Vi Polysaccharide Vaccine

Vi is the capsular polysaccharide of *S. typhi*, a group D *Salmonella*. The name Vi was given because the capsule is an essential virulence factor for the pathogenicity of *S. typhi*. Vi is composed of α (1→4) linked homopolymer of galacturonic acid with N- and O-acetylation at its O2 and O3 positions. The molecular weight of Vi is in the range of 500 kDa to 2000 kDa and is acid and heat stable. These characteristics enable Vi to be a useful vaccine. The protective mechanism of purified Vi is believed to be mediated by serum IgG that inactivates the inoculum upon contact on the epithelial surface. Serum antibodies likely also act upon *S. typhi* that gain access to the bloodstream.

Four efficacy trials in endemic regions: Nepal, South Africa, and China (2) demonstrated that Vi vaccine is safe and conferred ~65% to 70% efficacy against *S. typhi*. Vi vaccine is now licensed in more than 95 countries (20–22). Vi is not protected by patent rights, facilitating technology transfer to developing country producers. The technology for large-scale production of Vi is published in World Health Organization Technical Report Standardization and has been transferred to several countries where typhoid fever is endemic, including India, Vietnam, China, and Indonesia (27). Vi polysaccharide produced in these countries showed comparable safety and immunogenicity as Vi vaccine manufactured by pharmaceutical companies in industrialized countries (22,28). These locally produced Vi vaccines are available at lower prices than imported Vi vaccine in these endemic countries. Furthermore, due to its heat and acid stable nature, dried Vi polysaccharide bulk can be stored at −20°C for as long as 10 years and that makes stockpile of the vaccine feasible. Because of its simple chemical composition, the potency of Vi vaccine is regulated by molecular identification, which makes monitoring by regulatory authorities easy and unambiguous (27). For all these reasons, Vi vaccine is considered to be a suitable typhoid vaccine for public health program in developing countries.

Nevertheless, Vi vaccine also has limitations: reinjection is recommended every three years; it does not induce booster response upon reinjection; and finally, children under five years respond with low levels of Vi antibodies of shorter duration (29,30). Similar to other polysaccharide conjugate vaccines, these deficiencies were overcome by conjugating Vi to carrier proteins (10).

Vi-rEPA Conjugate Vaccine

Vi was conjugated to recombinant exoprotein A (rEPA) from *Pseudomonas aeruginosa*. A series of clinical studies of this conjugate vaccine confirmed its safety and improved immunogenic properties. The conjugate was shown to be safe and immunogenic in U.S. adults before clinical trials were undertaken in highly endemic areas in the Mekong Delta, Vietnam, where the annual attack rate for children under five is 0.5% (19,32). Clinical trials in adults, school-age children, and preschool (age 2–4 years) children demonstrated its safety and immunogenicity in the Vietnamese population (33). In school-age children, the Vi conjugate vaccine elicited significantly higher antibody levels than unconjugated Vi vaccine. A phase III double-blind, placebo-controlled, randomized trial in Vietnam in 11,091 two to five-year old children showed that the conjugate vaccine provided 89% protection over nearly four years of follow-up. The estimated protective level of serum anti-Vi IgG is 3.52 EU (approximately equivalent to 0.11 mg/mL anti-Vi IgG) (10).

The immunogenicity of Vi-rEPA is dosage-dependent; higher dosages elicited higher levels of Vi antibodies (34). Long-term follow-up of immunogenicity showed that adults injected 10 years earlier retained significantly higher levels of anti-Vi IgG than their prevaccination baseline.

Vi-rEPA Conjugate Preparation

Vi polysaccharide from *S. typhi* (provided by Sanofi Pasteur, Lyon, France) was covalently linked with purified carrier protein (rEPA) at the National Institutes of Health and designated as Vi-rEPA. Each injection (0.5 mL) contained 25 μg of Vi polysaccharide. Five clinical lots were prepared over the course of our clinical studies, and all elicited similar levels of immune response, indicating consistency in manufacture of Vi conjugates (10,33,34).

Phase I and II Clinical Trials in a High Endemic Area

Phase I and phase II studies showed that Vi-rEPA was safe and immunogenic in all groups of subjects ≥2 years of age (Table 1) (33). One injection of the Vi-rEPA in adults elicited a mean of 48-fold rise in serum anti-Vi IgG level six weeks later and remained at a GMT 10-fold above baseline at 26 weeks after immunization (119 EU vs. 9.62 EU; $p < 0.0001$) (33). The persistence of serum Vi antibody was evaluated again at 3 and 10 years after the injection; the antibody level declined slightly from the value recorded at 26 weeks (92.6 and 68.0 EU vs. 119 EU, $p > 0.1$).

A comparison of the immunogenicity of Vi and Vi-rEPA was conducted in school-age children, 50 in each group. Twenty-six weeks after one injection, the serum anti-Vi IgG level in children who received Vi was 13.4 EU versus 30.6 EU in children who got Vi-rEPA ($p < 0.001$).

To evaluate Vi-rEPA in younger children, 203 children two to four years of age were injected once or twice (6 weeks apart). There were no significant adverse reactions attributable to the vaccine. Six weeks after the first injection, 202/203

Table 1 Serum Anti-Vi IgG in Vietnamese Adults, School-Age Children, and Two- to Four-Year-Old Children Injected with Vi-rEPA Conjugate

			Anti-Vi IgG (ELISA units)					
Age (yr)	*N*	No. of inj.	Pre	6 wk[a]	10 wk	0.5 yr	3 yr[b]	10 yr
18–35	22	1 × conj	9.62	465	NA	119	92.6	68.0
5–14	55	1 × conj	0.67	169	NA	30.0	14.80	NA
2–4	48	1 × conj	0.19	77.2	54.3	20.4	4.83	
	52	2 × conj	0.18	69.9	95.4	30.6	4.56	
	50	1 × Vi	0.44	18.9	NA	13.4	NA	NA

[a]Blood samples taken and booster shot given.
[b]Anti-Vi IgG at third year versus Pre, $p < 0.001$.