result in comparison with isogenic wild-type strains. However, as clinical information with various engineered strains has accrued, it has become evident that the behavior of attenuated *S.* Typhimurium strains in mice does not adequately predict the behavior of homologous *S.* Typhi mutants in humans (58). Several examples can be cited where specific mutations that successfully attenuated *S.* Typhimurium for mice failed to adequately attenuate *S.* Typhi for humans (28,59,60). These observations underscore the critical importance of evaluating candidate live oral *S.* Typhi vaccine strains in carefully designed and executed clinical trials.

541Ty and 543Ty

Stocker et al. pioneered the concept of making auxotrophic mutants of *Salmonella* by inactivating genes encoding enzymes in the aromatic amino acid biosynthesis pathway (44,45), a strategy subsequently adopted and modified in the design of multiple other attenuated *S.* Typhi vaccine candidates that have been evaluated in clinical trials (54,59,61–64) (Table 1). For this reason, even though Stocker's original constructs (541TY and 543Ty) did not progress beyond phase I and were only minimally immunogenic, they are described here in some depth. Mutations in various *aro* genes described by Stocker render invasive *Salmonella* serovars nutritionally dependent on substrates (2,3 dihydroxybenzoate and para-aminobenzoic acid) that are not available in sufficient quantity within mammalian tissues; as a consequence, the vaccine strains remain viable but are severely inhibited in their ability to proliferate in the intracellular environment. Edwards and Stocker (45) constructed prototype strains 541Ty and 543Ty (a Vi-negative variant of 541Ty) from CDC 1080, a wild-type strain obtained from the collection of the Centers for Disease Control and Prevention (CDC). It may be of some relevance that the pathogenicity of this strain had never been directly tested in volunteers. In contrast, several other groups of investigators started with wild-type strain Ty2, the parent of Ty21a (25), in their attempts to engineer new attenuated strains (51,54,61,65). The pathogenicity of Ty2 was unequivocally established in experimental challenge studies in volunteers performed several decades ago (66), and this strain was thereupon used as the challenge organism to assess the efficacy of various live oral (67–69) and nonliving oral (70) typhoid vaccines.

Strains 541Ty and 543Ty also harbored a deletion mutation in *purA*, which results in a specific requirement for adenine (or an assimilable compound such as adenosine). A third mutation in *hisG*, leading to a histidine requirement, does not affect virulence but provided an additional biochemical marker to clearly differentiate the vaccine strain from wild *S.* Typhi. Strains 541Ty and 543Ty were quite well tolerated in dosages up to 5×10^{10} colony-forming units (CFU) in phase I studies but were notably less immunogenic than Ty21a in stimulating humoral antibody responses (46). For example, only 11% of subjects developed serum IgG anti-O antibodies.

Attenuated *Salmonella* Typhi Strain CVD 908

The first vaccine strain that proved to be well tolerated and impressively immunogenic following administration of a single oral dose in phase I clinical trials in humans is strain CVD 908 (60,62,71), which harbors precise deletion mutations in *aroC* and *aroD* (61) in the Ty2 background (Table 1). At a well-tolerated dose of 5×10^7 CFU, 92% of CVD 908 recipients manifested IgG O antibody seroconversions and showed evidence of priming of the intestinal immune system (IgA ASCs) (62). Moreover, vaccinees exhibited lymphoproliferative responses, and their PBMCs were shown to secrete cytokines (in particular, interferon-γ) upon exposure to *S.* Typhi flagella (72). CVD 908 also stimulates cytotoxic lymphocytes that recognize targets (Epstein-Barr virus–immortalized prevaccination B lymphocytes) expressing *S.* Typhi antigen on their surface (73).

Table 1 Attenuating Mutations Present in Recombinant Strains of *Salmonella* Typhi That Have Been Evaluated in Clinical Trials as Candidate Live Oral Vaccines

Mutated gene	Vaccine strain	Wild-type parent	Clinical phenotype	Immunogenicity phenotype	References
galE, *via*	EX645	Ty2	Not attenuated	Immunogenic	28
aroA, *purA*	541Ty	CDC 1080	Overly attenuated	Poorly immunogenic	45,46
aroA, *purA*, Vi-negative	543Ty	CDC 1080	Overly attenuated	Poorly immunogenic	45,46
aroC, *aroD*	CVD 906	ISP 1820	Insufficiently attenuated	Immunogenic	59
aroC, *aroD*	CVD 908	Ty2	Attenuated (but silent bacteremias at high dosage levels)	Highly immunogenic	60–62
aroC, *aroD*, *htrA*	CVD 908-*htrA*	Ty2	Attenuated	Immunogenic	49,79,81
aroC, *aroD*, *htrA*; P_{tac}-*tviA*	CVD 909	Ty2	Attenuated	Immunogenic	84
aroA, *aroD*	PBCC211	CDC 1080	Insufficiently attenuated	Immunogenic	64
aroA, *aroD*, *htrA*	PBCC222	CDC 1080	Insufficiently attenuated at high dosage level	Poorly immunogenic at well-tolerated dosage levels	64
cya, *crp*	X3927	Ty2	Insufficiently attenuated	Immunogenic	48,60,65
cya, *crp*, *cdt*	X4073	Ty2	Attenuated	Immunogenic	65,87,88
cya, *crp*, *cdt*	X8110	ISP1820	Attenuated (but silent bacteremias at high dosage levels)	Weakly immunogenic	89
phoP/phoQ	Ty800	Ty2	Attenuated	Immunogenic	51
phoP/phoQ, *aroA*	Ty445	CDC 1080	Overly attenuated	Poorly immunogenic	63
aroC, *ssaV*	M01ZH09	Ty2	Attenuated	Immunogenic	54