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DIARRHOEAL DISEASES CONTROL PROGRAMME

RESEARCH ON VACCINE DEVELOPMENT

GENERAL ASPECTS

The research component of the Diarrhoeal Diseases Control (CDD) Programme is primarily concerned with the development and evaluation of new or improved methods to prevent morbidity and mortality due to diarrhoeal diseases. An integral part of this effort is the development of effective and practical vaccines against specific diarrhoeal agents and important enteric infections. Although no safe and satisfactorily effective diarrhoea vaccines exist at present, there is good reason to believe that they can be developed. This optimistic view is based on (1) growing evidence that most intestinal infections caused by bacteria and viral agents evoke substantial, relatively long-lasting resistance to symptomatic reinfection, (2) a much improved understanding of the immune mechanisms that protect the intestine and how they can be most efficiently stimulated, (3) recent advances in biotechnology which permit the identification of important microbial antigens that evoke immune responses and the creation, by genetic manipulation or other means, of mutant and hybrid bacteria or viruses as potential live, avirulent vaccines, and (4) recent studies with volunteers and field trials which show that such vaccines can confer significant protection.

At present, five vaccines are being given priority for research support by the CDD Programme based upon the importance of the designated pathogens as causes of morbidity and mortality during the first years of life and/or their ability to cause serious epidemics. In the first category are rotaviruses and enterotoxigenic Escherichia coli, which together cause nearly half of the serious acute diarrhoeal episodes in children under 5 years of age. The second category includes Vibrio cholerae 01 and shigellae (especially S. dysenteriae type 1). The fifth organism, Salmonella typhi, causes significant morbidity and mortality in older children and young adults and can also give rise to serious epidemics. In the future, other agents may be added to this list, the most likely being enteropathogenic E. coli and Entamoeba histolytica.

It is likely that effective vaccines for enteric infections will be given orally and in most cases will consist of live, avirulent bacteria or viruses. Although the possibility of parenteral immunization cannot be entirely excluded, especially for infection caused by rotaviruses and E. histolytica, much basic and clinical research now indicates that protective immune mechanisms in the bowel mucosa, including both secretory IgA antibodies and cell-mediated immunity, are best stimulated by oral antigens, particularly live bacteria or viruses that colonize or penetrate the bowel mucosa and stimulate local, mucosa-associated lymphoid tissue.

Non-living antigens, such as killed bacteria or purified bacterial antigens, may also be used, but in most instances they appear to be less efficient as immunogens than live agents. It is possible that the efficacy of non-living antigens as oral vaccines can be improved by the use of adjuvants, and research is required in this direction. This should include efforts to develop immunogenic forms of non-immunogenic polypeptide antigens and to enhance the immunogenicity of poorly immunogenic proteins, polysaccharides, and antigen mixtures. Adjuvants are required that can enhance the induction of both humoral (secretory IgA) and cell-mediated immune responses in the intestine.

The anticipated use of live oral viral and bacterial vaccines raises important issues related to vaccine delivery. One concern is the need to develop simple and effective methods for preserving the viability of the vaccine. For rotavirus vaccine this will probably involve cold chain requirements similar to those for live viral vaccines used at present in the Expanded Programme on Immunization (EPI). For bacteria, it is likely that lyophilization will be required and methods will need to be developed for safe storage of lyophilized bacteria in individual doses, without loss of viability. A second concern is methods of maintaining vaccine viability during passage through the stomach. Most bacteria, and possibly rotaviruses, are acid-sensitive to such a degree that they must be protected from exposure to gastric acid to preserve their viability; some non-living antigens are also labile in the gastric environment and must be similarly protected. Research is required on simple methods, suitable for infants, children, and adults, that can be used to protect live vaccines and labile non-living vaccines during passage through the stomach. A third concern is that antidiarrhoeal vaccines should, if possible, be designed for delivery as part of existing national immunization programmes. This means that they should be effective when given with other EPI vaccines and not interfere with the efficacy of those vaccines. These properties would be particularly important for an oral rotavirus vaccine which, because of the epidemiology of the disease, may need to be coadministered with live oral poliomyelitis vaccine, possibly as a single combined vaccine. In addition to being effective, it would be absolutely essential that the rotavirus vaccine not interfere with the efficacy of oral poliomyelitis vaccine.

An initial requirement in vaccine development is the capacity for preliminary studies of the safety, immunogenicity, and efficacy of candidate vaccines in volunteers. To increase the availability of facilities for such studies, the CDD Programme, in cooperation with the United States Agency for International Development (USAID) and the International Development Research Centre (IDRC), Canada, is supporting the development of a Centre for the Trial of Vaccines against Infectious Diseases at the Faculty of Tropical Medicine of Mahidol University in Bangkok, Thailand. This is only the second such centre to be created in the world and the first in a developing country. Initial studies at the centre will begin in 1986.

RESEARCH ON SPECIFIC ANTIDIARRHOEAL VACCINES

Rotavirus vaccine

The vaccines most thoroughly studied to date are attenuated animal rotavirus strains. The Nebraska calf virus, attenuated by passage 147 times in tissue culture and designated RIT-4237, has provided 80-90% protection in Finnish infants over 6 months of age after 1 or 2 doses of 10^8 PFU; this protection appeared to last for at least 2 years and to be valid for disease caused by serotype 1 (and possibly serotypes 2 and 3) rotavirus. However, subsequent trials of the vaccine in The Gambia and Peru (supported by the Programme) and in Rwanda showed it to provide little or no protection, even when 3 doses were given with a buffer to prevent inactivation of the vaccine by gastric acid. The reasons for the failure of the vaccine in developing countries are not yet known, but may reflect over-attenuation of the virus by the large number of *in vitro* passages. A second vaccine now undergoing field trials in Finland, Peru, Sweden, USA, and Venezuela is a low passage Rhesus rotavirus (MMU 18006). This strain is considerably more immunogenic than RIT-4237 and thus can be used in much lower doses (e.g., 10^5 PFU), which has the advantage of appreciably reducing the cost. However, in contrast to RIT-4237 which caused no detectable side effects in any age group, MMU 18006 causes fever and occasionally mild diarrhoea in children above 5 months of age in developed

countries. On the other hand, it causes few or no side effects (and remains highly immunogenic) in younger infants, who are apparently protected from its residual virulence by persisting maternal antibodies. Five out of 6 ongoing trials with this vaccine (4 of which are supported by the Programme) are in infants under 5 months of age.

Other approaches also being taken to develop candidate rotavirus vaccines (some with support from the Programme) are as follows:

1. The Nebraska calf virus has been developed as a low passage vaccine (RIT-4256; 20 passages), as has an unrelated bovine strain (WC-3; 12 passages). Both are undergoing preliminary studies in humans.

2. Genetic hybrid strains that contain the human virus neutralization antigen and grow well in tissue culture have been derived from mixed cultures of human and animal rotavirus strains. Initial volunteer trials with some of these will begin shortly. Field trials to determine efficacy may begin within 8 months.

3. Virulent human rotavirus strains are being attenuated for use as vaccines by passage in tissue culture and cold adaptation.

4. Human rotavirus strains have been isolated from asymptomatic infants in nurseries for the newborn. These strains, which may be naturally attenuated, will soon be evaluated for safety in older infants.

5. Progress has been made in cloning into a bacterial vector DNA copies of the RNA gene that controls production of the rotavirus neutralization antigen. It is hoped that such bacteria will express the viral antigen and be useful as live oral vaccines to stimulate rotavirus immunity.

6. Finally, methods of preparing purified rotavirus antigens for use in parenteral or oral vaccines are being studied. These involve the cloning of appropriate rotavirus genes into cultured cells which would then be used as a source of protective antigens.

Typhoid fever vaccine

Currently available vaccines against typhoid fever need to be given parenterally and their acceptability is severely limited by a high incidence of untoward reactions. Therefore, the Programme has actively associated itself in efforts to develop an improved vaccine against typhoid fever. The major effort has been directed towards evaluation of the efficacy of the live oral typhoid vaccine, Ty21a, a chemically induced mutant able to survive only briefly in the intestinal tract. In the mid-1970s, this vaccine was shown in Egypt to be entirely safe and to evoke 96% protection against typhoid fever for at least 3 years when given in 3 doses in a liquid formulation with bicarbonate to schoolchildren. However, this formulation was impractical for routine use and other formulations were subsequently developed and evaluated in trials supported by the Programme in Santiago, Chile.

The results of these trials, summarized in Table 1, show that lyophilized vaccine given in plain gelatin capsules accompanied by capsules containing sodium bicarbonate to neutralize stomach acid was poorly effective (23-41% protection) even when 3 doses were given. When the vaccine was given in enteric-coated capsules, better results were obtained. While one dose was poorly protective, 2 doses gave about 50% protection for at least 3 years, and protection was progressively greater with 3 or 4 doses (data on 4 doses are not shown in the Table). However, the requirement of as many as 4 doses is considered impractical, and protection achieved by fewer doses did not reach the 70-80% range considered the minimum necessary for a vaccine to be of practical public health value. Accordingly, efforts are under way to develop a practical formulation that can be reconstituted in a liquid before being administered, as was the case with the vaccine used in the original trial in Egypt. It is planned to evaluate this new liquid formulation of Ty21a in Programme-supported field trials in Chile and Indonesia later in 1986.

The Programme is also supporting efforts to develop alternative live oral typhoid vaccine strains. Using recombinant DNA techniques, strains of S. typhi have been developed that are deficient in the genes controlling the biosynthesis of certain essential aromatic amines and purines. These strains, which are available as both Vi positive and Vi negative mutants, also appear in initial studies to be safe for humans and possibly more immunogenic than Ty21a. Field trials with these strains will be considered after initial safety tests have been completed later in 1986.

TABLE 1: SUMMARY OF EFFICACY OF LIVE ORAL Ty21a TYPHOID VACCINE IN CHILDREN AGED 5 TO 19 YEARS IN PLACEBO-CONTROLLED TRIALS IN SANTIAGO, CHILE

Vaccine formulation	Immunization schedule	Vaccine efficacy	Observation period
Gelatin capsules + bicarbonate	3 doses, 2-day intervals	23%	30 months
Gelatin capsules + bicarbonate	3 doses, 21-day intervals	41%	30 months
Enteric-coated capsule	1 dose	15%	44 months
Enteric-coated capsule	2 doses, 7-day intervals	50%[a]	44 months
Enteric-coated capsule	3 doses, 2-day intervals	67%	30 months
Enteric-coated capsule	3 doses, 21-day intervals	59%	30 months

[a] Protection averaged 59% for the first 24 months and 24% for the subsequent 20 months.

A third approach to the development of an improved typhoid vaccine is the study of purified Vi antigen given parenterally. The use of this antigen is based on evidence of its critical role in the pathogenesis of typhoid fever in mice and its lesser reactogenicity, compared with the traditional killed whole bacterial vaccine, when given parenterally. A trial to determine the efficacy of parenteral Vi antigen is under way in Nepal, with support from other agencies.

Cholera vaccine

The cholera vaccines that are available at present also need to be given parenterally and are of low efficacy. Two different approaches, both involving oral immunization, are being taken in efforts to develop a more effective vaccine. The first concerns non-living vaccines composed of whole bacteria or crude mixtures of bacterial products. In some cases, these are combined with antigens related to cholera toxin (e.g., the purified but non-toxic B-subunit, or procholeragenoid, the minimally toxic heat-induced aggregate of cholera toxin) so that both anti-bacterial and anti-toxic immune responses, which act synergistically in mediating protection, will be stimulated. One such vaccine, composed of killed V. cholerae alone or combined with purified B-subunit of cholera toxin, is now being field-tested at the International Centre for Diarrhoeal Disease Research, Bangladesh, with Programme support.

This study will determine whether the vaccine is protective against both cholera and disease caused by enterotoxigenic E. coli, some of which produce an enterotoxin closely related to cholera toxin. Results of the first six months of surveillance in this trial are shown in Table 2. These reveal marked protection by the combined vaccine and a lower level of protection by vaccine containing only killed bacteria. Further observation is continuing in order to determine the duration of protection. Although these results are very promising as regards development of an effective cholera vaccine, further research is required to determine the most practical formulation and immunization schedule for the vaccine. The Programme is also supporting studies in volunteers to determine the efficacy of a non-living vaccine composed of antigens from culture filtrates of V. cholerae. If initial studies are promising, further trials will be undertaken.

TABLE 2: EFFICACY AT 4-6 MONTHS OF ORAL IMMUNIZATION WITH KILLED WHOLECELL CHOLERA VACCINE WITH AND WITHOUT PURIFIED B-SUBUNIT[a]

Immunization	No. vaccinated[b]	No. with cholera	% efficacy
Wholecell/B-subunit	21 200	4	85
Wholecell	21 200	11	58
Placebo	21 200	26	-

[a] All vaccinees received 3 doses at approximately one month intervals. Each dose contained 2×10^{11} killed V. cholerae and 1 mg B-subunit.

[b] Approximate numbers.

A second approach to the development of cholera vaccine is the use of live, avirulent mutants of V. cholerae. A number of such mutants have been created using recombinant DNA methods to delete all or part of the genes controlling the production of cholera toxin. The resulting non-toxigenic strains (some of which produce only the B-subunit of cholera toxin) have evoked substantial protection in volunteers but, unfortunately, have also caused mild to moderate diarrhoea by mechanisms that are as yet undefined. Such strains are considered too reactogenic to be used as vaccines. Nevertheless, there is considerable hope that strains can be developed which will be immunogenic and entirely safe. Studies are at present under way to determine the mechanism by which non-toxigenic V. cholerae cause diarrhoea and to obtain mutants deficient in the responsible genes.

Other efforts to create live oral cholera vaccines involve the use of bacterial hybrids, created by the transfer of genes encoding putatively protective antigens (e.g., bacterial lipopolysaccharide, B-subunit of cholera toxin, or antigens involved in mucosal adhesion) into benign carrier bacteria (e.g., live oral typhoid vaccine strain Ty21a), that would colonize the bowel and thus help to deliver the antigen to responsive enteric lymphoid tissue. Such hybrid vaccines might simultaneously provide protection against two pathogens.

Shigella vaccines

The many earlier efforts to develop shigella vaccines failed to yield products that were both easy to administer and of high efficacy. New vaccines against shigella infection are now being developed using genetically manipulated bacteria. Plasmids encoding the production of a shigella antigen, and in some cases antigens associated with invasiveness, have been mobilized into benign carrier strains such as Ty21a typhoid vaccine strain or E. coli K-12, creating hybrid oral vaccines. The most advanced of these is a Ty21a-Shigella sonnei hybrid, which has been proved safe and protective in volunteers and will be field-tested in Chile, Israel, and Thailand later in 1986. The trials will determine whether the vaccine confers protection against both typhoid fever and S. sonnei infection. Other vaccines which may soon be tested are E. coli K-12 strains that harbour plasmids encoding the production of lipopolysaccharide of either S. flexneri 2a or S. dysenteriae type 1. The latter strain is of particular interest due to the importance of S. dysenteriae type 1 as a cause of severe

epidemic disease with high mortality rates, especially among small children. Finally, vaccine strains are being developed by creation of deletion mutants of S. flexneri with metabolic deficiencies similar to those already shown to be effective in creating live oral vaccines for salmonellosis in cattle.

In addition to the support it has given to some of the vaccine studies described above, the Programme is providing increased support for basic research to define virulence mechanisms and protective immunological processes relevant to vaccine development for shigellosis.

Enterotoxigenic E. coli (ETEC) vaccines

Efforts to develop vaccines against disease caused by ETEC are focusing on immunity mediated by antitoxin (anti-ST and anti-LT) and antibodies to superficial bacterial colonization factor antigens (CFAs). Both living and non-living vaccines are being developed. Non-living vaccines will include killed E. coli with differing CFAs on their surface. Immunity to LT will be stimulated by residual LT B-subunit trapped in bacterial periplasm or by addition of purified B-subunit of LT. A major problem is that the ST is not naturally immunogenic. One approach to solving this has been the development of synthetic ST linked to a synthetic fragment of the LT B-subunit, the latter acting both as a carrier for ST and to stimulate an anti-LT response. In animal studies, this vaccine has been shown to stimulate both anti-ST and anti-LT responses when given orally, and to protect against challenge with virulent ETEC that produce either toxin. Initial studies in volunteers are under way. A second approach will be the development of E. coli mutants that secrete a chimeric form of ST (ST linked to another protein) which may render ST antigenic. Efforts have also been made to induce immunity by feeding subjects purified CFA antigens. These proved to be immunogenic in animals but were ineffective in volunteers, perhaps owing to destruction of the antigens by gastric enzymes.

A second approach that may be more promising is the development of live attenuated vaccines by recombinant DNA methodologies. Volunteer studies have shown that oral immunization with non-toxigenic E. coli that produce a specific CFA confers substantial protection against challenge with unrelated toxigenic E. coli that produce the same CFA. Further research has developed methods of cloning the genes for known CFAs into non-pathogenic E. coli for use as candidate vaccines. The same methods are also being used to create strains that produce only the B-subunit of E. coli LT. Thus, live vaccines that evoke immunity to both CFAs and LT are available for further study. The shortcomings to this approach are (1) the lack of a means to evoke anti-ST immunity, and (2) a lack of knowledge regarding the CFAs produced by a substantial portion of ETEC strains. The success of this approach would appear to depend upon the identification of additional CFAs so that vaccine strains producing these antigens can be prepared.

Another approach to live vaccine development is the cloning of genes for critical protective antigens into carriers other than E. coli. This has been done by inserting genes for the LT B-subunit and CFA antigens into the Ty21a live oral typhoid vaccine strain.

CONCLUSION

Considerable progress has been made and further important advances are expected in the development of vaccines for diarrhoeal diseases and typhoid fever. With regard to the development of practical and effective vaccines within the next few years, optimism is greatest for rotavirus diarrhoea, typhoid fever, and cholera. However, substantial progress is also being made toward vaccines for shigellosis and enterotoxigenic E. coli diarrhoea. It is anticipated that some of these vaccines, when developed, will be incorporated into existing national immunization programmes (EPI).

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