levels of 10^7 to 10^9 CFU (64). No vaccinemias were detected in recipients of this further derivative, but some who ingested the highest dosage level developed fever, chills, and headache, leading to abandonment of further clinical trials.

X3927, X4073, and X8110

Curtiss et al. demonstrated that *cya* (encoding adenylate cyclase) and *crp* (cyclic AMP receptor protein) constitute a global regulatory system in *Salmonella* that affects many genes and operons. They showed that a *S.* Typhimurium strain that harbors deletions in *cya* and *crp* (48) was attenuated compared with its wild-type parent and oral immunization–protected mice against challenge with virulent *S.* Typhimurium. Curtiss and team thereupon constructed vaccine candidate strain X3927 (Table 1), a *cya,crp* double mutant of *S.* Typhi strain Ty2, for use as a live oral typhoid vaccine and as a live vector (65,86). In phase I clinical trials, Tacket et al. (60) demonstrated that X3927 was attenuated compared with wild-type but insufficiently so to serve as a live oral vaccine in humans, since occasional subjects developed high fever and typhoid-like symptoms. Several subjects also manifested vaccinemias.

In order to achieve a greater degree of attenuation, Kelly et al. (87) introduced into the *cya, crp* mutant a third deletion mutation in *cdt*, a gene that affects the dissemination of *Salmonella* from gut-associated lymphoid tissue to deeper organs of the reticuloendothelial system such as the liver, spleen, and bone marrow. The resultant *cya,crp,cdt* triple mutant strain, X4073 (Table 1), was fed to healthy adult North Americans, with buffer, in single doses containing 5×10^5, 5×10^6, 5×10^7, or 5×10^8 CFU. The strain was well tolerated except for one individual in the 5×10^6 CFU group who developed diarrhea (88). No subjects manifested vaccinemia. Four of five subjects who ingested 5×10^8 CFU exhibited significant rises in serum IgG O antibody and had ASCs that made IgA O antibody (88).

The Curtiss group next introduced the *cya,crp* and *cdt* mutations into the modern Chilean wild-type strain ISP 1820, resulting in vaccine strain X8110 (Table 1), which was fed to volunteers at dosage levels of 10^5, 10^6, 10^7, and 10^8 CFU (89). No subjects developed fever, one of four who ingested the highest dosage level developed diarrhea, and two of eight who received 10^{7-8}-CFU manifested vaccinemia (89).

Ty445 and Ty800

Hohmann et al. constructed two candidate *S.* Typhi strains harboring deletions in *phoP/phoQ* (51,63). Strain Ty445 (Table 1), which also harbors a deletion in *aroA*, was found to be overly attenuated and only minimally immunogenic (63). In contrast, strain Ty800 (Table 1), a derivative of Ty2 deleted only in *phoP/phoQ*, was generally well tolerated and immunogenic when fed in dosage levels from 10^7 to 10^{10} CFU in a small phase I clinical trial involving 11 subjects (51) (Table 2). At the highest dosage level, 1 of 3 vaccinees developed diarrhea (10 loose stools).

A double-blind, placebo-controlled phase II clinical trial of Ty800 at two dosage levels has been carried out in 183 healthy outpatient volunteers who received a single oral dose of 10^8 CFU ($N = 60$), 10^9 CFU, or placebo ($N = 63$). The vaccine was well tolerated compared with placebo, and a $\geq$4-fold increase in serum anti-LPS titers over predose level was observed in 36 of 55 recipients of 10^8 CFU (65.5%), and 44 of 55 who ingested 10^9 CFU ($p < 0.001$ versus placebo recipients) (ClinicalTrials.gov Identifier NCT00269295).

M01ZH09

Salmonella pathogenicity island 2 (SPI 2) encodes a type III secretion system that is necessary for *S.* Typhimurium to manifest full pathogenicity in the mouse model (90,91). *S.* Typhimurium strains harboring deletions in SPI2 do not manifest a full-blown systemic infection and show a diminished ability to replicate in macrophages. SPI2, which is activated under intracellular conditions, translocates effector proteins from the vacuole containing the *Salmonella* across the vacuolar membrane to the cytosol of the host cell (e.g., macrophages). *ssaV* forms part of the SSP2 secreton, the needle-like bacterial structure that transports proteins across the inner and outer bacterial membranes. *S. enterica* derivatives harboring mutations in *ssaV* are crippled in their ability to secrete SPI2 effector proteins. Deletion mutations in *aroC* and *ssaV* were introduced in wild-type strain *S.* Typhi strain Ty2 to derive vaccine candidate M01ZH09 (Table 1) (54).

In a small phase I clinical trial that included nine adult subjects, M01ZH09 was well tolerated and elicited anti-Typhi immune responses in the majority of vaccinees (Table 2). The vaccine strain was recovered from stool cultures of three subjects. Several phase II dose-ranging clinical trials were subsequently carried out in the United States and in Vietnam to assess the clinical acceptability and immunogenicity of M01ZH09 and to evaluate formulations (Table 2) (82,83). Three groups of 16 healthy adults each ingested a single dose containing 5×10^7 CFU, 5×10^8 CFU, or 5×10^9 CFU in $NaHCO_3$ buffer, while 12 subjects received placebo in a randomized, double-blind trial. The vaccine was well tolerated at all dosage levels, with only a few mild febrile responses. Bacteremia was not detected in any vaccine, and fecal shedding was abbreviated (82). All recipients of the 5×10^9 CFU dosage exhibited IgA anti-LPS ASCs, and 50% manifested rises in serum IgG anti-LPS antibody. In another phase II trial in U.S. adults, 32 subjects were randomly allocated to receive as single 5×10^9 CFU dose of M01ZH09 with or without $NaHCO_3$ buffer (that also contained ascorbic acid and aspartame) to neutralize gastric acid (83). The vaccine was well tolerated, although two subjects who received vaccine with buffer experienced diarrhea. Fecal shedding was abbreviated (seven days or less). Surprisingly, there was no difference in the rate or magnitude of immune responses between these groups (Table 2); 14 of 16 recipients of vaccine with buffer (88%) and 14 of 15 individuals who ingested vaccine without buffer (93%) exhibited rises in ASCs making IgA anti-LPS antibodies. Similarly, $\geq$4-fold rises in serum IgG anti-LPS antibodies were observed in 13 of 16 (81%) of vaccinees who got vaccine with buffer and in 11 of 15 (73%) who received vaccine without buffer.

The clinical trials in U.S. adults were followed by a phase II trial in 27 adults in Vietnam, which showed the vaccine to be as well tolerated and comparably immunogenic in that population as in U.S. adults. This was followed by a phase II study in Vietnamese children 5 to 14 years of age who received a single dose of vaccine ($N = 101$) or placebo ($N = 50$). The vaccine was again reported to be well tolerated and immunogenic in the children.

Salmonella Paratyphi A Strains CVD 1902 and CVD 1903

Given the ability of attenuated strains of *S.* Typhi to function as live oral vaccines that prevent typhoid fever and in view of the partial protection in some field studies conferred by oral Ty21a