and the spacing between the doses markedly influence the level of protection that can be achieved (1–5,31,33,34). In the first field trial of Ty21a in Alexandria, Egypt, six- to seven-year-old schoolchildren received three doses of vaccine (suspended in a diluent) on Monday, Wednesday, and Friday of one week (31); to neutralize gastric acid, the children chewed a 1.0-g tablet of $NaHCO_3$ several minutes before ingesting the vaccine or placebo. During three years of follow-up, 96% protective efficacy against bacteriologically confirmed typhoid fever was observed (31).

A practical formulation that has been a commercial product since the mid-1980s consists of lyophilized vaccine in enteric-coated, acid-resistant, capsules (1,2). In a randomized, placebo-controlled field trial in Santiago, Chile, three doses of this enteric-coated formulation given within one week provided 67% efficacy during the first three years of follow-up (2) and 62% protection over seven years of follow-up (35). Four doses of Ty21a in enteric-coated capsules given within eight days are significantly more protective than two or three doses (33). When the enteric-coated capsule formulation of Ty21a was licensed in the United States by the Food and Drug Administration in late 1989, it was with a recommended schedule of four doses given at an every other day interval; other countries use a three-dose immunization schedule.

In the mid-1980s, the Swiss Serum and Vaccine Institute (currently Berna Biotech, a Crucell Company) succeeded in preparing for large-scale field trials of a ''liquid suspension'' formulation of Ty21a that was amenable to large-scale manufacture. The new formulation consisted of two packets, one containing a dose of lyophilized vaccine organisms and the other containing buffer. To prepare a ''vaccine cocktail,'' contents of the two packets were mixed in a cup containing 100 ml of water and the resultant suspension was then ingested by the subject to be vaccinated. Another randomized, controlled field trial undertaken in Santiago, Chile (4) and a parallel trial carried out in Plaju, Indonesia (5) directly compared this new liquid formulation (that somewhat resembles what was used in the Alexandria, Egypt field trial) with the enteric-coated capsule formulation. In both the trials in South America and Asia, Ty21a administered as a liquid suspension was superior to vaccine in enteric-coated capsules; in the Santiago trial the difference was statistically significant (4). Moreover, Ty21a given as a liquid suspension protected young children as well as older children (4,5). In previous trials with enteric-coated vaccine, young children were not as well protected as older children (2). The liquid formulation was more practical for giving Ty21a to children <7 years of age and was strongly immunogenic in toddlers and preschool children (36,37). In contrast, an attempt to prepare a ''simple'' liquid suspension of Ty21a by emptying the contents of an enteric-coated capsule into milk containing 0.5 g of $NaHCO_3$ resulted in an ineffective mixture that was poorly immunogenic (38). Despite its pioneering role and many positive attributes (including excellent safety record, clinical acceptability, minimal reactogenicity, and stimulation of a moderate level of long-lived protection), recognized drawbacks of Ty21a include the lack of a molecular basis for its attenuation, relatively modest immunogenicity, and, most importantly, the need to administer at least three spaced doses to confer a moderate level of protection of extended duration (2,3,35). Accordingly, a new generation of live oral typhoid vaccines aims to be as well tolerated clinically as Ty21a, yet more immunogenic and protective, and to require administration of just a single oral dose.

Correlates of Protection of Ty21a

Although Ty21a is only modestly immunogenic and requires three or four spaced doses to elicit protection, the efficacy is surprisingly long lasting, enduring for at least five to seven years (35). Two immunologic assays were found to correlate with the protection conferred by different formulations and immunization schedules of Ty21a in field trials. These include serum IgG O antibody seroconversions (34) and enumeration of gut-derived IgA O antibody-secreting cells (ASCs) detected among peripheral blood mononuclear cells (39). The identification of these measurements as immunologic correlates of protection provided invaluable tools for use in clinical trials of new attenuated *S.* Typhi strains as possible live oral vaccines. More recently, it has been found that oral Ty21a is a potent stimulator of $CD8^+$ cytotoxic T cells (CTLs) that recognize cellular targets infected with *S.* Typhi, as well as interferon-γ-producing T cells (40,41). It is surmised that these T-cell responses also contribute to and correlate with protection, but this cannot be proven, since these assays were not available at the time of the field trials of Ty21a.

A NEW GENERATION OF ATTENUATED *SALMONELLA* TYPHI LIVE ORAL VACCINES

Investigators in various laboratories worldwide have undertaken to engineer new candidate vaccine strains that are as well tolerated as Ty21a yet more immunogenic, such that a single oral dose will elicit long-lived protective immunity. One early attempt was made to increase the immunogenicity of Ty21a by restoring its ability to express Vi antigen by introducing *viaB* (which encodes the enzymes required for synthesis of Vi polysaccharide) from wild-type strain Ty2 into the chromosome of Ty21a and demonstrating expression of Vi (42,43). Whereas the Vi-positive variant was well tolerated when fed to adult volunteers and most subjects who received three doses developed rises in serum IgG antibodies and IgA ASCs against *S.* Typhi O antigen, no subject manifested a rise in serum IgG anti-Vi or exhibited ASCs that secrete IgA anti-Vi (43).

Importance of the Wild-Type Parent Strain

Of the various attenuated vaccine strains that have been evaluated in clinical trials during the past two decades, each was derived from one of three wild-type parent strains, including Ty2, ISP1820, and CDC 1080. The choice of wild-type parent selected for attenuation influences the characteristics and nature of the ultimate live vaccine strain produced. Evidence for this derives from the different clinical acceptability of the resultant vaccine strains when identical mutations were introduced into distinct wild-type backgrounds (e.g., *aroC* and *aroD* into Ty2 vs. ISP1820).

Attenuating Strategies

Candidate vaccine strains have been prepared by inactivating genes encoding various biochemical pathways (28,44–47), global regulatory systems (48), heat shock proteins (49), other regulatory genes (50–52), and putative virulence properties (53–55). Elucidation of the sequences of the complete genomes of wild-type *S.* Typhi strain Ty2 (56) and CT 18 (57) has facilitated the development of attenuated *S.* Typhi strains.

The relative attenuating potential of various mutations has often been assessed preliminarily by feeding *S.* Typhimurium strains harboring these mutations to mice and observing the