ment 1.

Date NOV 13, 1981

ETHICAL REVIEW COMMITTEE, ICDDR,B.

E9

ipal Investigator Dr. J.R. Murphy and Dr. M.I. Huq

ication No. 81-048

e of Study Development of potential ve oral vaccine strains of Vibrio holerae

Trainee Investigator (if any) _____

Supporting Agency (if Non-ICDDR,B) Harvard Med Centre.

Project status:

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

cle 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)
NA 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)
NA Informed consent form for subjects
NA Informed consent form for parent or guardian
NA 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.

All laboratory stock culture and phages will be tested. Does not involve human subject.

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

Principal Investigator

Trainee

87-048
Rec'd: 8.12.87

SECTION I - RESEARCH PROTOCOL

1. Title : Development of potential live oral vaccine strains of Vibrio cholerae

2. Principal Investigator : Dr. J.R. Murphy

   Co-Investigator : Dr. W.B. Greenough
   Dr. M.I. Huq
   Dr. David Relman

3. Starting Date : January 1, 1982

4. Completion Date : June 30, 1983

5. Total direct cost : US $ 29,315.00

6. Scientific Program Head :

This protocol has been approved by the Disease Transmission Working Group.

Signature of Scientific Program Head : A Samady

Date : 23/11/81

7. Abstract Summary :

In recent years there has been increased emphasis on the development of live oral vaccine strains of Vibrio cholerae. Mutant strains which produce extremely low levels of Cholera enterotoxin or defective toxin have been isolated in several laboratories. These strains have not been completely genetically characterized because of the lack of appropriate genetic methodologies. Within the last year it has become apparent that infection of toxinogenic strains of V. cholerae with certain vibriophages results in a loss of toxinogenicity. These mutants, as they stand, are possible live oral vaccine candidates, or may become candidates after modification. These strains are particularly attractive for vaccine development for the following reasons: (a) deletion mutations cannot

revert to toxinogenicity, (b) Cholera toxin deletion mutants are, in general, prototrophic and (c) mimic their parental strains in all ways, except for their toxinogenicity. These mutant strains will be studied in the proposed protocol for candidate strain for oral vaccine.

8. Reviews :

a. Research Involving Human Subjects : _____

b. Research Review Committee : _____

c. Director : _____

d. BMRC : _____

e. Controller/Administrator : _____

INTRODUCTION

1. Objective:

The overall objective of this proposal is to isolate and characterize toxin deletion mutants in Bangladeshi, clinical V. cholerae strains using mutagenic vibriophages. Several vibriophages have now been shown to insert randomly into the Vibrio chromosome (1), inducing discrete deletions at or near the sites of insertion. In this fashion, stable vibrio mutants can be created in which all detectable toxin gene sequences have been deleted (2). These mutants, isolated in Dacca, will be attractive live oral vaccine candidates. In addition, the technology involved in such work will be transferred to permanent research staff at the ICDDR,B.

2. Background:

Immunity against Cholera has been found to be generated following convalescence from clinical disease or from inapparent infection. It has been seen from our studies in Matlab during recent years that clinical Cholera or asymptomatic infection with V. cholerae raises the serum vibriocidal titre. Levine et al. have shown that volunteers who have recovered from induced Cholera are resistant to subsequent challenge with virulent Cholera vibrios. Moseley et al. studied the distribution of antibody in the control population of a Cholera vaccine field trial in Matlab and the relation of antibody titres to the pattern of endemic Cholera. They observed that age-associated immunity is attributed to repeated antigenic stimuli provided by periodic natural exposure to the etiologic agent. In contrast, Feeley and Gangarosa have (4) observed that attempts to atimulate immunity artificially by means of various antigens (Intact killed bacteria, isolated somatic antigen, and Cholera entero-

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toxoid preparations) administered parenterally have had limited success; the immunity so generated is neither absolute nor long lasting.

During the disease itself, which may now be defined as an effective method of local immunization, the host is presented with a variety of Vibrio antigens, both known and occult. These include flagellar antigen, somatic (LPS) antigen, colonization factor antigen(s) such as Cholera lectin and outer membrane proteins, various vibrio extracellular enzymes such as proteases, mucinases and neuraminidases which map play important role in virulence, and lastly, the Cholera enterotoxin (choleragen). It is not known which of these play(s) a predominant role in the stimulation of immunity. Although it is not unlikely that local antibodies against the various antigens may act synergistically, individual components have been shown to be effective in experimental models. For example, as first demonstrated in mice by Fujita and Finkelstein and subsequently confirmed by other workers, peroral administration of various forms of purified Cholera enterotoxin antigen resulted in demonstrable immunity. Woodward et al. (5) used an avirulent, hypotoxigenic mutant M13 isolated by Finkelstein et al. ( ) and demonstrated that substantial immunity can be achieved in the absence of substantial amount of toxin antigen in the volunteers. Although the experiment established the feasibility of immunization of man with living attenuated Cholera vibrios, M13 had limitations, which restricted its further use.

Honda and Finkelstein reported in 1979 (3) the isolation of an avirulent mutant of V. cholerae using nitrosoguanidine which produced only the B subunit of the Cholera toxin. This $A^{-}B^{+}$ mutant, marked by Streptomycin resistance, was designated Texas star SR. Honda and Finkelstein reported that intestinal colonization of Texas star SR in Chinichillas resulted in immunity to challenge with

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wild type Cholera vibrios and demonstrated that antiserum to its isolated choleragenoid had high titre neutralizing antibody against the purified choleragen related to heat labile enterotoxin (LT) of E. coli. This mutant also has some problems, namely, its unclear genetic nature, its propensity to cause loose stools in some volunteers, and the lack of heterologous immunity it induces. It is unknown at what rate this NTG-mutant may revert to toxigenicity in a natural environment.

A randomly-inserting, mutagenic vibriophage, VcA1, has been described recently and is thought to be prevalent as a integrated, prophage (1). It induces a wide range of auxotrophic mutations at, or near, its site of insertion. Mekalanos et al. (2)have demonstrated that phages VcA1 and VcA2 induce deletions of toxin structural genes in V. cholerae as detected by loss of labelled LT-probe hybridization (Southern blotting method). These deletion mutants are stable and retain all other phenotypic characteristics. We now have an additional vibriophage, isolated from a Texas V. cholerae strain, which is capable of mutagenesis.

A toxin deletion mutant derived by phage mutagenesis of a virulent V. cholerae strain from Bangladesh may be the most suitable live oral vaccine candidate for introduction back into the Bangladesh environment. The strain will be adapted to the local environment and, in addition, local scientists will be integrally involved and responsible for the isolation and characterization of the strain.

B. SPECIFIC AIMS

1. To develop techniques of vibriophage mutagenesis of V. cholerae leading towards the genetic construction and development of Cholera tox deletion

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mutants for oral vaccine development in ICDDR,B facilities in Dacca.

2. To test the mutant strains thus obtained extensively for toxin production, virulence, genetic stability, and immunogenicity.

3. To train Bangladeshi scientists in techniques of phage genetics and genetic engineering so that research in these fields may continue independently in Dacca.

C. METHODS AND PROCEDURES

A suitable, virulent toxigenic clinical V. cholerae isolate will be selected and then subjected to infection by phages VcA1, VcA2, and E507. Nontoxigenic mutants will be screened for, using a ganglioside filter assay with LT probe (as per S. Moseley). Tox^- mutants will be purified. The presence of toxin gene sequences will be assessed using a Southern blot of endonuclease restriction DNA digests. Once potentially nontoxigenic, avirulent mutant strains are found, they will be tested on rabbits by either intestinal inoculation or by intragastric feeding; placebo-treated animals will act as controls. The animals will be challenged after one month with live V. cholerae virulent strains and the protection noted using already established techniques;vibriocidal antibody responses will also be noted.

The mutant strains obtained from this study will also be tested in an artificial tank containing local pond water covered with water hyacinth and the viability, reversion of toxinogenesis or any other transformation will be studied.

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D. SIGNIFICANCE

The currently available methods for the molecular genetic characterization of mutation in V. cholerae are insufficient. The development of potential live oral vaccine is therefore impeded. At present, we cannot make rational predictions about rates of reversion of candidate vaccine strain to wild type phenotype. The successful development of live oral vaccine strains and their introduction into Cholera endemic areas is dependent upon i) their efficacy and ii) their genetic stability. The understanding of the molecular genetic basis of all mutations introduced into candidate vaccine strains is essential. The use of phage-mediated deletion mutagenesis will insure genetic stability in a potential live oral cholera vaccine strain. The transfer of technical expertise to ICDDR,B scientists will allow active, continuation of this work in Dacca.

E. FACILITIES REQUIRED

a. Laboratory spaces - Bench spaces in Dacca is available - no extra space will be required. The initial phase of this research would be conducted in Boston for about 4-5 months. All chemicals and equipment to be used there are available through the NIAID grant for Dr. Murphy.

b. Appropriate vibriophages will be provided by Drs. Murphy and Relman.

c. Dark room and supplies for autoradiographic analysis - available in ICDDR,B.

F. COLLABORATIVE ARRANGEMENTS

This study will be a collaborative project between ICDDR,B Dacca and Harvard Medical School, Boston.

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Salary: Salary support for the ICDDR,B staff person while in Boston will come from Dr. Murphy's grant AI-13938.

Salary for Dr. Relman and Dr. Murphy while in Dacca is not required.

Housing: Dr. Murphy will assist in finding short-term housing for the ICDDR,B staff person in Boston.

Dr. Relman and Dr. Murphy will require Guest House accommodations while in Dacca.

Supplies: Dr. Murphy will provide all supplies for research conducted in Boston.

Research supplies for work done at the ICDDR,B will be provided by the ICDDR,B and Dr. Murphy.

Publications:

Publications resulting from research conducted will be co-authored by ICDDR,B contributors and contributors from Dr. Murphy's laboratory.

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SECTION III - BUDGET

1. PERSONNEL SERVICES

Name	Person month	Annual salary TK.	Project TK.	requirements US $
Dr. W.B. Greenough	-	-		-
Dr. J.R. Murphy	100% 3 months	-		-
Dr. M.I. Huq	15% 13 months			3,900.00
Dr. David Relman	100% 3 months	-		-
Mr. Q.S. Ahmed	35% 18 months	3,972.00	25,023.00	
Mr. A. Alim	25% 13 months	4,757.00	15,460.00	
Dr. A. Al-Mahmud	10% 9 months	8,164.00	7,347.00	

2. SUPPLIES AND MATERIALS

Media	900.00
Chemicals	850.00
Glass and Plastic ware	1,200.00
Millipore filter 0.22μ - 2000	825.00
Rabbit - 200	

3. EQUIPMENT

Millipore sweeney - 10	650.00
Double distillation apparatus - one	5,500.00
Controlled temperature incubator - 2	3,000.00

4. PATIENT HOSPITALIZATION

None

5. OUT PATIENT CARE

Not required

6. TRANSPORT

1500 miles @ Tk. 4.00	6,000.00

	TK.	US $
7. TRAVEL AND TRANSPORTATION OF PERSONS		
2 round trip BOSTON-DAC-BOSTON for Dr. Murphy and Dr. Relman		4,800.00
One round trip DACCA-BOSTON-DACCA for Mr. Shafi		1,200.00
8. TRANSPORTATION OF THINGS		
Cultures, media, chemicals from Boston		300.00
9. RENT COMMUNICATION AND UTILITIES		
Guest House charge for Dr. Murphy and Dr. Relman - total 90 days @ $30 per day		2,700.00
10. PRINTING AND REPRODUCTION		500.00
11. OTHER CONTRACTUAL SERVICES		
None		
12. CONSTRUCTION, RENOVATION, ALTERATIONS		
None		
Total:	Tk. 53,830.00 = $2,990.00	$ 26,325.00

Total budget requirement: $26,325.00 + $2,990.00
= $ 29,315.00

References

1. Johnson, S.R., Liu, B.C.S., and Romig, W.R. Auxotrophic mutations induced by Vibrio cholerae mutator phage VcA1. FEMS Microbiology Letters 11: 13-16, 1981.

2. Mekalanos, J.J., Moseley, S.L., Murphy, J.R., and Falkow, S. Isolation of enterotoxin structure gene deletion mutations in Vibrio cholerae induced by two mutagenic vibriophages. Proc. Natl. Acad. Sci. U.S.A., in press.

3. Honda, T., and Finkelstein, R.A. Seletion and characteristics of a Vibrio cholerae mutant lacking the A (ADP-ribosylating) portion of the cholera enterotoxin. Proc. Natl. Acad. Sci. U.S.A. 76: 2052-2056, 1979.

4. Feely, J.C. and Gangarosa, E.J. Field trials of cholera vaccine. In cholera and Related Diarrhoeas, 43rd Novel Symposium, Stockholm, 1978, pp.204-210 (Karger-Basel), 1980.

5. Woodward, W.E., Gilman, R.H., Hornick, R.B., Libonati, J.P. and Cash, R.A. Efficacy of a live oral cholera vaccine in human volunteers. Develop. Biol. Standard. 33: 108-112 (S. Karger-Basel), 1976.