Immunogenicity of *Salmonella typhi* Ty21a Vaccine for Young Children

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An attenuated *Salmonella typhi* Ty21a vaccine was administered to 18 infants and toddlers (≤24 months old) to determine its safety and immunogenicity. The vaccination (10^8 CFU per dose, three doses) was well tolerated. However, after the vaccination there was no evidence of a humoral or cellular immune response to *S. typhi*. The vaccine used was known to be immunogenic for older children and adults. The results support the view that the immunogenicity of Ty21a is age dependent.

Controlled clinical (6) and field (3, 11, 21) trials with school-age children and adults have established the *Salmonella typhi* Ty21a vaccine as effective in preventing typhoid fever. The vaccine is made of attenuated bacteria that are delivered orally. Ty21a engenders levels of protection from typhoid fever that are similar to those of parenteral vaccines (3, 11); however, Ty21a does not cause significant adverse reactions (6, 11). Such reactions are common and sometimes severe after the administration of parenteral typhoid vaccines (1, 8, 22).

It is established that DNA coding for protective antigens of various pathogenic microorganisms can be inserted into and successfully expressed by Ty21a and that the expressed gene products are immunogenic for humans (2, 5, 7, 14, 17–20). These results suggest that because Ty21a could serve both as a typhoid vaccine and as a vehicle for delivery of immunizations against unrelated pathogens. Because the majority of immunizations are administered to infants and toddlers, the advantage of attenuated *S. typhi* as a generic vaccination vehicle is in part a function of its capacity to successfully immunize very young children.

To determine whether Ty21a is safe and immunogenic for this age group, we administered the vaccine to infants and toddlers 6 to 24 months of age. Thirty-seven children (mean age ± standard deviation, 1.12 ± 0.48 years; 14 females) were enrolled from the population served by the Diagnostic Center of the Catholic University, Santiago, Chile. Eight adults (mean age ± standard deviation, 33 ± 9.0 years; four females) were enrolled as controls for the immunoassays. One adult had had two previous episodes of typhoid fever. The remaining seven adult controls denied previous typhoid fever or vaccination against typhoid fever. The vaccine utilized in this study was obtained from a lot used in field trials with school-aged children and adults (3, 11). The immunization regimen comprised three doses of either vaccine or placebo that were administered over an 8-day interval. For each dose of vaccine, a gelatin capsule containing bacteria was opened and emptied into 90 ml of cow’s milk to which 0.5 g of bicarbonate had been added. The placebo was milk with bicarbonate only. Cultures showed that Ty21a prepared in this way remained viable and that each dose contained 10^8 CFU.

The children were examined clinically before and 24 and 48 h after each dose of vaccine and on day 21 after the first dose. The evaluation included a physical exam, questioning of the mother, and obtaining a stool sample (from a diaper or rectal swab). The examinations were conducted by physicians at the clinic on days when vaccinations were administered or by nurses at the child’s home. Venous blood was collected immediately before and 21 days after the first dose of vaccine and placed into sterile tubes containing heparin for later use in immunoassays. The procedures for measuring anti-*S. typhi* antibodies (9, 12, 13, 15, 18), measuring *S. typhi*-specific lymphocyte response (13, 15, 16), and data processing and interpretation (13, 16) were described previously.

In some instances the levels of measured antibodies or lymphocyte replication were used to classify individuals as positive or lacking in an *S. typhi*-specific immune response. Classification as positive for the *S. typhi*-specific antibody response was determined as follows. When paired samples were available, positive was defined as a fourfold or greater rise in titer (11); when one sample was available, positive was defined as a titer of >20 or a net optical density (11) value of >0.07, >0.09, or 0.04 for immunoglobulin M (IgM), IgG, or IgA, respectively (see reference 15 for the derivation of these screening values). When paired samples were available for measures of lymphocyte replication response, positive was defined as a mean response to *S. typhi* Ty2 stimulation of ≥3,433 cpm in pretreatment samples and greater than this value for postvaccination samples. When

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single samples were available, positive was defined as >3,433 cpm. It has been established (16) that this screening value in this assay has a resolving power in the detection of prior exposure of adults to S. typhi antigen of 82% sensitivity and specificity.

The vaccine was well tolerated; 80% of the participants accepted ≥70 ml of formula at least one of the immunizations, and similar acceptance was found for the vaccine and placebo formulations ($P > 0.48$, Fisher exact test).

Children from both vaccinated and placebo groups presented with a variety of notable signs (Table 1). Of these, only one, a skin lesion, associated significantly with vaccination. The skin lesions appeared between 12 and 25 h after the first and/or second dose but not after the third dose of vaccine. These lesions were papules with erythema (2 to 4 mm in diameter) and were few in number (generally <10 per vaccinee); they were found on the trunk, face, and arms and resolved spontaneously by day 6 or 7 after the first vaccine dose. One vaccinee, 14 months old, was found to be febrile 18 h after the first vaccination dose. The fever persisted through 72 h. Samples of stool and blood were obtained at 24 and 48 h, and antibiotic therapy was begun at 48 h. The blood and stool cultures did not yield S. typhi. This child was not included in the rest of the protocol. The stool cultures from all volunteers at all sampled intervals were negative for S. typhi.

The criteria for inclusion in immunologic screening were completion of the immunization regimen where ≥70 ml of suspension was consumed for each dose and availability of prevaccination and 21-day postvaccination blood samples of adequate volume. Adjusting for the inclusion criteria and the individual dropped because of antibiotic treatment (see above) resulted in groups of 13 vaccine recipients and 16 placebo recipients.

Various tests for antibodies or lymphocytes specifically reactive with S. typhi were made (Table 2). No evidence was found of any vaccine-induced immune response to this organism.

Ingestion of Ty2la diluted in milk is established as a safe and immunogenic vaccination regimen for school-age children and adults (6, 21), and Chilean school-age children (6 to 9 years old) show 42% seroconversion after ingestion of Ty2la diluted in bicarbonate-containing milk (4). Thus, the finding that the vaccine was well tolerated was expected. However, it was surprising that this formulation failed to cause an anti-S. typhi immune response in infants and toddlers.

Because of the result presented above, two subsequent studies were made in which Ty2la was delivered to children 2 to 3.5 years of age (10). In the first study (with 24 vaccine recipients and 25 placebo recipients), a humoral immune response to S. typhi was found for 83% (33%) vaccine recipients (5 responders to O antigen [IgG], 3 responders to H antigen). In the second study (with 17 vaccine recipients and 24 placebo recipients), no humoral immune response followed vaccination. In these studies, diarrhea, fever, vomiting, or abdominal pain was observed on average in 4% of the recipients of the vaccine or placebo and no skin reactions were observed.

The composite result demonstrates that the Ty2la vaccine obtained from lots used successfully to immunize school-age children and adults (3, 4, 11) was markedly less immunogenic for children <5 years old. Because cultures established that sufficient viable bacteria were present in the milk and controls showed immunoassays to be functioning appropri-
ately, we conclude that the standard dose of Ty21a vaccine (which was determined from studies of school-age children) does not represent an immunogenic mass for the majority of very young children.

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