PD-ABB-948
6071

TAB-B

PROPOSAL

Submitted to:	Office of the Coordinator Research and University Relations Bureau of Science and Technology Agency for International Development Washington, D.C. 20523
Project Title:	Immunosuppression to Bacterial Vaccines in Schistosomiasis
Principal Investigator:	OLAKUNLE O. KASSIM, Ph.D., MPH Assistant Professor
Signature:	_____ Date: 06/04/84
Mailing Address:	Department of Microbiology Howard University College of Medicine 520 W Street, NW Washington, D.C. 20059
Project Duration:	September 1984 - September 1985
Direct Costs:	$30,808
University (Indirect) Costs:	$27,277
Total Project Costs:	$58,535
University Official for Fiscal Responsibility:	Caspa L. Harris, Jr., J.D. Vice President for Business and Fiscal Affairs
Signature:	Caspa L. Harris Date: 6/10/84 Asst Treasurer
Mailing Address:	Howard University 2400 Sixth Street, NW Washington, D.C. 20059

PROJECT RELEVANCE TO AID

Considerable efforts are being mounted by the World Health Organization, U.S. Agency for International Development (AID) and various governments of developing countries to immunize their young populations against diseases that are responsible for significant proportions of mortality and morbidity. Typhoid fever, whooping cough and pneumococcal meningitis are some of those diseases. But many of these countries are also endemic for schistosomiasis, malaria and other parasitic infections. It has been shown in some studies that acute falciparum malaria renders the host unresponsive to selected bacterial vaccines. This is a matter of serious concern in developing countries where immunization programs for various reasons have met with limited success. It has been speculated that the failure of those programs may in significant part be due to underlying immunosuppression that is attributable to schistosome and other parasitic infections. This study is, therefore, designed to determine whether immunosuppression induced by an infection of Schistosom mansoni in an experimental host could result in humoral and/or cellular unresponsiveness to whole cell vaccines of Bordetella pertussis, Salmonella typhimurium and Streptococcus pneumoniae. The three bacteria species have been chosen for this study because they are the etiological agents of whooping cough, gastroenteritis and bacterial meningitis, all of which are associated with mortality and morbidity rates in developing countries.

The significance of this study, therefore, lies in its elucidation of the immunosuppression that may otherwise render ineffective such vaccines that may be administered to children with acute or chronic schistosomiasis. The findings would, therefore, provide a strong basis for considering endemic parasitic disease prevalence as an important factor in the design and administration of immunization programs. AID has committed a significant portion of its resources to the control of communicable endemic diseases, through immunization and drinking water programs, and the findings from this study would undoubtedly enhance those objectives.

OBJECTIVES AND RATIONALE

The overall objective of this study is to determine whether immunosuppression induced by infection of Schistosoma mansoni in an experimental host could result in humoral and/or cellular unresponsiveness to whole cell vaccine of Bordetella pertussis, Salmonella typhimurium and Streptococcus pneumoniae. The experiments have therefore been designed with the following objectives:

- to determine the specific IgG, IgM and IgA antibody responses of S. mansoni-infected mice to whole-cell intraperitoneal vaccination (inoculation) of B. pertussis, S. typhimurium and Strep. pneumoniae

- to investigate the blastogenic responses to PHA, SEA and bacterial antigens during acute and chronic stages of S. mansoni infection (with or without bacterial immunizations)

- to compare the differences and/or similarities of the humoral and cellular responses during the acute and chronic stages of S. mansoni infection and

- to determine the kinetics of the responses over a 21-day period, which is long enough for observation of possible significant changes.

BACKGROUND

Schistosomiasis, malaria, whooping cough, bacterial meningitis and diarrheal diseases are a few of the diseases which are responsible for the high rates of infant mortality and adult morbidity in many developing countries. A recent survey undertaken by the World Health Organization (WHO) estimated that over 500 million people are exposed worldwide to the three species of human schistosomes (1). Both the vesicular and hepatosplenic forms of schistosomiasis represent chronic debilitaing diseases which may result in nephrotic syndrome, bladder calcification, pipestem liver fibrosis, hepatosplenomegaly and development of cor pulmonale (2,3). Whooping cough, due to Bordetella pertussis, is an acute respiratory disease that is characterized by paroxymal coughing. About 80% of all unimmunized children contract the disease, with a case fatality of 1-3% (4). Salmonella typhimurium, on the other hand, is associated with gastroenteritis and typhoid fever. The death rate from the disease varies from 2 to 10 percent. In addition to being associated with pneumonia, Streptococcus pneumoniae also causes meningitis, with case-fatality rates of 40-60% in many developing countries (5). Although diseases due to these bacteria have largely been brought under control in industrialized countries, they still represent significant causes of mortality and morbidity in developing countries. While a lot of research effort has been devoted to understanding the pathogenesis and immunology of the aforementioned diseases, they have been investigated as single disease entities, thus ignoring their potential interactions in affected hosts.

A number of reports have shown that in vitro proliferative responses of spleen cells to mitogens and parasite antigens were also depressed in human acute and experimental chronic schistosomiasis, and that normal responses could be restored with the removal of adherent phagocytic cells (6,7,8). Rocklin et al (7) reported a direct correlation of granuloma size with lymphocyte suppression of proliferation in autologous sera from patients with S. mansoni infection. Ottesen (8) followed some patients from the acute to the chronic stage of S. mansoni infection and found that adherent suppressor cells which were absent early in infection were present in the peripheral mononuclear cell population by the 20th month of infection. Keyes and Colley (9) also demonstrated a similar development of suppressor cell activity in spleen cells of mice with S. mansoni infection. It is important to keep in

mind in this review that while cellualr proliferative responses in schistosomiasis are depressed, there is no suppression of antibody production against specific parasite antigens. Analysis of our preliminary data from a cross-sectional study of Nigerian children with concurrent infections of falciparum malaria and schistosomiasis haematoboium shows that anti-P. falciparum merozoite IgG antibodies are significantly elevated in children with concurrent infections, at least suggesting that antibody projection to a malaria antigen was not depressed in the affected hosts (10). Nevertheless, sera from chronic malaria and schistosomiasis have been shown to have a suppressive effect on lymphocyte reactivity (8), indicating the involvement of serum factors in the depression of lymphocyte reactivity. While available evidence has demonstrated immunosuppression to various antigens and vaccines in human acute and experimental murine malaria (11,12,13), there is as yet no evidence in the literature to show whether similar suppression occurs or cannot occur in human or experimental schistosomiasis. Additional evidence of immunosuppression in parasitic diseases has come from the studies of Prost et al (14) who reported that only 7.1% of patients with onchocerciasis became immunized after an administration of tetanus toxoid vaccine in contrast to 44.5% in the control group. Humoral and cellular immunosuppression to tuberculin skin tests and BCG vaccination has also been reported in individuals with onchocerciasis (15).

The proposed study is, therefore, an attempt to examine the patterns of immune responses to selected bacterial vaccines in an experimental host during acute and chronic stages of S. mansoni infection. The host immune responses that will be examined include specific anti-bacteria IgG, IgM and IgA production or depression, and the kinetics of the humoral responses to each bacterial vaccine. Spleen cell proliferative rsponses to the bacterial antigens and the possible changes over time in those responses will also be investigated.

PROJECT FEASIBILITY AND SUMMARY OF PRELIMINARY INVESTIGATION

A preliminary phase of this project is currently being undertaken in our laboratory. This preliminary study has led to the determination of optimal bacterial antigen dosage for immunization of mice (without signficant mortality); antibody and enzyme conjugate concentrations for measurements of specific anti-S. mansoni and anti-bacteria IgG and IgM levels by the enzyme-linked immunosorbent assay (ELISA). The preliminary results have given us an indication of the pattern of antibody responses in S. mansoni-infected mice that were challenged with whole-cell bacterial vaccines. Preliminary studies on cellular immune responses of S. mansoni-infected mice are also being undertaken for delineation of optimal culture conditions. We therefore believe that the project is scientifically feasible within the three year period of project duration.

EXPERIMENTAL DESIGN AND METHODOLOGY

METHODS

Parasite Strain and Bacterial Cultures

The Puerto Rican strain of *Schistosoma mansoni*, routinely passaged in albino *Biomphalaria glabrata* snails and Swiss albino white mice will be used for the study. This is the strain that is currently being used in our preliminary studies. Six-week old female BALB/c mice will be used in all experiments, and they will be infected subcutaneously with 20 to 40 *S. mansoni* cercariae. Stock cultures of *Salmonella typhimurium*, *Bordetella pertussis* and *Streptococcus pneumoniae* have been obtained from the Clinical Bacteriology Laboratory of Howard University Hospital and from the Bureau of Biologics (Washington, D.C.). These cultures are intended for preparations of whole cell bacterial vaccines and bacterial antigens. The antigen preparations will be used in measuring serum antibody responses and in the lymphocyte transformation assays.

Soluble Egg Antigen of S. mansoni

About 3μg of lyophilized *S. mansoni* soluble egg antigen (SEA), prepared by the method of Boros and Warren (16) has been obtained through the auspices of Dr. K. E. Mott of the World Health Organization (Schistosomiasis and other Helminth Infections Division) and with the assurance of additional supplies whenever there are proven needs. Part of the antigen supply is currently being used for our preliminary studies.

Bacterial Whole Cell Vaccines and Extracted Antigens

Whole cell vaccines and extracted antigens of the three bacteria will be prepared as follows:

Bordetella pertussis

Working cultures of the bacteria will be grown from stock isolates on Bordet-Gengou potato-glycerol-blood (BG) agar at 37°C for 24 hrs. Colonies from the BG agar plates will be inoculated into 500ml of Stainer-Scholte medium, supplemented with bovine serum albumin (0.05mg/ml). After 16 to 24 hrs of culture at 37°C, the cells will be harvested by centrifugation at 10,000g for 15 min and washed 3x with cold PBS (pH 7.5). The cells will be suspended in PBS to a density of $1x10^{12}$ cells/ml, and the cell suspension will be divided into two separate flasks. Cells in one flask will be killed by treatment with 0.1% formaldehyde at 4°C for 48 hrs and then resuspended in PBS to $1x10^{10}$ cells/ml. Prior to being used, these whole cell vaccines will be heated at 56°C for 30 min to destroy heat-labile toxicity. Cells in the second flask will be sonicated for 10 min in an ice-cooled cell. After centrifugation at 100,000g for 30 min at 4°C, the supernatant will be retained as the antigen. Its protein concentration will be determinted by the Folin-Ciocalteau method and appropriate dilutions made before it is used in subsequent experiments.

Salmonella typhimurium

Working cultures will also be grown from a stock isolate on bismuth sulfite agar (BSA) at 37°C for 24 hrs. Bacterial colonies from the BSA culture will be inoculated into 500 ml of selenite broth and incubated at 37°C until a density of $1x10^{12}$ cells/ml of culture is reached. The cells will be harvested by centrifugation and the whole cell vaccine and antigen prepared as described above for B. pertussis.

Streptococcus pneumoniae

A 24 hr culture of Streptococcus pneumoniae isolate will also be grown in 500ml of brain-heart infusion broth. The cells will be harvested and the whole cell vaccine and antigen prepared as outlined above for B. pertussis.

Enumeration of Lymphocyte Subpopulations

Spleens removed from appropriate mice will be weighed, and spleen cell suspensions will be made by gentle teasing and pressing through a #100 stainless steel mesh into a cold RPMI 1640 medium, containing 100 units/ml penicillin, 100ug/ml streptomycin and 10% heat inactivated fetal calf serum. The cell suspensions will be washed in the same medium and diluted to a concentration of $1x10^8$ cells/ml. After determining the viability of the cells by trypan blue exclusion, aliquots of the cell suspensions will be enumerated for θ-bearing T cells and complement receptor-bearing B lymphocytes. The EAC rosetting procedure of Silveira et al (17) will be used for enumeration of B lymphocytes while the cytotoxic assay of Mandes et al (18) will be employed for T cells.

EXPERIMENTAL DESIGN

The experiments described in this section are designed to investigate the specific humoral and cellular responses to bacterial vaccines during the acute and chronic stages of S. mansoni infection in the mouse. The humoral responses that will be examined include the kinetics of specific anti-bacteria IgG, IgM and IgA production to whole cell vaccines of Bordetella pertussis, Salmonella typhimurium and Streptococcus pneumoniae. Spleen cells from S. mansoni infected and control mice will be used in the lymphocyte blastogenic assays in responses to sensitization with phytohemagglutin (PHA), S. mansoni soluble egg antigen (SEA) and extracted antigens of the three bacteria. The role(s) of adherent phagocytic spleen cells in the overall proliferative reponses will be determined with immune sera from S. mansoni infected mice and bacteria-vaccinated mice. The bactericidal properties of immune and control sera (for the 3 bacteria) will also be investigated.

Infection and Vaccination

A total of 450 six-week old female BALB/c mice will be used for the experiments. All mice will be maintained on Purina Chow and water ad libitum. About 230 of these mice mice will be infected subcutaneously

with 30 to 40 S. mansoni cercariae per mouse. At 7 and 18 weeks post infection, 100 of the infected mice will be randomly selected and divided into four groups. For the 7-week post infection, the groups will be designated "S. mansoni acute infection" groups I, II, III and IV, and "S. mansoni chronic infection groups" for the 18-week infected mice. Groups I, II and III mice of acute and chronic infections will receive intraperitoneally 0.5ml/mouse ($1x10^9$ cells/ml) of whole cell vaccine of B. pertussis, S. typhimurium and Strep. pneumoniae respectively. Group IV mice will receive 0.5ml/mouse of PBS. At the same 7 and 18 week periods, 100 of the noninfected mice will also be taken out of the mouse colony and divided into four groups designated "Bacteria-vaccine" groups I, II, III and IV. Groups I, II and III will also be given intraperitoneally 0.5/ml mouse of same concentration of whole cell vaccines of B. pertussis, S. typhimurium and Strep. pneumoniae respectively. Group IV mice will receive 0.5ml of PBS. At 0, 5, 10, 15 and 20 days post bacterial vaccination of each infection period, 5 mice from each group will be anaesthesized by ip injection of sodium pentobarbital and bled for serum at the retro-orbital plexus. Immediately after, spleen from these mice will be removed, weighed and the livers checked for presence of egg granulomas as a manifestation of infection. Spleen cell suspensions will be made and enumerated for percentages of T and B lymphocytes as described in Methods.

Humoral Immune Responses

Sera obtained at 0, 5, 10, 15 and 20 days post bacterial vaccination from all groups of mice will also be analysed by ELISA for specific IgG, IgM and IgA antibodies against the SEA and the bacterial antigens. A modification of the enzyme-linked immunosorbent assay (ELISA) of Voller et al (21) will be employed in the quantitation of the antibodies. The ELISA has been chosen for the antibody measurements because of its proven simplicity, sensitivity and reproducibility, and also because of its excellent performance in our ongoing preliminary studies. The assay will be performed as follows:
Briefly, 200ul of the appropriate concentrarion of the antigen in sodium carbonate bicarbonate coating buffer (pH 9.6) will be used to sensitize the wells of polyvinyl microtiter plates at 20°C for 4 hrs. After washing the plates with phosphate buffered saline (PBS) with Tween 20 (pH 7.4), duplicate 200µl serum dilutions (1/100) will be added to the test wells. The plates will be incubated at 20°C for 2 hrs to permit the antibody from the serum samples to absorb to the antigen bound in solid phase to the plastic plate. The plates will again be washed and 200µl of a 1/1000 alkaline phosphatase conjugated rabbit-anti-mouse IgG, IgM or IgA (Miles Laboratories) in PBS will be added to the wells and permitted to incubate at room temperature for 2 hrs. After a final wash, 200µl of para-nitrophenol phosphate (1mg/ml) in diethanolamine buffer (pH 9.8) will be added to each well. The reaction will be stopped after 30 min with 50µl of 3M NaOH. The absorbance will be read at 405 nm in an ELISA Reader. The intensity of the color reaction (OD) is directly proportional to the degree of antibody binding and will thus be a measure of anti-schistosomal or anti-bacterial IgG, IgM or IgA antibody. In order to demonstrate the quantitative differences of the specific antibody responses in the experimental animals, we will convert

the absorbance readings to proportional values of bound to added conjugated antibody, based on the total activity of the conjugate using the equation of Susuki and Damian (1981).

$$\% \text{ Ab} = \frac{\text{OD of test serum} - \text{OD without serum}}{\text{OD of entire conjugate substrate}}$$

Purified bacterial antigens such as pneumococcal polysaccharide (type III) and Sal. typhimurium O and Vi antigens will also be used in the antibody and blastogenic assays (22,23) and their specificity compared to those of the crude antigen extracts.

It is also expected that the ELISA measurements will help determine if an acute and/or chronic infection of S. mansoni may result in the suppression of overall humoral or class-specific antibody responses to the three bacterial vaccines. The results will also be used in determining the kinetics of the humoral responses over a 20-day period.

Lymphocyte Proliferative Responses

Lymphocyte responses to phytohemagglutin (PHA), S. mansoni soluble egg antigen (SEA) and extracted antigens of B. pertussis, S. typhimurium and Strep. pneumoniae will be determined with spleen cells obtained in the respective groups of mice. The 7-week post S. mansoni infection period represents the acute phase of infection and a stage that is characterized by intense egg production and large liver granuloma formations. The 18-week post infection period represents the chronic stage of the infection. This is a period of modulation of the immunopathological effects, particularly a decrease in egg granuloma size, diminution of portal pressure, splenomegaly, hepatomegaly, MIF and ESP production (19). It is also a period that is characterized by reduced lymphocyte responses to Con A and PHA mitogens (20). For these experiments, $5x10^5$ viable spleen cells from the sacrificed mice will be cultured in triplicate in RPMI 1640 medium in flat-bottom microtiter wells supplemented with 100 units/ml of penicillin 100 units/ml of streptomycin, 300µg glutamine and 10% inactivated fetal calf serum. Also added to the separate cultures will be 1µg/ml PHA, 8µg/ml (SEA) or the appropriate concentrations of the bacterial antigen extracts. Control cultures without any stimulating antigens or mitogens will also be included. Based on the results of our preliminary study, the cultures will be incubated in 5% CO_2-95% air at 37°C for 3 days (PHA) and 5 days for SEA and the bacterial antigen extracts. Six hours prior to harvesting, each culture will receive 1µCi/ml of triated thymidine (^{3}H-Tdr) (specific activity 2.0C/mM). After harvesting, the cells will be washed and processed in a solubilizing scintillation fluid and analysed for the amount of ^{3}H-Tdr incorporated in a spectrometer. The degree of lymphocyte response will be expressed at net counts per minute (cpm) of stimulated cultures minus the cpm of unstimulated control cultures.

The results of these experiments will be used in determining whether or not an acute or a chronic S. mansoni infection results in the suppression of cellular responses to any of the whole cell vaccines

of the 3 bacteria pathogens. Should there be a significant increase or decrease in the lymphocyte responses, the results will also help determine the kinetics of the response over a 20-day period after administration of the vaccines.

Roles of Adherent Spleen Cells and Immune Serum

It has already been demonstrated that removal of adherent phagocytic cells from spleen cell suspensions increases lymphocyte reactivity to parasite-specific S. mansoni antigens (20) and that addition of homologous immune serum to the lymphocyte cultures suppressed the lymphocyte response to PHA and parasite antigens (21). To determine if this phenomenon is parasite-specific and whether addition of immune serum from acute for chronic schistosome infection would depress proliferative responses of non-adherent spleen lymphocytes from S. mansoni infected mice, the following modifications will be made in the lymphocyte cultures. To study the role of adherent spleen cells, 100mg of sterile carbonyl iron powder will be added to $1x10^8$ spleen cells ($1x10^7$ cells/ml of RPMI-FCS) from S. mansoni infected mice and incubated for 1 hr at 37°C. The non-aherent spleen (NAS) cells will then be aspirated with a Pasteur pipette, and the incubating dish washed with RPMI 1640, with the washings added to the NAS cells. The aspirations and washings will be carried out over a magnet in order to retain the carbonyl-iron and associated phagocytic cells. The adherent cells will be fixed, stained with Wright's stain and counted under the microscope After determination of viability by trypan blue exclusion, $5x10^5$ cells of the NAS cells and unfractionated spleen cells from S. mansoni infected mice will be cultured in the presence of SEA and bacteria antigens. Control cultures without antigens will also be set up. The results will be compared to determine the specificity of lymphocyte response to homologous and heterologous antigens.

For the determination of specificity of chronic or acute serum on lymphocyte reactivity, two sets of spleen lymphocyte cultures from S. mansoni infected mice will be set up as outlined above, except that fetal calf serum supplement will be replaced by inactivated acute chronic serum (56°C, 1 hr). The first set of cultures will receive inactivated serum from S. mansoni-infected mice while the second set of cultures will be supplemented with inactivated serum from bacteria vaccinated mice. A set of control cultures, supplemented with fetal calf serum will also be set up. Each set of cultures, will then be stimulated with PHA, SEA and the bacterial antigens. ^{3}H-Tdr will be added and incubated as already described. The results will be compared to determine the specificity of homologous or heterologous serum in affecting homologous lymphocyte reactivity to parasite and bacterial antigens.

INTERPRETATION AND ANALYSIS OF RESULTS

Egg-induced granuloma formation represents the major element in the host's immune response to a schistosome infection. This is also the basis of the liver, spleen, intestinal and/or lung pathology induced by S. mansoni infection. Some investigations have shown that

the granulomatous reactions in experimental murine infections begin to modulate around the 12th week of infection, in addition to loss of foot-pad swelling and decreased lymphocyte blastogenic responses PHA and SEA (20). These observed phenomena are recognizable elements of immuno-suppression. The experiments outlined in this study have therefore been designed to investigate the effect of S. mansoni infection on the host's humoral and cellular responses and also compare these responses during the acute and chronic phases of the infection of whole cell vaccines of three pathogenic bacteria. It is possible that the host's humoral and cellular immune respones vary with each of the three bacteria. Important controls have been designed into the study with the inclusion of three separate groups of mice, one infected only with S. mansoni, another receiving whole-cell vaccines and the third control group given saline injection. Subtraction of the control effects would permit an objective interpretation of the results, following a statistical analysis of the data. Analysis of variance (ANOVA) and other appropriate stastistical tests will be used in comparing the data from the experimental and the control groups, and between the acute and the chronic phases of S. mansoni infection is parasite-specific or could also result in non-responsiveness to specific bacteria · accines.

LABORATORY ANIMALS

BALB/c female mice will be used in all experiments. They will be 6 to 8 weeks old at the time of purchase, which is expected to coincide with the period of primary exposure to the schistosome parasites. The mice will be housed in the Animal Room, which is maintained in strict sanitary conditions, and will be given food and water ad libitum. They will always be anaesthesized by ip injection of sodium pentobarbital prior to being bled at the retro-orbital plexus. The Biomphalaria glabrata snails, the intermediate hosts of S. mansoni, are fed on Romaine lettuce and are maintained in aerated aquaria, which are equipped with filters and whose water is changed every two weeks. Infected snails are kept in aluminum foil-covered aquaria, on which are displayed conspicuous labels of BIOHAZARD. An adequate amount of 95% ethyl alcohol in a spray bottle is kept at hand during artificial light-induced shedding of cercaria by infected snails. The alcohol is used both as a disinfectant and as a cercaricide.

TIME SCHEDULE

- preparation of whole cell vaccines and antigen extracts from B. pertussis, S. typhimurium and Strep. pneumoniae
- infection of BALB/c mice with S. mansoni and their subsequent immunization with whole cell bacterial vaccines
- determination of specific IgM, IgG and IgA concentrations by ELISA

- isolation and purification of homogenous bacterial antigens both for immunization of mice and sensitization of spleen lymphocytes

- comparison of isolated antigens for optimal immunization and sensitization

- determination of adequate concentrations of the bacterial antigens in the lymphocyte proliferation assays

- determination of lymphocyte proliferative responses to PHA, SEA and bacterial antigens

- analysis of data and preparation of manuscripts for publication

CURRICULUM VITAE

NAME: OLAKUNLE O. KASSIM

ADDRESS: Department of Microbiology
Howard University College of Medicine
520 W Street, NW
Washington, D.C. 20059

TELEPHONE: (202) 636-6284

EDUCATION:

1980	Master of Public Health (MPH), Epidemiology The Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland
1975	Ph.D., Parasitology University of Minnesota Minneapolis, Minnesota
1971	B.A. Biology/Chemistry Bethel College St. Paul, Minnesota

CONTINUING EDUCATION:

1983	Workshop on Atomic Energy Applications in Biomedical Research, Uniformed Services University of Health Sciences, Bethesda, Maryland
1978	Workshop on Bacterial Meningitis Institute of Health, Ahmadu Bello University, Nigeria
1976	Workshop on Enzyme-Linked Immunosorbent Assays: Application in Infectious Diseases Research National Institutes of Health, Bethesda, Maryland
1975	Certification: Registered Microbiologist in Pathogenic Bacteriology and Parasitology, American Academy of Microbiology, Washington, D.C.

EMPLOYMENT:

Assistant Professor, Department of Microbiology, Howard University College of Medicine, Washington, D.C. (September 1981-Present). Co-teach Pathogenic Bacteriology and Medical Microbiology. Research interests: Immunopathology and epidemiology of infectious diseases.

Assistant Professor, Department of Community Health and Family Practice (Division of International Health), Howard University (1980-1981).

Research Fellow, Division of Environmental Toxicology, Johns Hopkins School of Public Health, Baltimore, Maryland (1979-1980). Effects of chemical plasticizers on the complement system.

Research Fellow, National Institute for Medical Research, Lagos, Nigeria (1978-1979). Surveys and investigations of out-breaks of communicable diseases in rural areas; sero-epidemiology of falciparum malaria and urinary schistosomiasis in endemic areas; effects of malaria infection on complement components, C3, C4, C3b.

Post-doctoral Fellow, Laboratory of Parasitic Diseases, NIAID, National Institutes of Health (1975-1978). Studies on pathological characteristics of geographical strains of Schistosoma mansoni; enzymatic basis of resistance to schistosome infection of vector hosts.

Clinical Microbiologist, Metropolitan Medical Center, Minneapolis, Minnesota (1973-1975, part-time). Routine diagnosis of clinical specimens, antibiotic sensitivity studies and phage typing of Salmonella typhimurium.

Research Assistant, University of Minnesota, Minneapolis (Summer, 1973 & 1974), Epidemiological study of Schistosoma dermatitis (swimmer's itch) in Minnesota.

Laboratory Instructor, University of Minnesota, Minneapolis (1971-1975) Experimental Parasitology, Parasitic Protozoa, Microbiology and Comparative Physiology.

PUBLICATIONS:

Kassim, O.O. and E.D. Gilbertson. 1976. Hatching of Schistosoma mansoni eggs and observations on motility of miracidia. J. Parasit. 62:715-720.

Kassim, O.O. and C.S. Richards. 1978. Schistosoma mansoni: Lysozyme activity in Biomphalaria glabrata during infection with two strains of the parasite. Exptl. Parasit. 46:213-217.

Kassim, O.O. and C.S. Richards. 1978. Biomphalaria glabrata: Lysozyme activities in the hemolymph, digestive gland and headfoot. Parasit. 46:218-224.

Gilbertson, D.E., Kassim, O.O. and J.L. Stump. 1978. Studies on the biology of Bulimnea megasoma (Say) (gastropoda:pulmanata). J. Molluscan Studies, 44:145-150.

Kassim, O.O. and C.S. Richards. 1979. Radiosotope labelling of Schistosoma mansoni for in vivo studies in Biomphalaria glabrata. J. Invert. Pathol. 33:385-386.

Kassim, O.O. and C.S. Richards. Host reactions in Biomphalaria glabrata to miracidia, involving variations in parasite strains, numbers and sequence of exposure. Intern. J. Parasit. 9:565-570.

Kassim, O.O., A.W. Cheever and C.S. Richards. 1979. Comparison of infections with different strains of Schistosoma mansoni in mice. Exptl. Parasit. 48:220-224.

Ade-Serrano, M.A., Kassim, O.O. and Ejesie, C. 1981. Correlation of Plasmodium falciparum gametocytaemia with complement components (C3 & C4) titers in rural Nigerian school children. J. Clinic. Microbiol. 13:195-198.

Kassim, O.O. and G.C. Ejezie. 1982. ABO blood groups in malaria and schistosomiasis haematobium. Acta Tropica, 39:179-184.

Kassim, O.O. and M. Stek. 1983. Bacteriuria and haematuria in Schistosoma haematobium infection. J. Infect. Diseases 147:960.

Stek, M., Duncan, J.F., Kassim, O.O. and C.G. Santi. 1983. Prevalence of cytomegalovirus antibodies in Nigerian school children. Trans. Roy. Soc. Trop. Med. Hyg. 77:275-276.

Kassim, O.O. 1983. Genetic testing and chemical hypersusceptibility in the workplace. Urban Health 36-44 (Feb. 1983).

IN PRESS

Kassim, O.O. 1984. Proteinuria and hematuria in the diagnosis of infections due to Schistosoma haematobium. J. Clinic. Microbiol. (in press).

Kassim, O.O. 1984. Prevalence of hepatitis B antigenemia and antibodies in malaria and schistosomiasis. Infect. Immun. (in press).

Kassim, O.O. and Cote, T. 1984. Specific antibody responses in concurrent falciparum malaria and schistosomiasis haematobium. Am. J. Trop. Med. Hyg.

Stek, M., Jr., Kassim, O.O., Hansen, K. and Pelley, R.P. 1984. Comparison of an enzyme-linked immunosorbent assay using soluble egg antigen and a radioimmunoassay for the serodiagnosis of schistosomiasis haematobium. J. Trop. Med. Hyg.

BUDGET

A. PERSONNEL

Name	Position Title	% full-time effort/week	Salary
Dr. Kassim	Principal Investiagtor	50%	$ 0
-	Graduate Assistant	50%	$ 5,408

B. TRAVEL (Domestic)	500
C. EQUIPMENT & SUPPLIES	
ELISA Microplate Reader	5,000
Incubator	1,200
Refrigerator	850
Glassware	750
Chemicals & Reagents	2,500
D. ANIMALS	
i) Snail maintenance	260
Snail supplies	240
ii) BALB/c Mice	
Purchase costs	2,240
Maintenance costs	11,260
E. MISCELLANEOUS	
Data Analysis	200
Books, Journals and Publications	400
Total (Direct Costs)	$30,808
F. University Overhead (Indirect Costs) @ 90% Direct Costs	27,727
Total (Direct + Indirect Costs)	$58,535

BUDGET JUSTIFICATION

A. PERSONNEL

Principal Investigator: will be responsible for the direction and day-to-day activities of the project; will determine experimental procedures for the project and participate in their implementation; will analyse and interpret data and be responsible for their publication.

Graduate Assistant: will be a graduate student in a biomedical study area at Howard University and will assist in carrying out the day-to-day activities of the project as determined by the prinicpal investigator. With a pay-rate of $5.20 per hour (20 hrs/week), the total costs for the individual will come to $5,408 for the entire year.

B. TRAVEL

The $500 is budgeted for domestic travel and will enable the principal investigator to attend a scientific meeting, present, discuss and exchange ideas on the data generated from the study.

C. EQUIPMENT & SUPPLIES

The following equipment is available for the project:

Spectrophotometer (Gilford 240); Beckman Beta Liquid Scintillation Counter, Model LS250; Ultracentrifuge, Beckman L5-65; CO_2 Incubator (National Weinicke Co.); Freezer, Kelvinator Model UC-522; Standard Microscope (American Optical); Dissecting Microscope (American Optical); Balance, Ohaus Model 3I0; Tissue Homogenizer; Reagent Dispenser; and Water Bath. The scintillation counter, ultracentrifuge, CO_2 incubator and freezer are all located in the Department's Equipment Room, while the other equipment is located in my laboratory.

But the following items are also needed for a successful undertaking of the proposed project:

ELISA Microplate Reader ($5,000, Dynatech, Inc.) will be used for measurements of the antibody levels to the schistosome, malaria and bacterial antigens. The sensitivity, specificity and reproducibility of ELISA have been shown, in our own studies and those of other investigators, to be as good, if not greater than RIA. The relative attraction of the technique lies in its versatile application both in clinical and epidemiological studies, its ability to measure picogram amounts of circulating antigens and antibodies, and the relatively reduced time and costs required for its operation. The acquisition of the ELISA equipment would provide us with a convenient and relatively inexpensive system to quantitate antigen and specific antibodies in the proposed and future studies.

Glassware ($750): This includes beakers, flasks, petri dishes, test tubes and all the microtiter plates which will be required in all the cell culture and ELISA assays.

Refrigerator ($850): This item is required for refrigeration of a number of chemicals, reagents and buffers which cannot otherwise be stored at room temperature. It is also required for overnight low-temperature incubation of reaction mixtures in many of the assays outlined in the study.

Chemicals and reagents ($2,500): These include all the various chemicals and reagents such as the RPMI-1640 culture medium, CO_2 tanks, enzyme-antibody conjugates, triated thymidine, PHA, bacteriological culture media that are needed for culturing the three bacteria and for use in the lymphocyte proliferation assays.

D. ANIMAL COSTS

Biomphalaria glabrata snails: These snails are the intermediate host of *S. mansoni* and are used in maintaining the infection in our lab. They are fed *ad libitum* on Rommaine lettuce, which runs an average of $5 per week. The estimated lettuce supply is therefore $260 for an entire year. Cost of replacing filters and valves = $240/year.

BALB/c mice: These mice are a very suitable strain to carry the *S. mansoni* infections based on our experience. Source of supply: Charles River Animal Facility. *S. mansoni* soulble egg antigen (SEA) will be obtained from Dr. K. E. Mott of the World Health Organization in Geneva (Schistosomiasis and other Parasitic Diseases).

Acute Infection	
200 mice @ $5.60 per mouse	$1,120
Maintenance:	
200 mice @ $0.54 per day for 11 weeks =	$4,158
Chronic Infection	
200 mice @ $5.60 per mouse	$1,120
Maintenance:	
200 mice @ $0.54 per day for 21 weeks =	$7,102
Total	$13,500

E. MISCELLANEOUS

Two hundred dollars has been budgeted for the cost of computer analysis of the experimental data at the University Computer Center while $400 will cover the purchase of books and costs of publishing data from the project in scientific journals.

REFERENCES

1. Iarotski, L.S. and Davis, A. 1981. The schistosomiasis problem in the world: Results of a WHO questionnaire survey. Bull. WHO, 37:115-127.

2. Pugh, R.N.H., Jakubowski, A.W. and Gilles, H.M. 1979. Malumfashi Endemic Diseases Research Project. VI. Urinary schistosomiasis: abnormal urograms in infected males from the Malumfashi study area of northern Nigeria. Ann. Trop. Med. Parasit. 73:37-44.

3. Warren K.S. 1973. The pathology of schistosome infections. Helminth. Abstr. Series A, 42:59-633.

4. Mahieu, J.M., Muller, A.S., Voorhoeve, A.M. and Dicken, H. 1978. Pertussis in rural area of Kenya: Epidemiology and a preliminary report on a vaccine trial. Bull. WHO, 56:773.

5. Baird, D.R., Whittle, H.C. and Greenwood, B.M. 1976. Mortality from pneumococcal meningitis. Lancet ii, 1344-1346.

6. Todd, C.W., Goodgame, R.W. and Colley, D.G. 1979. Immune responses during human schistosomiasis mansoni. V. Suppression of schistosome antigen-specific lymphocyte blastogenesis by adherent/phagocytic cells. J. Immunol. 122:1440-1446.

7. Rocklin, R.E., Tracy, J.W. and Kholy, A.E. 1981. Activation of antigen-specific suppressor cells in human schistosomiasis mansoni by fractions of soluble egg antigens nonadherent to Con A sepharose. J. Immunol. 127:2314-2318.

8. Ottesen, E.A. 1979. Modulation of the host response in human schistosomiasis. I. Adherent suppressor cells that inhibit lymphocyte proliferative responses to parasite antigens. 123:1639-1644.

9. Keyes, G.S. and Colley, D.G. 1979. Immunoregulation in experimental schistosomiasis: In vitro induction and assay of spleen cell suppressor activity. J. Immunol. 122:2340-2344.

10. Kassim, O.O. and Cote, T. 1984. Specific antibody responses in concurrent falciparum malaria and schistosomiasis haematobium. Am. J. Trop. Med. Hyg. (Submitted)

11. Williamson, W.A. and Greenwood, B.M. 1978. Impairment of the immune response to vaccination after acute malaria. Lancet, 1:1328-1329.

12. Greenwood, B.M., Bradley, A.K., Blakebrough, I.S. and Whittle, H.C. 1980. The immune response to a meningococcal polysaccharide vaccine in an African village. Trans. Roy. Soc. Trop. Med. Hyg. 74:340-345.

13. McBride, J.S., Micklem, H.S. and Ure, J.M. 1977. Immunosuppression in murine malaria. I. Response to type III pneumococcal polysaccharide. Immunology, 32:635-644.

14. Prost, A., Schlumberger, M. and Fayat, M.T. 1983. Response to tetanus immunization in onchocerciasis patients. Ann. Trop. Med. Parasit. 77:83-85.

15. Rougemont, A., Boisson-Pontal, M.E., Gridel, P.G. and Sangare, S. 1977. Tuberculin skin tests and BCG vaccination in a hyperendemic area of onchocerciasis. Lancet, ii: 309.

16. Boros, D.L. and Warren, K.S. 1970. Delayed hypersensitivity-type granuloma formation and dermal reaction induced and elicited by a soluble factor isolated from Schistosoma mansoni eggs. J. Exp. Med. 132:488.

17. Silveria, N.P.A., Mendes, N.F. and Tolnai, M.E.A. 1972. Tissue localization of two populations of human lymphocytes distinguished by membrane receptors. J. Immunol. 108:1456.

18. Mendes, N.F., Miki, S.S. and Peixinho, Z.F. 1974. Combined detection of human T and B lymphocytes by rosette formation with sheep erythrocytes and zymosan - C3 complexes. J. Immunol. 113:531.

19. Boros, D.L., Pelley, R.P. and Warren, K.S. 1975. Spontaneous modulation of granulomatous hypersensitivity in schistosomiasis mansoni. J. Immunol. 114:1437-1441.

20. Colley, D.G. 1975. Immune responses to a soluble schistosomal egg antigen preparation during chronic primary infection with Schistosoma mansoni. J. Immunol. 115:150-156.

21. Voller, A. Bartlett, A. and Bidwell, D.E. 1976. Enzyme immunoassays for parasitic diseases. Trans. Roy. Soc. Trop. Med. Hyg. 70:98-106.

22. Carlson, B.A., Giebink, G.S., Spika, J.S. and Gray, E.D. 1982. Measurement of immunoglobulin G and M antibodies to type 3 pneumococcal capsular polysaccharide by enzyme-linked immunosorbent assay. J. Clin. Microbiol. 16:63-69.

23. Carlsson, H.E., Lindberg, A.A. and Hammarstrom, S. 1972. Titration of antibodies to Salmonella O antigens by enzyme-linked immunosorbent assay. Infect. Immun. 6:703-708.