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tachment 1.
ACE SHEET)

ETHICAL REVIEW COMMITTEE

Date January 04, 1993

incipal Investigator P. K. Bardhan — Trainee Investigator (if any)

plication No. 93-003F. Qadri — Supporting Agency (if Non-ICDDR,B) SAREC

tle of Study LOCAL AND SYSTEMIC TIBODY RESPONSE TO A PERORAL INACTIVATED TEC VACCINE

Project status:
(✓) : New Study
( ) : Continuation with change
( ) : No change (do not fill out rest of form)

rcle the appropriate answer to each of the following (If Not Applicable write NA).

Source of Population:
- (a) Ill subjects — (Yes) No
- (b) Non-ill subjects — (Yes) No
- (c) Minors or persons under guardianship — Yes (No)

Does the study involve:
- (a) Physical risks to the subjects — Yes (No)
- (b) Social Risks — Yes (No)
- (c) Psychological risks to subjects — Yes (No)
- (d) Discomfort to subjects — (Yes) No
- (e) Invasion of privacy — Yes (No)
- (f) Disclosure of information damaging to subject or others — Yes (No)

Does the study involve:
- (a) Use of records, (hospital, medical, death, birth or other) — (Yes) No
- (b) Use of fetal tissue or abortus — Yes (No)
- (c) Use of organs or body fluids — (Yes) No

Are subjects clearly informed about:
- (a) Nature and purposes of study — (Yes) No
- (b) Procedures to be followed including alternatives used — (Yes) No
- (c) Physical risks — Yes (No)
- (d) Sensitive questions — Yes (No)
- (e) Benefits to be derived — (Yes) No
- (f) Right to refuse to participate or to withdraw from study — (Yes) No
- (g) Confidential handling of data — (Yes) No
- (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — Yes (No)

5. Will signed consent form be required:
   - (a) From subjects — (Yes) No
   - (b) From parent or guardian (if subjects are minors) — Yes (No)
6. Will precautions be taken to protect anonymity of subjects — (Yes) No
7. Check documents being submitted herewith to Committee:
   - ___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
   - ✓ Protocol (Required)
   - ✓ Abstract Summary (Required)
   - ✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
   - ✓ Informed consent form for subjects
   - ___ Informed consent form for parent or guardian
   - ___ Procedure for maintaining confidentiality
   - ___ Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

agree to obtain approval of the Ethical Review Committee for any changes lving the rights and welfare of subjects before making such change.

Pradip Bardhan
Principal Investigator

Trainee

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# LOCAL AND SYSTEMIC ANTIBODY RESPONSE TO A PERORAL INACTIVATED ETEC VACCINE

| | | |
|---|---|---|
| Principal Investigator | : | P. K. Bardhan |
| Co-Principal Investigator | : | F. Qadri |
| Co-Investigator | : | Z. U. Ahmed |
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| ICDDR,B | : | R. B. Sack |
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| | | J. Holmgren |
| Starting Date | : | March, 1993 |
| Completion Date | : | 1 year |
| Funding Source | : | SAREC |
| Budget | : | $ 73,300 |

This protocol has been approved by the Laboratory Sciences Division.

R. Bradley Sack

Associate Director

Date :

## A. INTRODUCTION

### 1. Objective:

a) General Aim :

Developement of a safe and practical ETEC vaccine which provides effective protection against ETEC infections in children and adults.

b) Specific Aims :

(1) To determine the local and systemic antibody responses to a peroral ETEC vaccine consisting of a combination of formalin-killed ETEC expressing CFA/I, CFA/II and CFA/IV, and a recombinant cholera-toxin B-subunit (RCTB).

(2) To compare the response between healthy volunteers recieving the ETEC vaccine and persons convalescing from ETEC-induced diarrhoea.

(3) To ascertain whether peripheral blood antibody response after vaccination is a valid reflector of intestinal anitbody response after vaccination.

(4) To study whether this formulation of ETEC vaccine is safe for human use.

### 2. Background :

Diarrhoea caused by enterotoxigenic *Escherichia coli* (ETEC) is the commonest cause of acute diarrhoea in developing countries, in travellers to these areas, and in residents of high risk areas (Black et al,1980; Sack DA et al,1977; Sack RB et al,1975). In different hospital and clinic based studies, ETEC has been detected in upto 50% of diarrhoeal patients, and in one third to one half of patients with travellers' diarrhoea (Black et al,1986). It is estimated that ETEC accounts for more than one billion diarrhoeal episodes per year, and about one million deaths annually in children in developing countries (Svennerholm et al,1989). Control of ETEC in developing countries would require institution and maintenance of sanitary facilities and abundant water supply requiring a substantial increase in standards of living, events unlikely to occur in the immediate future. Thus any intervention, including vaccines, even if partially effective, would be of great public health benefit (Sack DA et al,1991). However, no vaccine against ETEC for human use is yet available. Important research advances regarding the prominant virulence antigens of ETEC has led to the development of candidate vaccines.

To cause disease, ETEC must be able to colonize the small intestine and elaborate a heat-labile enterotoxin (LT) and/or a heat-stable enterotoxin (ST) (Wanke et al,1989). The *E. coli* LT closely resembles cholera toxin structurally and functionally, cross-reacts with Cholera toxin, and can evoke an immune response, whereas ST acts through the guanylate cyclase-cGMP system and is non-immunogenic unless experimentally conjugated to a larger protein. ETEC strains may produce LT only, ST only or both ST and LT. ST only and LT/ST producing strains are important causes of diarrhoea in endemic areas, whereas LT only strains frequently cause travellers' diarrhoea. The proportion of ETEC strains with different enterotoxin profiles varies from country to country. In Bangladesh, the majority of the ETEC strains belong either to the ST/LT or ST-only groups (Black et al,1980).

Colonisation of ETEC is mediated by antigenically distinct fimbriae termed colonization factor antigens (CFA). In ETEC strains pathogenic to man, the three main CFA's are CFA/I, CFA/II, and CFA/IV (Thomas et al,1982; Gothefors et al,1985). CFA/I is a single homologous fimbrial antigen, but CFA/II comprises the coli-surface(CS) associated antigens CS1, CS2 and CS3, and CFA/IV the CS4, CS5 and CS6 antigens. About 50-70% of all clinical ETEC isolates express these antigens, although the proportion of these CFAs varies from country to country (Svennerholm et al,1989). In Bangladesh, 75% of ST/LT and ST-only ETEC strains were found to express CFAs, though none of the LT-only strains were found to bear CFAs (McConnel et al,1985). That protective immunity against ETEC can be achieved is suggested based on the following observations -

(1) A decrease in ETEC attack rate with increasing age, and higher number of asymptomatic cases in adults than in children suggests development of naturally acquired protective immunity.

(2) A similar inference can be drawn from the observation that a degree of resistance against ETEC infection develops amongst expatriates during prolonged residence in a high-risk area.

(3) Naturally acquired clinical ETEC diarrhoea evokes a significant intestinal and systemic antibody response, both antitoxic (against LT) and antibacterial (against homologous CFAs and LPS) (Stoll et al,1986).

(4) Experimental studies in animals as well as human volunteers have shown that oral or intraintestinal adminstration with ETEC strains expressing CFA/I, CFA/II or CFA/IV gave rise to substantial protective immunity against re-challenge with ETEC of homologous enterotoxin and CFA type.(Ahren et al,1985; Levine et al,1981).

(5) Naturally induced anti-LT antibody response (native ST is non-immunogenic) is mainly against the B-subunit of the toxin, which cross-reacts with B-subunit of cholera toxin. It was found that immunisation with cholera toxin B-subunit in humans confers short-term protection against diarrhoea due to LT-ETEC (LT only and LT/ST), particularly notable against diarrhoea with severe dehydration (protective efficacy 86%), (Clemens et al,1988).

(6) Colostrum containing anti-LT antibodies are protective against ETEC diarrhoea in animal models (Dorner et al,1980).

(7) Both anti-enterotoxin and anti-CFA antibodies can, independently of each other, protect against experimental homologous ETEC infection, and when present together, these two types of antibodies co-operate synergistically in protecting against ETEC disease (Ahrens et al, 1982).

Based upon the knowledge about the important protective antigens of ETEC and the immune mechanisms operating against ETEC infections, an effective ETEC vaccine may be constructed which should induce antibacterial, antitoxic and anti-colonisation immune responses in the intestine (Svennerholm et al,1989). Candidate vaccines should be tested for safety, immunogenicity and efficacy first in adult volunteers, and if found promising should then be evaluated in children (WHO,1990).

The anti-bacterial component of such a vaccine should contain *E. coli* strains expressing the commom CFAs and also the main O antigens associated with LT/ST ETEC. The toxoid component of such a vaccine should consist of LT B-subunit. Even better would be to have a nontoxic ST-B subunit conjugate that could induce both anti-ST as well as anti-LT immunity, but such a toxoid is not available yet. A vaccine containing chemically cross-linked toxoid vaccine containing synthetic St-peptide and E. coli LT B-subunit has been found to be able to induce significant immune response in animals against both the toxin components (Klipstein et al,1983).

Since the B-submit component is relatively expensive and rather advanced equipment is required for its production, cheap recombinant B-subunit (RCTB) has been developed. The production costs for the B Subunit component has been be drastically reduced from >1 US dollar to a few cents per dose, thus making it suitable for inclusion into the vaccine also in developing countries.

One candidate ETEC vaccine has been successfully developed in Goteborg, Sweden (Svennerholm et al,1989). Each dose contained $1x10^{11}$ formalin-killed *E. coli* expressing CFA/I, the different CS components of CFA/II and CFA/IV, and as enterotoxoid, 1 mg of purified cholera

toxin B-subunit. Strains belonging to common ETEC O-groups and expressing the different fimbrae in high concentrations were selected. Formalin inactivation caused complete killing without any significant loss of CFA antigenicity.

This vaccine has recently been given a clinical trial testing for safety and immunogenicity in 60 adult Swedish volunteers of both sexes (Svennerholm et al,1991). The vaccine was adminstered perorally twice or thrice; gut- derived immune respose was examined in intestinal lavage fluid and antibody secreting cells (ASC) in peripheral blood, and systemic response by specific antibody titres in serum samples.

The results indicated that there was a significant intestinal IgA antibody response against CTB and the CFAs in >80% of the vaccinees, and about half of the vaccinees also responded to the O78 antigen. The magnitude of the intestinal antibody responses against the vaccine antigens were comparable to the responses previously noted in patients convalescing from CFA-positive ETEC infection. The vaccine also induced significant ASC increases in the peripheral blood in >85% of the vaccinees, as well as marked serum antibody responses against CTB and CFAs in most of the volunteers. No significant side-effects that could be ascribed to the vaccine were observed in any of the 60 volunteers, although a few volunteers noted the kind of mild bowel discomfort occassionally observed after intake of buffered bicarbonate solution. These encouraging results suggest that it is possible to induce substantial antibody responses in the gut against the most important virulence factors of ETEC by only two doses of an inactivated per-oral vaccine, and prompts further evaluation of this vaccine in an ETEC endemic area like Bangladesh.

## 3. Rationale :

ETEC remains the commonest diarrhoeagenic organism worldwide, and is particularly common in relatively poor developing countries. In the absence of effective environmental and sanitary control measures which can be implemented in the immediate future, development of a useful vaccine against ETEC is of top priority. No such vaccine for human use is yet available. A candidate ETEC vaccine has been shown to be safe and immunogenic in Swedish healthy volunteers. This vaccine needs to be evaluated for safety, immunogenicity, and protective efficacy in an ETEC endemic country, i.e. in persons probably immunologically primed by ETEC antigens secondary to previous natural exposures.

Previous studies have shown that serum antibody responses, particularly against the whole cell component of the vaccine were rather modest, most probably because the vaccine predominantly induced an intestinal mucosal immune response. Recent studies indicate that differences exist between intestinal and peripheral blood immune re-

sponses after enteric immunisation, as noted after examining the specific gut-derived ASCs frim duodenal mucosa and peripheral venous blood (Quiding et al, 1991). Intestinal lavage is very useful for assessing intestinal immune response, but obtaining lavage sample is time consuming, laborious, and requires hospital facilities. Moreover, obtaining intestinal lavage or small intestinal mucosal specimens from children, which is the ultimate target population for enteric vaccines, is impractical. Thus, it is also very important to identify a proxy indicator (like peripheral specific ASCs) for intestinal immune responses to enteric vaccination.

## B. SIGNIFICANCE :

Diarrhoea due to ETEC is an important problem for most developing countries and for travellers to these areas. Progress towards development of vaccines against this disease has been slow, and as yet there is no ETEC vaccine available for human use. However, important recent results on the ETEC virulence antigens have led to the development of possible candidate vaccine An effective and practical ETEC vaccine will have immense public health significance. In a recently completed clinical trial among Swedish voluntee one such candidate vaccine has been found to be safe and capable of evoking substantial immune response against the important ETEC virulence antigens. Before evaluating the protective efficacy of this vaccine, it should be more thoroughly re-examined regarding the safety and immunogenicity in a populati residing in an ETEC endemic area. The present study, by addressing these issues, will allow further evaluation of this candidate vaccine.

## C. METHODS OF PROCEDURE

. Subjects:

A total of 40 individuals will be recruited to participate in he study:

) 20 healthy volunteers of either sex aged 18-35 years, without any nown history of cholera or other severe watery diarrhoea (requiring V fluid or hospitalisation) during the last 6 months.

) 20 patients of either sex , aged 18-35 years, suffering from acteriologically verified ETEC diarrhoea. They will be recruited rom the patients coming to the ICDDR,B treatment center, and should ulfill the following criteria -

i. To be culture positive for ETEC in stool

ii. No other diarrhoeagenic organism isolated from stool

iii. Duration of diarrhoea before hospitalisation <24 hrs

iv. To have a stool output of at least 1.0 liter during the first 24 hours of admission.

e patients will receive the standard rehydration fluids ( I.V. or S, as necessary) and also the normal diet. No antibiotics will be escribed.

All subjects will be fully informed about the purpose of the study, the procedures involved, and the possible side-effects in relation to the immunization and sampling of specimens.

Before entering into the study all subjects will be carefully examined by a physician. Presence of any one of the following will cause exclusion from the study -

- i. Hypertension
- ii. Heart disease
- iii. Epilepsy
- iv. Glaucoma
- v. Chronic renal disease
- vi. History of cholera/severe watery diarrhoea in the previous 6 months ( for the volunteers)
- vii. Pregnancy ( in case of women).

The Vaccine :

Each dose of the vaccine will contain a combination of formalin-killed 5 different strains of ETEC expressing CFA/I and the different sub-components of CFA/II and CFA/IV, totalling $1X10^{11}$ organisms, and 1 mg of RCTB. The vaccine preparation will be pre-tested in Sweden for sterility and antigenicity according to the European Pharmacopeia, before being released.

2. Immunizations:

The 40 subjects, divided into 2 groups, will be immunized as follows -

| Group | | Vaccination Route | No. of immunisations |
|---|---|---|---|
| I | Healthy volunteers | PO | 2 (15 days apart) |
| II | Clinical ETEC diarrhoea patients | - | - |

The antigen mixture will be administered as a drink in 150 ml of buffered $NaHCO_3$ solution.

Following administration of the vaccine, toxigenicity surveillance will be carried out daily for 5 days or until any symptom that can be related to the immunization have disappeared, recorded in pre-designed forms.

3. <u>Clinical Specimens</u>:

The clinical specimens to be collected from the study participants are :

(i) venous blood,
(ii) intestinal lavage fluid,
(iii) duodenal juice (using String capsule),
(iv) duodenal biopsies.

They will be obtained according to the following schedule :

| | 1st sampling | 2nd sampling |
|---|---|---|
| Group I (volunteers) | 1 day before 1st dose of vaccine | 7-9 days after 2nd dose of vaccine |
| Group II (patients) | On admission in hospital | 5-7 days after admission |

1. Venous blood (10 ml) will be collected into heparinised vials and then seperated by gradient centrifugation on Ficoll/Isopaque, the mononuclear cells will be collected for ELISPOT assay and plasma will be frozen in aliquots for determining specific IgG, IgA and IgM aginst the antigens of interest (CFA/I, CS1 - CS5, RCTB).

From all the other clinical samples (described as follows), IgA levels against RCTB, O antigens, CFA/1 and CS1 - CS5 will be determined.

2. The lavage specimens will be obtained by allowing the participants drink an isotonic (Macrogol) salt solution until a watery diarrhoea ensues.

INTESTINAL LAVAGE METHOD

Macrogol salt solution:

| | |
|---|---|
| NaCl | 1.46 g |
| $NaHCO_3$ | 1.68 g |
| KCl | 0.75 g |
| $Na_2SO_4$ | 12.89 g |
| Macrogol 3000 | 60.0 g |
| Aq dist | add 1000 ml |

This solution is commercially available .

Lavage procedure:

Subjects will be instructed to drink the Macrogol solution 200—250 ml every 10 minutes. Drinking will be usually continued for 2½—4 hours. There will be be access to a reserved toilet. The initial stools that are passed, usually 500—1000 ml, will be discarded. Thereafter, when the stools become "liquid", i.e. relatively clear, they will be collected. Directly after collection, each individual liquid stool specimens will be filtered through gauze, and Soybean Trypsin Inhibitor (Sigma) to a final concentration of 0.05 mg/ml and EDTA (Sigma) to a final concentration of 50 mM will be added. The enzyme-containing specimens will be added in an icebath. After collection of approximately 1000 ml of (individually filtered and enzyme treated ) liquid stool, the different specimens will be pooled and centrifuged at 700 x g for 15 minutes. 100 mM phenylmethylsulfonyl fluoride (PMSF) in 95% ethanol will be added to the supernatant to 1% (vol/vol), and the supernate will be centrifuged at 10000 x g for 15 minutes. 2% (weight/vol) sodium azide will be added to the supernatant to a final concentration of 0.02% (vol/vol) and again 100 mM PMSF in 95% ethanol will be added to a final concentration of 1% (vol/vol). The mixture will be aalowed to stand at room temperature for 15 minutes, and then BSA will be added to a final concentrate 1mg/ml. The aliquots will be frozen in big Petridishes at -30°C and then at -70°C, freeze-dried, weighed, and then stored until use.

In ETEC diarrhoea patients during the acute stage, intestinal lavage will not be necessary, instead approx. 1.0 l of stool will be collected and processed as described above.

3. Duodenal juice will be collected with the duodenal capsule (string test). After at least 2 hours fasting, the capsule will be swallowed and the proximal end of the nylon string taped to the cheek and left in place for 3-4 hours, after which the line will be pulled out. The participant will remain ambulatory and will be allowed to drink water. Only persons with a very sensitive gag reflex are bothered by the presence of the thread in the throat. Removal of the thread is mildly disagreeable, but is accomplished rapidly. The bile-stained alkaline mucus clinging to the distal 20-30 cm will be scraped off by squeezing the string between two fingers into a small container. Usually approx. 0.5 ml can be obtained, which will be heat-inactivated, aliquoted after centrifugation, and stored at -70°C.

4. Duodenal biopsies will be obtained from 10 volunteers and 10 patients to obtain intestinal lymphocytes (Quiding et al, 1991). Consents for endoscopy and duodenal biopsy will be obtained in seperate consent forms. The biopsies will be obtained through endoscope using standard endoscopic biopsy- forceps; local anaethesia will be used. The duodenal biopsy samples will be rinsed thoroughly

with 0.01M phosphate buffer, 0.15M NaCl, pH 7.4, and cut into 0.1 x 0.1-mm pieces using tissue-choppers. After 2 washings with chilled Hepes- buffered Hank's balanced salt solution containing 1mM $CaCl_2$ and 10mM dithiothreitol (extraction buffer), the fragments will be dispersed in extraction buffer at 4°C with *B. thermoproteolyticus* thermolysin (Nordstrom et al, 1990). After 30 minutes extracted cells will be isolated by filtering through 150-µm nylon mesh. After further extraction with collagenase/dispase, filtration, pellation with centrifugation and washing, the cells will be incubated at 37°C for 30 minutes with deoxyribonuclease in Iscove's medium, and single cell suspecsions will be finally obtained by filtration through a 50-µm nylon mesh. String test for obtaining duodenal juice will not be performed on those subjects consenting to endoscopy and duodenal biopsy, as duodenal juice can be obtained during endoscopy.

## 4. <u>Laboratoy Assays</u>

Titres of IgG and IgA will be determined in serum samples, whereas only IgA antibodies will be assayed in the intestinal lavage and duoedenal juice samples. Antibodies to the different colonization antigens (CFA/1, CFA/II and CFA/IV) will be determined by ELISA, using purified CFAs as solid phase antigens. CFAs will be purified by the method of Evans et al (1979), and ELISA-coating concentrations of all the CFAs will be 1 µg/ml. Antibodies to O-antigens will be assayed by ELISA. O-antigens of the vaccine strains will be used as the solid phase antigens when analysing smples from the volunteers, whereas in case of samples obtained from the patients, the O-antigen for each test will be prepared from the predominat faecal ETEC isolate of the respective patient to be used as the solid phase antigen. The O antigens will be prepared by hot phenol-water extraction followed by extensive dialysis against water and then freeze-drying of the water phase; the coating concentration will be 100 µg/ml.

Anti-CTB antibody will be studied by means of $GM_1$-ELISA.

Total IgA content will be determined by microtitre ELISA; IgA titres will be expressed as a ratio of the total IgA content of each specimen. All ELISA determinations will be tested for specificity. The methods are described in detail by Stoll et al (1986).

Testing procedure for Lavage:

The samples will be tested 5-fold concentrated (as compared to that of the original lavage fluid). Total and specific IgA content will be analysed in specimens using appropriate ELISA methods, and the ratio of specific IgA and total IgA in each specimen will be determined.

Detection of antibody-secreting cells

Peripheral blood mononuclear cells (MNC) will be isolated from heparinized venous blood by gradient centrifugation on Ficol/Isopaque (Pharmacia, Uppasala, Sweden), and will be assayed for numbers of total and specific antibody-secreting cells, ASCs, by a two-colour micromodification (Czerkinsky, 1988) of the original enzyme-linked immunospot (ELISPOT) assay (Czerkinsky, 1983). Individual wells of nitrocellulose-bottomed 96-well plates (Millititer HA, Millipore Corp., Bedford, MA) will be coated with 0.1 ml of purified CFA/1 (10 µg/ml), CFA/II (20 µg/ml) or GM1 ganglioside (3 nmoles/ml), diluted in PBS, overnight at 4°C. After PBS-washes, the wells coated with GM1 will be further exposed to CT (2.5 µg/ml) for 2 h at 37°C. Following three additional washes with PBS, 0.1 ml of the MNC suspensions will be added to all wells, containing various numbers of cells ($5x10^4$-$1x10^6$), and the plates will be incubated for 3 h at 37°C in a humidified atmosphere supplemented with 7.5% $CO_2$. Thereafter, a mixture of two affinity purified goat anti-human immunoglobulin antibodies with distinct isotype specificities, one conjugated to alkaline phosphatase (ALP) and the other to horseradish perosidase (HRP) will be added to the wells. Plates will be incubated overnight with the enzyme-conjugated anti- immunoglobulins at 4°C, and will be thereafter developed by the sequential addition of the appropriate enzyme chromogen substrates. Spots, corresponding to the zones of antibodies secreted by individual cells, will be enumerated in quadruplicate wells under low magnification (x40), and data will be adjusted to numbers of spot-forming cells per $10^7$ MNC.

Total IgA-, IgG-, and IgM-secreting cells will similarly be enumerated in wells previously coated with affinity purified goat antibodies to the $F(ab')_2$ fragment of human IgG.

Total and specific antibody secreting cells obtained from the duodenal mucosa will be enumerated from the singlr cell suspensions in the same way as the peripheral blood lymphocytes.

D. DATA ANALYSIS :

The data obtained through the various assays will be analyzed and described with regard to clarifying the following points -

1. Frequency and magnitude of antibody response after immunization
   (a) by identifying significant titre rises (>=2 fold) in lavage and serum samples individual cases

   (b) by comparing mean antibody titres pre- and post- vaccination

   (c) by comparing ASCs pre- and post-immunization.

2. Comparison of the antibody and ASC response of the volunteers after immunization with that of the convalescent ETEC patients.

3. Comparison and correlation between immune response detected by the different methods (peripheral blood, lavage, duodenal juice and duodenal mucosa).

4. Frequency and magnitude of untoward effects (if any) attributable to the vaccine.

## E. FACILITIES REQUIRED :

Laboratory and office space is available. Data analysis facilities avilable in the centre is also adequate. However, space in the hospital for intestinal lavage needs to be arranged.

## F. COLLABORATIVE ARRANGEMENTS :

This study is being planned as a collaborative study between the ICDDR,B and the Department of Medical Microbiology, University of Göteborg, Sweden.

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WHO Memorandum (1990). Development of vaccines against cholera and diarrhoea due to enterotoxigenic Eschericia coli : Memorandum from a WHO meeting. Bull WHO 68:303-12.

BUDGET

| Personnel | | |
|---|---|---|
| Dr. P. K. Bardhan | 20% | 3,000 |
| Dr. F. Qadri | 15% | 3,100 |
| Dr. Z. U. Ahmed | 10% | 2,600 |
| Clinical Fellow | 100% | 2,500 |
| Sr. Research Officer | 100% | 11,000 |
| Laboratory Attendant | 100% | 2,000 |
| Cleaner | 25% | 500 |
| | | 24,700 |
| Patient Hospitalisation<br>30 pts x 5 days x $30/day | | 4,500 |
| Endoscopic Examinations<br>40 x $30 | | 1,200 |
| Equipments : | | |
| Virtis 2.0 L Freeze-drying Flask | 24 | 3,600 |
| Liquid Nitrogen Tank | 1 | 1,500 |
| 7.5% $CO_2$ Incubator | 1 | 2,000 |
| Wooden Commodes (Lavage Collection) | 2 | 400 |
| Laboratory Supplies : | | |
| ELISPOT Plates | 250 | 4,000 |
| ELISPOT Filtrate Collection Trays | 250 | 2,500 |
| Conjugates ( 1 ml) | 120 | 2,500 |

| Item | Quantity | Amount |
|---|---|---|
| Plasticware | | 5,000 |
| Immuno-chemicals and bio-chemicals | | 4,000 |
| Media, Sera, etc. | | 500 |
| Liquid Nitrogen | | 500 |
| **Laboratory Tests :** | | |
| Stool C/S (Cholera, Shigella, Salmonella, ETEC, Campy) | 50 | 1,750 |
| Stool M/E | 50 | 250 |
| Stationaries and Office-Supplies | | 250 |
| Local Transport | | 500 |
| International Travel<br>Goteborg-Dhaka-Goteborg Air-Ticket<br>Perdiem and honororium in Dhaka | | 9,500 |
| Data Analysis | | 1,000 |
| Printing and publications | | 500 |
| Medical Illustration | | 500 |
| Staff Clinic | | 250 |
| Volunteers | | 1,650 |
| Communications (Fax, Telex, etc.) | | 250 |
| | | 73,300 |

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Title: Local and systemic antibody response to a peroral inactivated ETEC vaccine

Summary of Referee's Opinions: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

Rank Score

| | High | Medium | Low |
|---|---|---|---|
| Quality of Project | ✓ | | |
| Adequacy of Project Design | ✓ | | |
| Suitability of Methodology | ✓ | | |
| Feasibility within time period | ✓ | | |
| Appropriateness of budget | | ✓ | |
| Potential value of field of knowledge | | ✓ | |

CONCLUSIONS

I support the application:

a) without qualification ☑

b) with qualification ☐

- on technical grounds ☐
- on level of financial support ☐

I do not support the application ☐

Name of Referee: Ivan Ciznar PhD, DrSc.

Signature: [signature] Date: October 22, 1992

Position:

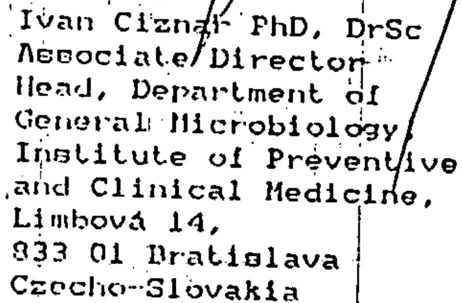
Institution: Institute of Preventive and Clinical Medicine, Limbová 14,
833 01 Bratislava
Czecho-Slovakia

Comments

The proposal "Local and Systematic Antibody Response to a Peroral Inactivated ETEC Vaccine" is focusdd to evalution of safety, immunogenicity and protective efficacy of the vaccine in an ETEC endemic country. The results obtained with this vaccine in volunteers in non-endemic country showed that the vaccine was safe and immunogenic. However interpretation of such data for endemic country would not be correct. Moreover protective efficacy data are absolute must for approval of any vaccine. Thus the aims of the proposal are clearly specified, the rationale based on recent information related to data from previous studies. There is no doubt that the study has a silnificance of a substancial merit. Methods are carefully selected. The project is feasible within the time interval and quantity of work proposed by the protocol.

Criticizm: - Looking at the bugdet, the specific aims and methods it seems to me that 15 % of a laboratory scientist participation in this project is too low. It is obvious that the major task in this project will be in laboratory analysis and evaluation of data (see specific aims). I would suggest to increase it to 25 %.

- Bugdet item includes international travel Göteborg - Dhaka - Göteborg. I would like to see under this item also international travel Dhaka - Göteborg - Dhaka in order to give a chance to a Bangladeshi scientist to get in touch with atmosphere of a high standard laboratory in Göteborg and to transfer new methods, approaches and organization into the ICDDR,B labs.



October 22, 1992

Ivan Ciznar PhD, DrSc
Associate Director
Head, Department of
General Microbiology
Institute of Preventive
and Clinical Medicine,
Limbová 14,
833 01 Bratislava
Czecho-Slovakia

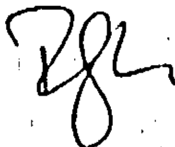
# FACSIMILE TRANSMISSION

Division of Viral Diseases
Center for Infectious Diseases
Centers for Disease Control
1600 Clifton Road, N.E.
Atlanta, Georgia 30333

| | |
|---|---|
| . Moyenul Islam<br>Associate Director<br>atory Sciences Division<br>R,B | From:<br>Roger I. Glass, M.D., Ph.D.<br>Chief, Viral Gastroenteritis Unit<br>Respiratory and Enterics Branch |
| : 880-2-883116 | FAX: (404) 639-3645 |
| es: 2 | Date: 10. 30 92 |

)r. Islam:

the review on "Local and systemic antibody response..." I apologize for the delay in getting you.



Toxigenic *E coli* remains one of the most important pathogens among Bangladeshi children. Research and local systemic antibody responses to a peroral ETEC vaccine parallels the approach used successfully in the development of an inactivated oral cholera vaccine. The preparation being examined has already been tested in Swedish volunteers and has been found to be free of adverse side-effects and immunogenic. Since Swedes have little previous exposure to toxigenic *E coli* the current proposal allows the vaccine to be examined in an endemic setting. The laboratory techniques are most suitable to this investigation, and bring to ICDDR,B the competent and skilled collaborators who will make it effective. The Swedish collaborators have a long history of productive work with ICDDR,B and this protocol continues this tradition. The protocol should be supported as it provides an important area of investigation for which ICCDR,B could be key.

[signature]
Atlanta, Ga 30333
Oct 30, 1990

Response to Dr. Ivan Ciznar

1. The time to be spent by the laboratory scientist is mostly for supervision and trouble-shooting, as most of the actual bench-work will be carried out by a senior research officer (100% time budgeted).

2. It was decided that instead of sending someone to Goteborg to get acquinted with advanced techniques, it would be better to bring a scientist from Goteborg to ICDDR,B for transfering new methods so that several persons can learn the relevant techniques.

Response to Dr. Roger Glass

No specific comments.