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Re-examination of immune response and estimation of anti-Vi IgG protective threshold against typhoid fever-based on the efficacy trial of Vi conjugate in young children

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Abstract

Background—The capsular polysaccharide of *Salmonella enterica* serovar Typhi, Vi antigen, is an essential virulence factor and a protective antigen. Similar to other polysaccharide vaccines, the protective action of Vi, both to the polysaccharide alone or when presented as a conjugate, is mediated by serum IgG Vi antibodies. The evaluation of Vi capsular polysaccharide based vaccines to prevent typhoid fever would be significantly facilitated by the identification of a “protective level” of serum antibodies to Vi antigen.

Methods—The protective level of anti-Vi IgG against typhoid fever was derived from the protective efficacy and immune response of a Vi-rEPA conjugate vaccine efficacy trial. The estimation was derived by two methods: correlation of the percent efficacy and the antibody distribution profile in the vaccine group at a given period of observation, and use of the relative ratio of anti-Vi IgG levels between the vaccine and placebo groups greater or equal to the Relative Risk of typhoid fever used in the efficacy determination.

Results—Both methods predicted a similar range of a minimum protective level of anti-Vi IgG between 1.4 and 2.0 µg/ml (short term threshold). When applying a protective threshold of 10 µg/ml at 6 months post immunization, an IgG level in excess of 1.4 µg/ml was achieved by 90% of children at 46 months post immunization, consistent with an 89% level of protection over the duration of the study. We thus suggest that the proportion of children with Vi IgG > 10 µg/ml (long term threshold) 6 months after immunization may reflect the proportion protected over at least a 4 year period.

Conclusion—The current assignment of an anti-Vi IgG protective level may be of value when evaluating vaccine performance of future Vi conjugate vaccines.

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1. Introduction

Typhoid fever caused by infection with the bacterium *Salmonella enterica* serovar Typhi (*S. Typhi*) remains a global health problem [1,2]. Vi polysaccharide vaccine was licensed in 1990s and demonstrated ~70% efficacy in high endemic regions [3-5]. The new generation of Vi conjugate vaccine was shown to be safe, and provided greater than 90% protection to immunized children when administered concomitantly with infant routine vaccines [6-8]. The WHO and GAVI are joining forces to accelerate Vi conjugate vaccine availability for distribution in immunization programs where the typhoid burden is high and is unlikely to be reduced in the short term by sanitation improvement [9,10]. A serological surrogate for new vaccines based on the existing Vi conjugate efficacy trial, both the polysaccharide and the conjugate vaccines, offers an estimation of potency and may serve as a surrogate to reduce the lengthy and financial demanding clinical efficacy trials otherwise required for licensing [8,11,12].

Efficacy data from a Vi-rEPA phase III clinical trial and the duration of its protective action provided useful information to estimate the protective activity [6,7,13,14]. Even though a variety of possible mechanisms of protection for typhoid vaccines have been proposed, the protective function of Vi antibody is well established based on the licensed Vi polysaccharide vaccine in several efficacy and post licensure demonstration studies [2,4,5,8].

The first serological correlate of protection for typhoid with Vi polysaccharide comes from a randomized controlled trial and concluded that a level of 0.6–1.2 µg/ml Vi antibody was the serological correlate of protection at three years after immunization with Vi polysaccharide [15,16]. This value was based on the radioimmunoassay measurement of total Vi antibodies. In a putative agreement conveyed by the newly licensed meningococcal and pneumococcal conjugate vaccines, the major class of antibodies that correlates with protection is serum IgG [17-19]. During the Vi-rEPA efficacy trial, there were two estimates of the anti-Vi IgG protective level based on the geometric mean (GM) level in the vaccine group: it was first proposed to be 8.7 µg/ml based on the 27 months surveillance and subsequently lowered to 4.3 µg/ml at 46 months [6,7]. These values were extracted from the GM titers of the younger children in the study (2–3 years old) without further analysis of the antibody distribution.

In this report, we re-examine the immune response data collected through the entire period of the Vi-rEPA efficacy trial and apply two analytical methods to estimate the anti-Vi IgG protective level.

2. Materials and methods

2.1. Efficacy trial of protective efficacy

The study was based on the protective efficacy and serological data collected during the Vi-rEPA efficacy trial in 2–5 years old Vietnamese children in Mekong Delta (NIH Protocol ID numbers: OH98-CH-N0001, OH98-CH-N0002; FDA BB Investigation New Drug (IND) number 6990) [6,7]. Briefly, this randomized, placebo controlled, double-blinded efficacy trial was conducted in approximately 11,000 children, each receiving 2 injections 6 weeks apart. A typhoid case was defined by a positive blood culture for *S. Typhi*. Active surveillance was performed for 27 months and followed by passive surveillance for another 19 months. At 42 months, a cross-over vaccination was offered to the placebo group [7,8].

The protective efficacy of the Vi-rEPA conjugate during three periods was calculated based on the blood positive typhoid incidence in the vaccine and placebo groups during 1–12, 13–27 and 28–46 months, as well as the entire 1–46 months using the formula:

$$E = \text{Vaccine efficacy} = 100 \times [1 - \text{Relative Risk}]$$

where Relative Risk = typhoid incidence in Vi-rEPA vaccinees/typhoid incidence in controls.

Blood samples were collected randomly from vaccinees monthly for the 46 months period. Serum anti-Vi IgG were assayed by enzyme-linked immunosorbent assay (ELISA) and expressed in ELISA units (EU) relative to a standard arbitrarily assigned a value of 75 units. This unit was converted to weight unit (1 EU = 1.24 µg/ml) against a calibrated human anti-Vi IgG reference standard [14]. The minimum detectable level was 0.05 µg/ml.

The confidence limits were calculated by the method of Miettinen and Nurminen [20]. The Chi-square test or when appropriate, Fisher's exact test was used for the comparison of categorical variables. Logarithms of the antibody concentrations were used in all calculations. Antibody levels at 6 months intervals were expressed in geometric means (GM) and 25–75th percentiles. The comparisons of GM were performed with the unpaired or paired *t*-test. At the end of each observation period, antibody levels were calculated from 10th to 50th percentiles.

2.2. Estimation of anti-Vi IgG protective level

Two methods were used to estimate the anti-Vi IgG protective level. In the first method we re-examined the serological response during the 46 months of the Vi-rEPA efficacy trial. The kinetic profile of anti-Vi IgG levels throughout the entire period was analyzed and percentile distribution between 10th and 50th, median and GM of the response at various time points were calculated. The efficacies of the vaccine at 12, 27 and 46 months following vaccination were used as the benchmark for the estimation of the protective antibody level. If the efficacy equaled *E* at a given period, we can assume that the antibody value corresponding to the $(1 - E)$ th percentile in the vaccine group, adjusted for those in the control group, is an estimation of a non-protective cut-off level. However since the

pharmacokinetics of antibodies are complicated and the most rapid rise and decline both occurred during the first year post-immunization, the estimates based on this method will be influenced by the period of when the data were taken.

The second method is similar to the one used in Klugman et al. [16]. Since the vaccine efficacy E is inversely proportional to the Relative Risk of typhoid incidence in the vaccine and control groups, if we assume that Vi antibodies confer protection, then the incidence of antibodies above a particular antibody level X µg/ml can be indicative of the minimum protective level. The antibody incidence relative ratio RR is defined as:

$$RR(X) = \left[\frac{\text{incidence in vaccine group}}{\text{incidence in control group}} \right] \quad \text{having antiVi IgG} \geq X \text{ } \mu\text{g/ml.}$$

RR is calculated based on the serological data in the efficacy trial during 5 sampling periods from 6 to 42 months, results are plotted against selected X values (0.05–10.0 µg/ml). The reason we chose 42 instead of 46 months was because at 46 months the antibody levels in the control group had an elevated immune response due to the cross-over injection.

The minimum protective level was determined by aligning the RR with the corresponding efficacy Relative Risk. In the relative ratio method the antibody level at which the slope of RR curve begins to change is identified and the X value at the inflection point is then assigned as the cut off level for protection.

2.3. Application of the estimated protective level to Vi-rEPA dosage study

We re-examined the antibody distribution collected at Vi-rEPA dosage study (NIH IRB number OH00-CH-N003; FDA BB IND number 6990) [21]. Children 2–5 years old, 76–80 per group, were injected twice at 6 weeks apart with the investigational vaccine Vi-rEPA at various dosages of Vi per injection: full (25 µg), half (12.5 µg) or 1/5th (5 µg). The anti-Vi IgG levels were measured at 10 and 52 weeks, the GM anti-Vi IgG and the proportion of children in each group with higher than the protective level proposed in the original article (4.3 µg/ml) were compared to the one based on the current estimate [21].

3. Results

3.1. The efficacy of protection

As reported there was no statistically significant difference in the protective efficacy of Vi-rEPA among the three observation periods during the 46 months of surveillance. (Table 1) [6,7]. The efficacy was the highest at 94% (CI 84–99) during the first year and declined to 87% (CI 79–97) in the intermediate period and 82% (CI 62–99) in the last 19 months. The GM anti-Vi IgG of the vaccine group can serve as the preliminary estimates of the protective level without further analysis and the values ranged from 23.15 µg/ml at 12 month to 4.80 µg/ml at 46 months. At 46 months, the GM of the younger vaccinees (2–3 years old children at the time of immunization) was 4.3 µg/ml (3.5 ELISA units), and was the final proposed protective level from the efficacy study [7].

3.2. Estimation of protective level based on antibody percentile distribution

The anti-Vi IgG response at different periods during the Vi-rEPA efficacy trial was expanded from 10th to 50th percentiles (Table 2). During the first 12 months, the efficacy was 93.9% and the antibody level corresponding to the lowest 6.1st percentile among the vaccinated children was 5.41 µg/ml. After adjustment for the background (GM in the control group), the estimated protective level was 5.05 µg/ml at 12 months. Similarly, the protective levels estimated at 30 months (13.1st percentile) and at 46 months (17.6th percentile) were 2.27 µg/ml and 1.18 µg/ml respectively. The average of all 3 estimates was 2.83 µg/ml. It is common that the most rapid rise and decline of anti-Vi IgG levels occurred during the first year. The kinetics of the latter periods is closer to the steady state and the averaged protective level of the 30 and 46 months was 1.73 µg/ml. The last serological landmark is at the end of the 46 months surveillance, where the efficacy was 89% and the 11th percentile anti-Vi IgG equaled 1.40 µg/ml.

3.3. Estimation of protective level based on the antibody incidence relative ratio between vaccine and placebo groups

To apply the second method, the serological correlate of the antibody-incidence relative ratios (RR) is plotted against selected anti-Vi IgG cut off values (*X* axis) (Fig. 1). At 6 and 12 months the RR values were the highest and the corresponding curves showed steep decline. In contrast curves of the later periods showed a more flat shape.

The efficacy Relative Risk was used as the selection criterion for the target RR value (Table 1). During the Vi-rEPA efficacy trial there were very few cases of typhoid fever in the vaccine group and the calculated Relative Risk range from 16.5 in the first period to 5.7 in the last period; these values are too high in comparison with the observed RR (Fig. 1).

We used the alternative method by locating the inflection point from each curve and identify the corresponding antibody concentration on the *X*-axis. It is noticed that, independent of the time of sampling, the slope of the curves showed sharp decline at approximately 1.4–2.0 µg/ml.

Together, these data suggest that levels of anti-Vi IgG in excess of 1.4 µg are likely protective if measured 4 years post immunization, and a lower 90% confidence interval of 10 µg/ml measured at 6 months.

3.4. Application of the estimated protective level to Vi-rEPA dosage study

The anti-Vi IgG levels elicited in children enrolled in the dosage study were measured at 10 and 52 weeks [21]. The proportion of children elicited higher than the estimated protective level was compared among different dosage groups. Based on the current estimates, a more conservative cutoff value 2.0 µg/ml was chosen to compare with the original proposed 4.3 µg/ml (Table 3). The GM showed a significantly lower antibody response in the group receiving the lowest dosage when compared with those who received higher dosages (16.45 and 14.02 vs 7.97, $P < 0.0001$). Similar inferiority was found when using the GM based protective level (4.3 µg/ml); nearly a quarter of the children in the lowest dosage group (23%) did not reach this level. However when we lowered the cut off thresholds from 4.3 to

2.0 µg/ml, nearly all children achieved this newly proposed protective level at 10 or 52 weeks and there was no difference among the 3 dosages.

4. Discussion

The current estimated anti-Vi IgG protective level, derived from either methods, lies at 1.4 µg/ml at 46 months after immunization, which is lower than the original proposed value based on the GM level at the end of the efficacy study [7]. This level is closer to the 0.6–1.2 µg/ml total Vi antibody, measured at 3 years post immunization, from Vi polysaccharide vaccine study in South Africa despite the difference in the methods of titration [16]. This level is higher than the protective IgG levels found in other polysaccharide vaccines. For Hib the correlate of long term protection has been set at 1 µg/ml (compared to 10 µg/ml in this study) and short term protection at 0.15 µg/ml (compared to 1.4 µg/ml in this study) [22]. For pneumococcal conjugate vaccine the correlate of protection has been estimated at 0.35 µg/ml [23]. The protective thresholds of absolute IgG concentration determined for meningococcal serogroup A is 2.0 µg/ml and this value is adopted for other meningococcal serogroups [24]. Our data suggest that protection from typhoid fever may require higher levels of circulating IgG than are needed for protection of infants from Hib, pneumococcal or meningococcal disease, but as responses to conjugates are age dependent this difference may be more of a function of age than an actual biological difference.

Based on the proposed anti-Vi protective threshold we have re-examined the serological response in young children receiving different dosages of Vi-rEPA. Here we found that young children received either full or a fractional dose of Vi-rEPA all retained protective level of anti-Vi IgG 1 year after vaccination. This finding may be of importance for future Vi conjugate formulation. The optimal dosage in the marketed vaccines is usually derived from fine balances of many factors including protective efficacy, number of doses, age related immunogenicity, cost and possible overloading of immunogens [2,8,10].

The correlation between the protective efficacy and immunogenicity established from a randomized, placebo controlled, double-blind trial can provide invaluable resources for potency estimation of vaccines [6,7]. The performance of future Vi conjugate vaccine can be judged by serologic parameters as surrogates for efficacy data and in many instances shorten the licensing pathway [8-10]. As observed here the pharmacokinetics of antibody response is complicated, the initial fast elevation also followed by a rapid declination during the first couple of months. In order to make valid comparisons at a reasonable duration; we have chosen a 6 month post immunization comparison, with a protective cut off of 10 µg/ml, for future clinical trials. Also noted here, long-term antibody persistent data provide valuable information on the duration of the vaccine protection.

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Fig. 1.

Incidence relative ratio (RR) of anti-Vi IgG from Vi-rEPA conjugate efficacy trial in 2–5 years old children. The relative antibody ratio (RR) of anti-Vi IgG in children receiving Vi-rEPA or saline at different time periods after the first injection: ◆ 6 months, ■ 12 months, × 24 months, ▲ 30 months, * 42 months. The X-axis indicates the cutoff anti-Vi IgG in µg/ml. The Y-axis indicates the relative ratio of incidence between the vaccine group and the control group having the anti-Vi IgG level higher than the cutoff point X µg/ml.

Table 1

Efficacy of Vi-rEPA conjugate in 2–5 years old children.^a

Duration (months) ^a	Number of typhoid cases ^b		Efficacy (95% CI)	1/(Relative Risk)	Anti-Vi IgG ^c at end of the period GM, µg/ml (25–75)%	
	Vi-rEPA (N = 5525)	Saline (N = 5566)			Vaccine group	Control group
1–12	2	33	93.9 (84–99)	16.5	23.15 (12.8–40.4)	0.38 (0.21–0.64)
13–27	3	23	86.9 (79–97)	7.7	6.89 ^d (4.00–12.2)	0.71 ^d (0.19–3.10)
28–46	3	17	82.4 (62–99)	5.7	4.80 (2.53–10.5)	1.00 (0.37–2.8)
1–46	8	73	89.0 (76–97)	9.1	–	–

^aChildren 2–5 years old were injected twice at 6 weeks apart with the investigational vaccine Vi-rEPA. Active surveillance performed from 1 to 27 month and passive surveillance from 28 to 46 months [7,8].

^bCase defined by blood culture positive collected from patients during the period. *N* represents number of volunteers at the beginning of the efficacy study.

^cAnti-Vi IgG measured at the end of the observation period, number of samples collected; *n* ~ 42–64.

^dGM at 30th months.

Table 2

Time course and percentiles of anti-Vi IgG response of children 2–5 years old in Vi-rEPA efficacy trial.

Time (n)	Anti-Vi IgG levels post immunization (µg/ml)									
	0 mo (43)	1 mo (43)	6 mo (30)	12 mo (30)	24 mo (30)	30 mo (31)	36 mo (73)	42 mo (42)	46 mo (53)	46 mo (52)
GM	0.12	87.42	27.75	23.15	13.33	6.89	5.07	4.54		4.80
<i>Centiles</i>										
5%	0.05	24.84	8.36	5.31	1.91	1.07	0.37	1.02		0.41
10%	0.06	36.60	10.75	5.96	4.92	2.29	0.66	1.31		1.39
15%	0.07	42.00	11.95	7.27	7.02	2.91	1.92	2.00		1.62
50% (median)	0.13	81.79	34.1	23.62	10.85	7.45	6.78	4.12		5.62
Control (GM)	0.20	0.25	0.81	0.38	0.71	0.47	0.98	0.99		47.29 ^a
Efficacy				93.9		86.9%		82.4%		

^aControl group received cross-over one injection of Vi-rEPA at 42 months.

At 12 months, 6.1% 5.41 µg/ml; minus control group 0.38 µg/ml = 5.03 µg/ml.

At 30 months, 13.1% 2.74 µg/ml; minus control group at 0.47 µg/ml = 2.27 µg/ml.

At 46 months, 17.6% 2.17 µg/ml; minus control group at 42 months 0.99 µg/ml = 1.18 µg/ml.

Table 3

Anti-Vi IgG levels in children 2–5 years old immunized with various dosage^a of Vi-rEPA in compared to estimated protective levels.

Dosage ^a	Anti-Vi IgG (GM, µg/ml) ^b		
	0 weeks	10 weeks	52 weeks
25.0 µg	0.16	126.90	16.45
(n)	(78)	(77)	(77)
% (>2.0 µg/ml)	0	100	99
% (>4.3 µg/ml)	0	100	95
12.5 µg	0.18	92.58	14.02
(n)	(80)	(80)	(79)
% (>2.0 µg/ml)	0	100	100
% (>4.3 µg/ml)	0	100	95
5.0 µg	0.21	53.29	7.97
(n)	(76)	(76)	(75)
% (>2.0 µg/ml)	3	100	100
% (>4.3 µg/ml)	0	100	77

^a Children 2–5 years old, 76–80 per group, were injected twice at 6 weeks apart with the investigational vaccine Vi-rEPA at various dosages of Vi per injection: full (25 µg), half (12.5 µ) or 1/5th (5 µ).

^b The GM and the proportion of children in each group had higher than the original estimated protective level (4.3 µg/ml) or the current estimate (2.0 µg/ml) were compared. Statistics, 16.45 and 14.02 vs 7.97, $P < 0.001$.