tachment 1.
ACE SHEET)

ETHICAL REVIEW COMMITTEE, ICDDR,B. Library Date 21/7/93

Principal Investigator DR. FIRDAUSI QADRI Trainee Investigator (if any)

Application No. 93-024 Supporting Agency (if Non-ICDDR,B)

Title of Study Local and systemic immune response in patients in a diarrhoeal idemic due to Vibrio cholerae 0139

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

Circle 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 NA

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

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 NA
(c) Use of organs or body fluids Yes No NA

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 NA
(d) Sensitive questions Yes No NA
(e) Benefits to be derived Yes No NA
(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 NA

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.

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

Firdausi Qadri
Principal Investigator

Trainee

ENTERED 2 1 JUN 1993

APPLICATION FOR PROJECT GRANT

1.	PRINCIPAL INVESTIGATOR	:	Firdausi Qadri
2.	OTHER INVESTIGATORS		
	COINVESTIGATORS	:	Tasnim Azim M. John Albert Ann-Mari Svennerholm
	CLINICIANS	:	M.A. Salam Ali Miraj Khan
	SCIENTIFIC COORDINATOR	:	R. Bradley Sack
3.	TITLE OF PROJECT	:	Local and systemic immune response in patients in a diarrhoeal epidemic due to *Vibrio cholerae* 0139
4.	STARTING DATE	:	As soon as possible
5.	COMPLETION DATE	:	Two years
6.	TOTAL BUDGET REQUESTED	:	US$ 161,983
7.	FUNDING SOURCE	:	To be named
8.	HEAD OF PROGRAMME	:	Dr. R. Bradley Sack Associate Director Laboratory Sciences Division

R. Bradley Sack

9. AIMS OF PROJECT

a) **General aim**

The aim of this protocol is to examine the cellular and humoral immune responses, both systemic (peripheral blood) and local (saliva), in adult humans with diarrhoea due to the new strain of *Vibrio cholerae* 0139 and

compare the response with patients with diarrhoea due to *V. cholerae* 01, Inaba/Ogawa serotypes.

b) Specific aims

1) To compare the presence of specific antibody secreting cells (ASC) of different isotypes (IgA, IgG, IgM) in the peripheral blood of patients with diarrhoea due to *V. cholerae* 0139 and *V. cholerae* 01.

2) To determine the antibody response (IgA, IgG isotypes) to different antigens (LPSs, cholera toxin, adhesion antigens) in plasma and saliva and correlate it with the presence of ASC in peripheral blood.

3) To compare the levels of antibacterial antibody levels in serum (vibriocidal assay) in patients with diarrhoea in the study group against *V. cholerae* 0139 and *V. cholerae* 01 Inaba/Ogawa serotypes.

4) To identify possible alterations in the lymphocyte responses in the study groups using phenotyping with specific monoclonal antibodies (proportions of CD3, CD4, CD8, CD20 and CD25).

5) To measure the levels of Interleukin-2 (IL-2) and interferon Y (IFN-Y) in the study groups.

c) Rationale

Epidemics and pandemics of cholera are known to be caused by strains of *V. cholerae* 01 of either Classical or El Tor biotypes and Inaba or Ogawa

serotypes. However, the current epidemic sweeping Bangladesh and India is known to be caused by *V. cholerae* non 01. It has been assigned the serogroup 0139 with the suggested name of "Bengal". No non-01 *Vibrio* has ever been reported to cause large epidemics of severe clinical cholera-like disease. This, therefore, seems to be a new variant of *V. cholerae* 01 which has just emerged, about which very little is known. A study of the immune responses generated in these patients and their link to the various antigens on the bacteria is necessary to our understanding of the disease and production of vaccines to effectively immunize against the disease.

d) Significance

This recent outbreak of diarrhoea due to new serotype *V. cholerae* 0139 raises the question of whether the antigenic components that are present in the field-tested oral cholera vaccine or new experimental vaccines are adequate for protection against the new serotype. There is also a need to know if other components have to be added to available vaccines to make them more effective against possible outbreaks involving conventional strains as well as the *V. cholerae* 0139. A study that will link the immune response of patients in this new outbreak to the antigenic determinants on the epidemic strain will contribute significantly towards the understanding, containment and prevention of any future epidemics due to the new serotype of *V. cholerae*. The results obtained in this study may lead to the development of a new and more effective vaccines against *V. cholerae* 01 and/or *V. cholerae* 0139.

10. ETHICAL IMPLICATIONS

The following groups of patients will be studied:

Disease condition	No. of* patients	Age of patients (yrs)	Samples	Source of patients
Watery diarrhoea due to *V. cholerae* 0139	30	18-40	Peripheral blood Serum Saliva Stool	ICDDR,B
Diarrhoea due to *V. cholerae* 01 (Inaba/Ogawa)	30	18-40	Peripheral blood Serum Saliva Stool	ICDDR,B
**Healthy controls (of similar socioeconomic status as patients, non-diarrhoeal)	60	18-40	Peripheral blood Serum Saliva Stool	Volunteers

*Both male and female

** Controls must be without any history of diarrhoea for at least the last 2 months. They should also have no history of illness (fever, cold, cough) in the last one month. Volunteers should preferably be people who are not working in the ICDDR,B hospital or laboratories. They will be recruited by investigators and technicians involved in the study after having signed a consent form. The volunteers will be chosen so that they are of the same socioeconomic status as the patients.

The required sample, n, for estimating different immunological parameters for each of the groups has been obtained using the following equation:

$$n = \frac{\alpha^2 \delta^2}{\epsilon^2}$$

Where, α is the value of normal variate for which the estimated value will be within $\pm\epsilon$ of the population value with a probability of $(1-2\alpha)$. The variance of different immunological markers have been considered as before (T. Azim,

P.I. of ongoing protocol # 89-014) and it has been found that a sample size of 30 is sufficient to limit the error within 20% of the population parameter with 95% confidence level.

The following samples will be obtained each time:

Peripheral blood (10 ml) (for differential leucocyte, mAbs, Hb, estimation of lymphocytes, plasma)	:	On day of diagnosis (i.e. d1) on day 4, and on day 10 on day 21
Peripheral blood (2 ml) for cytokine assays in EDTA vials	:	As above
Peripheral blood (2 ml) for serum	:	As above
Saliva (1-2 ml)	:	As above

In controls, only a single set of samples will be collected.

Adults coming to the ICDDR,B Clinical Research Centre (CRC) with acute watery diarrhoea of not more than 24-h duration resembling cholera will initially be enrolled in the study. Samples will be taken from patients culture-proven positive for *V. cholerae* 01 (Inaba/Ogawa) and *V. cholerae* 0139. Peripheral blood (14 ml) and saliva samples (1-2 ml) will be collected on confirmation of disease, on day 1 (day of diagnosis), 4, 10 and 21 days after onset of diarrhoea, as shown above. Such volumes of blood will not be harmful for the patients. The patients will be clinically evaluated by a daily physical examination and will have a standard six-hourly monitoring of oral temperature, pulse rate and respiration. The haemoglobin percentage, total and differential leucocyte counts will be measured. The study will not interfere with the management and treatment of the patients and none of the procedures will be harmful. Written consent will be obtained from the

patients and controls. Patients will be released from the hospital on day 4 if diarrhoea is controlled and requested to return for two follow-ups, 6 days and 17 days from discharge.

The clinical aspects of the study, such as patient enrollment and management, will be carried out by the clinicians as per the schedule followed in the CRC, ICDDR,B.

11. BACKGROUND, RESEARCH PLAN AND BIBLIOGRAPHY

A) BACKGROUND

Epidemic of cholera due to *V. cholerae* 0139 synonym Bengal

Recently a cholera outbreak of epidemic proportions in Bangladesh (Albert *et al.*, 1993) is in progress, and it is now estimated that nearly 200,000 people have so far been affected with approximately 2000 deaths (Government of Bangladesh, Epidemic Surveillance). A similar outbreak has also been reported from India (Ramamurty *et al.*, 1993). This is the first time that a *V. cholerae* non-01 serogroup has caused such large epidemics of cholera-like illness. Results suggest that there are major difference between the new serotype and other *V. cholerae* non-01 strains that have been studied previously (Shimada and Sakazaki, 1979). The latter, in general, are known to cause sporadic cases of diarrhoea and have not been associated with large epidemics and outbreaks such as the present one. The *V. cholerae* non-01 strains infrequently produce little, if any, cholera toxin, as opposed to the current epidemic strain, which produces large amounts of cholera toxin as evidenced directly, by laboratory tests (Albert *et al.*, 1993a, 1993b; Shimada *et al.*, 1993) and indirectly, from the severity of the dehydration seen in patients in the ICDDR,B CRC and Matlab DTC. In addition to cholera toxin,

strains of *V. cholerae* 0139 have been found to express mannose-sensitive haemagglutinin (MSHA) (Holmgren *et al.*, personal communication) and a fimbrial antigen described by Ehara *et al.* (Ehara *et al.*, 1991). These two antigens are also present in *V. cholerae* 01. The clinical severity parallels that caused by *V. cholerae* 01. Another interesting observation is that the epidemic, in contrast to earlier outbreaks, has affected the adult population more than children, thus presenting as cholera does historically when striking an immunologically virgin population. It is likely that the recent epidemic has affected a population that did not have immunity to the new strain. The epidemic strain is thus different from *V. cholerae* 01 and also unrelated to the known 137 serogroups of *V. cholerae* non-01. Therefore, it has been assigned to a new serogroup 0139 with the suggested name of "Bengal" to symbolize its first isolation from the coastal areas of Bay of Bengal (Shimada *et al.*, 1993).

Cholera due to *V. cholerae* 01 and the humoral immune response

Cholera is endemic in Bangladesh and shows two peaks, one in the hot season (March, April, May and June) and the other in the post-monsoon season (September, October, November and December). It is an important cause of illness and death in Bangladesh and other developing countries of the world and currently involves both hemispheres in the 7th pandemic. It is now understood that an oral cholera vaccine which will stimulate intestinal immunity can provide protection against the disease (Holmgren *et al.*, 1989). The B subunit-whole cell oral cholera vaccine, which contains the B subunit of the cholera toxin and heat- and formalin-killed whole cells composed of four different strains belonging to Inaba/Ogawa serotypes and the classical and El Tor biotypes, has been field-tested in Bangladesh (Clemens *et al.*, 1990).

Studies have shown that serum IgA and IgG anti-toxin and vibriocidal antibodies can be used as indirect measures of local immune response to immunization (Jertborn *et al.*, 1986, 1991). These antibodies can be correlated to the presence of secretory IgA (sIgA) antibodies against toxin and bacteria in intestinal fluids after oral cholera immunization.

The titres of secretory IgA antibody to toxin and to *V. cholerae* 01 LPS in intestinal lavage fluid detected by ELISA, have been found to correlate closely with toxin neutralizing and vibriocidal activities in serum. However, lavage sampling is cumbersome and not always suitable for the evaluation of intestinal immune responses to cholera. It is also possible to study the response in extraintestinal fluids such as breastmilk (Glass *et al.*, 1983) and saliva (Svennerholm *et al.*, 1984). A significant increase of anti-toxin titre in saliva was detected in Swedish volunteers orally immunized with the B subunit-WC vaccine (Jertborn *et al.*, 1984).

Results of studies on the antibody response in extraintestinal fluids have suggested that breastmilk and saliva may be useful for monitoring gut mucosal responses to naturally occurring cholera, although it may be less sensitive for reflecting intestinal immunity after artificial immunization (Jertborn, 1986).

In cholera the potent protective antigens are the toxin, and the lipopolysaccharides. The anti-toxin immunity is directed against the B subunit of the toxin (Svennerholm *et al.*, 1984) while immunity against the bacteria includes antibodies against the LPS. In addition to these components, other cell surface colonization factors, which facilitate the adherence of the bacteria to the small intestine may contribute to further antibacterial immune mechanisms of protection. Other factors that may be

important are (a) the pilus associated mannose-sensitive haemagglutinin (MSHA) (Jonson *et al.*, 1989), which is expressed in the El Tor biotype of *V. cholerae* 01 strains, (b) the toxin coregulated pilus antigen (TCP) (Taylor *et al.*, 1987), and (c) a fimbrial antigen demonstrated by Ehara *et al.* (1991). The immune responses to these antigens need to be studied in patients with cholera.

Cellular immune response in cholera

Cholera is a noninvasive disease. Antibodies present in the mucosal surfaces of the gut can protect against development of the disease. Protective immunity is dependent on the stimulation of the mucosal immune system of the gut. It is for this reason that both secretory immunoglobulins and cell-mediated immune responses in the mucosal surfaces of the gut need to be studied. Specific sIgA antibodies against bacterial antigens and the cholera toxin may give protection to the host by interfering with the adhesion of the bacteria and by neutralizing the cholera toxin before it binds to the intestinal receptors. Direct measurement of mucosal immune responses requires collection of saliva, breastmilk or intestinal fluids.

Until recently, knowledge of the lymphocytes involved in generation of the secretory immunoglobulins in the gut in cholera was limited. However development of improved techniques for the isolation of viable lymphoid cells from small mucosal biopsies (Quiding *et al.*, 1991) and antibody secreting cells in the peripheral blood using the sensitive enzyme-linked immunospot assay (ELISPOT) (Czerkinsky *et al.*, 1988) have made it possible to study cells involved in the secretory antibody response. The ASC in the peripheral blood have been found to be directly related to the degree of stimulation of the

intestinal mucosal immune response in enteric diseases (van de Verg *et al.*, 1990; Quiding *et al.*, 1991; Wenneras *et al.*, 1992; Orr *et al.*, 1992; Losonsky *et al.*, 1993). The direct correlation of the ASC to the gut mucosal response is based on the observations that antigen sensitized B lymphocytes from the gut-associated lymphoid tissues enter the circulation and preferentially home on the intestinal mucosa as well as the salivary and the mammary glands (McDermott *et al.*, 1979). In these locations, the B cells further differentiate into antibody secreting plasma cells which can then be detected in the circulation. A rise in the levels of specific ASC can be detected in the peripheral blood 5 to 7 days after vaccination or natural disease in enteric infections. However, the primary or secondary ASC response to *V. cholerae* LPS and CT were found to be of limited value in predicting vaccine efficacy of the live *V. cholerae* Inaba CVD 103-HgR vaccine although useful information could be obtained using this assay for studying the mucosal priming of vaccinees (Losonsky *et al.*, 1993).

Immune response in patients due to *V. cholerae* 01 0139

In this protocol, we plan to study the immune responses of patients with cholera due to *V. cholerae* 0139 and compare it with that of patients affected with cholera due to *V. cholerae* 01 Inaba/Ogawa serotypes, and test samples against a battery of antigens both homologous and heterologous. The study of the ASC in peripheral blood will be used to assess the magnitude of the immune response to CT, the different lipopolysaccharides and to other purified fimbrial colonizing antigens. The ASC response will be compared with the antibody response in these patients. A study of the levels of IL-2 and interferon-Y will also be assessed in the different study groups and compared with normal controls. It is known that humoral immunity is generated

following *V. cholerae* infection, and it appears that CD4 T lymphocytes play a role in host defense against cholera enterotoxin-induced diarrhoea due to *V. cholerae* 01 (Horniqvist *et al.*, 1991). The activation of all these lymphocytes may be associated with changes in the numbers of different populations of lymphocytes and as there is a link between the mucosal and systemic immune responses, an assessment of lymphocyte populations in the blood may give a clue as to the dynamics of the cellular immune response. Again, as T lymphocyte activation may be associated with the release of IL-2, there may be increased levels of IL-2 locally and systemically. Similarly other cytokines, such as IFN-Y, may also be secreted. IFN-Y secreting cells have been shown to increase in duodenal mucosa but not in peripheral blood of vaccinees (Quiding *et al.*, 1991). However, the immune response in patients is higher than in vaccinees and the severe illness being seen in this epidemic justifies measurement of such cytokines in blood.

B) RESEARCH PLAN

The following investigations will be carried out:

a) Bacteriology

Stools from patients and controls will be cultured to isolate *V. cholerae* 01 (El Tor biotypes only which are more common), *V. cholerae* 0139 and any copathogens, if any. *V. cholerae* strains will be stored at -70°C in trypticase soy broth (TSB) containing 15% glycerol.

b) Extraction and purification of LPS

LPS will be prepared (Westphal *et al.*, (1965) from a representative strain of the *V. cholerae* 0139 as well as from *V. cholerae* 01

Inaba/Ogawa serotypes (El Tor biotype). LPS will also be purified from a nonpathogenic *E. coli* strain to serve as a negative control in the experiments.

c) Peripheral blood

Blood (10 ml) will be obtained by venipuncture and centrifuged on Ficoll-Paque for separation of plasma and mononuclear cells. 2 ml of blood will be collected in EDTA-containing tubes for cytokine assays. Plasma will be stored at -70°C for cytokine assays. Serum will be collected from 2 ml of peripheral blood for the vibriocidal assays.

i) Peripheral blood mononuclear cells: Cells will be phenotyped by indirect immunofluorescence to ascertain the proportion of T cells (CD3), T cell subsets (CD4 and CD8), B cells (CD20) and receptor for IL-2 (CD25) using monoclonal antibodies.

ELISPOT: Procedures developed for determining ASC response using ELISPOT using *V. cholerae* 01 antigens, such as CT (Wenneras *et al.*, 1992) and LPS (Losonsky *et al.*, 1993), will be applied. The presence of antibody secreting cells will be assayed using peripheral blood mononuclear cells (1×10^5 and 1×10^4 cells per well) in nitrocellulose backed microtitre plates (in Quiding *et al.*, 1991) in quadruplicate wells. Antigens that will be used in the assay include LPSs (4 different LPS) from the different bacteria (10 µg/ml coating dose). In addition, outer membrane protein antigens and heat-killed whole bacterial extracts will also be tested to optimize detection of the ASC response. MSHA, TCP and the fimbrial antigen described by Ehara *et al.* (Ehara

et al., 1991) will be obtained from respective researchers from the field (TCP and MSHA from J. Holmgren, University of Goteborg, Sweden, the fimbrial protein from M. Ehara, Nagasaki University, Japan) and used in appropriate concentrations. Anti-CT responses will be assayed using the GM1 ganglioside in the first layer and CT in the second layer in ELISPOT (Wenneras *et al.*, 1992).

ii) Saliva: Saliva samples will be collected by having patients expectorate into sterile containers. All samples will be obtained an hour after a meal in the morning. The sample will then be heat-inactivated, centrifuged and stored at -20°C.

ELISAs will be carried out with plasma and saliva samples using antigens similar to those used in the ELISPOT. Anti-toxin antibodies (CT) will be assayed using the GM1, ELISA technique. Specific antibodies of IgA, and IgG isotypes will be determined. Samples from the patients in different groups and controls will all be tested against a battery of different antigens (LPSs, CT, MSHA, TCP, fimbrial antigen, *V. cholerae* 01 and 0139). Immunoblot assays will be carried with selected antigens and serum samples based on ELISA and ELISPOT assays. Total IgA in saliva samples will be determined using ELISA (Schultsz *et al.*, 1992).

Cytokine assays: Levels of IFN-Y (Quiding *et al.*, 1991) will be carried out using monoclonal antibody based ELISA and IL-2 will be assayed using an IL-2 dependent murine cell-line (Teranishi *et al.*, 1984) or ELISA (Endogen, USA). Both cytokines will be assayed using plasma samples stored at -70°C.

Vibriocidal assays: Antibacterial antibodies will be determined (McIntyre, 1964) using two-fold dilution of serum and representative strains of the different groups of *V. cholerae*.

d) Data analysis

Statistical differences between antibody, ASC, lymphocyte phenotyping and cytokine levels between the two groups of patients (those affected by *V. cholerae* 0139 or *V. cholerae* 01 Inaba/Ogawa) will be determined using the Wilcoxon's rank sum test. Comparisons will be made between the two patient groups and compared to the control group.

C) BIBLIOGRAPHY

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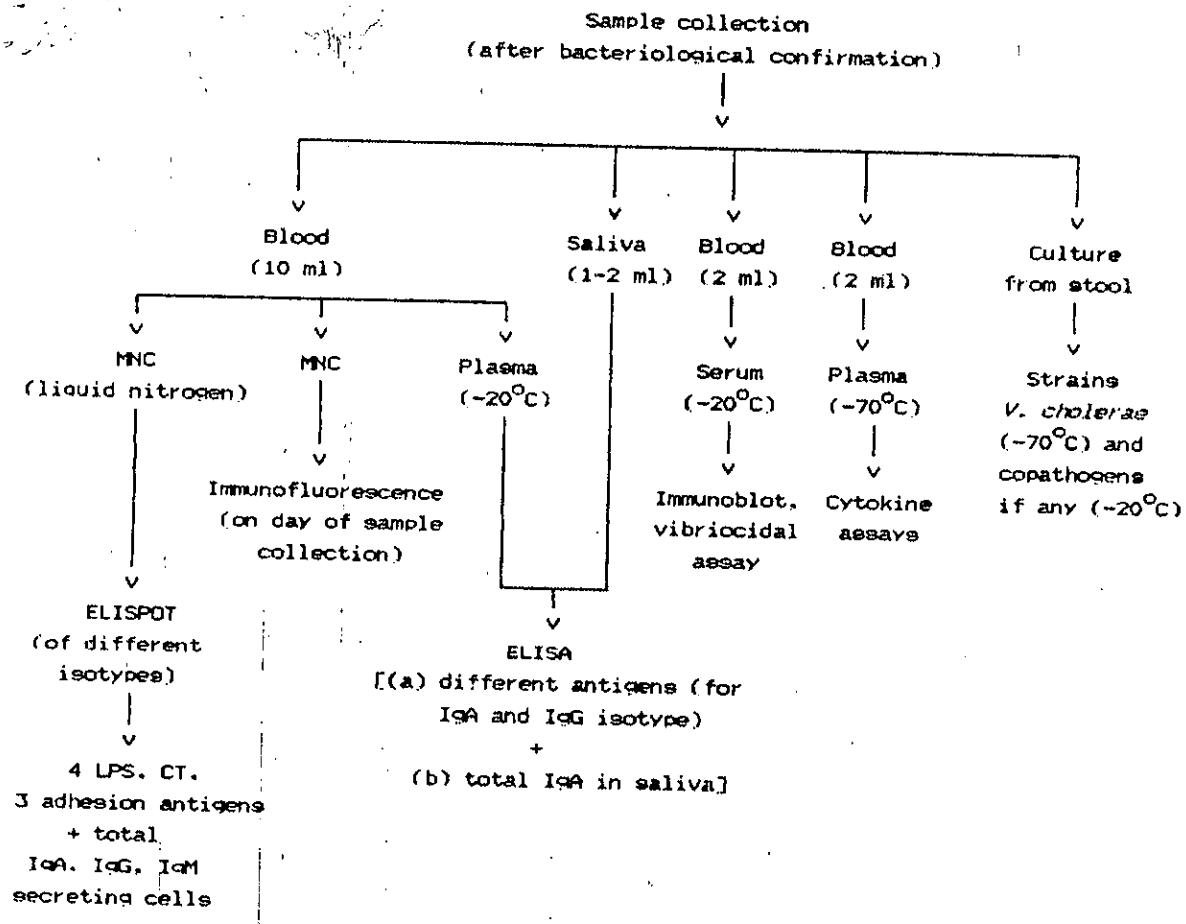
12. FLOW CHART

Sample collection and sequence of work



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Figures in parentheses indicate storage temperature.

MNC = mononuclear cells

Sequence of work

1) Collect specimens, process and store at appropriate temperatures

2) Standardize ELISA with different antigens and complete assays

3) Standardize ELISPOT assays with different antigens and complete assays

4) Set-up assays for cytokines and determine levels in different study groups

5) Analyze data using the Wilcoxon's rank sum test

13. ITEMIZED SPECIFIC TASKS FOR EACH LISTED INVESTIGATOR

Dr. Firdausi Qadri (100%)

- ELISA, ELISPOT, cytokine assays, vibriocidal assays and study of virulence properties of strains
- Supervise work in the lab and coordinate specimen collection from patients
- Analyze data and write reports on results obtained

Dr. Tasnim Azim (25%)

- Phenotyping of MNC by immunofluorescence, ELISPOT and cytokine assays

Dr. Ann-Mari Svennerholm

- Scientific and academic feedback

Dr. M.J. Albert (5%)

- Coordination and academic feedback.

Dr. M.A. Salam
Dr. Ali Miraj Khan

- Patient enrollment, clinical management and follow-up

Research Officer (2) (100%)

- Carry out tests specified for the protocol involving both microbiological and immunological techniques

14. BUDGET

Detailed budget

Year 1

1. Personnel

Dr. Firdausi Qadri (NOD, S-7)	100%	US$ 17,604	
Dr. Tasnim Azim (NOB, S-3)	25%	-	
Research Officer (GS-VI, S-1)	100%	5,892	
Research Officer (GS-V, S-1)	100%	4,548	
Lab Attendant (GS-II, S-1)	100%	2,412	
Dr. M.A. Salam	10%	-	
Dr. Ali Miraj Khan	10%	-	
		----------	US$ 30,456

2. Patient hospitalization

*80 patients who will complete 5 days stay in hospital	= 80 x 5 x 19	7,600	
20 patients who are initially enrolled but dropped for any reason	= 20 x 2 x 19	760	
Reimbursement for wage loss	= 80 x 4 x 5	1,600	
		----------	9,960

*10 extra to be enrolled from each group to ensure complete collection of samples

3. Laboratory costs

380 stool dark-field examination (80 patients x 4 = 320 + 60 controls = 380)	= 380 x 2	760	
380 stool culture all plates	= 380 x 7.5	2,850	
Stool microscopy	= 380 x 2	760	
Blood for TC, DC, and ABO typing haematocrit	= 380 x 6.5	2,470	
		----------	6,840

4.	Supplies and materials		
	Office supplies	1,000	
	Materials -		
	ELISAs for six different antigens (CT, LPS - 01 and 0139, MSHA, TCP, fimbrial antigen in plasma and saliva) - 320 x 6 x 3 (triplicate), icluding ELISA plates and conjugates	10,000	
	Plasticware (including disposable pipettes, centifuge tubes, cryo vials, storage vials	10,000	
	Microbiological and cell culture media, foetal bovine serum, other biochemicals	3,000	
	Liquid nitrogen, carbon dioxide for incubator	2,000	
			26,000
5.	Interdepartmental (Bio-Engineering/Maintenance)		2,500
6.	Miscellaneous		
	Printing and publications	600	
	Medical illustration	500	
	Staff clinic	350	
	Communication (fax, telex, etc.)	240	
			1,690
			77,446
7.	Capital		
	Biohazard hood (Class-II)	7,000	
	Table-top centrifuge	5,500	
	Water-bath	2,500	
	Personal computer	2,900	
	Laser printer	2,900	
			20,800
	TOTAL for year 1		US$ 98,246

Year 2

1. Personnel

Dr. Firdausi Qadri (NOD, S-8)	100%	US$ 17,780	
Dr. Tasnim Azim (NOB, S-4)	25%	2,821	
Research Officer (GS-VI, S-2)	100%	6,481	
Research Officer (GS-V, S-2)	100%	5,002	
Lab Attendant (GS-II, S-2)	100%	2,653	
Dr. M.A. Salam	10%	-	
Dr. Ali Miraj Khan	10%	-	
			US$ 34,737

2. Supplies and materials

Cytokine assay kit for IL-2 and IFN-Y for (380 x 2 samples for plasma)	10,000	
ELISPOT assays for 6 antigens as for ELISA including cost of nitrocellulose backed ELISPOT plates	8,000	
Plasticware and glassware	3,000	
Microbiological media, cell culture media, foetal bovine serum and other biochemicals	2,000	
Liquid nitrogen	2,000	
		25,000

3. Interdepartmental (Bio-Engineering/Maintenance) 2,500

4. Miscellaneous

Medical illustration	500	
Data analysis	1,000	
		1,500
TOTAL for Year 2		US$ 63,737

Total budget for 2 years

Year 1	=	US$ 98,246
Year 2	=	US$ 63,737
GRAND TOTAL		US$ 161,983

JUSTIFICATION FOR CAPITAL BUDGET

Biohazard hood

The study will involve extensive use of biohazard hood. At present there is only one hood in the Immunology Lab which is already being used for three different protocols and is overloaded.

Centrifuge

The only table-top centrifuge, which is 6 years old, in the Immunology Lab is similarly under extensive use and requires frequent maintenance and repairing.

Water-bath

As above, we are now using a water-bath that has been locally put together by our Bioengineering Cell staff to somehow carry out our work and replacement is needed.

Personal computer and printer

The computers (processor 8088) and printers (dot matrix) now in use in the Divison are all outdated and defective resulting in slowing down of work. The hardwares are also not compatible with newer versions of softwares.

FQ:mh/B4A:LSIRESP.PRT

ENGLISH CONSENT FORM

IMMUNE RESPONSE STUDIES IN PATIENTS IN THE DIARRHOEAL EPIDEMIC

ou are suffering from a diarrhoeal disease which may be cholera or very ch like cholera. The germ that usually causes this kind of dehydrating arrhoea is well known and is called *V. cholerae* O1. However a new kind germ is now causing the same kind of illness. We know very little of e new germ that causes this disease and in order to understand more about is illness we will need to study such patients. For this purpose we seek ur assistance in collecting blood, saliva and stool samples from you. out 14ml of blood (2½ teaspoon) and 1 to 2 ml of saliva (about ½ aspoon) will be collected from you four times during the study. These ll be one day after admission, 3 days later, 6 days and 17 days after at. Stool samples will also be collected on these days. The collection these samples will not be harmful to you in any way. During the study u will have to stay in the hospital of four days and will be discharged the 4th day. We will request you to come back to the hospital 6 days d 17 days after discharge, when we will again collect samples of blood, liva and stool from you.

e tests that we will carry out will be very helpful in understanding the ason why such a severe epidemic of cholera is occurring in Bangladesh. so, this study may help development of vaccine to prevent infection from is germ.

u are to decide if you want to participate in the study. Even if you do t agree to participate, you will receive the standard treatment of this spital. Even after initial participation in the study you have the right withdraw yourself at any time at your will. If you decide to withdraw om the study you will not miss the opportunity of getting standard eatment from this hospital.

you agree to participate in the study, please put your signature or your ft thumb impression at the specified space below.

ank you for your cooperation.

gnature/left thumb pression of Patient	Date
gnature of Investigator	Date
gnature of witness	Date

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, ঢাকা, বাংলাদেশ

সম্মতি পত্র-

বাংলাদেশে বর্তমানে কলেরার ন্যায় নতুন এক ধরণের রোগজীবানু (Bacteria) মহামারী আকারে দেখা দিয়েছে। মারাত্মক জলশূন্যতা সৃষ্টিকারী এই অনুজীব (Microbe) সম্পর্কে আমাদের জ্ঞান সীমাবদ্ধ। দেশ, জাতি তথা মানবজাতির বৃহত্তর স্বার্থে এ রোগের গবেষণা অপরিহার্য। আপনি বর্তমানে কলেরা সদৃশ এই নতুন ধরণের ব্যাকটেরিয়া দ্বারা আক্রান্ত। আপনার এ রোগ ও তার সংক্রমন সম্পর্কে জানতে হলে আপনার দেহ থেকে ১৪ মি: লি: (২½ চা চামচ) রক্ত, ২ মি: লি: (½ চা চামচ) মুখের লালা এবং অল্প পরিমান পায়খানা তিনবার সংগ্রহ করতে হবে। হাসপাতালে ভর্তি হবার ১ দিন পর ১ম বার, ৩ দিন পর ২য় বার, ৬ দিন পর ৩য় বার এবং ১৪ দিন পর ৪র্থ বার নমুনা সংগ্রহ করতে হবে।

আপনাকে হাসপাতালে ভর্তি হবার ৪ দিন পর ছুটি দেওয়া হবে। এ সময় আপনাকে অনুরোধ করা হবে ৭ দিন পর হাসপাতালে আসতে এবং তখন যথানিয়মে ৩য় বারের মত নমুনা সংগ্রহ করা হবে। এই নমুনা সংগ্রহে কোন ক্ষেত্রেই আপনার দৈহিক ক্ষতি হবে না।

গবেষণায় অংশগ্রহণ সম্পূর্ণ আপনার ইচ্ছার অধীন। আপনি অংশগ্রহণ না করলেও হাসপাতালের সুচিকিৎসা পাবেন। এমনকি আপনি আংশিকভাবে অংশ নিয়েও যেকোনো সময় নিজের নাম প্রত্যাহার করে নিতে পারেন এবং তাতে আপনি হাসপাতালের চিকিৎসা থেকে বঞ্চিত হবেন না। আপনি যদি গবেষণায় অংশগ্রহণে রাজী হন তবে নীচের নির্দিষ্ট স্থানে আপনার সম্মতিসূচক স্বাক্ষর / বৃদ্ধাঙ্গুলীর ছাপ প্রদান করুন।

আপনার সহযোগিতার জন্য আপনাকে ধন্যবাদ।

সম্মতি দাতার স্বাক্ষর / বৃদ্ধাঙ্গুলীর ছাপ — তারিখ —

গবেষকের স্বাক্ষর — তারিখ —

সাক্ষীর স্বাক্ষর — তারিখ —

INTERNATIONAL CENTRE FOR DIARRHOEA DISEASE RESEARCH, BANGLADESH

CONSENT FORM FOR VOLUNTEERS (CONTROL)

We are conducting a study on patients who are suffering from cholera. The germ which usually causes this dehydrating diarrhoea is well known and is called *Vibrio cholerae* 01. However, a new kind of germ which is called *V. cholerae* 0139 is also causing a similar kind of disease. We know very little about the new germ that causes this disease and in order to understand more about this disease, we will study patients. For this purpose adults without cholera will also need to be studied. The study will help us to better understand the disease. We would like to enroll you in the study. For this purpose we will take 14 ml (2½ teaspoonful) of blood, saliva (1-2 ml) and stool (5-10 g) from you. These samples will be taken only once from you where we will look for protective factors. None of these procedures will be harmful to you. If you agree to participate in the study, please sign or put your left-thumb impression below.

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Signature of left-thumb impression of guardian | Date

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Signature of investigator | Date

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Signature of witness | Date

আন্তর্জাতিক উদ্গাময় গবেষণা কেন্দ্র, বাংলাদেশ

স্বেচ্ছাসেবকের সম্মতিপত্র

আমরা কলেরা রোগীর উপর গবেষণা করছি, যে জীবাণু (ব্যাকটেরিয়া) জলশূন্যতা সৃষ্টিকারী উদারাময়ের কারণ উহা খুবই পরিচিত এবং ইহাকে ভিবরিও কলেরী ও-ওয়ান (V. Cholerae 01) বলা হয়। ইহা ছাড়া নতুন এক ধরণের ব্যাক্টেরিয়া যেটা ভিবরিও কলেরী ও-ওয়ান সিরোটাইপ (V. Cholerae 0139) নামে অভিহিত। উহাও ঠিক একই ধরণের ডায়রিয়া রোগ সৃষ্টি করে। এই নতুন অনুজীবটি (Microb) সম্পর্কে আমরা বিশেষ কিছু এখনও জানিনা। সেই রোগজীবাণু সম্পর্কে ভালভাবে জানার জন্য রোগী নিয়ে গবেষণা প্রয়োজন। সে জন্য প্রাপ্তবয়স্ক যারা কলেরায় আক্রান্ত নয় তাদেরকে এই গবেষনায় অন্তর্ভুক্ত করা হবে। এই গবেষণা রোগটি সম্পর্কে অনেক অজানা তথ্য দিবে। আমরা আপনাকে এই গবেষণায় তালিকা ভুক্ত করতে চাই আর এ জন্য আপনার নিকট থেকে ১৪ মিঃ লিঃ (২½ চা চামচ) রক্ত, ১-২ মিলিঃ মুখের লালা এবং অল্প পরিমান (৫-১০ গ্রাম) পায়খানা কেবল মাত্র একবারই সংগ্রহ করবো। এই নমুনা সংগ্রহে আপনার কোনো ক্ষতি হবে না এবং ইহা দ্বারা রোগ প্রতিরোধ শক্তি (Protective factor) পরীক্ষা করা হবে। আপনি যদি এই গবেষণায় অংশ গ্রহণে সম্মত হন তা'হলে অনুগ্রহ পূর্বক নীচে আপনার স্বাক্ষর / বৃদ্ধাঙ্গুলীর ছাপ যথা স্থানে প্রদান করুন।

সম্মতি দাতার স্বাক্ষর / আঙ্গুলের ছাপ — তারিখ —

গবেষকের স্বাক্ষর — তারিখ —

সাক্ষীর স্বাক্ষর — তারিখ —