

Vaccination against typhoid fever: present status

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Typhoid fever remains an underestimated important health problem in many developing countries, causing more than 600 000 deaths annually in the world. Because of the reactogenicity of the parenteral, killed whole-cell vaccine, research has been oriented towards vaccination orally using live organisms and purified antigen. Live vaccine Ty21a, given by the oral route, has been extensively tested in several studies in developing countries. Its liquid formulation was the most effective, providing more than 60% protection after 7 years of follow-up. A Vi polysaccharide vaccine has been elaborated and provided more than 65% protection; after 3 years of follow-up the Vi antibody level was still at a high level.

These two vaccines are therefore candidates for use in public health control programmes. Before such use, however, they need further evaluation for safety and protective efficacy when administered to the EPI-targeted age groups. The question of whether typhoid fever vaccines interfere with the response to simultaneously administered measles vaccine must also be studied.

New live vaccines, given by the oral route in one dose, have been constructed through genetic engineering. The first results are promising, but they must be improved before use in a large-scale study. These strains could be used as live vector to deliver foreign antigens to the intestinal mucosa.

Historical overview

Typhoid fever (TF) remains an important public health problem in many developing countries. It has been estimated that about 16 millions cases occur annually in the world, with more than 600 000 deaths. Human beings are the only reservoir and host for this enteric fever which is caused by *Salmonella typhi*, formerly called *Bacillus typhosus*, *Eberthella typhosa* and *Salmonella typhosa*. Paratyphoid fever is different from TF and is due to *S. paratyphi* A or *S. paratyphi* B.

In 1829 C. A. Louis (25) in Paris described typhoid, clearly separating it from other fevers, and related the clinical features to lesions in the intestines, mesenteric lymph nodes and spleen. Bretonneau in France and Smith in the USA recognized the spread of the disease by contagion and the immunity conferred by illness. In 1873 Budd (2) in England provided evidence that bowel discharges were the main, waterborne, mechanism of infection, and in 1880 Eberth (5) discovered the etiologic agent in tissues from a patient infected with TF.

In 1884 Gaffky (10) first cultivated and isolated *S. typhi* in pure culture from the spleens of infected patients. In 1896, Pfeiffer & Kolle (33) in Germany and Wright (49) in England prepared the first vaccine for human use with heat-killed organisms, and demonstrated that antibodies could passively protect guinea pigs against experimental infection. That same year Widal (46) reported that convalescent-phase serum mixed with *S. typhi* led to the sticking together of organisms in clumps and losing their motility. Thus was born the term "agglutinins" and the classic serological test for diagnosis of infection by *S. typhi*.

Epidemiology and clinical aspects

Epidemiology

Typhoid fever continues to be a global health problem. It is difficult to estimate its worldwide impact because the clinical picture resembles many other febrile infections, and because of the limited capacity for bacteriological diagnosis in most areas of the developing countries owing to lack of funds. However, it has been possible to estimate the prevalence of TF in the world (Table 1) (6, 16). For example, in Indonesia there were a mean of 900 000 cases per year and more than 20 000 deaths; 3–19-year-olds accounted for 91% of typhoid cases, with an attack rate of blood-culture positive TF of 1026 per 100 000 per year.

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Table 1. Estimated number of typhoid cases and deaths in the world

Countries in:	Population ($\times 10^6$)	Incidence/ $10^5/\text{yr}$	Total cases	No. of deaths
Africa	531	500	2 655 000	130 000
Asia	2 662	500	13 310 000	440 000
Latin America	397	150	595 500	10 000
Oceania	5	150	7 500	124
Developed world	1 131	2	22 620	74
Total	4 726		16 590 620	580 198

In the endemic areas of South America the age-specific incidence was:

- low in under-3-year-olds. However, an epidemiological study in Chile (7), based on the systematic collection of a single blood culture from all children younger than 24 months of age who were presented to health centres with fever, regardless of their other clinical symptoms, showed that 3.5% had an unrecognized bacteraemic infection due to *S. typhi* or *S. paratyphi* (in none was enteric fever suspected on clinical grounds);
- high, with a peak among schoolchildren aged 5 to 19 years;
- low in adults over 35 years of age.

Humans are the only natural hosts and reservoir. The infection is transmitted by ingestion of faecally contaminated food, vegetables or water, the highest incidence occurring where contaminated water supplies serve a large population. Epidemiological data suggest that waterborne transmission of *S. typhi* is usually a result of small inocula, whereas foodborne transmission is associated with large inocula and high attack rates.

The hypothesis that vegetables (irrigated with untreated waste waters) and fruit (freshened with contaminated river water) represent important vehicles of transmission in Chile explains the following epidemiological observations:

- the seasonal appearance of TF (in the summer when there is no rain and irrigation is used);
- the low reported incidence of TF in young children (since raw vegetables are not an important food for them);
- the high incidence of TF in high socioeconomic neighbourhoods (where salads are eaten in restaurants and at home);
- the low incidence of TF in areas (e.g., the lakes region) with all-year-round rain so that irrigation is not needed.

Levine et al. (20) studied the role of chronic TF carriers in Santiago, Chile, and found a crude rate of 694 carriers per 100 000 inhabitants. From the prevalence of cholelithiasis in Santiago and the presence of *S. typhi* in bile from patients undergoing cholecystectomy, they calculated that 29 594 women and 4 575 men were chronic carriers. These authors conducted family studies because they were interested in determining whether chronic carriers were present in the households where there were children with TF, and whether risk factors could be identified for persons with typhoid, as compared with persons in uninfected households. Their conclusion suggests that chronic carriers within the household do not play an important role in transmission of typhoid in Santiago.

In contrast, typhoid in developed countries is transmitted when chronic carriers contaminate food vehicles through absence of hygiene.

Clinical aspects

S. typhi and *S. paratyphi* A and B are invasive bacteria that reach the reticuloendothelial system where, usually after 10–14 days of incubation, they lead to systemic illness. TF is an acute infection leading sometimes to severe forms. Two complications, intestinal perforation and haemorrhage, occur in 0.5–1% of cases. In Indonesia, a severe form of TF has been described with cerebral dysfunction, delirium and shock. For a long time TF was often confused with other prolonged febrile syndromes, particularly typhus fever of rickettsial origin. The classic clinical description of TF usually begins with malaise, anorexia, myalgia, fever of 39–40°C, abdominal discomfort, headache, and hepatosplenomegaly. A bronchitic cough is common in the early stage of the illness. During the period of fever 25% of white patients show exanthem (rose spots) on the chest, abdomen and back. Constipation is typical in older children and adults, while diarrhoea may occur in younger children. The leukocyte count is often below 4500/mm³. Paratyphoid fever is similar to TF but usually a milder disease clinically. In most countries in which these diseases have been studied, the ratio of disease caused by *S. typhi* to that caused by *S. paratyphi* is about 10 to 1.

Patients who are positive for HIV are at significantly increased risk for infection with *S. typhi* and *S. paratyphi* (12).

Pathogenesis of the etiological agent

Usually human hosts ingest the causative organisms in contaminated water or food. The inoculum size and the type of vehicle in which the organisms are ingested greatly influence both the attack rate and the incubation period. Clinical illness appeared in 98% and 89% of volunteers who had ingested res-

pectively 10^9 and 10^8 pathogenic *S. typhi* in 45 ml of skimmed milk. Doses of 10^5 caused TF in 28% to 55% of volunteers, whereas none of 14 persons who ingested 10^3 organisms developed clinical illness.

After ingestion, the typhoid organisms pass through the pylorus to the small intestine, rapidly penetrating the mucosal epithelium to reach the lamina propria where an influx of macrophages ingest the bacilli but generally do not kill them. Some bacilli remain within the macrophages of the small intestine lymphoid tissue. Other typhoid bacilli are drained into the mesenteric lymph nodes where further multiplication and ingestion by macrophages take place. It is believed that the main route by which typhoid bacilli reach the blood stream is by lymph drainage from the mesenteric nodes, after which they enter the thoracic duct and then the general circulation. As a result of this primary bacteraemia, the pathogens reach an intracellular haven (24 hours after ingestion) through the organs of the reticuloendothelial system where they reside during the incubation period (usually 10–14 days, but varying with the size of the inoculum from 3 days to 3 months). Clinical illness is accompanied by a fairly sustained secondary bacteraemia.

S. typhi, the causative agent of TF, is in group D *Salmonella* according to the classification by Kauffman & White. Its antigenic formula, established on the basis of its somatic (O) and flagellar (H) antigens, is [O 9, 12, (Vi), d]. It is motile, with a peritrichous flagella (H - d antigen), which is also encountered in approximately 80 other bioserotypes of *Salmonella*. Strains, freshly isolated from patients, possess on their surface a polysaccharide capsule Vi antigen (related to virulence), which prevents O antibodies from binding to the O antigen. Boiling removes the Vi antigen (thermolabile), which is also present in *Citrobacter*, *S. dublin* and *S. paratyphi C*.

Immunology

The circulating, secretory and cell-mediated immune response is stronger overall after natural infection than after vaccination (27, 29) and includes both prominent serum and cell-mediated components. Parenteral, killed whole-cell (WC) vaccines elicit a serum response equal to a natural infection, but not a comparable cell-mediated response. With the live oral vaccines the opposite is true. Described below are the immune responses after vaccination and the so-called "herd immunity".

Immune response after vaccination

Because of the complex nature of the pathogenesis of *S. typhi* clinical infection, a protective role is probably played:

- by the secretory intestinal antibody in preventing mucosal invasion;
- by the circulating antibody against bacteraemic organisms; and
- by cell-mediated immunity in eliminating intracellular bacilli.

The immune response depends on the nature of the vaccine. With parenteral vaccines the circulating antibody response is substantial and presumably provides the predominant protective effect. In contrast, with live oral vaccines the circulating antibody response is modest, but a vigorous cell-mediated immune response occurs, increasing the protection conferred by the vaccine.

With parenteral whole-cell vaccine, elicitation of serum H antibodies and sometime Vi antibodies (47) correlates with protection, whereas O antibodies do not. In contrast, with live oral vaccines, the cell-mediated immune response seems to be directed towards the O and H antigen and not towards the Vi antigen.

Circulating antibodies. With parenteral, acetone or heat phenol inactivated vaccines the O antibodies are IgM (LPS (lipopolysaccharide antigen) is T independent), while the H antibody response is initially IgM and then becomes IgG. With purified Vi polysaccharide vaccine the response depends on the preparation of the antigen (41). Oral, killed WC vaccine stimulates meagre serum O, H, or Vi antibody responses. Attenuated strains elicit relatively weak serum antibody responses that are intermediate between those after parenteral killed and oral killed vaccines.

The serum antibody response has been most extensively studied with vaccine strain Ty21a. Furer and Germanier (11) noted that Ty21a grown in the presence of galactose, which leads to bacilli bearing smooth LPS, was highly protective, whereas vaccine grown in the absence of galactose, which leads to rough bacilli, was not. He noted a significantly greater seroconversion of O antibody in recipients of vaccine grown in the presence of galactose. Serum levels of IgG and IgA antibodies to *S. typhi* O antigen have been measured before and after the vaccination of healthy Chileans who received Ty21a in one of two formulations, and in various immunization schedules (enteric-coated and gelatin + NaHCO_3 vaccines). An ELISA method showed, among recipients of enteric-coated capsules, a strong correlation between the seroconversion rate of IgG O antibody and vaccine efficacy in the field. Thus, while serum O antibody is not believed to be the operative mechanism of immunity elicited by attenuated strains, it clearly correlates, in this case, with protection.

Secretory antibody response. The intestinal secretory antibody response of any of the vaccines (paren-

teral, killed oral, live oral) has not been studied in a large number of recipients. However, several studies have shown that local antibody (IgAs) to O antigen was stimulated following oral vaccination with live oral vaccines, particularly in individuals from endemic areas.

Mucosal tissues contain their own local immune system, working in separation from the generalized immune system (14), but activated lymphocytes from the gut can disseminate immunity to other mucosal and glandular tissues. An important basis for local immunity is the migration of specific, antigen-activated B and T cells from Peyer's patches (PP) to the intestinal lamina propria and epithelium. Antigen (or bacteria administered orally) is taken up in the PP by modified epithelial cells, called "M" cells, and then transported to the lymphoid tissue of the PP which contain B and T cells as well as antigen-presenting cells (APC = macrophages or dendritic cells). After antigen-induced proliferation and partial differentiation, both B and T cells enter the regional mesenteric lymph nodes, and then after further differentiation they are transported through the thoracic duct into the circulation.

As these cells have surface determinants — so-called adressesins, which are specific for lymphocyte homing receptors on endothelial cells — in mucosal and glandular tissues, they will return to and extravasate into these tissues. Most of the B and T cells activated in the intestine migrate to the lamina propria of the intestine while another population of T cells moves to the intestinal epithelium, but a substantial proportion (10–25%) end up in mucosal tissues outside the intestine.

B cells in the lamina propria synthesize the IgA molecule as a dimer. This dimeric IgA is then transported into the gut lumen by a "lock and key" interaction between the J chain on the IgA dimer and the secretory component (SC) receptor present on the basolateral membrane of the enterocyte. At the apical surface, the IgA molecule is exocytosed, and the SC is cleaved to deliver the classical IgAs molecule with a small piece of SC remaining in the membrane.

Kantele (17) studied the human immune response (in persons vaccinated 2 years previously by oral Ty21a vaccine) to a secondary immunization by the same oral vaccine by enumerating the specific antibody-secreting cells (ASC) in the peripheral blood. This study shows the presence of immunologic memory in respect of the human ASC response, and confirms the separate nature of ASC and serum responses. Serum antibody responses were not seen in any of the vaccinees after secondary immunization, whereas after primary immunization 60% of these subjects responded.

T-cell response. Cell-mediated responses have been measured, following vaccination with parenteral, killed, WC vaccines or live oral vaccines (9, 31). The assays utilized have included lymphocyte replication, inhibition of mononuclear cell migration in the presence of soluble antigen, or inhibition of growth of *S. typhi* by mononuclear cells. Live oral vaccines stimulate the more potent T-cell immune response (40), which appears to be largely directed against the O antigen. Following oral immunization with *S. typhi* Ty21a (39), or O901 (27), or 541Ty (24), the appearance of a potent plasma-dependent mononuclear cell inhibition of the growth of *S. typhi* has been observed. Preliminary evidence showed that the necessary component in plasma is immunoglobulin and that IgA is most effective.

Herd immunity

An indirect protective effect has been observed in control groups during field trial studies of Ty21a. In fact, analysis of the incidence rate of typhoid fever in the placebo control group in the first field trial of Ty21a in Area Norte, Santiago, Chile, provided some interesting data on what might possibly occur following a systematic wide-scale application of Ty21a live oral vaccine in TF control programmes (21).

The incidence rate in the randomized control group in the first year of surveillance was 227 cases per 100 000 schoolchildren. Surveillance of the second typhoid season in Area Norte took place after most of the children in the adjacent area, Occidente, had been given vaccines as part of the second field trial of Ty21a. The incidence fell to 139 cases per 100 000, a decrease of 39%. Shortly before the third typhoid season of surveillance began in Area Norte, almost 200 000 children in Areas Sur and Central were given 2, 3 or 4 doses of vaccine. In this third year of surveillance the incidence in the placebo group fell again by 50% (70 cases). A rate so low has not been encountered in Area Norte for decades. The fourth year of surveillance in Area Norte field occurred during a year when no further trials were carried out in Santiago. The incidence of TF in the placebo group did not fall further; in fact, it increased by 30%. Shortly before the fifth year of surveillance in Area Norte, 90 000 additional Santiago children were entered into a trial, 88% of whom received three doses of vaccine, the others receiving placebo. Approximately 80% of these children were in Area Sur Oriente, the rest were young children in Area Norte who entered school after initiation of the 1982 vaccine trial in Area Norte. During this fifth year of surveillance, the incidence of TF in the placebo group again showed a decrease, dropping by 40% from the incidence recorded in the previous year.

The trial in Area Occidente began one year after the trial in Area Norte. A similar, but less pronounced, change in incidence was seen in the placebo group during four years of surveillance in the same period.

Vaccines

Despite effective treatment for typhoid fever (26), it has been reported that some *Salmonella* strains presented resistance to several antibiotics (35). It is therefore necessary to prepare vaccines which could be used as a public health tool in developing countries. The various vaccines that have been utilized to prevent TF can be divided according to their mode of administration and their composition.

Vaccines given by parenteral and aerosol routes

Killed organisms or subunit immunizing antigens have been used.

(1) Vaccines composed of killed organisms

Parental, killed WC vaccine inactivated by heat, phenol, or acetone has been used since 1896. Between 1960 and 1970 WHO sponsored a series of field trials. The first, held in Yugoslavia showed that a fluid, heat-inactivated, phenol-preserved parenteral vaccine was superior in protective efficacy when compared with an alcohol-inactivated and preserved vaccine. After this trial the Walter Reed Army Institute of Research prepared for WHO two lyophilized vaccines for use in other field trials (45). These included a heat phenol-inactivated vaccine (L) and an acetone-inactivated vaccine (K) tested in randomized, controlled, double-blind trials in Yugoslavia (50) and Guyana. In addition, the K vaccine was evaluated for efficacy in Poland and the L in Russia.

Results are presented in Table 2. The K vaccine was found to provide significantly more protection than the L vaccine.

Although protective, killed WC vaccines are rarely used in systematic TF control programmes because of adverse reactions.

(2) Vaccines composed of chemically defined subunits

Many attempts have been made to prepare extracts and sonicates of *S. typhi*. The various subunit immunizing agents, which were called "chemical" vaccines include the following:

- freeze and thaw extract vaccines;
- trypsinized extract vaccines;
- purified Vi polysaccharide (PS) vaccines; the technique of extraction denatures the PS. Robbins et al. (34) utilized a non-denaturing technique of extraction to prepare an effective vaccine.

Some chemical vaccines have been used by either the parenteral or aerosol route.

(3) Vaccine composed of Vi polysaccharide

History. The Vi polysaccharide of *S. typhi* is a homopolymer of N-acetyl-galacturonic acid which covers the bacteria as a capsular antigen and correlates with its virulence. A poor serological response to Vi antigen has been shown in acute TF which contrasts with the very high response in most chronic carriers. Vi protected the O antigen of *S. typhi* from agglutination by O antibodies, and it was proposed that Vi antibodies played an important role in protection against TF.

Landy (19) proposed to assess the role of Vi as a protective antigen in injecting it in a very highly purified form. But the technique employed was denaturing and the vaccine failed to protect volunteers.

Table 2. Field trials of two doses of lyophilized acetone (K) and heat phenol (L) inactivated vaccines

Sites and dates	Age in years	Vaccines	No. of vaccinees	Duration of survey (years)	Incidence/10 ⁵ /yr	Efficacy (%)
Yugoslavia 1960-63	2 to 50	K	5 028	2.5	318	79
		L	5 068	2.5	727	51
		Control	5 039	2.5	1488	
Guyana 1960-67	5 to 15	K	24 046	7	67	88
		L	23 431	7	209	65
		Control	24 241	7	602	
Poland 1961-64	5 to 14	K	81 534	3	7	88
		Control	83 734	3	47	
Russia 1962-65	7 to 15	L	36 112	2.5	55	66
		Control	36 999	2.5	162	

Wong (48) purified the Vi polysaccharide by a non-denaturing technique with hexadecyltrimethylammonium bromide, which he used as a parenteral vaccine. More recently this work was extended by Robbins who prepared with Mérieux the "Thyphim Vi" vaccine.

Safety and immunogenicity studies with purified Vi antigen. Tacket (41) evaluated immunogenicity of two nonadenated Vi lots prepared at NIH Bethesda (USA) or at the Merieux Institute (France). The former contained 5% and the latter 0.2% of contaminating LPS. Both lots elicited high titres of Vi antibody in about 90% of recipients. However, the less pure lot also stimulated O antibody in more than 80% of vaccinees. In contrast, the 99.8% pure preparation was well tolerated and stimulated O antibody in fewer than 20% of vaccinees. Moreover, Tacket (42) showed that the Vi antibodies generated in the vaccinees persisted at least three years.

Field trials with purified Vi vaccine. Two randomized controlled field trials were initiated in Nepal (1) and in South Africa (18) to assess the safety and efficacy of the candidate Vi vaccine produced by the Merieux Institute. Control groups received anti-meningococcal vaccine in South Africa and anti-pneumococcal vaccine in Nepal. In both trials the vaccine was well tolerated. In Nepal (1), a single 25mg intramuscular dose provided 72% protection for at least 17 months against culture-confirmed TF in subjects aged 5 to 44 years (incidence in control, 655/100 000 per year). Similar results were obtained in a field trial in South African schoolchildren (6 to 14 years old) in whom a single 25 mg dose conferred 64% protection (16) against culture-confirmed TF for at least 21 months (see Table 3). The study in Nepal utilized active surveillance methods in which health workers visited the households of participants every two days to detect typhoid cases. The African trial used a combination of active and passive surveillance methods.

Table 3. Culture-confirmed TF in persons immunized with Vi or control (pneumo or meningo) polysaccharide antigens

Site and vaccine	No. of vaccinees	No. of typhoid cases	Incidence/10 ⁵ /yr	Efficacy (%)
Nepal:				
Vi	3457	9	260	72
Pneumo	3450	32	930	
South Africa:				
Vi	5692	16	280	64
Meningo	5692	44	850	

The results of these two trials, where surveillance is being continued to determine the duration of immunity, clearly establish the efficacy of typhoid vaccines based on *humoral immunity to the Vi antigen*.

A safety and immunogenicity study has been conducted in 158 children aged 2 to 10 years old. The first results, by age group, demonstrated a clear augmentation in the production of Vi antibodies by age; only two children did not seroconvert. Indonesian children less than two years of age have been inoculated with one dose of pure Vi polysaccharide; results from this study are currently being investigated.

It is known that highly significant protection against *S. typhi* can be exhibited in the absence of Vi antibody, since the protective oral vaccine Ty21a lacks Vi antigen and therefore does not stimulate Vi antibody. This raises the question of whether maximal protection against TF might be obtained by combining a vaccine that stimulates Vi immunity with a live oral vaccine, such as Ty21a, which elicits humoral and cell-mediated immunity against non-Vi antigens (21).

Vi vaccine is currently licensed by Merieux Institute ("Thyphim Vi") in Chile, Congo, Côte d'Ivoire, France, Republic of Korea, Netherlands, Peru, Philippines, Togo and the United Kingdom.

Vi polysaccharide conjugate vaccines. In an attempt to increase the immunogenicity of Vi as a parenteral vaccine, Szu et al. (37, 38) conjugated Vi polysaccharide to tetanus toxoid, diphtheria toxoid and cholera toxin, conferring T-dependent properties on the polysaccharide. The candidate conjugate vaccine elicited higher levels of serum antibodies than purified Vi alone in two animal species, mice and rhesus monkeys. Immunized animals responded to a booster dose with conjugate vaccine, by exhibiting further increases in Vi antibody titre. In contrast, booster doses of purified Vi polysaccharide failed to increase the level of Vi antibody.

Potential characteristics of the Vi conjugate vaccine are:

- they are more immunogenic in young animals, but require multiple doses to achieve maximal antibody titres;
- they will most probably require a cold chain to maintain their stability, probably more than with purified Vi; and
- the cost will be substantially higher than that of a simple polysaccharide.

Clinical studies with such conjugates are in progress.

Vaccines given by the oral route

(1) Oral, killed whole-cell vaccines

It has been known already for some time that killed WC *S. typhi* can be given safely by the oral route without eliciting adverse reactions, in contrast to when administered parenterally. However, it has provided little protective efficacy in field trial studies.

(2) Oral, live attenuated vaccines

Attenuated *S. typhi* strains that have been studied are described below.

Streptomycin-dependent *S. typhi*. This strain was obtained by repeated cultivation in the presence of streptomycin, and was therefore unable to proliferate in the absence of streptomycin. It was shown to be safe and effective as a live oral vaccine in studies on volunteers. However, in subsequent studies, protection was not conferred when the vaccine was administered with reconstituted lyophilized organisms. Although these studies were abandoned, experience with the streptomycin-dependent strains paved the way for other attenuated *S. typhi* strains to be used as safe and effective live oral vaccines.

galE mutant Ty21a. In 1975, Germanier & Furer (11) described strain Ty21a, a mutant of *S. typhi* Ty2 which carries a mutation in the *galE* gene, resulting in the absence of activity of the enzyme uridine diphosphate (UDP) galactose-4-epimerase (Fig. 1). Derived by chemical mutagenic activity of nitrosoguanidine it is entirely deficient in activity of the *galE* gene product, UDP-galactose-4-epimerase which is responsible for the interconversion of UDP-galactose and UDP-glucose. But the nonspecific action of nitrosoguanidine produced other changes in addition to the *galE* mutation, e.g., inability to produce H₂S, several nutritional auxotrophies (growth rate approximately half that of the parent Ty2), and lack of Vi antigen.

Galactose residues are an important component of the smooth LPS O antigen in wild type *S. typhi*. They come from the action of UDP galactose-4-epimerase. When grown in the absence of galactose, Ty21a does not express smooth O antigen and is not immunogenic. In the absence of UDP galactose-4-epimerase, the galactose residues can be obtained through the exogenous pathway which leads to an accumulation of galactose-1-phosphate and UDP galactose. In the absence of UDP galactose-4-epimerase activity, this accumulation of intermediate metabolites leads to bacterial death by lysis, which has been presumed to account for the failure to recover vaccine organisms from coprocultures of persons who ingested the usual dose of 1 to 5 × 10⁹ organisms.

In preliminary safety and immunogenicity studies in adult North Americans, Ty21a (grown in a low concentration of galactose) was well tolerated, even with oral doses as high as 10¹¹ organisms and was immunogenic (23).

Field trial in Egypt

The first field trial of efficacy was conducted from 1978 to 1980 in Alexandria, where approximately 32 000 schoolchildren aged 6 to 7 years were randomized to receive three doses (10⁹ organisms) of vaccine or placebo administered every other day. Each dose of lyophilized vaccine was reconstituted in the field with a diluent to create a liquid suspension and was ingested by the child 1 minute after chewing a 1.0 g tablet of NaHCO₃. During three years of passive surveillance, 22 bacteriologically confirmed cases of TF were observed in the control group but only 1 case in the vaccinated group (96% efficacy).

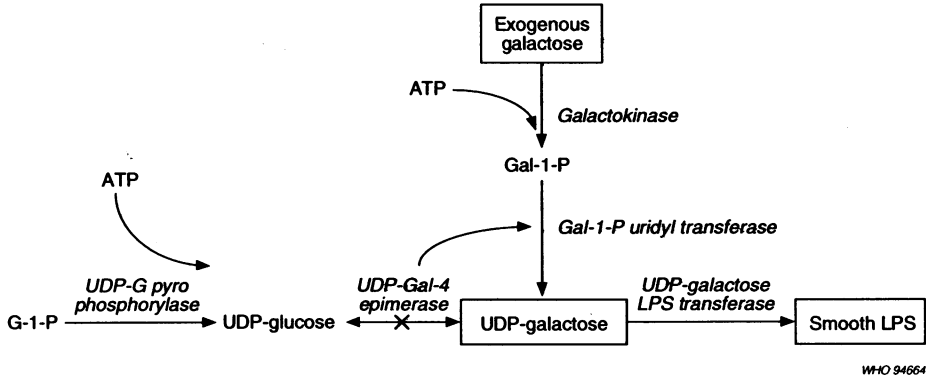
After these encouraging results the Swiss Serum and Vaccine Institute in 1981 prepared a commercial formulation of Ty21a in gelatin capsules containing a dose of lyophilized vaccine together with two additional gelatin capsules, each containing 0.4 g of NaHCO₃. Later they produced enteric-coated capsules (using hydroxy-propylmethyl-cellulose phthalate) to make the capsules acid-resistant. The latter resist gastric acid for at least two hours, but dissolve within 10 minutes in artificial intestinal fluid of pH 6 or more.

Field trial in Chile

Despite the efficacy demonstrated in volunteers and in Egypt, several practical points still needed to be resolved before Ty21a could be considered for widespread use in public health, such as:

- What was the efficacy of Ty21a when administered as enteric-coated capsules without pre-treatment with NaHCO₃?
- Could fewer doses than those used in Alexandria provide a satisfactory level of protection?
- What level of protection would Ty21a provide in areas where the incidence of TF is much higher than the 44 to 50 cases per 100 000 population per year observed in Alexandria?
- What was the efficacy of the commercial formulation (gelatin capsules containing NaHCO₃) compared with the lyophilized vaccine which was marketed after the Egyptian trial?
- Could a longer interval between the doses enhance the immunogenicity of the vaccine?

Fig. 1. Schema of the incorporation of exogenous galactose by the *galE* mutant. In the absence of UDP-galactose-4-epimerase, there is an accumulation of galactose-1-phosphate and UDP-galactose (from the exogenous pathway).



— Could an immunological assay be identified that would correlate with levels of vaccine efficacy in a field trial, which could be used to predict the effect of changes in formulation and immunization schedules?

Many of these points were successfully investigated in a series of four field trials of vaccine efficacy carried out in Chile with Ty21a vaccine (22).

Three of the four studies were conducted with placebo groups. The first two field trials were initiated in the Northern and Western administrative areas of Santiago in 1982 and 1983. The third began in the Southern and Central administrative area of Santiago in 1984. The last was conducted in the South Eastern and Northern administrative area of the town. Santiago was selected because of the combination of high endemicity of TF (annual incidence exceeded 150 per 100 000), an excellent health care infrastructure, and a long history of school-based vaccination programmes.

Only bacteriologically confirmed cases (i.e., where *S. typhi* was isolated from blood, bone marrow, or bile-stained duodenal fluid) were used in reckoning vaccine efficacy.

Area Occidente (western) field trial (1983–86). More than 140 000 children were randomized to one of five groups that received vaccine or placebo as follows:

- Group 1: three doses of vaccine in enteric-coated capsules with two days' interval.
- Group 2: three doses of vaccine with NaHCO₃, gelatin formulation, with two days' interval.
- Group 3: three doses of vaccine in enteric-coated capsules, with an interval of 21 days between the doses.

- Group 4: three doses of vaccine with NaHCO₃, gelatin formulation, with an interval of 21 days between the doses.
- Group 5: three doses of placebo with 2 days' interval.

The vaccine contained 1 to 3 × 10⁹ viable organisms per dose. Since TF exhibits marked activity in the summer months (November to April), the vaccinations were carried out from May to October.

The main points of the results obtained after 36 months of follow-up (Table 4) can be summarized as follows:

- the enteric-coated formulation was significantly superior to the gelatin NaHCO₃ capsules;
- increasing the interval between doses to 21 days offered no advantage over administering all three doses within a week; and
- the level of protection (over 60% vaccine efficacy) conferred by the best regimen, given two days apart, persisted for at least seven years (current surveillance time).

Area Norte (northern) field trial (1982–86). A total of 92 356 schoolchildren were randomized in three groups as follows:

- Group 1: two doses of Ty21a vaccine in enteric-coated capsules (1 to 3 × 10⁹ organisms per dose).
- Group 2: one dose of vaccine and 1 dose of placebo identical in appearance.
- Group 3: two doses of placebo.

The doses of vaccine were given to the children 1 week apart.

The main points are as follows:

Table 4. Comparison of the efficacy of two different formulations administered in different immunization schedules in Santiago

	Enteric-coated capsules		Gelatine capsules + NaHCO ₃		
	Long interval ^a (n = 21 598)	Short interval ^a (n = 22 170)	Placebo (n = 21 906)	Long interval ^a (n = 21 541)	Short interval ^a (n = 22 379)
No. of cases	34	23	68	46	56
Incidence ^b	157.4	103.7	310.4	213.5	250.2
Efficacy (%)	49.3	66.6		31.2	19.4

^a Long interval = 3 doses, 21 days apart; short interval = 3 doses, 2 days apart.

^b Incidence per 100 000 population.

- two doses of enteric-coated vaccine provided protection (52% to 71%) for a period of two years, which then dropped to 22% in the third year and was nonexistent in the fourth year of surveillance; and
- a single dose of vaccine in enteric-coated capsules provided low levels of protection for two years, which dropped in the third year of surveillance.

Thus, Ty21a (in enteric-coated formulation) in one or two doses provides insufficient levels of protection.

Area Sur and Area Central field trial (1984–87). This field trial was mainly set up to assess the feasibility of using Ty21a as a public health tool in large-scale school-based immunization programmes, and also to determine if administration of four doses of vaccine within an 8-day vaccination period could enhance protection. Some 190 000 children were randomized to receive two, three or four doses of Ty21a vaccine in enteric-coated capsules (1 to 3×10^9 organisms per dose), within a period of eight days (8). The results of three years of surveillance show that the incidence of TF in recipients of three doses of vaccine was only slightly lower than that in children who received two doses. In contrast, the incidence of TF after four doses was significantly lower than the rates in children who received 2 or 3 doses. At the request of one of the ethical groups that reviewed this field trial proposal, a placebo control group was removed.

Area Sur Oriente field trial (1986). In 1986 a fourth field trial was initiated in the Area Sur Oriente where 90 000 children received three doses of Ty21a or placebo within one week in either enteric-coated capsules or a liquid formulation. The results show that the liquid formulation of Ty21a, similar to that which was used in Egypt, is superior to enteric-coated capsules. A similar field trial has been conducted in Indonesia where the liquid formulation conferred slightly greater protection than the enteric-coated formulation.

Regarding laboratory correlates of protection, it has been shown that the seroconversion rate of IgG ELISA *S. typhi* O antibody increases with each additional dose (from one to four) of Ty21a ingested within eight days and that the seroconversion rate roughly correlates with the level of protection encountered in field trials. This antibody production is not believed to mediate protection *per se* but it serves as a useful correlate to compare dosage regimens.

Comparison of the Egyptian and Chilean field trials. Data from the two sites are compared in Table 5. Surveillance was conducted during a period of 36 months in Alexandria and 60 months in Santiago. The 66% protection for at least five years conferred by three doses of Ty21a in enteric-coated capsules given within one week in the Area Occidente field trial contrasts with the 96% efficacy recorded in Alexandria. The following explanations can be proposed:

- (1) *Human genetic differences.* The immune response to *Haemophilus influenzae* type b purified polysaccharide exhibits genetic restriction. It is possible that the Egyptian children mount better immune responses to Ty21a than Chilean children, based on genetic differences (13).
- (2) *Antigenic differences* in circulating *S. typhi* strains. It is theoretically possible that antigenic differences exist among *S. typhi* strains, and that Ty21a provided better protection against the strains prevalent in Alexandria than against those in Santiago.
- (3) *Epidemiological factors.* Epidemiological data suggest that water-borne transmission of *S. typhi* is usually a result of small inocula, whereas food-borne transmission is associated with large inocula and high attack rates. Typhoid is presumed to be water-borne in Alexandria, and is usually food-borne in Santiago. The lower efficacy of Ty21a in Santiago might be explained by exposure to larger inocula than in Alexandria.

Table 5. Efficacy of three doses in liquid formulation or in enteric-coated capsules given within a week in Egypt (Alexandria) and Chile (Santiago)

	Egypt: liquid formulation		Chile: enteric-coated capsules	
	Vaccinees (n = 16 486)	Placebo (n = 15 902)	Vaccinees (n = 22 170)	Placebo (n = 21 906)
No. of cases	1	22	35	101
Incidence	6	138	158	461
Efficacy (%)	95.6 (77-99) ^a		66 (50-77) ^a	
P-value		<0.001		<0.0000001

^a 95% confidence interval.

(4) *Formulation*. In Alexandria, children ingested a freshly reconstituted liquid suspension from a lyophilate, whereas in Santiago the children ingested lyophilized organisms contained within enteric-coated capsules. Vaccine organisms may be more viable when reconstituted *in vitro* before feeding than if they must emerge from the lyophilized state in the intestinal environment, where they are exposed to bile acids, enzymes, and degraded food. Moreover a liquid suspension allowed the vaccine organisms to be in contact with the tonsils, a lymphoid organ.

Field trial in Indonesia

This study (36) was designed to determine the protective efficacy of Ty21a under the conditions of intense transmission found in Indonesia and to compare, under these conditions, the protective efficacy of Ty21a given in solution with NaHCO₃ with that of Ty21a given in enteric-coated capsules. A total of 20 543 subjects (aged 3 to 44 years) were randomized to receive either three doses of enteric-coated capsules containing placebo or live Ty21a, or three doses of lyophilized placebo or live Ty21a reconstituted with phosphate buffer. During 30 months of follow-up, the rate of blood-culture-positive TF among the controls was 810 per 100 000 per year, 379 per 100 000 per year for recipients of liquid formulation, and 468 per 100 000 per year for those who received enteric-coated capsules. The liquid formulation was found to be most effective (Table 6). The protective efficacy was 53% for the liquid formulation and 42% for the enteric-coated capsules in subjects aged between 3 and 19 years, who accounted for 91% of the TF cases and had an attack rate of blood-culture-positive TF of 1206 per 100 000 per year. One explanation for the lower protective efficacy of the vaccine in Indonesia than in Chile is that immunity was overcome by more frequent inoculations of greater numbers of bacteria with intense transmission of TF. Field studies suggest that protective efficacy could fluctuate with the attack rate.

As regards paratyphoid fever, neither formulation protected against infection with *S. paratyphi* A. In the Santiago field trial, one dose of Ty21a vaccine resulted in 22% efficacy against *S. paratyphi* B and two doses resulted in 54% efficacy.^a

Mutant prepared by genetic engineering. Although Ty21a has proved to be safe and efficacious, it suffers from some drawbacks, including the requirement for multiple doses to stimulate protection, the fragility of the vaccine strain in the fermentation and lyophilization processes of large-scale manufacture, and the fact that a nonspecific method of mutagenesis resulted in multiple genetic changes in Ty21a. Biotechnology is being applied to develop new attenuated strains of *S. typhi* that might serve as oral vaccines. It is hoped to obtain successful immunization with just a single oral dose.

Mutation affecting regulatory pathways

Investigators at Washington University, led by Curtiss, have constructed candidate vaccines based primarily on inactivating the *cya* (adenylate cyclase), and *crp* (cAMP receptor protein) genes which form a global regulatory system in *Salmonella*. In phase I studies a strain (X3927) with these mutations in *cya* and *crp* caused febrile adverse reactions. Curtiss and colleagues thereupon introduced an additional putatively attenuating mutation into the gene encoding a deep tissue invasion factor (*cdt*) in strain X3927, resulting in strain X4073. The latter mutation was intended to diminish or prevent invasion of the vaccine strain beyond the intestinal lymphoid tissue to deeper organs of the reticuloendothelial system. When fed to volunteers at dosages of 10⁵ and 10⁶ organisms, this vaccine was found to be non-reacto-

^a Ty21a vaccine is licensed by Berna and other laboratories ("Vivotif") and by Sclavo ("Neotif") in 28 countries. Three enteric-coated capsules are given on day 1, day 3, and day 5.

Table 6. Comparison of protective efficacy of Ty21a oral vaccine in different formulations (liquid, enteric-coated capsules (ECC), and gelatin capsules), compared with placebo, in Egypt, Chile and Indonesia.

	Vaccine formulation	Efficacy	Efficacy compared	Placebo ^a
Egypt	Liquid + NaHCO ₃	96%	—	50
Chile (Occidente)	ECC/gelat. caps	66% ECC, 19% gelat.	ECC > gelatin	110
Chile (Oriente)	Liquid/ECC	35% ECC, 76% liquid	Liquid > ECC	100
Indonesia	Liquid/ECC	42% ECC, 53% liquid	Liquid > ECC	810

^a Figures are the incidence of TF, among those using placebo, per 100 000 per year.

genic and to induce serum anti-O antibodies and antibody-secreting cell (ASC) responses to the O and H antigens that were somewhat better than those seen after administration of Ty21a. Moreover, the vaccine organism could not be recovered in blood cultures. However, febrile adverse reactions (accompanied by bacteraemia) and loose stools were observed in some individuals who received this vaccine strain used as a live vector carrying a plasmid encoding a hepatitis B virus antigen.

Miller (28) from Boston constructed a mutant strain *PhoP*. It is a derivative of *S. typhimurium* with mutation on the virulence regulon which is composed of the *PhoP* (transcriptional regulator) and *PhoQ* (environmental sensor) proteins, and the genetic loci which they regulate positively (*pags* for activated genes) and negatively (*prgs* for *PhoP* repressed genes). Three regulated loci (*pagC*, *pagD* and *prgH*), when singly mutated, affect the virulence of *S. typhimurium* for mice. The *phoP*, *phoQ*, *pagC* and *pagD* genes are highly conserved between *S. typhimurium* and *S. typhi*.

Mutation affecting biosynthetic pathways

Another approach involves mutations in genes affecting biosynthetic pathways that render the strain dependent for growth on substrates that are unavailable in mammalian tissues. The deletion mutation of gene *Aro* creates a requirement for several aromatic compounds including *p*-amino-benzoic acid (PABA) for synthesis of folates, and dihydroxy benzoic acid (DHB) for synthesis of iron chelator enterochelin, which are not mammalian metabolites. A second deletion mutation, at gene *pur A*, causes a specific requirement for adenine (or an assimilable compound such as adenosine). These nutritional requirements render the strain unable to sustain growth in mammalian tissues.

This approach was chosen by Levine and co-workers (23) at the Center for Vaccine Development in Baltimore to construct strain 541Ty (Aro⁻, pur⁻, Vi⁺) and a Vi-negative variant strain 543Ty (Aro⁻, pur⁻, Vi⁻). In a clinical evaluation for safety and

immunogenicity, these two strains caused no adverse reactions in 37 adult American volunteers who ingested doses as high as 10¹⁰ vaccine organisms with buffer. A good cellular immune response was obtained (24). However, only meager humoral responses were induced in a small percentage of volunteers. The mutation in two separate biosynthetic pathways in strains 541Ty and 543Ty apparently resulted in overattenuation. On the basis of these observations new constructs have been prepared that involved mutations in only a single biosynthetic pathway.

Levine, Hone and co-workers then created an attenuated *S. typhi* vaccine candidate, CVD 906 (15), by introducing precise deletion mutations in two genes *aroC* and *aroD* involved in the amino acid biosynthesis pathway. These mutations render the *S. typhi* nutritionally dependent on substrates PABA and DHB, which are lacking or available in only low concentrations in human tissues. CVD 906 is a derivative of wild type *S. typhi*, ISP 1820. When given in one dose of 5 × 10⁷ CFU it elicited a good humoral and mucosal immune response in 80% of U.S. volunteers; no Vi antibodies have been recovered in sera and 2 out of 9 volunteers got diarrhoea. Because of its reactogenicity, another candidate vaccine, CVD 908, has been constructed on a similar model from a less invasive strain (44). CVD 908, based on *aroC* and *aroD* mutations of the parent strain Ty2, has been tested in human volunteers at the University of Maryland. First results showed no side-effects at a single dose of 5 × 10⁴ to 5 × 10⁸ organisms and a good response in IgA anti-LPS antibody-secreting cells. However, systematic daily culturing of blood from vaccinees during the first 12 days following ingestion of CVD 908 vaccine resulted in isolation of the vaccine strain from the majority of volunteers (50% at 10⁶ to 100% at 10⁸) on one or more days from the fourth to the tenth day after vaccination. None of these vaccinees manifested adverse reactions. CVD 908 was also well tolerated by 24 subjects when it was utilized in a dose of 10⁷ organisms as a live vector expressing foreign antigens of *Plasmodium falciparum* or of pathogenic bacteria.

As CVD 908 vaccine enters phase II clinical trials to assess its safety and immunogenicity in large numbers of subjects, attention will be paid to determine if there is any clinical significance associated with the isolation of vaccine organisms from blood cultures during the 4–10 days after vaccination. Further studies are planned to examine whether such a bacteraemia constitutes a major safety concern.

Typhoid fever control

Potential role of vaccination

Many questions should be posed by public health authorities before deciding to vaccinate a large number of people. Which groups should be vaccinated? Which immunization schedules should be used? What is the conservation time of the vaccine and the duration of protection? What kind of recommendations should be given?

Groups to be vaccinated. Of the new typhoid vaccines previously described, only Ty21a and Vi vaccines are currently available for practical use. Ty21a in enteric-coated capsules and purified Vi polysaccharide are already licensed in many countries. Little is known about the safety and immunogenicity of both these vaccines in infants. Murphy (30), in a preliminary study in Chile, has shown that Ty21a is not always well tolerated in infants and toddlers, particularly those aged 6 to 24 months, and elicits a much smaller serum antibody response compared with that in older children. However, Murphy used a particular formulation of Ty21a, i.e., by opening the coated capsules and diluting the powder in distilled water (dilution in milk was impossible). Olanratmanee (32) and Cryz (4) studied the safety and immunogenicity of Ty21a in a liquid formulation in Thai children aged 4 to 6 years of age. The immune response to Vi vaccine is currently being studied in children aged 6 months to 4 years, the first results showing a good immune response in all children during the first 3 months of follow-up, with a slight decrease in the level of antibodies in the youngest children.

If these two vaccines are to be incorporated into national programmes their use may be focused on school-age children who account for the highest incidence of TF and are amenable to school-based immunization programmes; both vaccines are protective in this age group. However, in some countries this age group may not be well represented in the school population. In industrialized countries, Ty21a or Vi vaccines may be recommended to prevent TF among clinical microbiologists and to protect travellers above 5 years of age.

Cold chain. Ty21a must be kept refrigerated and requires a cold chain; freezing will not harm the vac-

cine. In contrast, the Vi PS vaccine is not adversely affected by elevated temperatures in tropical areas and probably requires no cold chain.

Immunization schedules. *Oral vaccine Ty21a:* three oral doses of Ty21a in enteric-coated capsules are given on day 1, day and day 5. The level of protection can be increased by administration of a fourth dose of enteric-coated vaccine or by using a liquid formulation on day 7. The oral route is usually well accepted and facilitates mass administration by non-professionals.

Parenteral Vi vaccine: A single parenteral inoculation with purified Vi vaccine gave similar levels of protection for at least 2–3 years after vaccination (efficacy, 65–70%). It acts as a T-cell independent antigen and did not develop increased titres of Vi antibody after a booster dose.

Durability of protection. According to the results of field trials, three doses of Ty21a in enteric-coated capsules provided the same level of protection (>60%) for at least seven years. No large follow-up report has been published on the level of protection of the Vi vaccine; however, a good anti-Vi antibody level was recovered three years after vaccination (42).

Recommendations. A meeting (in Geneva, October 1993) of the Task Force (J. Sadoff, J. Clemens, J. Holmgren, M.M. Levine and J. Mekalanos) from the WHO/UNDP Programme for Vaccine Development evaluated the currently available anti-typhoid vaccines, defined their use in public health, and made the following recommendations:

(1) The reactogenicity of the current heat-phenol killed typhoid vaccine negates its usefulness as a public health tool. Countries wishing to incorporate vaccination against typhoid as a routine control measure should strongly consider use of either Vi polysaccharide or Ty21a vaccines. Current evidence overwhelmingly supports use of the liquid formulation rather than enteric-coated capsules of the Ty21a vaccine.

(2) For routine vaccination programmes, school-based vaccination may be appropriate in some areas; where there is limited school attendance the incorporation of typhoid vaccination into the current EPI schedule may be preferred. Unfortunately, the latter strategy cannot be pursued until a suitable, safe and protective vaccine is found for administration to the EPI-targeted age groups.

(3) The decision to incorporate vaccination against typhoid into a country's immunization programme should ideally be based on careful consideration of

the local epidemiology of typhoid, including age-specific incidence and subpopulations at particularly high risk, as well as quantitative analysis of the costs and benefits of the typhoid vaccine to be included.

(4) Further research, supported by WHO, is required for both Ty21a and Vi vaccines on the following issues:

(a) More information is required about the immunogenicity and safety of these vaccines when administered to infants at the age scheduled for measles vaccine (9 or 12 months in most typhoid-endemic areas). Such studies should not only evaluate immune responses to each vaccine at this age, when compared with administration at an older age (e.g. 24 months), but also assess whether the typhoid vaccine interferes with responses to simultaneously administered measles vaccine.

(b) If studies outlined in (a) yield encouraging results, the efficacy of the typhoid vaccines, co-administered with measles vaccine, should be formally evaluated in a population with endemic typhoid.

(c) Because the duration of protection conferred by Vi vaccine is uncertain, information about this parameter is needed from field trials conducted in all age groups.

(d) Because epidemiological idiosyncrasies of different field sites, such as the intensity of *S. typhi* infections, may modify the level of protection for any given vaccine, the efficacy of Ty21a and Vi vaccines from different field trials should be compared when administered contemporaneously to the same population.

(e) Because Ty21a and Vi vaccines appear to protect via different immunological mechanisms and because each vaccine confers only moderate protection, it is of great interest to assess whether combined administration of the vaccines improves protection. This assessment should ideally be undertaken with a formal field trial.

(f) For countries deciding to incorporate Ty21a or Vi vaccine in public health control programmes, phase-4 evaluations of safety and efficacy, using observational designs such as case-control studies, should be strongly encouraged.

(5) Commercial producers of Vi and Ty21a typhoid vaccines should be encouraged to work with typhoid endemic countries to enable local production of the vaccine with suitable quality control procedures.

Conclusion

Because of the reactogenicity of the parenteral, killed whole-cell typhoid vaccine, research was

directed towards oral vaccination using live organisms, which have been effective in public health programmes. The widespread application of Ty21a, one such oral vaccine, can result in a "herd immunity" effect.

Some new vaccine strains prepared by genetic engineering could improve the currently obtained results. For example, it would be interesting to get an immunizing regimen that could elicit Vi antibody besides the non-Vi humoral and cellular immune response stimulated by an attenuated *S. typhi*. A Vi-positive mutant of Ty21a (3, 43), when given by the oral route, was built for that purpose, but was unable to elicit Vi serum antibodies as in naturally infected patients with *S. typhi* (26% of them developing Vi seroconversion). However, protective efficacy (provided either by Ty21a vaccine or by Vi vaccine) suggests combined immunization using oral Ty21a and parenteral purified Vi.

Résumé

La vaccination antityphoïdique: situation

La fièvre typhoïde, important problème de santé publique pour de nombreux pays en développement, a toujours été sous-estimée. Elle est responsable de plus de 600 000 décès par an. A cause des réactions secondaires entraînées par le vaccin tué administré par voie parentérale, les recherches ont été orientées vers la vaccination par voie orale à l'aide d'antigènes purifiés ou de bactéries entières. Le vaccin Ty21a, donné par voie orale, a été largement utilisé au cours de plusieurs études dans les pays en développement. Sa présentation sous forme liquide s'est révélée la plus efficace: il confère une protection supérieure à 60% après 7 années de suivi. Un vaccin polysaccharidique Vi a été mis au point et la protection est supérieure à 65% après 2 années de suivi. Trois ans après vaccination, les anticorps anti-Vi atteignent toujours un niveau élevé.

Ces deux vaccins sont candidats à un usage en santé publique. Cependant, avant d'être utilisés dans ce but, leur innocuité et leur immunogénicité devront être évaluées à l'âge auquel les vaccins du PEV sont donnés. De plus, il faudra également vérifier que l'administration du vaccin antityphoïdique n'interfère pas avec la réponse immunitaire stimulée par le vaccin antirougeoleux.

De nouveaux vaccins vivants administrables par voie orale ont été mis au point par génie génétique. Les premiers résultats sont très prometteurs, mais il faut encore les améliorer avant qu'ils puissent être utilisés à grande échelle. Ils

pourront également servir de vecteurs vivants pour produire des antigènes au niveau de la muqueuse intestinale, entraînant ainsi une réponse immunitaire de cette muqueuse.

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