ocument 1. ICDDR,B LIBRARY Library (2) Date 24.02.86
DHAKA-12
ETHICAL REVIEW COMMITTEE, ICDDR,B.

rincipal Investigator DR. FIRDAUSI QADRI Trainee Investigator (if any) 22

pplication No. 86-008 Supporting Agency (if Non-ICDDR,B) UNDP

itle of Study EXPRESSION OF ANTIGENS RELATED TO OMP IN SHIGELLA GROWN IN DIFFERENT CONDITIONS

Project status:
(x) New Study
( ) 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). NA

Source of Population: NA
(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: NA
(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.

NO HUMAN SUBJECT IS INVOLVED IN THE STUDY

(PTO)

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

Firdausi Qadri
Principal Investigator

FEB 26 1986

Trainee

SECTION I - RESEARCH PROTOCOL

1. TITLE : Expression of antigens related to OMP in *Shigella* grown in different conditions

2. PRINCIPAL INVESTIGATOR : Dr. Firdausi Qadri,
Department of Biochemistry
University of Dhaka

COINVESTIGATOR : Dr. Ivan Ciznar, ICDDR,B

3. STARTING DATE : March, 1986

4. COMPLETION DATE : February, 1987

5. TOTAL DIRECT COST : US$ 35,767

6. SCIENTIFIC PROGRAM HEAD: Dr. Ivan Ciznar
This protocol has been approved by the Host Defense Working Group

Signature of Program Head Ivan Ciznar

Date Jan. 30, 1986

7. ABSTRACT SUMMARY

Shigella dysenteriae type 1 will be grown in synthetic, semisynthetic and nutritionally rich media. Outer membrane proteins will be extracted and their banding pattern analyzed by polyacrylamide electrophoresis. Antigenicity of OMP will be assessed by means of western blotting and by cross immunoelectrophoresis with the help of rabbit antisera prepared against whole-cells and purified OMP. Antigenic composition will be correlated with tests of invasiveness by Sereny and in HeLa cells. Antisera against OMP of different origin will

be used for neutralization of invasiveness. Investigators expect that such oriented study will help to clarify whether different OMPs could be presented *in vivo* and whether there is a direct relation between antigenicity and invasiveness. It is also expected that OMPs from *Shigella* grown in different environment would be used in subsequent studies for detection of antibodies in patients with dysentery. This is an important problem to be solved in a vaccine development program.

8. REVIEWS:

a. Ethical Review Committee _____

b. Research Review Committee _____

c. Director _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. OBJECTIVE

a) To analyze composition and antigenicity of OMP from Shigella grown in different environment

b) To correlate the antigens of OMP with invasiveness of Shigella

c) To find out whether antibodies against particular OMP's antigens can inhibit invasiveness.

2. BACKGROUND

Infections caused by Shigella dysenteriae type 1, as well as by other serotypes are major cause of debilitation and death in children of developing countries.

Understanding of pathogenesis and immunity in shigellosis has been considered as an important step in development of efficient preventive and therapeutical interventions. While a substantial progress has been achieved in elucidation of virulence factors associated with plasmids and chromosomal loci in Shigella dysenteriae type 1 (Watanabe et al, 1984), as well as other Shigellae (Sansonetti et al, 1983; Sansonetti et al, 1982; Maurelli et al, 1985), a lot remains to be clarified regarding immune mechanisms. Apparent complexity of factors determining virulence of Shigellae has been the main problem in the vaccine development program. All Shigella vaccine developed in the past failed to

elicit effective immunity (Levine et al, 1983) perhaps for the main reason, they did not contain the main protective antigens in a proper form and concentration.

Recent genetic studies clearly showed that OMPs are the substances of the pathogen which could play an important role in virulence and pathogenicity of Shigella (Hale et al, 1983) and as it was shown later, the OMPs also carry serotype and group specific antigens (Maurelli et al 1985).

It has been known that the expression of antigens depends not only on the genetic determinants in plasmids or chromosomal loci but also on environment and condition in which the pathogen multiplies (Achtman, 1980; Kabir, 1980; Kawooka et al, 1983; Foo et al, 1984). Therefore, we could draw the operational hypothesis that different OMPs may be expressed under different environmental conditions and could also carry different antigens. Whether all the antigens associated with OMPs are related to the invasiveness of Shigella dysenteriae type 1 is not known. Obviously, such data would help substantially in assessment of protective role of the OMPs in vaccine. Therefore, we intend to use antisera with antibodies against different OMPs in inhibition of Shigella dysenteriae type 1 invasiveness in Sereny test and in Hela cells model.

3. RATIONALE

Development of efficient vaccine against Shigella dysenteriae type 1 infection depends on knowledge of protective antigens of the pathogen. There are several components of Shigella with a potential to carry protective antigens. Recently OMPs have drawn a lot of attention in this regard. Before construction of a Shigella vaccine, it would be very helpful to know whether OMP antigens and specifically which of them stimulate production of antibodies efficient in inhibiting of invasiveness. Such antigens could be synthesized under specific environmental conditions, reflecting conditions in vivo. The goal of this study is to bring a new knowledge on this topic.

B. SPECIFIC AIM

1) To identify OMP antigens of Shigella dysenteriae type 1 grown in different media.

2) To correlate invasiveness with antigenic composition of OMPs.

3) To assess the protective role of OMPs antigens by inhibition of Sereny test and Hela cells invasion with rabbit antisera against specific antigens.

C. MATERIAL AND METHODS

Strains

Two Shigella dysenteriae type 1 strains will be used in the study. These will be selected after checking the invasive properties and plasmid profile. The strains positive in Sereny test (Sereny, 1955) and having a defined plasmid profile will be further analyzed. Plasmid DNA will be detected by electrophoresis in agarose gel as described by Kopecko et al (1980).

Media

The strains will be cultivated at 37°C in Penassay (PA) or brain-heart-infusion (BHI) broth used as nutritionally rich media. Minimal synthetic medium will consist of inorganic salts and glucose as described in Protocol No. 84-033. Semisynthetic medium will have the same composition as synthetic plus 2% of low molecular fraction of casamino acid (Difco).

OMPs

Outer membrane proteins will be extracted by the procedure of Johnson et al.

Assays

SDS-PAGE and western blotting will be performed as described by Towbin et al (1979). Cross immunoelectrophoresis and cross and intermediate gel will be performed as described by Kroll (1973).

Preparation of rabbit antisera

Adult albino rabbits will be immunized with whole-cell preparations as well as with OMPs. Immunization schedule will start with 10^5 cells or equivalent of OMPs preparations. The rabbits will receive totally five doses (from 10^5 to 10^9) in three-day intervals. One week after the last dose, the serum will be collected and stored at $-40^{\circ}C$.

Biological tests

Guinea pigs keratoconjunctivitis test will be done by Sereny (1955) and invasion of HeLa cells by Oaks et al (1985).

D. SIGNIFICANCE

The proposed study is expected to clarify whether OMP-related antigens are protective and whether they are equally expressed in cells growing under different environment. Identification of such antigens would help to better assessment of the immune response of man during the natural infection against Shigella infection. Such information is necessary for understanding of immune mechanism and vaccine development against shigellosis.

E. FACILITIES REQUIRED

No additional facilities will be needed.

F. COLLABORATION

This protocol is collaborative one between Department of Biochemistry, University of Dhaka, and ICDDR,B. Dr. Firdausi Qadri will carry on the main part of the study in facilities available at ICDDR,B laboratories. We expect that such collaboration would be a base for establishment of solid program leading to M.S. degrees for students of Dhaka University.

REFERENCES

1. Foo, et al. Type 1 Pilli (FI) of porcine enterotoxigenic E. coli: Vaccine trial and test for production in the small intestine during disease. Inf Immun, 43, 1-4, 1984.

2. Hale, T.L., et al. Characterization of virulence plasmids and plasmid associated outer membrane proteins in Shigella flexneri, Shigella sonnei and E. coli. Inf Immun, 40, 340-350, 1983.

3. Johnson, K.H., and Gotschlich, E.C. Isolation and characterization of the outer membrane of Neisseria gonorrhoeae. J Bacteriol, 119:250-257, 1974.

4. Kroll, J. Crossed immunoelectrophoresis. p81. In Exelsen H.H. A manual of quantitative immunoelectrophoresis. Blackwell Scientific Publications, Oxford, 1973.

5. Levine, M.M., et al. New knowledge on pathogenesis of bacterial enteric infections as applied to vaccine development. Micr Rev, 47, 510-550, 1983.

6. Maurelli, A.T., et al. Cloning of plasmid DNA sequences involved in invasion of HeLa cells by Shigella flexneri. Inf Immun, 49, 164-171, 1985.

7. Sansonetti, P.J., et al. Involvement of a plasmid in the Invasive Ability of Shigella flexneri. Inf Immun, 35, 852-860, 1982.

8. Sansonetti, P.J., et al. Alterations in the pathogenicity of E. coli K12 after transfer of plasmid and chromosomal genes from Shigella flexneri. Inf Immun, 39, 1392-1402, 1983.

9. Towbin, H., et al. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. Procedure and some applications. Proc Natl Acad Sci, USA, 76, 4350-4354, 1979.

10. Watanabe, H., et al. Small virulence plasmid of Shigella dysenteriae 1 strain W 30864 encodes a 11.000 dalton protein involved in formation of specific LPS side chains of serotype 1 isolates. Inf Immun, 46, 55-63, 1984.

ICDDR,B
BUDGET PROPOSAL
(In US $)

AREA DESCRIPTION

Program Name: HOST DEFENSE WORKING GROUP

~~Project~~/Protocol/~~Branch~~ Name: Expression of antigens related to OMP in Shigella grown in different conditions

Principal Investigator/~~Branch Head~~/~~Program Head~~: Dr. Firdausi Qadri

Budget Code: ..... Estimated Beginning Date: March, 1986

Protocol No: ..... Estimated Ending Date: February, 1987

EXPENSE CATEGORY		*Column A: Total Project Cost	Column B	Column C	Column D: Proposed 1986
A/C No.	Description / Refer Page				
3100	Local Salaries 2	8,372			
3200	Intl. Salaries 8	14,640			
3300	Consultants 14	-			
3500	Travel Local 15	-			
3600	Travel Intl. 16	2,800			
3700	Supplies & Mat. 17,18	7,605			
3800	Other Costs 19	400			
4800	Inter Deptl. Ser. 20	1,950			
	Total Direct Operating Cost	35,767			
0300	Capital Expenditure Refer Page 21	-	**	**	
	TOTAL DIRECT COST	35,767			

* Refers to entire life of project.
** For Finance use only.

Budget86.1-3
AZIZ-5.

PERSONNEL REQUIREMENT-(LOCAL STAFF) 1986

	No.of Positions	No. of Man Months	$ Amount
. Direct Project/Protocol/Branch Staff at 1-1-1986 Sourced from Page 3	1.7	20.4	3,680
dd:			
. New Recruitments Sourced from Page 4	1.0	12.0	4,692
. ~~Staff~~ allocated from other area Sourced from Page 5	-	-	-
(i) Sub Total	2.7	32.4	8,372
ess:			
. Separations Sourced from Page 6	-	-	-
. ~~Staff~~ allocated to other area Sourced from Page 7	-	-	-
(ii) Sub Total	-	-	-
(i)-(ii) TOTAL	2.7	32.4	* 8,372

*Agrees with Page 1 A/C No.3100 Column D

LOCAL STAFF (EXISTING) AT 1.1.1986

Job Designation	Level	A No. of Positions 30.9.85	B No. of New Recruits Oct.-Dec.85 (+)	C No. of Separations Oct.-Dec.85 (-)	D No. of Positions at 1.1.86 (A+B-C)	E Man Month at 1.1.86 (D x 12)	F ** Rate per Month	G $ Amount (E x F)
Research Officer	GS-5				1.0	12.0	210	2,520
Lab Technician	GS-4				0.5	6.0	151	906
Laboratory Attendant	GS-1				0.2	2.4	106	254
				TOTAL	*	*		*3,680

** See computer printout for average rate per month.

* AGREES WITH PAGE 2 ROW A

Job Designation	Level	Start Date	No. of Posit.	No. of Man Months	Rate/ Month	$ Amount
Assistant Scientist	NO-B	1-3-86	1.0	12.0	391	4,692
		TOTAL	* 1.0	* 12.0		* 4,692

** See annexure A for a list of rates.

*** Calculate man month from the month of joining upto the end of December 1986 i.e., if joining month is March 1986 then man month will be 10 for one person. If no. of persons is more than one, say 4 then man month will be (10x4)=40.

* AGREES WITH PAGE 2 ROW B

PERSONNEL REQUIREMENT-INTERNATIONAL STAFF-1986

	No.of Positions	No. of Man Months	$ Amount
irect. Project/Protocol/Branch taff at 1.1.1986 Sourced from Page No. 9	0.2	2.4	14,640
ew Recruitments Sourced from Page No. 10	-	-	-
taff allocated from other area Sourced from Page No. 11	-	-	-
(i) Sub-Total	0.2	2.4	14,640
eparations Sourced from Page No. 12	-	-	-
taff allocated to other area Sourced from Page No. 13	-	-	-
(ii) Sub-Total	-	-	-
TOTAL	0.2	2.4	14,640

INTERNATIONAL STAFF (EXISTING) AT 1.1.1986

Job Designation	Level	A No. of Positions 30.9.85	B No. of New Recruits Oct.-Dec.85 (+)	C No. of Separations Oct.-Dec.85 (-)	D No. of Positions at 1.1.86 (A+B-C)	E Man Month at 1.1.86 (D x 12)	F ** Rate per Month	G $ Amount (E x F)
Senior Scientist	P5				0.2	2.4	6,100	14,640
				TOTAL	0.2	2.4		14,640

* AGREES WITH PAGE 8 ROW A

** Contact your Associate Directors or Finance (Ext.280) for rates.

Budg 186.9

TRAVEL PLAN (INTERNATIONAL)-1986

Job Designation	Purpose of Travel	Travel From-To-From	Estim. days of travel (A)	** Perdiem Rate (B)	** Perdiem Amount (C=AxB)	*** Transportation Air (D)	*** Transportation Ground (E)	**** Other Cost (F)	Total $ Amount (C+D+E+F)
Senior Scientist	Congress	Dhaka-Europe-Dhaka	10	100	1,000	1,500	150	150	2,800
							TOTAL	*	2,800

** Check with Finance Ext. 281 for perdiem rate.
*** Check with Travel Ext.234 for transportation cost.
**** Includes excess baggage, embarkation fee, travel tax etc.

*AGREES WITH PAGE 1 A/C 3600 COLUMN D

Budget86.16

SUPPLIES AND MATERIALS-1986

A/C Code	Description	$ Amount
3701	Drugs (used for medication in the hospitals and field stations)	-
3702	Glassware (bottle, beaker, cylinder, petridish, aluminium seal, stopper, stopper, tube etc.)	200
3703	Hospital Supplies (bandage, gauze, binde, bowl, catheter, cotton, needle, syringe, solution, leukoplast, towel etc.)	100
3704	Stationery and Office Supplies (Battery, book register, binders, files, pencil, fastener, paper, ribbon, stapler etc.)	300
3705	Chemicals and Media (Acid, reagent, dextrose, sodium, bactoagar etc.)	1,000
3706	Materials for Uniform (Cloth, button etc. required for making uniforms)	100
3707	Fuel, Oil and Lubricants (Diesel, mobil, petrol, kerosene etc.)	-
3708	Laboratory Supplies (Aluminium foil, bags, blade, brush, cap, container, film X-Ray etc.)	1,000
3709	Housekeeping Supplies (Aerosol, battery, wiping cloth, duster, lock and key etc.)	100
3710	Janitorial Supplies (Bleaching powder, brush, detol, detergent, insecticide, soap etc.)	50
	(Contd. to Page No. 18)	
	Sub-total	2,850

SUPPLIES AND MATERIALS-1986

(Cont'd. from Page Nos. 17)

A/C Code	Item Description	$ Amount
	Brought Forward	2,850
3711	Tools and Spares (Automobile spares, tyres, tubes, battery, stores required for maintenance services etc.)	-
3712	Non-stock Supplies (Materials not normally kept in stock and purchased only against specific requisitions)	3,000
	Sub-Total	5,850
3713	Freight and other Charges Add 30% to above sub total	1,755
	TOTAL	7,605

AGREES WITH PAGE 1 A/C 3700 COLUMN D

Note:- For rates, please contact Supply Ext.260 (add 10% to rates for inflation)

OTHER COST-1986

A/C Code	Accounts Description	$ Amount
3800	Repairs and Maintenance (Maintenance and repairs of vehicles, equipments, furniture and building)	100
3900	Rent, communicatiou and utilities (Postage, telephone, telegram, electricity etc.)	100
4100	Bank charges	-
4200	Legal and professional expenses (Professional membership,fee, legal fee, audit fee etc.)	-
4300	Printing and Publication (Printing of forms, books, journals, reprints etc.)	200
4400	Entertainment, Hospitality & Donation (Guest house accommodation, donations, hospital food, lunch, refreshment etc.)	-
4500	Service Charges (Porter, labour, washing, laundry and other misc. exp.)	-
4600	Staff Development and Training (Training course fee, training materials, stipend,scholarship, subsistance paid to the staff)	-
	TOTAL	400

AGREES WITH
PAGE 1
A/C No.3800
COLUMN D

**INTERDEPARTMENTAL SERVICES-1986

A/C Code	Service Area	$ Amount
4801	Computer	-
4802	Transport Dhaka	-
4803	Transport Matlab	-
4804	Water Transport Matlab	-
4805	Transport Tekner	-
4806	Xerox and Mimeograph	500
4807	Pathology	-
4808	Microbiology Lab	50
4809	Biochemistry	-
4810	X Ray	-
4811	T.V. Clinic	-
4812	Media	200
4813	Patient hospitalization for study	-
4814	Animal Research	1,000
4815	Medical Illustration	100
4817	Telex	100
4818	Out Patient Care	-
4830	Transport Subsidy	-
	TOTAL	$ 1,950

** See annexure B for rates.

AGREES WITH PAGE 1 A/C 1300 COLUMN D