Vaccines for Bacterial Diarrhoeas

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The bacteria that cause human diarrhoea, dysentery and/or enteric infections can be categorised as belonging to five different groups according to the basis of their pathogenic mechanisms.[1]

1. Bacteria which attach to the mucosa and elaborate exo-enterotoxin without invading the underlying tissue or causing recognisable histopathological lesions. Representatives of the bacteria in this group are *Vibrio cholerae* and enterotoxigenic *Escherichia coli* (ETEC).

2. Bacteria which adhere to the epithelium, produce toxin and cause dissolution of the brush border of the enterocyte without further invasion. Enteropathogenic *E. coli* is the respresentative organism of this group.

3. Some bacteria such as the *Shigella* species invade the mucosa and proliferate within the enterocytes causing cell dysfunction and death.

4. Certain bacteria pass through the mucosa to the lamina propria and mesenteric lymph nodes where they proliferate. These bacteria, including the *Salmonella* species (other than *Salmonella typhi* and *S. paratyphi* A and B). *Campylobacter jejuni* and *Yersinia enterocolitica*, in spite of inducing a chemotactic response of the polymorphonuclear leukocytes, rarely cause bacteraemia or systemic infection.

5. Bacteria which translocate through the epithelium followed by generalised infection. *S. typhi* and *S. paratyphi* A and B represent this group.

Advanced knowledge on the pathogenesis of bacterial enteropathogens has led to the application of the information to vaccine development. The idea is to prepare an immunising agent which would induce the production of an immunological factor that may interfere with a particular step of the pathogenic mechanisms.

Efforts were undertaken long ago to develop vaccines against certain bacterial enteropathogens such as *V. cholerae* and *S. typhi*. However, similar effort have not yet begun for many diseases caused by such pathogens as *Compylobacter* and *Yersinia* because research is just beginning to uncover more information about the pathogeneses, antigenic structures and immune responses to these infections.

CHOLERA VACCINES

Cholera vaccines have been used for more than 100 years for protecting individuals, preventing the importation of the disease into countries and/or controlling epidemics. In the past, most national cholera control programmes usually included mass immunisation of the people. However, the attitude towards the use of cholera vaccines has changed markedly during the past 15 to 20 years as it became evident that cholera vaccine has only limited effectiveness. At present, it is not recommended that international travellers be vaccinated against cholera. In endemic areas, especially among people at high risk such an prisoners and military personnel at installations where hygiene is very poor, mass immunisation has to be repeated every six months or once a year.

Cholera vaccines which have been or are currently being developed may be classified as follows:

1. **Parenteral vaccines**
   - Parenteral killed bacteria vaccine (PKB)
   - Parenteral toxoid vaccine
2. **Oral vaccines**
   - Killed antigen vaccines
     - Oral toxoid vaccine
     - Killed bacteria-B subunit vaccine (KB-B)
     - Killed bacteria procholeragenical vaccine (KB-P)
     - French vaccine
     - TM MU vaccine
   - Live mutant vaccines
     - Chemically mutagenised strains (Texas-Star SR)
     - Genetically engineered strains

Parenteral killed bacteria vaccine (PKB)

PKB vaccine, the currently available cholera vaccine, consists of killed *V. cholerae* of both biotypes and both serotypes in a concentration of about 2×10^{11} cells per ml. The vaccine is given subcutaneously in a dose of 0.5 ml. As a result of various field trials it was found

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that, although PKB vaccine provides significant protection, the protection is neither stable nor long-lasting. The filed trials revealed that the protective rate was about 50 per cent and the protective period about six months. Besides, the protection was found to be poorest in smaller children, the age group with the highest rate of cholera attack. It was also observed that PKB vaccine did not prevent infection with *V. cholerae*, thus the vaccine is not an efficient tool for effective cholera intervention. Moreover, most of the PKB vaccinees suffered from adverse reactions, e.g. headache, fever, and swelling with inflammation and pain at the site of inoculation because the vaccine contains a large amount of endotoxin.[2,3]

Parenteral toxoid vaccine

Because of the limited effectiveness of PKB vaccine, attempts had been made to provide protection by inducing antitoxic immunity. Since cholera toxin is the important virulent factor in cases of cholera, it was thought that stimulation of an intense antitoxic response in the host could conceivably confer good protection against the disease. However, a field trial of one of the parenteral toxoid vaccines resulted in unsatisfactory protection.[4]

Cholera is a mucosal infection. Its pathogenic mechanisms are confined only to the intestinal lumen and mucosal surfaces.[5] It is also known that intestinal immune response is best stimulated by antigen presented locally to the intestine. Thus, current approaches to immunoprophylaxis against cholera are based on the development of oral vaccines.

Oral cholera vaccine candidates are of two types: the killed antigen preparations and the live mutants.[1]

Killed antigen preparations

1. Killed bacteria-B subunit combination (KB-B). KB-B is a combined vaccine consisting of purified B subunits and killed *V. cholerae* of both biotypes. Each dose contains 5 mg of B subunit and about 2 x 10^{11} killed whole vibrios (5 x 10^{10} each of heat–killed classical Inaba and Ogawa cells and 10^{11} formalin-killed El Tor, Inaba cells). The vaccine was tested on American volunteers by giving them three consecutive doses orally on days 0, 14 and 28. Each volunteer was given a 300 mg tablet of cimetidine to suppress gastric acidity three hours prior to vaccination and 1g of sodium bicarbonate one minute before ingesting the vaccine. The combined vaccine was found to induce a good seroconversion rate. The efficacy of the vaccine in the challenge study was found to be about 64 per cent (4 out of 11 vaccinees developed diarrhoea while all of the controls – non vaccinees – developed cholera after being challenged with *V. cholerae* which was carried out one month after the last dose of vaccine had been administered).

2. Killed whole V. cholerae oral vaccine. This type of oral vaccine consists of killed *V. cholerae* alone. It also was tested on American volunteers in an immunisation schedule similar to the one for KB-B vaccine. The volunteers also received the cimetidine and sodium bicarbonate treatment. Challenge study revealed a 56 per cent efficacy rate for this type of vaccine.

3. Killed bacteria - procholeragenoid oral vaccine (KB-P). Procholeragenoid is the cholera enterotoxin the toxicity of which is reduced by heat. Each dose of KB-P vaccine contains a total of about 2 x 10^{11} *V. cholerae* organisms of both biotypes and serotypes. In tests, three oral doses were given orally to American volunteers. For the first and second doses 50 μg of procholeragenoid were administered; for the third one, 200 μg. Prior to taking the vaccine, the volunteers ingested cimetidine and sodium bicarbonate. Challenge study revealed a 25 per cent protection rate among the vaccinees. However, the rest of the volunteers developed milder diarrhoea than non-vaccinated individuals.

4. French oral vaccine.[6] Produced by the Pasteur Institute in Paris, this vaccine is a crude fraction of the *V. cholerae* O_1 cell wall. Preliminary safety tests have shown that this antigen is safe when given orally. It stimulates the production of serum vibriocidal antibody. The results of a preliminary trial in Zaire in 1983 suggest that this oral vaccine has protective properties when given in two doses, eight days apart.

5. TM–MU Vaccine. This combined vaccine was developed at the Faculty of Tropical Medicine, Mahidol University, Thailand. It consists of *V. cholerae* lipopolysaccharide, cell-associated haemagglutinin prepared from El Tor vibrios and procholeragenoid (a gift from Dr. Furer of the Swiss Serum Laboratory, Switzerland). The vaccine is aimed at eliciting three functional types of antibodies. The first type is agglutinating antibody which agglutinates the vibrios, thus rendering them non-motile and unable to penetrate the mucus gel to the underlying epithelium. The second type is anti-adhesive antibody (anti-cell-associated haemagglutinin) which interferes with the adherence of the vibrios to the epithelium, thereby preventing successful colonisation. The third type elicited by procholeragenoid antitoxin which prevents the binding of the cholera toxin to the Gm1 receptor on the enterocyte membrane. Without binding of the B subunit to the specific receptor, the toxic portion of the toxin molecule cannot exert its function and diarrhoea will not occur. This combined vaccine has been tested in experimental animals with satisfactory protective activity.[7,8]

Live oral mutants

1. Chemically mutagenised vaccine (Texas-Star SR). Texas-Star SR is an $A^- B^+$ *V. cholerae* mutant derived from El Tor Ogawa strain 3084 by mutagenesis with nitroso-

guanidine. This mutant was tested on American volunteers as a prototype vaccine prior to the advent of genetically engineered strains. The immunogenicity and protective activity of the vaccine were moderate. However, the vaccine has many drawbacks which include mild diarrhoea in about 25 per cent of recipients. Because the strain is derived from chemical mutagenesis, in which case the precise genetic lesion(s) responsible for the attenuation of the mutant and other genetic changes are not known, it is possible that the mutant may revert to a wild type. In addition to these factors, the immunogenicity of the parent Ogawa 3084 strain has not yet been tested in volunteers.[9-13]

2. Oral vaccines prepared from genetically engineered V. cholerae strains.[14] Attenuated *V. cholerae* strains, in which the genes encoded for A and B subunits or the A subunit alone were deleted, have been prepared by Karper *et al* by recombinant DNA technology. These mutants were derived from pathogenic classical or El Tor strains, the pathogenicity and immunogenicity of which had been well studied in human volunteers.

2.1 A^-B^- El Tor Inaba strain JBK 70. This strain was prepared by recombinant DNA technique from the El Tor Inaba N16961 strain. Its preparation involved the deletion of genes encoded for the production of both the A and B subunits of cholera toxin. Other gene loci of the strain were unchanged. Volunteers were given doses of 10^6, 10^8 or 10^{10} viable JBK70 cells following the ingestion of 2 g of sodium bicarbonate. Ten vaccinees, who received a single dose of JBK70, and eight controls (non-vaccinees) were challenged on one month later with 10^6 pathogenic El Tor N16961 cells. Only one of the 10 vaccinees developed cholera compared with seven of the eight controls.

2.2 A^-B^+ classical Ogawa CVD101. The strain was derived from classical Ogawa 395. Ninety-four per cent of the genes encoding the A1 subunit were removed from the parent 395 strain by recombinant DNA techniques. The resulting A^-B^+ mutant has been named CVD101. Volunteers were given doses of 10^8, 10^7, 10^6, 10^5 or 10^4 organisms orally after ingesting sodium bicarbonate. A proportion of all volunteers developed mild diarrhoea, which tended to be more prominent in those who received 10^8 or 10^7 vaccine organisms. However, the oral vaccine yielded a 90 per cent seroconversion rate. A study to test the efficacy of CVD 101 has not yet been carried out.

2.3 A^-B^+ classical Inaba CVD103. This mutant is an A^-B^+ variant of classical Inaba 569B. The organisms were administered to volunteers and were found to cause no adverse reaction in vaccinees. The efficacy of this type of vaccine has not yet been studied.

Oral vaccines prepared from genetically engineered mutants stimulate high antibody levels. Some are protective after a single dose. However, recipients have experienced a high rate of adverse reactions. Besides cholera toxin, both JBK70 and CVD 101 produce a Shiga-like toxin and a haemolysin that has cytotoxic and enterotoxic activity. It remains to be seen whether deletion of one or both of the genes encoded for the two virulence properties will further attenuate the two strains without reducing their immunogenicity and protective ability.

TYPHOID VACCINES

The pathogenic mechanisms of typhoid may be summarised as follows:

Ingested typhoid bacilli which survive gastric acidity in the stomach and arrive at the small intestine will penetrate the intestinal mucosa into the lamina propria. There, they cause an intense chemotactic response of macrophages; upon arrival, the non-immune macrophages ingest the organisms. However, some of the organisms remain in the intestinal lymphoid tissue while others enter the mesenteric lymph nodes, lymph drainage, thoracic duct and blood circulation, respectively. Primary bacteraemia ensues. As a consequence of this bacteraemia, the pathogens are distributed to various organs of the reticulo-endothelial system where the bacteria reside and multiply. After an incubation period of about 10 to 14 days clinical typhoid fever appears.[15-17]

Typhoid vaccines, like those for cholera had been developed a long time ago; however, the ones presently available are not satisfactory. The parenteral whole-cell vaccines confer variable protective efficacy depending on the method of inactivation used in preparing the vaccines. They cause a high rate of adverse reactions. The protective efficacy is often overcome when ingestion of the infective organisms is in large amounts. Parenteral cell-free vaccines have been found to be even less effective than the whole-cell ones. Although non-reactogenic, the efficacy of inactivated whole-cell oral vaccines in man has never been demonstrated in field trials or in volunteer challenge studies.

Live oral vaccines

1. Streptomycin-dependent S. typhi vaccine. This was the first live oral typhoid vaccine to be tested in man. In volunteer studies, the vaccine proved to be safe, but failed to induce protection against clinical infection.[18]

2. Ty21a oral vaccine.[19-22] Prepared from the so-called "gal E" mutant of *S. typhi*, this typhoid vaccine is derived from an attenuated strain characterised by a defect in the enzyme "uridine diphosphate galactose-4-epimerase" which is responsible for the biosynthesis of UDP-galactose from UDP-glucose. The activity of two other enzymes of the mutant, namely the galactokinase and galactose-1-phosphate uridyl transferase, is reduced to 20 per cent of that of the wild type. In

the wild type, galactose is incorported into the LPS via UDP-galactose, thus the incomplete LPS is formed in the Ty21a mutant. As a result, the Ty21a is neither virulent nor immunogenic. However, when the galactose is supplied exogenously such as in the human intestine, then smooth type LPS is produced and the strain becomes immunogenic. Exogenous galactose taken up by Ty21a, besides being incorporated into the cell wall, also accumulates in the cytoplasm because of a lack of galactokinase and galactose-1-phosphate uridyl transferase. Accumulation of galactose produces lysis of the cell before causing disease.

The safety of Ty21a as an oral vaccine for man has been demonstrated in volunteers and in school children in endemic areas. The vaccine protects against challenge with virulent *S. typhi* in human volunteers. It has also been shown in a field trial to be highly protective for school children living in endemic areas.

VACCINES FOR ENTEROTOXIGENIC *ESCHERICHIA COLI* (ETEC)

In order to produce diarrhoea, the ETEC which reach the small intestine have to colonise the intestinal epithelium using a colonisation factor.[23] After successful colonisation, they produce enterotoxin(s) which can be either heat-labile (LT) or heat-stable (ST) polypeptides or both. So far, three types of colonisation factors are known in human ETEC, namely colonisation factors 1 (CFA/I), CFA/II and E 8775. The heat-labile enterotoxin of ETEC has biological properties similar to cholera toxin. It is a large, immunogenic polypeptide product of genes encoded in a transferable plasmid. The heat-stable toxin is a hapten which causes diarrhoea by converting intracellular GTP to cGMP.[1,24-26]

Current approaches to immunoprophylaxis against ETEC infection involve vaccines that stimulate antitoxic and/or anti-adhesion immunity. All of the vaccines are at the developmental stage.[1,27,28] They include the followings:

1. Vaccines prepared from purified CFA fimbriae. The aim is to use this type of vaccine to produce antibodies to colonisation factor(s). Protection tests performed in animals indicated that CFA antigens are immunogenic and safe; however, the protection is fimbriae type-specific.

2. Vaccines prepared from detoxified toxin(s). Experiments on pregnant sows have shown that LT given parenterally elicited significant rises in the level of LT antitoxin in their milk. Piglets suckled on the immunised sows were significantly protected against diarrhoea due to LT^+ ETEC.

The problem with using LT or ST as an oral immunising agent involves finding the means to detoxify these toxins; the use of formaldehyde or glutaraldehyde to treat LT tends to abolish its immunogenicity.

Besides being toxic (which means that a suitable method is required for detoxification), ST by itself is not immunogenic. The molecule has to be conjugated to a pre-existing antigen in order to become an immunogen. The carrier proteins which are likely to be used in the preparation of ST-carrier conjugate are either the B subunit of LT or IgG molecules of the host.

3. Procholeragenoid vaccine. Since LT polypeptides of ETEC have biological activity and molecular similarity to cholera toxin, procholeragenoid (heat-treated cholera toxin, the toxicity of which has been much reduced) has been used as an oral vaccine against LT^+ ETEC. However, procholeragenoid by itself retains a certain degree of toxigenicity and often causes diarrhoea in vaccinees when given orally. Besides, LT^+ ETEC represents only one third of the ETEC organisms that cause clinical diarrhoea, the remaining two thirds being caused by ST^+ ETEC. Vaccines prepared from procholeragenoid seem, therefore, to be of less importance in protecting against ETEC diarrhoea.

Along this line, there are also attempts to use the B subunit of LT as immunogen. Recombinant DNA technology is also being used to produce large quantities of LT. When it becomes available, one could also prepare "procoligenoid" and test it as a vaccine for LT^+ ETEC.

VACCINES FOR ENTEROPATHOGENIC *E. COLI* (EPEC)

EPEC requires two virulent factors in order to be pathogenic.[1,32] These factors are:

1. Adhesin, which is encoded by genes in the 55 to 72 megadalton plasmids.

2. Cell-associated protein toxin, which seems to be identical to the neurotoxin-cytotoxin-enterotoxin of *Shigella* species.[33]

Upon arrival at the epithelium of the small intestine, the EPEC adhere (using an adhesive factor produced by genes encoded in the 55 to 72 megadalton plasmids) and produce a toxin which destroys the brush border of the enterocytes. The precise biochemical events by which EPEC causes diarrhoea are unclear.

Vaccines against EPEC include:

1. Sodium deoxycholate Boivin antigen extract vaccine prepared from strains O_{111}, and O_{55} by Mochmann *et al.*[29]

2. Sodium deoxycholate Boivin preparation produced from strains O_{111}, O_{55} and O_{86} by Rauss and coworkers.[30,31]

3. Formaldehyde-killed whole-cell O_{111}, O_{55} and O_{86}.

4. Attenuated streptomycin-dependent O_{111}.

The latter two vaccines have not yet been submitted to any field trial for testing their efficacy. The Boivin antigen vaccines gave unsatisfactory protection in field trials. These vaccines are not pre-

sently in routine use anywhere in the world.

Since the adhesive property of EPEC is now known to be controlled by genes encoded in the 55 to 72 megadalton plasmids, it may be possible to identify the phenotypes of these genes. It has been speculated that such products might be a fimbriae type or an outer membrane protein. When such product(s) is (are) known, it should be possible to prepare oral vaccine(s) against EPEC consisting of the purified antigen(s).

VACCINES AGAINST SHIGELLOSIS

To manifest its pathogenicity, *Shigella* organisms must possess the following virulence factors.[1,34,35]

1. Specific, smooth LPS antigen.
2. Genes that enable the organisms to invade epithelial cells and proliferate therein.
3. Elaboration of toxin after enterocyte invasion and proliferation.

The importance of specific smooth LPS as one of the virulence properties of *Shigella* is best studied in *Shigella sonnei.* Phase 1 *Shigella sonnei,* which have smooth, complete LPS with 2-amino-2-deoxy-L-altruonic acid, are virulent. Phase II organisms, which are rough, do not have this structure and they are not pathogenic. The change of a phase I to a phase II type of organism is controlled by a 120 megadalton plasmid.[35]

S. dysenteriae and *S. flexneri* are known to exhibit colonial variation. While translucent colonies are virulent, the opaque ones are not (although both types of colonies have smooth LPS). It was found that in virulent organisms glycosyl residue is attached to rhamnose in the core polysaccharide of the LPS molecule. All evidence suggests that the chemical structure of the LPS is important with regard to the virulence of *Shigella* organisms.[1]

Besides, virulent *Shigella* must have the ability to invade intestinal epithelial cells and proliferate within these cells. The process of cell invasion by *Shigella* has been studied extensively. It is now known that the *Shigella* use their outer membrane protein encoded by a 140 megadalton plasmid to attach to the enterocytes. After attachment, the bacteria produce a cell-free heat-labile extracellular product which stimulates phagocytosis of the usually non-phagocytised epithelial cells. When the organisms are in the enterocytes, they multiply and produce Shiga-toxin which is neurotoxic (it causes paralysis in mice), cytotoxic (it causes He La cell death *in vitro*) and enterotoxic (it causes fluid accumulation in a rabbit-ligated intestinal loop). Diarrhoea and epithelial cell death ensue.

Many *Shigella* vaccines have been developed.[1] These include:

1. Killed parenteral whole-cell vaccine
2. Formalin-treated *Shigella* toxoid vaccine
3. Attenuated mutant vaccines which include colonial mutants, a streptomycin-dependent mutant, a non-invasive *Shigella-E. coli* hybrid and an *E. coli* mutant expressing the O antigen of *Shigella.*

New knowledge about the pathogenic stages of *Shigella* infection, i.e. enterocyte invasion, toxin production and the roles of large plasmids, is currently becoming available. Such information may be applied to the development of a *Shigella* vaccine. In fact, vaccine consisting of outer membrane protein, which the organisms use to attach themselves to the enterocytes, has been developed. It is hoped that such a vaccine would induce the production of immunological factor(s) which would in turn prevent the attachment of the pathogens to the epithelium.

The Ty21a– *S. sonnei* hybrid was also produced by recombinant DNA technique. This was achieved by transferring the 120 megadalton plasmid, which controls the phase variation of *S. sonnei* into Ty 21a (the oral vaccine strain for typhoid). The resultant hybrid (*S. typhi* 5076-1C) expresses both *S. typhi* and *S. sonnei* O antigens. When the strain was given orally to laboratory animals, they produced antibodies to both O antigens. Also, the vaccine gave no reactogenicity. Experiments on humans are on the way.[1]

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