ttachment 1.

Date 14.3.1981

ETHICAL REVIEW COMMITTEE, ICDDR,B.

rincipal Investigator Leif Gothefors Trainee Investigator (if any) _____

pplication No. 81-014(P) Supporting Agency (if Non-ICDDR,B) _____

itle of Study Influence of WCV on the immunogenicity of B-subunit given by the oral route

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

ircle 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 NA
   (c) Physical risks — Yes No NA
   (d) Sensitive questions — Yes No NA
   (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 NA

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.

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

Principal Investigator

Trainee

81-014(P)
rec'd 30/3/87

SECTION I - PILOT STUDY

1. Title: INFLUENCE OF WHOLE CELL CHOLERA VACCINE (WCV) ON THE IMMUNOGENICITY OF B-SUBUNIT GIVEN BY THE ORAL ROUTE

2. Investigators: L. Gothefors, A.M. Svennerholm

3. Starting date: 1st May 1981

4. Completion date: 31st July 1981

5. Total direct costs: US$ 2665

6. Scientific Program Head:

This protocol has been approved by the combined Pathogenesis Therapy and Host Defence Working Groups.

Signature of Scientific Program Head : W.B.

Date : 24/3/87

7. Abstract Summary:

A limited protocol is proposed to evaluate if cholera whole cell vaccine has a negative influence on the immunogenicity of the cholera toxin B subunit given at the same time. Twenty-four female volunteers will be immunized orally or parenterally and the immune response will be evaluated from serum and saliva samples taken before and 14 days after immunization.

8. Reviews:

a. Ethical Review Committee: _____

b. Research Review Committee: _____

c. Director: _____

d. BMRC: _____

e. Controller/Administer: _____

SECTION II - PLAN OF PILOT STUDY

A. INTRODUCTION

1. Objectives: To compare the serum and salivary antitoxin antibody response after oral administration of B subunit alone and in combination with oral or parenteral whole cell vaccine.

2. Background information: In the initial B subunit study (protocol No.79-009) 12 women were given an oral immunization with 500 µg of B subunit each. Eleven of these women responded with a significant IgG antibody response in serum (sevenfold geometric mean increase) and in 7 there was a significant IgA antibody response (three-fold mean increase). A peroral booster immunization did not increase either the IgG or the IgA antibody responses in serum further. Also in intestinal lavage a single oral immunization resulted in significant titer increases in most of the cases. Thus, 9 of 11 volunteers responded with an IgA increase in intestine (as a mean ten-fold) and 8 of 11 with a similar IgG increase. A booster immunization induced titer increases of about similar magnitude and duration as did the primary immunization. Furthermore, this study showed that there was a statistically significant correlation between IgA antibody responses in intestinal lavage and in saliva.

Within the present protocol (80-029) 8 women have been given one oral immunization with 500 µg of B subunit together with WCV ($5x10^{10}$ killed Inaba and Ogawa vibrios). Preliminary analyses of the antibody responses

in serum and intestinal lavage from these women have been undertaken using the same methodology that was used in the previous study. The results from the present study show one interesting difference from those obtained in the 1979 trial. Thus, none of the women receiving the combined vaccine responded with a significant antitoxin antibody response in serum after a single immunization. The same was true for the responses in intestinal lavage. Thus, a significant IgA antibody response was only found in lavage of one of the women after a primary immunization.

Interestingly, 6 of 8 responded with a significant IgA antitoxin antibody response after the booster immunzation. This secondary response was of similar magnitude and duration as noted in intestine in the study 1979.

There are several possibilities for this discrepancy between the responses after a single immunization in the present and the previous study. One possibility might be that the degree of natural priming has varied from one year to another, so that the women in the 1979 study were more efficintly primed. Comparison of serum samples from the two studies, however, showed very similar preimmunization levels in the two study groups. Another possibility might be that the whole cell vaccine has a negative influence on the immunogenicity of the B subunit (antigen competition? Immunosuppression? etc). If this is true 500 µg of B subunit may be a too small dose for priming but may be effective for boosting.

B. SPECIFIC AIMS

To investigate, in concurrently tested study groups, whether whole cell vaccine has a negative influence on an orally given B subunit.

If this pilot-study confirms the results from 1979 and 1980, i.e., WCV has a suppressing effect on B subunit, no further studies of this kind are needed.

If the pilot-study contradicts older results or is difficult to evaluate, a larger study has to be undertaken in the autumn 1981 where the immune response is evaluated in 3 groups orally immunized with a) WCV, b) WCV + B subunit and c) B subunit. In such a study lavages have to be done.

C. METHODS OF PROCEDURE

1. Subjects: Twenty-four female volunteers will be sought for in Nandipara. They must fulfil the following criteria:

   a) to be healthy

   b) to be non-pregnant (only the I.M. Group)

All subjects will be fully informed about the purpose and procedure of the study. Informed consent will be required from every patients.

2. Immunizations: The women will be divided in three groups (8 in each):

   a) 500 µg B subunit P.O.

   b) 500 µg B subunit P.O. + 5×10^{10} killed vibrios (WCV) P.O.

   c) 500 µg B subunit P.O. + 6×10^{9} killed vibrios (WCV) I.M.

The oral WCV contains 5×10^{10} vibrios in 2.5 ml; the parenteral contains 8×10^{9} per ml.

3. Clinical specimens: Serum and saliva samples will be taken on day 0 and on day 14.

   The methods for sampling and handling of the specimens are the same as previously described.

4. Laboratory assays: Serum samples will be analyzed for antitoxin and antibacterial antibodies of IgG and IgA classes, while saliva is analyzed for the same antibodies but only of IgA class.

5. Data analysis: The data obtained will be compared with those obtained in the 1979 and 1980 studies with regard to the following questions:

   a) Does oral WCV suppress the antibody response to B subunit?

   b) If so, does also parenterally given WCV have this effect?

D. SIGNIFICANCE

Results of animal studies indicate that antitoxic and antibacterial antibodies in the gut cooperate synergistically in the host resistance to cholera.

After the encouraging results from administration of oral B subunit it was a logical approach to give the B subunit in combination with a whole cell vaccine. When, however, our preliminary analysis indicate that none of the women receiving the combined vaccine responded with a significant antitoxin antibody response in serum and lavage after a single immunization, we must ask ourselves: Is this combination useful? We have also to realize that our knowledge of local immunity is very limited; more basic information on intestinal immunity in humans is badly needed before we consider the use of oral enteric vaccines.

E. FACILITIES REQUIRED

Transport will be needed to and from Nandipara. Laboratory specimens will be handled by the Immunology Branch. The clinic in Nandipara needs a face-lift, but no new facilities are required.

F. COLLABORATIVE ARRANGEMENTS

Department of Microbiology, Goteborg will collaborate as earlier.

ABSTRACT SUMMARY

1. The population to be studied includes village people from Nandipara, representative of those who would benefit from a potential cholera vaccine.
2. The risks related to this study are minimal. The antigens have already been extensively used in Sweden and Nandipara with no untoward effects. The blood samples are of small volume.
3. Surveillance by a physician will be maintained after giving the antigens.
4. The volunteers are identified only by study number.
5. Signed formal consent will be obtained.
6. No interview.
7. The individual subject may benefit if the antigen provides some protection against cholera. The society in general would benefit if a successful cholera vaccine is developed.
8. The project will require specimens of blood and saliva. It will not require hospital or other records.

PERMISSION FORM - IMMUNIZATION WITH A COMBINATION OF WHOLE CELL CHOLERA VACCINE AND B SUBUNIT ANTIGEN

The International Centre for Diarrhoeal Disease Research, Bangladesh is carrying out research to determine the immune response to a material which may some day be used as a cholera vaccine. This material, which is composed of common whole cell vaccine and a natural toxoid (B subunit antigen) will stimulate the body to make protective substances against cholera, but it has no harmful activity. We would like you to participate in this study. If you decide to participate, you can expect the followings:

1. You will have the vaccine(s) given on one occasion, either by injection or by mouth.
2. We will collect samples of saliva and blood at two times.
3. You will be visited after your vaccine is administered to look for any reaction.
4. You do not have to join the study. If you decide not to join, you will still be elligible for care at ICDDR,B. You may also decide to withdraw after entering the study and this will not affect any medical care you might require now or later on.
5. Your medical records will be kept confidential.

If you decide to join the study, please sign here -

_____

BUDGET SUMMARY

		Dollars
1.	Personnel Services	1050
2.	Supplies and Materials	400
3.	Equipment	0
4.	Hospitalization costs	0
5.	Outpatient care	0
6.	ICDDR,B Transport	15
7.	Travel	1000
8.	Transport of things	200
9.	Rent/Communication	0
10.	Printing	0
11.	Contractual services	0
	Total :	2665

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সম্মতি পত্র

(সম্পূর্ণ কোষশূন্য সাব ইউনিট' সম্বন্ধে টিকা)

বাংলাদেশ আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র কলেরা জীবাণু সম্পূর্ণ কোষ হতে তৈরী এন্টিজেনের সংগে 'বি সাব ইউনিট' যুক্ত করে একটি নূতন টিকা আবিষ্কারের জন্য গবেষণা করছে। শরীরে এই ঔষধের প্রতিক্রিয়া নিরূপণ করাই এই গবেষণার উদ্দেশ্য। এই ঔষধ শরীরে কলেরা রোগ প্রতিরোধ শক্তির সৃষ্টি করে এবং এর কোন ক্ষতিকর প্রতিক্রিয়া নাই। আশা করি আপনি আমাদের এই গবেষণায় অংশ গ্রহণ করবেন। আপনি যদি অংশ গ্রহণে রাজী থাকেন তাহলে নিম্নে বর্ণিত নিয়মগুলো লক্ষ্য করুন:

১। আপনাকে একবার মাত্র টিকা নিতে হবে। ঔষধটি খেতে হতে পারে অথবা ইনজেকশন ও হতে পারে।

২। আমরা দুইবার আপনার থুথু ও রক্তের নমুনা সংগ্রহ করব।

৩। টিকা দেবার পর আমরা ঔষধের প্রতিক্রিয়া দেখার জন্য আপনার মৌজ-খবর নেব।

৪। গবেষণায় অংশগ্রহণ না করলেও আমরা এই কেন্দ্রে আপনার প্রয়োজনীয় চিকিৎসা করব। গবেষণা চলাকালীন যে কোন সময়ে আপনি চাইলে গবেষণা পরিত্যাগ করতে পারবেন। তাতে এখন এবং পরে আপনার প্রয়োজনীয় চিকিৎসার কোন ত্রুটি হবে না।

৫। গবেষণায় কাগজ-পত্রাদি গোপন রাখা হবে। আপনি অংশ গ্রহণ করতে চাইলে এখানে সই করুন
