gave rise to the locally produced antibodies. The duration of protection afforded by antibodies of this origin might be different from that of the serum derived ones.

The fall in serum IgA level after 6 months may imply that production of IgA in the intestine decreased at that time as it is believed that most of serum IgA are produced in the intestine (Pierce and Koster, 1981). This decrease in IgA synthesis (using serum IgA as an indicator) may be so much so that no more effective intestinal IgA was available any longer or it may merely means that the IgA produced in the lamina propria were concentrated by the epithelial cells carried secretory components and finally all of them being secreted into the intestine as secretory IgA. If this latter hypothesis holds true, it is possible to speculate that after 6 months of the vaccination, protective antibodies in the form of secretory IgA are still available in the intestinal tract.

Protection against pathogens in the intestinal tract by H-antibodies was found in cholera (Steele *et al.*, 1975). It is not know whether similar situation arises in typhoid infection. H-antibodies to typhoid bacilli sustained at high levels for longer than one year in the sera of the vaccinated individuals. Unfortunately, classes of those H-antibodies were not determined. Therefore, it is not known how much IgG antibodies were in the immune sera.

Based on IgG anti-O agglutinating antibody and fro mELISA IgA found in sera of vaccinated volunteers, it is reasonable to

believe that the heat-killed, phenolized parenteral typhoid vaccine could confer protection for at least 6 months.

SUMMARY

The heat-killed, phenolized parenteral typhoid vaccine was tested in informed volunteers. Assessment for its immunogenicity was performed using Widal test and enzyme-linked immunosorbent assay (ELISA). The anti-H antibody, which is a marker of the vaccine antigenicity peaked at one month after the vaccination and appeared throughout the one year course of the study. The anti-O antibody peaked at 7th day after vaccination and lasted only for 6 months. Classes of specific antibodies were determined by ELISA using single extracted lipopolysaccharide from *Salmonella typhi* 0901 as antigen. The possible protective role of serum derived intestinal IgG and IgA were discussed. Based on the agglutinating antibodies, the results indicate that the heat-killed, phenolized typhoid vaccine conferred at least 6 months protective period.

REFERENCES

BAILEY, W.R. and SCOTT, E.G., (1974). Diagnostic microbiology : a textbook for the isolation and identification of pathogenic microorganisms, (4th ed.), Mosby Company.

BENESON, A.S., (1964). Serological responses of man to typhoid vaccine. *Bull. WHO., 30* : 353.

COLLINS, F.M., (1970). Immunity to enteric infection in mice. *Infect. Immun. 1* : 243.

CVEJETANOVIC, B. and VEMURA, K., (1965). The present status of field and laboratory studies of typhoid and paratyphoid vaccines. *Bull. WHO., 32* : 29.

HEJFEC, L.G., SALMIN, L.V., LEJTMAN, M.Z., KUZMINOVA, M.L., VASILEVA, A.V., LEVINA, L.A., BENCIANOVA, T.G., PAVLOVA, E.A. and ANTONOVA, A.A., (1966). A controlled field trial and laboratory study of five typhoid vaccines in the USSR. *Bull. WHO., 34* : 321.

HEJFEC, L.G., LEVINA, L.A., KUZMINOVA, M.L., SLAVINA, A.M., DROZD, A.K., TONOJAN, J.A., KAENMAN, L.I., LEVITES, N.E., ABIDOV, L.A., ABIDOV, A.Z. and GONEAROVA, L.F., (1969). A controlled field trial to evaluate the protective capacity of a single dose of acetone-killed agar grown and heat-killed broth grown typhoid vaccines. *Bull. WHO., 40* : 903.

MACKANESS, G.B., BLANDEN, R.V. and COLLINS, F.M., (1966). Host-parasite relation in mouse typhoid. *J. Exp. Med., 124* : 573.

PIERCE, N.F. and KOSTER, F.T., (1981). Stimulation of intestinal immunity in acute enteric infections in children. Holme, Holmgren, Merson and Mollby (ed.). Elsevier-North-Holland Biomedical Press, Amsterdam, New York, Oxford.

SANGPETCHSONG, V. and CHAICUMPA, W., (1982). Characterization of *Salmonella* antigens. *ASEAN J. Clin. Sci., 3* : 37.

STEELE, E.J., CHAICUMPA, W. and ROWLEY, D., (1975). Further evidence for cross-linking as a profective factor in experimental cholera : properties of antibody fragments. *J. Infect. Dis., 132* : 175.

WHO, (1979). Intestinal immunity and vaccine development. *Bull. WHO., 57* : 719.

YUGOSLAV TYPHOID COMMISSION, (1964). A controlled field trial of the effectiveness of acetone dried inactivated and heat-phenol inactivated typhoid vaccines in Yugoslavia. *Bull. WHO., 30* : 623.