Attachment 1.
(FACE SHEET)

Date 23.12.90

ETHICAL REVIEW COMMITTEE, ICDDR,B.

SSS

Principal Investigator TASNIM AZIM Trainee Investigator (if any) _____

Application No, 90-020 Supporting Agency (if Non-ICDDR,B) _____

Title of Study THE ROLE OF IMMUNE (DYS) FUNCTION IN PERSISTENT DIARRHOEA OF CHILDHOOD

Project status:
(✓) 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).

1. Source of Population:
   (a) Ill subjects (Yes) No
   (b) Non-ill subjects (Yes) No
   (c) Minors or persons under guardianship (Yes) No
2. 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)
3. 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
4. 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.

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

Tasnim Azim A-032078

Principal Investigator Trainee

90-020
23/12/90

APPLICATION FOR PROJECT GRANT

1.	PRINCIPAL INVESTIGATOR	:	Dr. Tasnim Azim
	COINVESTIGATORS	:	Dr. Laila Noor Islam Dr. Firdausi Qadri Mr. M. A. Wahed Dr. Michael Louis Bennish Dr. Jena Derakhshani Hamadani
2.	TITLE OF THE PROJECT	:	The role of immune (dys)function in persistent diarrhoea of childhood
3.	STARTING DATE	:	As soon as possible
4.	COMPLETION DATE	:	3 years from starting date
5.	TOTAL BUDGET REQUESTED	:	US$ 206,988
6.	FUNDING SOURCE	:	
7.	PROGRAMME COORDINATOR	:	Associate Director Laboratory Sciences Division

8. AIMS OF THE PROJECT

a) General aim

To identify immune abnormalities that may possibly play a role in precipitating persistent diarrhoea; this will be done by comparing the immune response of children who develop persistent diarrhoea with those who recover from the acute illness.

b) Specific aims

1) Identification of possible alterations in granulocytic and lymphocytic populations and defence mechanisms in diarrhoeal children which may preclude recovery and lead to diarrhoeal persistence.

2) Assessment of the role of cytokines in diarrhoeal persistence in children.

3) Insights into the immunopathogenesis of persistent diarrhoea in general and in Bangladeshi children in particular.

c) Significance

This study will assess whether immunological abnormalities play a role in precipitating persistent diarrhea in children by carrying out a systematic investigation of granulocytic and lymphocytic responses and cytokine levels.

8. ETHICAL IMPLICATION

The following children will be studied:

Disease condition	No. of children	Age of children (months)	Source of children
Acute watery diarrhoea	~150	7-24	ICDDR,B
Persistent diarrhoea	30	7-24	ICDDR,B
Malnutrition			
1st degree	30	7-24	ICDDR,B
2nd degree	30	7-24	ICDDR,B
3rd degree	30	7-24	ICDDR,B and Dhaka Shishu Hospital
Healthy controls	30	7-24	ICDDR,B and Dhaka Shishu Hospital

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 $_{\alpha}$ is the value of normal variate for which the estimated value will be within $\pm\varepsilon$ of the population value with a probability of $(1-2\alpha)$. We have considered the variances of different immunological markers and found that a sample size of 30 is sufficient to limit the error within 20% of the population parameter with 95% confidence level.

Children between the ages of 7 mths-2 yrs who have been suffering from watery diarrhoea for 7±1 days will be enrolled in the study. A rough estimate suggests that around 20% of these children will develop persistent diarrhoea. Therefore, 150 children with acute diarrhoea will have to be enrolled initially to obtain 30 with persistent diarrhoea. However, since this is a rough estimate, the number may vary.

The children will be clinically evaluated daily by a physical examination, measurements of height, weight and temperature. Microscopic examination for cellular elements in stool will be carried out. The haemoglobin percentage and total and differential leucocyte count will be measured in blood. The nutritional status of the children will be assessed by calculating weight for age and will be classified as follows:

1st degree malnutrition	90-75% of NCHS 50th percentile
2nd degree malnutrition	75-60% of NCHS 50th percentile
3rd degree malnutrition	<60% of NCHS 50th percentile

Control children will be matched for age and nutrition and will therefore include children with 1st, 2nd or 3rd degree malnutrition. Healthy children without malnutrition will also be included. Children with malnutrition will be obtained from the nutrition rehabilitation unit of ICDDR,B while healthy

controls will be obtained from Dhaka Shishu Hospital or siblings of patients at ICDDR.B.

Samples of blood, saliva and stool will be collected from all children. Seven mls of venous blood will be drawn at initial enrollment and 14-15 days after the onset of diarrhoea, if diarrhoea persists. Stool and saliva will be taken on the same day; saliva will be collected at least 1 hr after the last feed using a clean plastic pipette. In addition, delayed type hypersensitivity (DTH) will be tested on the forearm of the children. Control children will provide single samples of blood, stool and saliva and undergo one test for DTH.

The study will not interfere with the management and treatment of the children and none of the procedures will be harmful. A written consent will be obtained from the guardian.

9. BACKGROUND

One of the definitions of persistent diarrhoea is the passage of an increased volume of watery stool for a period longer than 2 weeks (Walker-Smith, 1988). A wide range of clinical and pathological conditions are covered by this definition, however, this study will concentrate only on those cases where persistent diarrhoea follows an episode of acute diarrhoea.

Studies in rural and urban Bangladeshi children under 4 years with diarrhoea reveal that 4% of these are persistent (Henry, 1987). Most children admitted to the Treatment Centre of the ICDDR,B are severely ill with malnutrition, malabsorption and often secondary infections such as bronchopneumonia; a fatal outcome in these children is not uncommon. The cause/causes of development of

persistent diarrhoea is/are unknown. Some conditions have however been associated with persistent diarrhoea including:

1. Infections

There are reports of isolation of bacterial, parasitic and viral pathogens from cases of persistent diarrhoea. They include *Shigella* spp., *Campylobacter jejuni*, *E. coli*, *Giardia lamblia*, rotavirus and *Cryptosporidia*. However, Shahid *et al.* (1988) have shown that the incidence of isolation is similar to that of acute diarrheoa. Furthermore, in many cases no pathogen can be isolated; Roy *et al.* (1989) isolated a pathogen in only 1/26 patients with persistent diarrheoa. These findings suggest that although enteric pathogens are instrumental in some cases of persistent diarrhoea, there may be an underlying condition preventing recovery from the acute illness.

2. Carbohydrate malabsorption

Transient carbohydrate malabsorption may accompany acute bouts of diarrhoea due to a decrease in disaccharidase enzymes and/or a decrease in surface villous area (Phillips *et al.*, 1980). The reason for persistence of carbohydrate malabsorption is not understood again suggesting an underlying pathophysiology of absorption.

3. Cow's milk and soy protein intolerance

Cow's milk allergy is characterised by an early age onset (mean age is 9 weeks) (Kuitunen et al, 1975). It causes a spectrum of problems including persistent diarrhoea. Diagnosis is made by an acute challenge of milk which precipitates the symptoms and the child improves when cow's milk is withdrawn.

Some of these children may also be sensitive to soy protein (Whitington and Gibson, 1977). In Malaysia, cow's milk allergy has been reported to be relatively high (Iynykaran et al, 1979). Although the condition does occur in Bangladesh there is no official estimation of its incidence rate.

Certain risk factors for persistent diarrhoea have also been identified such as nutritional status, environment and breast feeding. Studies in Bangladesh have shown that 28% of all deaths in children under 5 years are associated with persistent diarrhoea and malnutrition (Fauveau, 1986). However, the exact relationship between malnutrition and persistent diarrheoa is not clear. Black *et al.* (1984) have shown that children with malnutrition have diarrheoa for longer durations than well nourished children but Koster *et al.* (1987) found no correlation between the two. Snyder and Merson (1982) have shown that persistent diarrhoea is more common in children living in poor environmental conditions which may be related to contamination of water and weaning foods (Black *et al.*, 1982). Moreover, in children under 2 years, the incidence of persistent diarrhoea is less if they are breast fed which further strengthens the relationship between faecal contamination of food and persistent diarrhoea. On the other hand, persistent diarrhoea is not limited to children living in poor environmental conditions (Roy *et al.*, 1989).

Thus, no specific cause can be attributed to persistent diarrhoea. Because of the association of persistent diarrhoea with malabsorption and which can lead to malnutrition, it is treated as a nutritional disorder. The management of persistent diarrhoea, therefore, concentrates largely on dietery and empirical antimicrobial therapy. Although treatment with different diets can be effective it is only partially successful; studies on dietery therapy (Roy *et al.*, 1989) and nutrient absorption (Roy *et al.*, 1990) at the Treatment

Centre for ICDDR,B stress the need for more intensive analyses of the condition. There are ongoing studies on enzyme (amylase) levels, more effective diets and on the microbiology of the gut with particular reference to enteric pathogens at ICDDR,B. However, there are no studies addressing the role of immunopathology in precipitating persistent diarrhoea.

Very little is known about the role of the immune status of the child in the development of persistent diarrhoea. Malnutrition is frequently associated with persistent diarrhoea and the nutritional status is a well recognised determinant of immunecompetence. It is therefore expected that children with persistent diarrhoea will have lowered immunity. However, their lowered immunity may not entirely be related to nutritional status. A preliminary prospective study carried out on Bangladeshi children identifies susceptibility to frequent attacks of diarrhoea of longer duration in children with decreased cell mediated immunity (Koster *et al.*, 1987). Regression analyses of the data reveals that the development of prolonged diarrhoea is related to decreased immunity rather than malnutrition. Based on these preliminary findings, our hypothesis is that immunecompetence could be an important determinant in the development of persistent diarrhoea. This study will investigate this hypothesis by comparing the immune responses of children who recover from acute diarrhoea with those children in whom diarrhoea persist. The following questions will be addressed in this study:

1. Is there an underlying cellular immune defect which is associated with persistence of diarrhoea?

The cellular immune response is mediated by T and B lymphocytes both of which arise from stem cells in the bone marrow. T lymphocytes mature in the thymus from where two populations emerge, CD2+, CD3+, CD4+ (helper/suppressor-

inducer) and CD2+, CD3+, CD8+ (suppressor/cytotoxic) cells. These mature T lymphocytes are functionally competent and on exposure to antigen can mediate help or suppression of other cells or effect cytotoxicity. Studies from our Centre (Koster *et al.*, 1987) which show that decreased cell mediated immunity causes children to suffer from prolonged bouts of diarrhoea, measured cell mediated immunity by delayed type hypersensitivity responses (DTH). In DTH, antigenic peptides are presented by antigen presenting cells and recognised by CD4+ T lymphocytes in association with MHCII antigens. On activation, these cells secrete soluble factors which recruit CD8+, cytotoxic T lymphocytes and macrophages to destroy the antigen. Decreased DTH responses therefore suggest either a decrease/lack of mature T lymphocytes or an inability of mature T lymphocytes to respond to antigen. Inability to respond to antigen may be due to an increase in suppressor cells or the release of inhibitory factors. We will investigate these hypotheses by assessing the percentage of mature CD3+ T lymphocytes in the peripheral blood and the percentages of CD4+ and CD8+ T lymphocytes. In addition, the proliferative responses to known T lymphocyte mitogens will be tested.

B lymphocytes develop in the bone marrow and mature B lymphocytes expressing surface IgM (sIgM) enter the circulation. On exposure to antigen, sIgM+ B lymphocytes become activated, switch to other Ig isotypes, proliferate and give rise either to memory cells or Ig secreting plasma cells. Activation of B lymphocytes may be T lymphocyte-dependent or -independent. T lymphocyte-dependent activation of B lymphocytes relies on help from CD4+ T lymphocytes. If CD4+ T lymphocytes are decreased in number or unable to function, T-dependent B lymphocyte proliferation will be reduced or absent and this can be measured *in vitro* by assessing proliferation in response to T lymphocyte-dependent B lymphocyte mitogens.

2. Do dysfunctional granulocytes contribute to the development of persistent diarrhoea?

Like lymphocytes, granulocytes arise in the bone marrow from stem cells and mature into functional granulocytes under the influence of various haemopoietic factors. From the bone marrow granulocytes enter the circulation and become available for defence against external antigen. For optimal defence, neutrophils must first adhere to endothelial cells, migrate through the blood vessels, engulf the antigen and degranulate. As in many cases of persistent diarrhoea microorganisms can be isolated, it is possible that the granulocytes in these children have a functional defect. Hill et al (1977) found that in children suffering from recurrent episodes of otitis media and chronic diarrhoea there is a decrease in neutrophil chemotaxis. We will examine the functional responses of neutrophils to known neutrophil stimulants and relate functional changes, if any, to the development of persistent diarrhoea.

3. Are there altered levels of circulating cytokines such as interleukin 1 (IL1) and tumour necrosis factor (TNFα)?

IL1 is a pleiotropic cytokine secreted by many cell types most notably macrophages. There are 2 types of IL1- IL1α and ILß. Although IL1ß is predominant, the activities of IL1α and IL1ß are almost overlapping. IL1 has a wide range of effects and mediates systemic acute phase responses, as well as local tissue inflammation. Thus, it induces fever, hypoglycaemia, acute phase protein synthesis, stimulates lymphocytes and the production of granulocytes, elicits production of other cytokines including colony stimulating factors, etc. The release of IL1 is stimulated by microorganisms, endotoxin, antigen-antibody complexes, cytokines such as TNF and complement components such as C5a. Therefore, infection, trauma or any inflammatory

process will stimulate IL1 production any of which may be operative in persistent diarrhoea.

TNFα (cachectin) is similar to IL1 in its actions and production. TNFα is a critical mediator of septic shock syndrome. Its secretion is stimulated by endotoxin and C5a. TNFα may also, therefore, be increased in persistent diarrhoea.

In addition to answering the above specific questions, the study will provide a better understanding of the immunopathogenesis of persistent diarrhoea in children from developing countries who are not only nutritionally compromised but also subject to repeated infections. Because of the unique location of ICDDR,B - in a country in which the incidence is reasonably high - and the Centre's technical competence and availability of basic equipment, it is the most likely place in which some of these questions can be answered.

RESEARCH PLAN

Tests will be carried out on

i) Peripheral blood

ii) Saliva and stool

iii) Delayed type hypersensitivity (DTH)

a) Peripheral blood

Peripheral blood will be used for experiments on serum, plasma, lymphocytes (mononuclear cells) and granulocytes. Seven mls of peripheral blood will be required from children and this will be obtained by venepuncture. In those cases where less blood is obtained, some tests will not be carried out. The plan is shown below:

PERIPHERAL BLOOD (7-10 ml maximum)

—> 1.0 ml
1. C_3, C_4 levels
2. Protein fractions

2 ml for cytokines <—

4 ml in heparin containing tube
separated on Ficoll-Hypaque

—> PLASMA will be collected from above the band of mononuclear cells and used for:
1. IgG, IgM and IgA levels
2. CRP, iron, transferrin or ferritin, zinc
3. Ig to diphtheria and tetanus
4. Autologous plasma for use in other tests

GRANULOCYTES will be obtained from the pellet after removal of RBC by hypotonic lysis and Dextran sedimentation. Cells will be used for:

1. Phagocytosis —> 1×10^6 cells
2. Chemotaxis —> 1×10^6 cells
3. Polarisation —> 2×10^6 cells

MONONUCLEAR CELLS
will be obtained by collecting the band at the interface and used for:

1. Resting DNA synthesis —> 2×10^5 cells
2. PHA stimulation —> 1.0×10^6 cells
3. PWM stimulation —> 4.0×10^5 cells
4. Con A stimulation —> 4.0×10^5 cells
5. Phenotyping by immunofluorescence —> 2.0×10^6 cells

Research plan

a) Serum

1. C3, C4 levels will be measured by a discrete analyser (COBAS BIO). This will be done to control for malnutrition where C3 levels may be lowered.

2. Protein fractions will be estimated by electrophoresis.

b) Plasma

1. IgG, IgA, IgM will be measured by COBAS BIO. Besides providing us information of possible immunoglobulin (Ig) alterations in persistent diarrhoea, any change will reflect alterations in cellular immune responses.

2. Igs to diphtheria (neutralisation test) and tetanus (ELISA) will be asssessed if the children have been immunised against these antigens. These specific Igs will be a measure of the overall immune status of the children and complement DTH tests.

3. C reactive protein will be measured by COBAS BIO to assess the extent of underlying inflammation.

4. Zinc (by an atomic absorption spectrophotometer), iron and transferrin/ferritin (by COBAS BIO) will be measured as these have profound effects on immunity and may be lowered in malnutrition.

5. Cytokines

For the assay of these cytokines, 2 ml of blood will be added to a purple top EDTA containing vacutainer tube to which the protease inhibitor aprotinin has been added (to inhibit enzymatic

degradation of cytokines). The tube will be immediately put on ice, and then centrifuged at approximately 3000 rpm for 5 minutes. The plasma (excluding the buffy coat) will be aspirated, and transferred to a 1.5 ml Eppendorf tube, and then be subjected to a hard-spin (1,000 rpm) in a microfuge in order to completely sediment any remaining white cells or platelets. The supernatants will then again be aspirated, placed in storage vials, and stored at -70°C. The vials will be transported to Tufts University, Boston for assaying cytokine levels. Assay of IL1α and IL1ß and TNF will be done using a radioimmunoassay method. Sensitivities of the assay are typically 80 pg/ml (IL1ß) 40 pg/ml (IL1α) and 10 pg/ml (C/TNF). There is no crossreactivity of the antibodies used in these assays with other leukocyte-derived factors.

Cytokine levels are important indicators of underlying tissue damage and their measure, therefore, has considerable significance in this study. However, as these tests are expensive and have to be carried out in the US, they will be excluded from the study, if the budget provided is inadequate.

c) Mononuclear cells

1. Resting DNA synthesis will be measured by incubating mononuclear cells with ^{3}HTDR for 3 hrs. to assess purification.

2. Phytohaemagglutinin (PHA) (a T lymphocyte mitogen) stimulation will be measured by culturing cells with PHA in the presence of autologous plasma, heterologous plasma or calf serum for 72 hours and assessing proliferation by ^{3}HTDR incorporation.

3. Pokeweed mitogen (PWM) (A T-dependent B lymphocyte mitogen) stimulation will be measured by culturing cells with PWM or Con A in the presence of autologous plasma, heterologous plasma or calf serum for 5 days and assessing proliferation by ^{3}HTDR incorporation. A similar experiment will be conducted with conconavalin A (con A) (a T lymphocyte mitogen). Where cell numbers are inadequate, PHA stimulation will be excluded from the study.

4. Phenotyping by indirect immunofluorescence will be carried out for determining proportions of T cells, B cells and T cell subsets (T helper cells or CD4 and T suppressor/cytotoxic cells or CD8) using monoclonal antibodies UCHT1 (a kind gift of Prof. P.C.L. Beverley), B1 (CD20) (commercial source), anti-CD4 (a kind gift of Dr. Q. Sattentau) and UCHT4 (from Prof. P.C.L. Beverley) respectively.

d) Granulocytes

1. Phagocytic index - neutrophils will be incubated with baker's yeast suspension and pooled human serum from 6 healthy controls for 60 minutes. Cells will then be centrifuged and resuspended in a drop and a smear made on a glass slide and stained wih Wright's stain. Ingested yeast in 50 neutrophils will be counted under a microscope.

2. Neutrophil chemotaxis will be measured by using a Boyden chamber. Neutrophils will be placed in the upper chamber, the chemotactic

peptide N-formyl-Met-Leu-Phe (FMLP), layered in the lower chamber and PBS (phosphate buffer saline) added to both. After incubation for 30 minutes, the filters will be removed, fixed in methanol and stained in haematoxylin and then counted under a microscope.

3. Morphological polarisation of neutrophils-neutrophils will be incubated with FMLP for 30 mins at 37^{o}C. The cells will then be fixed with glutaraldehyde, washed and scored for the proportion of neutrophils deviating from spherical morphology.

b) Saliva and stool

Saliva collected will be heat-inactivated and centrifuged. ELISAs will be carried out on the clear supernatant to measure total Ig and IgA. Igs to polio virus will also be tested by a neutralisation assay if the children have been immunised against polio.

Stool samples will be homogenised by mixing thoroughly in phosphate buffered saline and centrifuged. ELISA to determine Ig levels will be carried out on the clear supernatant.

These tests will be a measure of the response of the mucosal immune system.

c) Delayed type hypersensitivity (DTH)

DTH will be tested using a Multitest CMI kit whereby 7 antigens and a control will be introduced intradermally into the forearm using a multiple puncture device. An induration of 2mm or more diameter after 48 hrs will be counted as a positive reaction. The antigens that will be tested include:

1. Tetanus antigen	550,000 Merieux units/ml
2. Diphtheria antigen	1100,000 Merieux units/ml
3. Streptococcus antigen (group C)	2,000 Merieux units/ml
4. Tuberculin antigen	300,000 IU/ml
5. Glycerin control : solution of glycerin to 70% weight/volume	
6. Candida antigen (albicans)	2,000 Merieux units/ml
7. Trichophyton antigen (mentagrophytes)	150 Merieux units/ml
8. Proteus antigen (mirabilis)	150 Merieux units/ml

The t-test will be used to calculate the statistical significance of each measure, comparing the treatment group with the control group.

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11. PUBLICATIONS OF INVESTIGATORS (last five years)

a) Dr. Tasnim Azim

1. Azim T, Allday MJ and Crawford DH. (1990) Immortalization of Epstein-Barr virus-infected CD23-negative B lymphocytes by the addition of B cell growth factor. J. Gen. Virol., 71:665-671.

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b) Dr. Laila N. Islam

1. Islam LN and Wilkinson PC. (1989) Evaluation of methods for isolating human peripheral blood monocytes. J. Immunol. Meths. 121:75-84.

2. Wilkinson PC. and Islam LN. (1989) Recombinant IL-4 and IFN-γ activate locomotor capacity in human B lymphocytes. Immunol, 67:237-243.

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c) Dr. Firdausi Qadri

1) Yasmeen T and Qadri F. 1984. Purification of alkaline phosphatase from human placenta. J Chromatog, 315:425.

2) Qadri F. 1985. The reactive triazine dyes--their usefulness and limitation in protein purifications. Trends in Biotechnol, 3:7.

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Dr. M. L. Bennish

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12. FLOW CHART

Study population		No. of children	Time (3 years)
Acute diarrhoea		~150	Children will be enrolled whenever available, preferably by 1½ years
Persistent diarrhoea		30	- as above -
Malnutrition:	1st degree	30	Samples will be obtained from children as and when they are available
	2nd degree	30	
	3rd degree	30	
Healthy controls		30	- as above -

13. ITEMISED SPECIFIC TASK FOR EACH LISTED INVESTIGATOR

Investigator/Task	Percentage of time involved
a) Dr. T. Azim	25%
1. Lymphocyte studies 2. DTH studies 3. Measurement of antibodies to diphtheria, tetanus and polio 4. Overall coordination	
b) Dr. L. N. Islam Granulocyte studies	50% (of a 12-hour/week consultancy)

c) Dr. F. Qadri — 10%

Estimation of Ig levels in plasma, saliva and stool

d) Mr. M. A. Wahed — 5%

Estimations of Ig levels, serum complement levels, iron, transferrin/ferritin, CRP and zinc levels in plasma by COBAS BIO and AAS

e) Dr. J. D. Hamadani — 10%

Clinical assessment and management of patients at ICDDR,B

f) Dr. M. L. Bennish

Organise cytokine assays in the US

13. BUDGET

(in US$)

	1st year	2nd year	3rd year	Total
PERSONNEL				
Dr. T. Azim	6,000	6,600	14,519	27,119
Dr. L. N. Islam	1,100	1,221	2,500	4,821
Mr. M. A. Wahed (5%)			803	803
Technician (2)	4,950	5,445	12,100	22,495
	12,050	13,266	29,992	55,238
SUPPLIES AND CHEMICALS				
Plastics	6,000	7,000	8,000	21,000
Chemicals	6,000	6,600	7,500	20,100
Media, serum & other reagents	8,500	9,500	10,000	28,000
	20,500	23,100	25,500	69,100
HOSPITAL EXPENSES	3,000	3,400	3,750	10,150
MISCELLANEOUS, including mail, transport, fax, library, etc.	4,500	5,000	5,500	15,000
CYTOKINE ASSAYS		3,500	4,000	7,500
10% added costs	9,000	10,000	11,000	30,000
TRAVEL (to Tufts University for transporting plasma)			3,500	3,500
CAPITAL EXPENDITURE				
Instruments and equipments including Co_2 incubator, $-20^{o}C$ freezer $4^{o}C$ refrigerator and maintenance	10,000	3,500	3,000	16,500
TOTAL : US$	59,050	61,766	86,172	206,988

TA:mh/T1:CYTOKIN3.PRT

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH

CONSENT FORM

Your child is suffering from acute watery diarrhoea from which most children recover, but in a few, the diarrhoea persists. We do not know why some children develop persistent diarrhoea. As it causes considerable illness, sometimes death, we are carrying out a study to investigate its cause. We hope such a study will help us better understand the illness and thereby lead to its better treatment. We would like to enroll your child in this study. For the purpose of the study we require 7 mls of blood from your child, and a sample of saliva and stool. We will also carry out a skin test by pricking the forearm of your child at 8 small sites to assess his/her ability to combat infections. If the skin test is negative, we will vaccinate your child with the vaccines available at the Clinical Research Centre, ICDDR,B. If your child is still ill after another week, we will repeat all the samplings and tests. None of these procedures are harmful and they will not interfere with the usual care and treatment that is normally provided.

Your child will receive the usual care and treatment provided by the hospital irrespective of whether he/she participates in the study or not. If at any time you wish to withdraw your child from the study, you are free to do so. All the information obtained during the study will be confidential and, if you wish to know the results, they will be provided to you on request when they become available.

If you agree to let your child participate in the study, please sign or put your left thumb print impression below.

Signature (or left-thumb print of guardian)	Date
Signature of investigator	Date
Signature of witness	Date

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH

CONSENT FORM

We are conducting a study on children suffering from persistent diarrhoea. Persistent diarrhoea is a little understood condition in which diarrhoea persists for 2 weeks or more. As it causes considerable illness, sometimes death, we are investigating into its nature and cause. We hope such a study will help us better understand the illness and thereby lead to its better treatment. For this study we need to test not only children with persistent diarrhoea but also children without diarrhoea. We would, therefore, like to enroll your child in the study.

For the purpose of the study we require 7 mls of blood from your child and a sample of saliva and stool. We will also carry out a skin test by pricking the forearm of your child at 8 small sites to assess his/her ability to combat infections. The skin test will be read after 48 hours and if it is negative, we will vaccinate your child with the vaccines available at the Clinical Research Centre of ICDDR,B.

None of these procedures are harmful. All the information obtained during the study will be confidential and,if you wish to know the results, they will be provided to you on request when they become available.

If you agree to let your child participate in the study, please sign or put your left thumb print below.

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Signature (or left-thumb print of guardian) Date

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Signature of investigator Date

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Signature of witness Date

Project title: The role of immune (dys)function in persistent diarrhoea of childhood.

Principal Investigator(s): Drs. T. Azim, L.N. Islam, F. Qadir, M.L. Bennish and Mr.M.A. Wahed.

Summary of Referee's Opinions: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

	Rank Score		
	High	Medium	Low
Quality of Project	✓		
Adequacy of Project Design	✓		
Suitability of Methodology	✓		
Feasibility within time period	✓		
Appropriateness of Budget	✓		
Potential value to field of knowledge	✓		

CONCLUSIONS

I support the application:

a) without qualification ☑

b) with qualification:
- on technical grounds ☐
- on level of financial support ☐

I do not support the application ☐

Name of Referee: DOROTHY H. CRAWFORD

Position: PROFESSOR of MICROBIOLOGY

Institution: LONDON SCHOOL of HYGIENE and TROPICAL MEDICINE

DHCrawford
Signature

1/9/90
Date

DETAILED COMMENTS

Please briefly provide your opinions of this proposal, giving special attention to the originality and feasibility of the project, its potential for providing new knowledge and the justification of financial support sought; include suggestions for modifications (scientific or financial) where you feel they are justified.

(Use additional pages if necessary)

This research project aims to study the state of the immune system in children in Bangladesh with persistent diarrhoea and compare this with control children. The study described will yield general information about lymphocyte and neutrophil function, secretary antibody production and cytokine levels. The study has been well designed and makes best use of the small amounts of material available. It remains within the bounds of feasibility and should yield important results within the allotted timespan. These results will make a considerable contribution to our knowledge in this important field of research. I feel that the financial support is fully justified and I would wholeheartedly support the funding of this research project in full.

Project title: The role of immune (dys)function in persistent diarrhoea of childhood.

Principal Investigator(s): Drs. T. Azim, L.N. Islam, F. Qadri, M.L. Bennish and Mr. M.A. Wahed.

Summary of Referee's Opinions: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

	Rank Score		
	High	Medium	Low
Quality of Project	✓ to ✓		
Adequacy of Project Design	✓		
Suitability of Methodology	✓ Mostly		
Feasibility within time period	✓		
Appropriateness of Budget	✓		
Potential value to field of knowledge	?		

CONCLUSIONS

I support the application:

a) without qualification ☐

b) with qualification:
- on technical grounds ☑ Fairly minor. See overleaf
- on level of financial support ☐

I do not support the application ☐

Name of Referee: PROFESSOR P. C. WILKINSON

Position: Professor

Institution: Immunology Dept., University of Glasgow

Peter C Wilkinson
Signature

31st August 1990
Date