

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator DR. AMAR BIN ASHRAF Trainee Investigator (if any) \_\_\_\_\_

Application No. 85-0287 Supporting Agency (if Non-ICDDR,B) \_\_\_\_\_

Title of Study Immune response in human typhoid fever and its role in the pathogenesis of typhoid perforation Project status:  
(X) New Study  
( ) Continuation with change  
( ) No change (do not fill out rest of form)

Give 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:
  - NA 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
  - NA Informed consent form for parent or guardian
  - Procedure for maintaining confidentiality
  - NA 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. 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.

(PTO)

To obtain approval of the Ethical Review Committee for any changes in the rights and welfare of subjects before making such change.

Ashraf  
Principal Investigator

\_\_\_\_\_  
Trainee

85-028P  
22/8/85

SECTION-I - RESEARCH PROTOCOL

- 1. Title : Immune response in human typhoid fever and its role in the pathogenesis of typhoid perforation
- 2. Principal Investigator : Dr. Amar Bin Ashraf  
Coinvestigators : Dr. Ivan Ciznar, Dr. Ansaruddin Ahmed, Dr. Asma Khanam, Mr. Akbar Ali, Dr. R. N. Majumder  
Consultant : Dr. Md. Shahid Hossain (Consultant, DMCH)  
Consultant : Dr. T. C. Butler
- 3. Starting Date : As soon as approved by ERC
- 4. Completion Date : Six months from ERC approval date
- 5. Total direct cost : US\$ 4,502
- 6. Scientific Program Head : Dr. Ivan Ciznar

This protocol has been approved by the Host Defense Working Group. (Acting)

Signature of the Scientific Program Head : Man. bin Ashraf

Date : 22/8/85

7. Abstract summary

Ten adult patients, male or female, 20-30 age group, with typhoid fever and ten adult patients, male or female, with typhoid gut perforation will be studied to compare the cell-mediated, as well as humoral immune response in both group of patients. Peripheral blood will be obtained to assess immune responses. The tube dilution test (Widal test) will be employed to measure the humoral immune response. On the other hand,

lymphocyte stimulation test will be employed to assess the cell-mediated immune response. In this test, T-cell blastogenesis in presence of phytohaemagglutinin, LPS and Barber's protein of S. typhi will be measured by Tritiated thymidine uptake. Control will be kept for each sample. The same test will be repeated with convalescent blood, i.e. one week after recovery.

8. Reviews

- a. Ethical Review Committee \_\_\_\_\_
- b. Research Review Committee \_\_\_\_\_
- c. Director \_\_\_\_\_

## SECTION-II - RESEARCH PLAN

### A. INTRODUCTION

#### 1. Objective

The objective of this study is to evaluate the immune response in typhoid fever with particular emphasis on the role of cell-mediated immunity in the pathogenesis of typhoid gut perforation.

#### 2. Background

Typhoid fever remains a notable cause of morbidity and mortality in developing countries like Bangladesh. Most of the case fatalities are due to typhoid perforation. One study in ICDDR,B by Dr. T. Butler, et al (1984), based on clinical records of 323 patients with typhoid fever have shown 4.6% patient developed intestinal perforation. Case-fatality rates were 6 of 9 patients treated medically and 1 of 4 patients treated surgically (2).

A literature review of 57,864 cases of typhoid fever in developing countries in antibiotic era revealed that perforation developed in 2.5% patients, a percentage that was similar to the incidence of 2.8% reported in pre-antibiotic era (2). These results indicate that antibiotic therapy did not help much in reducing case fatality due to typhoid perforation. These results also demand further study of the pathogenesis of typhoid fever.

Several studies have suggested that patients who do not develop cell-mediated immunity have an increased incidence of complications and relapse in typhoid fever (13).

Cell-mediated immune mechanism appears to be important in host resistance to infection with Salmonella. It has been found that about one-third of patients who are hospitalized because of salmonellosis have some type of major underlying diseases, such as leukemia, lymphoma, systemic lupus erythematosus or aplastic anaemia (6).

A major characteristic of typhoid is the activation of macrophages. Phagocytosis is a major host defense mechanism and substances released from macrophages probably play a significant role in the pathogenesis of the disease (1). Diarrhogenic strains of Salmonella evoke a polymorphonuclear cellular response when they reach the lamina propria.

Prostaglandins released from the inflammatory exudate stimulate the adenylate cyclase-cyclic AMP system which enable the epithelial cells to actively secrete fluid and electrolytes (4).

On the contrast, typhoid bacilli when penetrates the gut lining and reach the lamina propria, they evoke a mononuclear rather than the polynuclear leukocytic response due to lack of enterotoxin activity (4). Thus, they also do not activate the adenylate cyclase system through prostaglandins since neutrophil leukocyte response that would release these chemicals is not evoked (4). There are some evidence to suggest that mononuclear cellular response could be due to small amounts of endotoxin released at the site of bacterial multiplication (7).

Immune cells probably play a critical role in the host's response to S. typhi infection, but this response has not been studied adequately in human.

Kumar, et al (1974) using 'leukocyte migration inhibition test' (LMIT) as measure of 'cell-mediated immune response' (CMI response) showed that a CMI response develops during typhoid fever. In their study of 22 patients, they also noted a correlation between an adequate CMI response and the absence of complications like intestinal haemorrhage, paralytic ileus, intestinal perforation, relapse (11).

Bala Krishna Sarma, et al (1977) showed that 67% of 60 adults had a positive CMI response which was higher in the second and third weeks of illness than in the first week, a negative CMI response was found in the 1st week in patient with no complication (1).

On the contrast, one study in Sri Lanka by V. Thevanesam, et al (1981) using the same LMIT as a measure of CMI response did not find any correlation between the development of adequate CMI response and the occurrence of complications though they have confirmed a positive CMI response in typhoid fever (14).

From the above background, it is clear that there is paucity of studies designed to prove the correlation between altered cell- and antibody-mediated immune responses and typhoid perforation. We want to do this in the present project using assays quantifying both immune responses (in which LMIT will not be used).

#### B. SPECIFIC AIMS

1. To compare the cell-mediated, as well as humoral immune response in perforated and non-perforated cases of typhoid fever

2. To determine whether there is a correlation between immune deficiency or altered immune response and typhoid perforation

C. METHODS OF PROCEDURE

Selection of Patients

Ten adult patients, either sex, aged 20-30 years, with typhoid fever without perforation and ten adult patients, either sex, with typhoid perforation will be included in this study.

Patient's Sample

a) From typhoid fever without perforation

10 ml of peripheral blood\* will be taken twice, during acute and convalescent stages, from selected cases of typhoid patients routinely admitted at ICDDR,B Hospital or Dhaka Medical College Hospital with the consent of respective consultant concerned.

Diagnosis will be established by positive blood culture.

b) From typhoid fever with perforation

10 ml of peripheral blood will be drawn twice, during acute and convalescent stages, i.e. one week after recovery. Patients will be selected from routine typhoid perforation cases admitted at the Dhaka Medical College Hospital with the consent of respective consultant concerned.

As typhoid perforation usually occurs around 3rd week when there is less possibility of blood culture to be positive, diagnosis

---

\* 10 ml blood will be taken in addition to the amount of blood required for routine test

will be established by either positive blood culture or positive swab culture taken from site of perforation, or intestinal biopsy\* with significant Widal test and typical clinical features.

#### Assessment of humoral immune response

Widal test will be done to see antibody titre against O, Vi, H antigens.

#### Assessment of cell-mediated immune response

Cell-mediated immune response will be assayed by lymphocyte stimulation test. In this test, a pure lymphocyte fraction obtained from peripheral blood by Ficoll-hypaque density gradient method will be cultured with phytohemagglutinin (PHA), Barber's protein and LPS of S. typhi and blastogenesis will be assayed by tritiated thymidine uptake. Control will be kept for each sample.

Lymphocytes from normal persons will show 50% or more conversion to blast forms, whereas, cells from individuals with cell-mediated immune deficiency will demonstrate little or no transformation of lymphocytes in culture.

#### D. SIGNIFICANCE AND RATIONALE

There has been no significant decrease in the frequency of typhoid gut perforation with the advent of specific antibiotic therapy in comparison to that seen in pre-antibiotic era. And most of the case fatalities are

---

\* Intestinal tissue for biopsy will be taken only from resected portion of gut at the time of its surgical repair. This is a routine procedure at Dhaka Medical College Hospital.



due to the typhoid gut perforation. This result necessitates a search for etiology of typhoid perforation. Previous studies suggest that immune mechanism probably play a critical role in the pathogenesis of the disease. This study will help us to better understand the role of immune system in the pathogenesis of the typhoid fever. It will particularly help to evaluate the possible correlation between immune response and the incidence of typhoid gut perforation.

E. FACILITIES REQUIRED

The Biochemistry Branch and Host Defense Laboratory have adequate facilities to support this pilot study.

F. COLLABORATIVE ARRANGEMENTS

Collaborative arrangements with the Dhaka Medical College Hospital will be pursued. Since P.I. is a graduate of that institution, it will be easier. Peripheral blood, as well as biopsy materials (for confirmation of diagnosis) of perforated group of patients will be obtained from that institution with the consent of respective consultant concerned.

G. ANALYSIS OF DATA

1. Data sheets containing information on history and laboratory data will be kept for all cases
2. Statistical correlation will be examined by Fisher's Exact Test and Student's T-test

#### ABSTRACT SUMMARY

1. The subject population of this study will be adult, male or female, 20-30 age group, with typhoid fever. As most of the typhoid perforations occur around this age group. Two groups of patients will be included in the study, typhoid fever without any complication and typhoid fever with intestinal perforation.
2. As no test will be done on any of these patients, except peripheral blood drawing, the study does not pose any risk to the patient.
3. Sterile technique will be adopted during blood drawing.
4. All patients enrolled in the study will be assigned a study number, and this number will be used during analyzing the information. No confidential information will be used.
5. Informed consent will be obtained from the subjects. No information will be withheld from the subjects.
6. Not applicable.
7. No direct benefit to the individual subject. Society will be benefitted as we shall possibly gain a better understanding of the pathogenesis of typhoid perforation which is the single-most important cause of mortality in typhoid fever.
8. 10 ml of peripheral blood will be obtained.

## REFERENCE

1. Balakrishna Sarma VN, Malanviya AN, Kumar R, Ghai OP and Bakhtary MM. Development of immune response during typhoid fever in man. Clin Exp Immunol (1977), 18:35-9
2. Butler T, Knight J, Nath SK, Speelman W, Roy SK, and Azad MAK. Typhoid fever complicated by intestinal perforation: A persisting fatal disease requiring surgical management. Rev Inf Dis (1983); 7: 244-55
3. Chowdhury MR: Modern Medical Microbiology, 1st edition
4. Fegin and Cherry. Text Book of Pediatric Infectious Diseases. Vol I
5. Ganguly NK, Anand IS, Khanna AK, Kohli RS, and Wahi PE. T-cells and T-cell subsets in rheumatic heart disease. Ind J Med Res 76 (1982); 854-8
6. Harrison's Principle of Internal Medicine, Edition 10th:963
7. Hornick PB, and Greisman SE. On the pathogenesis of typhoid fever. Arch Intern Med; 138:357-9
8. Hornick RB, Greisman SE, Woodward TE, DuPont HL, Dawkins AT, and Snyder MJ. Typhoid fever: Pathogenesis and Immunologic Control. Part-I, N Eng J Med; 283(13):686-91; Part-II, N Eng J Med; 283(14):39-46
9. Hudson and Hay FC. Practical Immunology, 2nd Edition; 301-2, 285-8, 26-31

10. Ivan Roitt, Basic Immunology, 3rd Edition
11. Kumar R, Malaviya AN, Murthy RGS, Venkataraman M, and Mohapatra.  
Immunological study of typhoid: Immunoglobulins C<sub>3</sub>, antibodies and leukocyte migration inhibition in patient with typhoid fever and TAB vaccinated individuals.
12. Sodmann and Sodmann, Pathologic Physiology: 128-33
13. Strickland Hunter's Tropical Medicine, Sixth Edition
14. Thevanesam V, Areculeratne SN, Weliangge LV, and Athunda PI. Cell-mediated and humoral response in human typhoid fever. Trop Geogr Med (1982); 34:13-7

## SECTION-III - BUDGET

A. DETAILED BUDGET1. PERSONNEL SERVICES

<u>Name</u>	<u>Position</u>	<u>Time Effort</u>	<u>Project Requirement</u>	
			<u>Taka</u>	<u>Dollar</u>
Dr. Amar Bin Ashraf	Principal Investigator	70%	-	-
Dr. Ivan Ciznar	Coinvestigator	5%		1,000
Dr. Ansaruddin Ahmed	Coinvestigator	10%	6,000	-
Dr. Asma Khanam	Coinvestigator	5%	2,000	-
Dr. R. N. Majumder	Coinvestigator	5%	1,000	-
Mr. Akbar Ali	Coinvestigator	5%	2,000	-
Dr. Shahid Hossain	Guest Investigator		-	-
Dr. T. C. Butler	Consultant		-	-
Laboratory Technician (one)		100%	6,000	-
			17,000	1,000

2. SUPPLIES AND MATERIALS

- Clinical supplies: needles, gloves, syringes, test tubes			-	300
- Lab test:				
Blood culture	50 x Tk.150		7,500	-
Widal test	50 x Tk. 50		2,500	-
Biopsy	25 x Tk.150		3,750	-
Swab culture	30 x Tk.150		4,500	-
- Reagents and chemicals			-	2,000
- Tissue culture media			-	500
- LPS and Barber's protein preparations			-	500
			18,250	3,300

3. LABORATORY EQUIPMENT

Available at the Centre

	<u>Project Requirement</u>	
	<u>Taka</u>	<u>Dollar</u>
4. <u>HOSPITALIZATION</u>	-	-
5. <u>OUTPATIENT CARE</u>	-	-
6. <u>ICDDR,B TRANSPORT</u> 50 trips to Dhaka Medical College Hospital	10,000	-
7. <u>PRINTING AND REPRODUCTION</u>	3,000	-
8. <u>CONSTRUCTION, RENOVATION</u>	-	-
TOTAL:	48,250	4,300
Conversion rate US\$ 1 = Tk.26	48,250	1,856
TOTAL:		\$ 6,156

Total direct cost (excluding Personnel Services):-	ISS 6,156
Less	1,654
	<u>ISS 4,502</u>

B. BUDGET SUMMARY

	<u>Project Requirement</u> <u>Dollar</u>
1. PERSONNEL SERVICES	( 1,654 ) <i>excludes of this is a pilot project.</i>
2. SUPPLIES AND MATERIALS	4,002
3. LABORATORY EQUIPMENT	-
4. HOSPITALIZATION	-
5. OUTPATIENT CARE	-
6. ICDDR,B TRANSPORT	384
7. PRINTING AND REPRODUCTION	116
8. CONSTRUCTION AND RENOVATION	-
TOTAL	<u>\$ 6,156</u>

Total cost : US\$ 6,156

Less Personnel Services : 1,654

Total Project Cost : US\$ 4,502

CONSENT FORM

You are suffering from typhoid fever. This disease can be dangerous if complications develop. The single-most important complication is intestinal perforation that may lead to death. Our present knowledge cannot explain why certain individual with typhoid fever develops intestinal perforations and others do not in spite of same treatment. We are searching for an answer. We need your help.

If you agree, we shall include you in our study. We shall take a detailed history from you concerning your disease-related problems and shall record your laboratory findings.

We shall take twice 10 ml of peripheral blood from you using sterile technique to assess your immune mechanism. The amount of blood drawn will not cause you any harm.

This study will not hamper or alter your treatment. You will continue to receive usual treatment. You are free to give consent for the study. Even if you do not give consent for the study, you will get the usual and appropriate treatment.

We shall let you know the reports of the investigations done if and when you desire so. We shall answer any question you have concerning this study.

If you agree to participate in this study, please sign your name here.

\_\_\_\_\_  
Investigator's signature

Date \_\_\_\_\_

\_\_\_\_\_  
Signature/left-hand thumb impression

Date \_\_\_\_\_



টাইকয়েড কুরে রোগ প্রতিরোধ কক্ষতার উমিকা এবং অন্ত্র ছিপ্র উপসর্গের  
সহিত ইহার সফলক নিয়মক পরীক্ষা ।

সমুতি পর ।

আপনি টাইকয়েড কুরে আশনু, এই রোগ খুবই মারাত্মক হতে পারে যদি উপসর্গ  
বা জটিলতা দেখা দেয় । বিশেষ করে অন্ত্র ছিপ্র হওয়া সত্বেকে পুরস্কপূর্ণ উপসর্গ বা  
জটিলতা সেটার কারণে এমন কি মৃত্যু পর্যন্ত হতে পারে । একই রকম চিকিৎসা সত্বেও  
কোন কোন টাইকয়েড রোগীর অন্ত্র ছিপ্র হয়, অকমেই হয়না - এর কারণ কি ? এই  
প্রশ্নের উত্তর আমরা আজো নিতে পারিনি সঠিক ভাবে । এই প্রশ্নের উত্তর অনুসন্ধানের রত  
আমরা । আর তাই আপনার সক্রিয় সহযোগিতা আমাদের কাম্য ।

আপনি অনুমতি নিজে আমরা আপনাকে আমদের পরীক্ষার অনুষ্ঠান করব । আমরা  
এই রোগ সফলকিত আপনার সব সমস্যা শুনব । আপনার যাবতীয় পরীক্ষার ফলাফল সিনিয়র  
করে রাখব । আপনার রোগ প্রতিরোধ কক্ষতা পরিমাণের জন্য আমরা আপনার হাতের রক্ত নালী  
থেকে দুই বার দশ সিসি রক্ত নিব, সফূর্ণ জীবানু মুক্ত প্রস্তুতিতে । এই পরিমাণ রক্ত নিয়ে  
আপনার কোন জট হওয়ার সম্ভাবনা নেই ।

এই পরীক্ষার জন্য আপনার চিকিৎসার কোন ব্যাঘাত অথবা কোন রকম পরিবর্তনের  
ও প্রয়োজন হবে না । আপনাকে অন্যদের মতোই একইভাবে চিকিৎসা করা হবে । এই  
পরীক্ষায় অংশ গ্রহন করা না করা একানুই আপনার ইচ্ছার উপর । কোন বাধ্যবাধকতা নাই ।  
আপনি এই পরীক্ষায় অংশ গ্রহন করতে রাজী না হলেও আপনার সঠিক চিকিৎসা চলবে ।  
আপনার পরীক্ষা লব ফলাফল আপনি চাইলে জাতিতে পারবেন । আমাদের এই পরীক্ষা সফলক  
যদি আপনার কোন জিজ্ঞাস্য থাকে, তার ও উত্তর দেব আমরা ।

আপনি যদি এই পরীক্ষায় অংশ নিতে রাজী থাকেন তাহলে বীচ আপনার শাকর  
অথবা টিপসহি দিন ।

পবেষকের শাকর

শাকর/টিপসহি ( নাম সূক্ষ্মপুঞ্জিত )

তারিখ :

তারিখ :

মেডিকেল কলেজ হাসপাতাল  
ঢাকা, বাংলাদেশ



MEDICAL COLLEGE HOSPITAL  
DHAKA, BANGLADESH

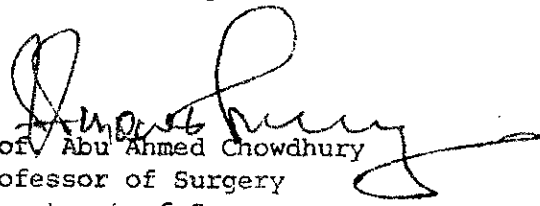
August 12, 1985

The Associate Director  
Host Defense Working Group  
International Centre for Diarrhoeal  
Disease Research, Bangladesh  
Dhaka

Dear Sir

Apropos our discussion with Dr. Amar Bin Ashraf, we agree to collaborate, as required, with International Centre for Diarrhoeal Disease Research, Bangladesh, for Dr. Amar Bin Ashraf's protocol "Immune response in human typhoid fever and its role in the pathogenesis of typhoid perforation."

Yours faithfully

  
Prof. Abu Ahmed Chowdhury  
Professor of Surgery  
Department of Surgery  
Dhaka Medical College and Hospital