

FD-ABL-401
95497



U.S. AGENCY FOR
INTERNATIONAL
DEVELOPMENT

AUG 31 1993

Ms. Donna V. Helm
Director
Research Administration
The Johns Hopkins University
615 N. Wolfe Street
Baltimore, Maryland 21205

ENTERED

SEP 30 1993

Section

SUBJECT: Grant No. HRN-5600-G-00-3021-00

Dear Ms. Helm:

Pursuant to the authority contained in the Foreign Assistance Act of 1961 and the Federal Grant and Cooperative Agreement Act of 1982, as amended, the Agency for International Development (hereinafter referred to as "A.I.D.") hereby provides to The Johns Hopkins University (hereinafter referred to as "Johns Hopkins" or "Grantee") the sum set forth in Section 1C.2. of Attachment 1 of this Grant to provide financial support for the program described in Attachment 2 of this Grant entitled "Program Description."

This Grant is effective as of the date of this letter and funds obligated hereunder shall be used to reimburse the Grantee for allowable program expenditures for the period set forth in Section 1B. of Attachment 1 of this Grant.

The total estimated amount of this Grant is the amount set forth in Section 1C.1. of Attachment 1, of which the amount set forth in Section 1C.2. is hereby obligated. A.I.D. shall not be liable for reimbursing the Grantee for any costs in excess of the obligated amount. However, subject to Section 1C.4. of Attachment 1, additional funds may be obligated by A.I.D. until such time as the obligated amount may equal the total estimated amount of this Grant.

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This Grant is made to the Grantee on the condition that the funds will be administered in accordance with the terms and conditions as set forth in the attachments listed under my signature below, which together constitute the entire Grant document and have been agreed to by your organization.

Please acknowledge receipt and acceptance of this Grant by signing all copies of this Cover Letter, retaining one copy for your files, and returning the remaining copies to the undersigned.

If you have any questions, please contact Ms. Kim Randall of my staff at (703) 875-1155.

Sincerely yours,

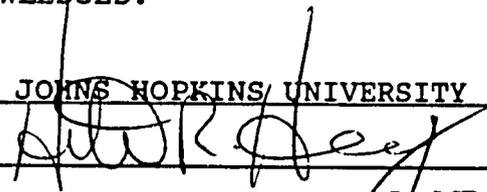


Michael Gushe
Grant Officer
Chief, OP/A/HRN
Division A
Office of Procurement

Attachments:

- 1. Schedule
- 2. Program Description
- ~~3. Standard Provisions~~
- ~~4. Special Provision entitled "Restrictions on Lobbying" (>\$100K)~~

ACKNOWLEDGED:

 THE JOHNS HOPKINS UNIVERSITY
 BY: 
 TYPED NAME: Herbert R. Hansen, Jr., MBA, CPA
Sr. Associate Dean for
 TITLE: Finance and Administration
 DATE: 9/8/93

FISCAL DATA

A. GENERAL

- A.1. Total Estimated A.I.D. Amount: \$149,884
- A.2. Total Obligated A.I.D. Amount: \$86,855
- A.3. Cost-Sharing Amount (Non-Federal): \$10,490
- A.4. Other Contributions (Federal): \$ N/A
- A.5. Project No.: 936-5600
- A.6. A.I.D. Project Office: R&D/R, Howard Minners
- A.7. Funding Source: A.I.D./W
- A.8. Tax I.D. No.: 1520595110
- A.9. CEC No.: 00-191-0777
- A.10. LOC No.: 72-00-1325

B. SPECIFIC

- B.1.(a) PIO/T No.: 936-5600-3692845
- B.1.(b) Appropriation: 72-1131021.1
- B.1.(c) Allotment: 341-36-099-04-20-31
- B.1.(d) BPC: DDVA-93-16950-KG11
- B.1.(e) Amount: \$41,855

- B.2.(a) PIO/T No.: 936-5600-3692846
- B.2.(b) Appropriation: 72-1131021.1
- B.2.(c) Allotment: 341-36-099-04-20-31
- B.2.(d) BPC: DDVA-93-16950-CG11
- B.2.(e) Amount: \$45,000

ATTACHMENT 1

SCHEDULE

1A. PURPOSE OF GRANT

The purpose of this Grant is to provide financial support for the program described in Attachment 2 of this Grant entitled "Program Description."

1B. PERIOD OF GRANT

1B.1. The effective date of this Grant is the date of the Cover Letter and the estimated completion date is September 1, 1996. Funds obligated hereunder (see Section 1C.2. below) shall be used to reimburse the Grantee for allowable program expenditures incurred by the Grantee in pursuit of program objectives at any time during the period beginning on the effective date of this Grant and ending on the estimated completion date.

1B.2. However, because this Grant is incrementally funded (see Section 1C.4. below), funds obligated hereunder are only anticipated to be sufficient for program expenditures through August 31, 1994

1C. AMOUNT OF GRANT AND PAYMENT

1C.1. The total estimated amount of this Grant for its full period, as set forth in Section 1B.1. above, is \$149,884.

1C.2. A.I.D. hereby obligates the amount of \$86,855 as partial funding of the total estimated amount set forth in Section 1C.1. above for program expenditures during the indicated period set forth in Section 1B. above. Notwithstanding said total estimated amount, A.I.D. shall not be liable for reimbursing the Grantee for any costs in excess of the obligated amount, except as specified in paragraph (f) of the Standard Provision of this Grant entitled "Revision of Grant Budget" (see also Section 1C.4. below).

1C.3. Payment shall be made to the Grantee in accordance with procedures set forth in the Standard Provision of this Grant entitled "Payment - Letter of Credit," as shown in Attachment 3.

1C.4. As indicated in Section 1C.2. above, this Grant is partially funded. Until such time as the obligated amount (see Section 1C.2. above) shall equal the total estimated amount

(see Section 1C.1. above) of this Grant, additional increments of funds may be obligated by A.I.D. under this Grant (by a Grant modification), subject to availability of funds, possible evaluation of the program, program priorities at the time, and the requirements of the Standard Provisions of this Grant entitled "Revision of Grant Budget" and, if applicable (see Section 1K.2. for applicability) "Cost Sharing (Matching)," as set forth in Attachment 3.

1C.5. The total estimated amount of the program described in Attachment 2 of this Grant is \$160,374, of which A.I.D. may provide the amount specified in Section 1C.1. above, and the Grantee will provide \$10,490 in accordance with Section 1L. below.

1D. GRANT BUDGET

1D.1. The following is the Budget for the total estimated amount of this Grant (see Section 1C.1. above) for its full period (see Section 1B. above). The Grantee may not exceed the total estimated amount or the obligated amount of this Grant, whichever is less (see Sections 1C.1. and 1C.2., respectively, above). Except as specified in the Standard Provision of this Grant entitled "Revision of Grant Budget," as shown in Attachment 3, the Grantee may adjust line item amounts as may be reasonably necessary for the attainment of program objectives. Revisions to the budget shall be in accordance with Section 1C. above and the Standard Provisions of this Grant entitled "Revision of Grant Budget" and, if applicable "Cost Sharing (Matching)."

1D.2. Budget

| <u>Cost Element</u> | <u>A.I.D.</u> | <u>Grantee/ Others (Non-Fed)</u> | <u>Grantee/ Others (Federal)</u> | <u>Total</u> |
|---------------------|---------------|--|--|---------------|
| Salaries & Wages | \$ 63,759 | \$ 8,100 | \$ - 0 - | \$ 71,859 |
| Fringe Benefits | 8,630 | 2,390 | - 0 - | 11,020 |
| Travel & Per Diem | 23,166 | - 0 - | - 0 - | 23,166 |
| Other Direct Costs | 22,381 | - 0 - | - 0 - | 22,381 |
| Indirect Costs | <u>31,948</u> | <u>- 0 -</u> | <u>- 0 -</u> | <u>31,948</u> |
| TOTAL | \$149,884 | \$ 10,490 | \$ - 0 - | \$160,374 |

1D.3. Inclusion of any cost in the budget of this Grant does not obviate the requirement for prior approval by the Grant Officer of cost items designated as requiring prior approval by the applicable cost principles (see the Standard Provision of

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this Grant set forth in Attachment 3 entitled "Allowable Costs") and other terms and conditions of this Grant, unless specifically stated in Section 1I. below.

1E. REPORTING

1E.1. Financial Reporting

1E.1.(a) Financial reporting requirements shall be in accordance with the Standard Provision of this Grant entitled "Payment - Letter of Credit," as shown in Attachment 3. If a Standard Form 269 is required by the aforesaid Standard Provision, the "Long Form" of said form shall be used.

1E.1.(b) All financial reports shall be submitted to A.I.D., Office of Financial Management, FA/FM/CMPD/DCB, Room 700 SA-2, Washington, D.C. 20523-0209. In addition, three copies of all financial reports shall be submitted to the A.I.D. Project Office specified in the Cover Letter of this Grant, concurrently with submission of the Quarterly Technical Reports (See Section 1E.2. below).

1E.1.(c) The frequency of financial reporting and the due dates of reports shall be as specified in the Standard Provision of this Grant referred to in Section 1E.1.(a) above.

1E.1.(d) The Grantee's financial reports shall include expenditures of A.I.D. Grant funds provided hereunder, as well as non-federal matching funds and any other contributions in accordance with Section 1L. below.

1E.2. Program Performance Planning and Reporting

1E.2.(a) Quarterly Reports

The Grantee shall submit five (5) copies of brief quarterly program performance reports, which coincide with the financial reporting periods described in Section 1E.1. above, to the A.I.D. Project Office specified in the Cover Letter of this Grant. In addition, two copies shall be submitted to A.I.D., POL/CDIE/DI, Washington, DC 20523-1802. These reports shall be submitted within 30 days following the end of the reporting period, and shall briefly present the following information:

1E.2.(a)(1) A comparison of actual accomplishments with the goals established for the period, the findings of the investigator, or both. If the output of programs can be readily quantified, such quantitative data should be related to cost data for computation of unit costs.

1E.2.(a)(2) Reasons why established goals were not met, if applicable.

1E.2.(a)(3) Other pertinent information including the status of finances and expenditures and, when appropriate, analysis and explanation of cost overruns or high unit costs. See also Section 1I.4. of this Grant.

1E.2.(b) Special Reports

Between the required program performance reporting dates, events may occur that have significant impact upon the program. In such instances, the Grantee shall inform the A.I.D. Project Officer as soon as the following types of conditions become known:

1E.2.(b)(1) Problems, delays, or adverse conditions that will materially affect the ability to attain program objectives, prevent the meeting of time schedules and goals, or preclude the attainment of work units by established time periods. This disclosure shall be accompanied by a statement of the action taken, or contemplated, and any A.I.D. assistance needed to resolve the situation.

1E.2.(b)(2) Favorable developments or events that enable time schedules to be met sooner than anticipated or more work units to be produced than originally projected.

1E.2.(b)(3) If any performance review conducted by the Grantee discloses the need for change in the budget estimates in accordance with the criteria established in the Standard Provision of this Grant entitled "Revision of Grant Budget," the Grantee shall submit a request for budget revision to the Grant Officer and the A.I.D. Project Officer specified in the Cover Letter of this Grant.

1E.2.(c) Environmental Impact

If it appears that outputs of this project will result in an adverse environmental impact, the Grantee shall notify the A.I.D. Project Officer prior to implementation, in order to allow for orderly preparation of an environmental impact statement. The Grantee shall assure that appropriate U.S. Government, A.I.D., and/or host country procedures are followed.

1E.2.(d) Technical and Research Reports and Publications

The Grantee shall summarize technical and research activities of the project in reports, and distribute such reports to the appropriate USAID Missions, developing countries, and host

country and international institutions in order to encourage use of the technology developed. Such reports will be completed within 60 days after completion of the activity. Journal articles and other publications are encouraged. See also the Standard Provision of this Grant entitled "Publications" (if the Grantee is a U.S. organization) or "Publications and Media Releases" (if the Grantee is a non-U.S. organization).

1E.2.(e) Final Report

Within 90 days following the estimated completion date of this Grant (see Section 1B. above), the Grantee shall submit five (5) copies of a final report to the A.I.D. Project Office specified in the cover letter of this Grant. In addition, two copies shall be submitted to A.I.D., POL/CDIE/DI, Washington, DC 20523-1802. It will cover the entire period of the Grant and include all information shown in this Section 1E.2., specifically including, but not necessarily limited to: (1) a summarization of the program's accomplishments or failings; (2) an overall description of the activities under the program during the period of this Grant; (3) a description of the methods of work used; (4) comments and recommendations regarding unfinished work and or program/continuation and direction; and 5) A fiscal report that describes in detail how the Grant (and any matching) funds were used.

1E.2.(f) Trip Reports

Within 30 days following the completion of each international trip, the Grantee shall submit 3 copies of a trip report summarizing the accomplishments of the trip to the A.I.D. Project Officer specified in the cover letter of this Grant. If several individuals are travelling together to one site, a single report representing the group will suffice. The report shall include the purpose of the trip, technical observations, suggestions and recommendations, overall impressions of the site situation (if appropriate), and a list of persons visited with their title and organization affiliation.

1E.2.(g) Care of Laboratory Animals

If the Standard Provision entitled "Care of Laboratory Animals" applies to this Grant (see Section 1K. for applicability), the Grantee shall include the certificate required by paragraph (c) of said Standard Provision in all of its reports which pertain to the use of laboratory animals.

1E.2.(h) Research Involving Recombinant DNA

If any research involving recombinant DNA is being funded hereunder, the Grantee shall comply with the reporting requirements set forth in Section 1I.5. of this Grant.

1F. TITLE TO PROPERTY

Title to property acquired hereunder shall vest in the Grantee, subject to the requirements of the Standard Provision of this Grant entitled "Title To and Use of Property (Grantee Title)" regarding use, accountability, and disposition of such property, except to the extent that disposition of property may be specified in Section 1I. below.

1G. PROCUREMENT AND (SUB)CONTRACTING

1G.1. Applicability

This Section 1G. applies to the procurement of goods and services by the Grantee (i.e., contracts, purchase orders, etc.) from a supplier of goods and services (see the Standard Provisions of this Grant entitled "Air Travel and Transportation," "Ocean Shipment of Goods," "Procurement of Goods and Services," "AID Eligibility Rules for Goods and Services," and "Local Cost Financing"), and not to assistance provided by the Grantee (i.e., a subgrant or [sub]agreement) to a subrecipient (see the Standard Provision of this Grant entitled "Subagreements").

1G.2. Requirements

1G.2.(a) In addition to other applicable provisions of this Grant, the Grantee shall comply with paragraph (b)(1) of the Standard Provision of this Grant entitled "AID Eligibility Rules for Goods and Services," concerning Grants funded under the Development Fund for Africa (DFA) and Grants with a total procurement value of less than \$250,000 under this Grant. However, paragraph (b)(1) of the Standard Provision entitled "AID Eligibility Rules for Goods and Services" does not apply to:

1G.2.(a)(1) The restricted goods listed in paragraph (a)(3) of the Standard Provision entitled "AID Eligibility Rules for Goods and Services," which must be specifically approved by the Grant Officer in all cases, except to the extent that such approval may be provided in Section 1I.3. below;

1G.2.(a)(2) Paragraph (d) of the Standard Provision entitled "AID Eligibility Rules for Goods and Services" pertaining to air and ocean transportation, to which the Standard Provisions entitled "Air Travel and Transportation" and "Ocean Shipment of Goods" apply, respectively;

1G.2.(a)(3) Paragraph (c) of the Standard Provision entitled "AID Eligibility Rules for Goods and Services;"

1G.2.(a)(4) Construction implemented by U.S. firms, regardless of dollar value, which requires that at least 50% of the supervisors and other specified key personnel working at the project site must be U.S. citizens or non-U.S. citizens lawfully admitted for permanent residence in the United States; and

1G.2.(a)(5) Engineering services, regardless of dollar value, which shall be limited to the United States (Geographic Code 000).

1G.2.(b) Paragraph (b)(2) of the Standard Provision entitled "AID Eligibility Rules for Goods and Services" does not apply.

1G.3. Approvals

Inclusion of costs in the budget of this Grant for the purchase of nonexpendable equipment obviates neither the requirement of Section J.13. of OMB Circular A-21 (for educational institutions) or Section 13 of Attachment B of OMB Circular A-122 (for nonprofit organizations other than educational institutions) for prior approval of such purchases by the Grant Officer, nor any other terms and conditions of this Grant, unless specifically stated in Section 1I.2. below.

1G.4. Title to Property

See Section 1F. above.

1H. INDIRECT COST RATES

1H.1. Pursuant to the Standard Provisions of this Grant entitled "Negotiated Indirect Cost Rates - Predetermined" and "Negotiated Indirect Cost Rates - Provisional (Nonprofits)," a predetermined indirect cost rate or rates shall be established for each of the Grantee's accounting periods which apply to this Grant. Payments on account of allowable indirect costs shall be made on the basis of such predetermined rates. The rate(s) for the initial period and the base(s) to which it is (they are) applied is (are) as follows:

| <u>Type</u> | <u>Rate</u> | <u>Base</u> | <u>Period</u> |
|-----------------------|-------------|-------------|---------------------|
| On-Campus/Home Office | 32.0% | 1/ | 07/01/93 - 06/30/95 |
| Off-Campus/Off-Site | 18.0% | 1/ | 07/01/93 - 06/30/95 |

1/ Base of Application: Modified Total Direct Costs

1H.2. Rates for subsequent periods shall be established in accordance with the Standard Provision of this Grant entitled "Negotiated Indirect Cost Rates - Predetermined."

1I. SPECIAL PROVISIONS

1I.1. Limitations on Reimbursement of Costs of Compensation for Personal Services and Professional Service Costs

1I.1.(a) Employee Salaries

Except as the Grant Officer may otherwise agree in writing, A.I.D. shall not be liable for reimbursing the Grantee for any costs allocable to the salary portion of direct compensation paid by the Grantee to its employees for personal services which exceed the highest salary level for a Foreign Service Officer, Class 1 (FS-1), as periodically amended.

1I.1.(b) Consultant Fees

Compensation for consultants retained by the Grantee hereunder shall not exceed, without specific approval of the rate by the Grant Officer: either the highest rate of annual compensation received by the consultant during any full year of the immediately preceding three years; or the maximum rate of a Foreign Service Officer, Class 1 (FS-1) (as periodically amended), whichever is less. A daily rate is derived by dividing the annual compensation by 2,087 and multiplying the result by 8.

1I.2. Equipment and Other Capital Expenditures

1I.2.(a) Requirement for Prior Approval

Pursuant to Sections 1D.3. and 1G.3. above and the Standard Provisions of this Grant entitled "Allowable Costs" and "Revision of Grant Budget," and by extension, Section J.13. of OMB Circular A-21, the Grantee must obtain A.I.D. Grant Officer approval for the following:

1I.2.(a)(1) Purchase of General Purpose Equipment, which is defined as an article of nonexpendable tangible personal

property, the use of which is not limited only to research, medical, scientific, or other activities [e.g., office equipment and furnishings, air conditioning equipment, reproduction and other equipment, motor vehicles, and automatic data processing equipment, having a useful life of more than two years and an acquisition cost of \$500 or more per unit);

1I.2.(a)(2) Purchase of Special Purpose Equipment, which is defined as an article of nonexpendable tangible personal property, which is used only for research, medical, scientific, or other technical activities, and which has a useful life of more than two years and an acquisition cost of \$1,000 or more per unit); and

1I.2.(a)(3) Other Capital Expenditures, which is defined as the cost of the asset, including the cost to put it in place).

1I.2.(b) Approvals

In furtherance of the foregoing, the Grant Officer does hereby provide approval for the following purchases, which shall not be construed as authorization to exceed the total estimated amount or the obligated amount of this Grant, whichever is less (see Section 1C. above):

N/A

1I.2.(c) Exception for Automation Equipment

Any approval for the purchase of automation equipment which may be provided in Section 1I.2.(b) above or subsequently provided by the Grant Officer is not valid if the total cost of purchases of automation equipment (e.g., computers, word processors, etc.), software, or related services made hereunder will exceed \$100,000. The Grantee must, under such circumstances, obtain the approval of the Grant Officer for the total planned system of any automation equipment, software, or related services.

1I.2.(d) Compliance with A.I.D. Eligibility Rules

Any approvals provided in Section 1I.2.(b) above or subsequently provided by the Grant Officer shall not serve to waive the A.I.D. eligibility rules described in Section 1G. of this Grant, unless specifically stated.

1I.3. Restricted Goods

Pursuant to Section 1G. above and paragraph (a)(3) of the Standard Provision of this Grant entitled "AID Eligibility Rules for Goods and Services," the Grant Officer's approval is required for purchase of the restricted goods described therein. In furtherance thereof, the Grant Officer does hereby provide such approval to the extent set forth below. The Grant Officer's approval is required for purchases of such restricted goods if all of the conditions set forth below are not met by the Grantee. Any approval provided below or subsequently provided by the Grant Officer shall not serve to waive any terms and conditions of this Grant unless specifically stated.

1I.3.(a) Agricultural Commodities

Agricultural commodities may be purchased provided that they are of U.S. source (generally, the country from which the commodities are shipped) and origin (generally, the country in which the commodities are mined, grown, or produced) and purchased from a U.S. supplier, except that wheat, rice, corn, soybeans, sorghums, flour, meal, beans, peas, tobacco, hides and skins, cotton, vegetable oils, and animal fats and oils cannot be purchased under any circumstances without the prior written approval of the Grant Officer. However, if this Grant is funded under the Development Fund for Africa (DFA) (see Section 1G.2.[b][4] above), procurement of agricultural commodities from Special Free World countries (Geographic Code 935) is authorized, except that procurement of agricultural commodities outside the United States must have the advance written approval of the Grant Officer when the domestic price of the commodity is less than parity, unless the commodity cannot reasonably be procured in the U.S. in order to meet the needs of the project

1I.3.(b) Motor Vehicles

Motor vehicles, if approved for purchase under Section 1I.2.(b) above or subsequently approved by the Grant Officer, must be of U.S. manufacture and must be of at least 51% U.S. componentry. The source of the motor vehicles, and the nationality of the supplier of the vehicles, must be in accordance with Section 1G.2. above. Motor vehicles are defined as self-propelled vehicles with passenger carriage capacity, such as highway trucks, passenger cars and busses, motorcycles, scooters, motorized bicycles, and utility vehicles. Excluded from this definition are industrial vehicles for materials handling and earthmoving, such as lift trucks, tractors, graders, scrapers, and off-the-highway trucks. However, if this Grant is funded

under the Development Fund for Africa (DFA) (see Section 1G.2.[b][4] above), procurement of motor vehicles from Special Free World countries (Geographic Code 935) is authorized; provided, however, that procurement of non-U.S. vehicles shall be held to an absolute minimum.

1I.3.(c) Pharmaceuticals

Pharmaceuticals may be purchased provided that all of the following conditions are met: (1) the pharmaceuticals must be safe and efficacious; (2) the pharmaceuticals must be of U.S. source and origin (see Section 1G. above); (3) the pharmaceuticals must be of at least 51% U.S. componentry (see Section 1G. above); (4) the pharmaceuticals must be purchased from a supplier whose nationality is in the U.S. (see Section 1G. above); (5) the pharmaceuticals must be in compliance with U.S. Food and Drug Administration (FDA) (or other controlling U.S. authority) regulations governing United States interstate shipment of pharmaceuticals; (6) the manufacturer of the pharmaceuticals must not infringe on U.S. patents; and (7) the pharmaceuticals must be competitively procured in accordance with the procurement policies and procedures of the Grantee and the Standard Provision of this Grant entitled "Procurement of Goods and Services."

1I.3.(d) Pesticides

Pesticides may only be purchased if the purchase and/or use of such pesticides is for research or limited field evaluation by or under the supervision of project personnel. Pesticides are defined as substances or mixtures of substances: intended for preventing destroying, repelling, or mitigating any unwanted insects, rodents, nematodes, fungi, weeds, and other forms of plant or animal life or viruses, bacteria, or other micro-organisms (except viruses, bacteria, or other micro-organisms on or living in man or other living animals); or intended for use as a plant regulator, defoliant, or dessicant.

1I.3.(e) Rubber Compounding Chemicals and Plasticizers

Rubber compounding chemicals and plasticizers may only be purchased with the prior written approval of the Grant Officer.

1I.3.(f) Used Equipment

Used equipment may only be purchased with the prior written approval of the Grant Officer.

1I.3.(g) Fertilizer

Fertilizer may be purchased if it is either purchased in the U.S. and used in the U.S., or if it is purchased in the cooperating country with local currency for use in the cooperating country. Any fertilizer purchases which do not comply with these limitations must be approved in advance by the Grant Officer. However, if this Grant is funded under the Development Fund for Africa (DFA) (see Section 1G.2.[b][4] above), procurement of fertilizer from Special Free World countries (Geographic Code 935) is authorized; provided, however, that procurement of more than 5,000 tons of non-U.S. fertilizer must have the advance written approval of the Grant Officer.

1I.4. Limitation on Use of Funds

1I.4.(a) The Grantee shall not utilize funds provided by A.I.D. for any testing or breeding feasibility study, variety improvement or introduction, consultancy, publication, conference or training in connection with the growth or production in countries other than the United States of an agricultural commodity for export which would compete with a similar commodity grown or produced in the United States.

1I.4.(b) The reports described in Section 1E.2. shall contain a statement indicating the projects or activities to which United States funds have been attributed, together with a brief description of the activities adequate to show that United States funds have not been used for the purpose in Section 1I.4.(a) above.

1I.4.(c) The Grantee agrees to refund to A.I.D. upon request an amount equal to any United States funds used for the purposes prohibited by Section 1I.4.(a) above.

1I.4.(d) No funds provided by A.I.D. under this Grant shall be used to provide assistance, either directly or indirectly, to any country ineligible to receive assistance pursuant to the Foreign Assistance Act as amended, related appropriations acts, or other statutes and Executive Orders of the United States (also see the Standard Provision of this Grant entitled "Ineligible Countries").

1I.5. Compliance With Federal Guidelines and Regulatory Procedures Pertaining to Recombinant DNA

1I.5.(a) The Grantee shall implement any research activities under this Grant which involve recombinant DNA in accordance with:

1I.5.(a)(1) The National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules;

1I.5.(a)(2) Procedures issued by the U.S. Department of Agriculture (USDA), the Environmental Protection Agency (EPA) or other appropriate Federal agency;

1I.5.(a)(3) A.I.D.'s environmental procedures; and

1I.5.(a)(4) Such other Federal guidelines and procedures as may apply during the course of research.

1I.5.(b) The Grantee cannot commence testing in any foreign location until written approval for such testing is obtained from the A.I.D. Project Officer and the government of the country where testing is planned. Testing shall be conducted in accordance with all applicable regulations of that country.

1I.5.(c) In addition, and prior to commencement of any such testing, the Grantee shall make a judgement and communicate same to the A.I.D. Project Officer as to whether the regulations, procedures, or facilities of the country in question are adequate to ensure testing in an environmentally sound manner. In the event such judgement is that they are not, the Grantee and the A.I.D. Project Officer will consult and agree on the conditions to be applied to the testing which will have such environmental effect.

1I.5.(d) Reports submitted to A.I.D. under this Grant will address regulatory issues as noted above related to the activity.

1I.6. Defense Base Act (DBA) Insurance and/or Medical Evacuation Services

Pursuant to Section J.16. of OMB Circular A-21 (for educational institutions) or Section 18 of Attachment B of OMB Circular A-122 (for nonprofit organizations other than educational institutions), the Grantee is authorized to purchase DBA insurance and/or medical evacuation services under this Grant. If DBA insurance and/or medical evacuation services are purchased, it may be purchased from the insurance company or agent with which A.I.D. has a contract to provide DBA insurance and/or medical evacuation services for A.I.D. contracts; provided that such insurance company or agent offers such DBA insurance/medical evacuation services at the same rates such insurance/services are provided under A.I.D. contracts. The Grant Officer will provide the name, address, and telephone number of such insurance company or agent upon request.

1I.7. Disposition of Property

With reference to Sections 1G.4. and 1I.2.(b) above, disposition of nonexpendable property acquired hereunder shall be as follows:

N/A

1I.8. Compliance with Federal Guidelines and Regulatory Procedure

a. The Grantee will implement this research activity in accordance with all relevant guidelines for U.S. Government funded research such as:

- (1) The National Institutes of Health (NIH) guidelines for the ethical treatment of human subjects;
 - (2) Guidelines for the handling of radioactive materials;
 - (3) NIH and USDA guidelines for the handling of pathogenic microorganisms;
 - (4) USDA-AOHIS procedures for animal and plant health inspection;
 - (5) The National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules;
 - (6) Procedures issued by the USDA, EPA, or other appropriate Federal agency, regarding testing of genetically engineered organisms;
 - (7) U.S. State Department's and A.I.D.'s environmental procedures; and
 - (8) Such other Federal guidelines and procedures as may apply during the course of research.
- b. All existing comparable guidelines of the host country in which the research is actually located must be followed also.
- c. Reports submitted under this activity to A.I.D. will address the cited regulatory issues. All

modifications of protocols affecting these regulatory concerns must be reported. The investigators are responsible for reporting any difficulties encountered in implementing these protocols.

11.9. Laboratory Safety and Hazard Containment

Research will be conducted following the protocols described in ATTACHMENT 2, (which is the original proposal or subsequent amendments or letter from the Principal Investigator), which insure the safety of persons involved in the research. Notwithstanding, the research must be conducted following procedures issued by the U.S. Government and those issued by the government of the host country for the containment of these hazards.

If the protocols involving laboratory safety and hazard containment are revised, they must be re-reviewed by the investigator's institutional review committee(s) that approved the original protocol, and the Project Officer and Office of Research must be informed in writing before the revised protocols are used. The revised procedures must be consonant with the guidelines of the country in which the laboratory is located and of the United States. Copies of the approval the revised protocols by the investigator's institutional review committee(s) should also be provided the Project Officer and the Office of Research.

Similarly, the research will be conducted in the facilities described in Attachment 2. If the research is moved to new facilities, or the facilities are modified in such a way to affect safety or hazard containment, a description of the new facilities must be provided to the Project Officer and to the Office of Research before the research is affected. Any applicable institutional reviews of the facilities must be repeated, and the re-certification must be provided to the Project Officer and the Office of Research.

11.10. Human Subject

Research will be conducted following the protocols described in Attachment 2 (which is the original proposal or subsequent amendments of letter from the Principal Investigator), which insures the well-being and informed consent of human subjects. It will also be conducted in accord with the applicable procedures issued by the U.S. Government to insure ethical treatment of human subjects, and by those issued by the government of the host country in which the human subjects are to be involved.

If the protocol(s) involving human subjects is revised, it must be re-reviewed by the investigator's institutional ethical review committee, and the Project Officer and Office of Research must be informed in writing before the revised protocol(s) is used. The revised procedures must be consonant with the guidelines of the host country and the United States. If the patient's informed consent form is revised, a copy of the new form must be submitted to both the Project Officer and the Office of Research. A copy of the approval of the revised form by the investigator's institutional ethical review committee must also be provided to the Project Officer and the Office of Research.

In addition and prior to commencement of any experimentation involving human subjects, the Grantee shall make a judgment and communicate the same to A.I.D. as to whether the regulations, procedures or facilities of the country in question are adequate to ensure the safety and free and informed consent of the human subjects. In the event such judgment is that they are not, the Grantee and A.I.D. will consult and agree on the protocol to be applied to insure the safety and free, informed consent of the subjects.

1J. RESOLUTION OF CONFLICTS

Conflicts between any of the Attachments of this Grant shall be resolved by applying the following descending order of precedence:

- Attachment 1 - Schedule
- Attachment 3 - Standard Provisions
- Attachment 4 - Special Provision entitled "Restrictions on Lobbying"
- Attachment 2 - Program Description

1K. STANDARD PROVISIONS

The Standard Provisions set forth as Attachment 3 of this Grant consist of the following Standard Provisions denoted by an "X" which are attached hereto and made a part of this Grant:

1K.1. Mandatory Standard Provisions For U.S., Nongovernmental Grantees

- (X) Allowable Costs (November 1985)
- (X) Accounting, Audit, and Records (August 1992)
- (X) Refunds (September 1990)
- (X) Revision of Grant Budget (November 1985)

- (X) Termination and Suspension (August 1992)
- (X) Disputes (August 1992)
- (X) Ineligible Countries (May 1986)
- (X) Debarment, Suspension, and Other Responsibility Matters (August 1992)
- (X) Nondiscrimination (May 1986)
- (X) U.S. Officials Not to Benefit (November 1985)
- (X) Nonliability (November 1985)
- (X) Amendment (November 1985)
- (X) Notices (November 1985)
- (X) Metric System of Measurement (August 1992)

**1K.2. Additional Standard Provisions For U.S.,
Nongovernmental Grantees**

- (X) OMB Approval Under the Paperwork Reduction Act (August 1992)
- (X) Payment - Letter of Credit (August 1992)
- () Payment - Periodic Advance (January 1988)
- () Payment - Cost Reimbursement (August 1992)
- (X) Air Travel and Transportation (August 1992)
- (X) Ocean Shipment of Goods (August 1992)
- (X) Procurement of Goods and Services (June 1993)
- (X) AID Eligibility Rules for Goods and Services (August 1992)
- (X) Subagreements (August 1992)
- (X) Local Cost Financing (June 1993)
- (X) Patent Rights (August 1992)
- (X) Publications (August 1992)
- (X) Negotiated Indirect Cost Rates - Predetermined (August 1992)
- () Negotiated Indirect Cost Rates - Provisional (Nonprofits) (August 1992)
- () Negotiated Indirect Cost Rates - Provisional (For-Profits) (August 1992)
- (X) Regulations Governing Employees (August 1992)
- () Participant Training (August 1992)
- () Voluntary Population Planning (June 1993)
- (X) Protection of the Individual as a Research Subject (August 1992)
- () Care of Laboratory Animals (November 1985)
- (X) Title To and Use of Property (Grantee Title) (November 1985)
- () Title To and Care of Property (U.S. Government Title) (November 1985)
- () Title To and Care of Property (Cooperating Country Title) (November 1985)
- (X) Cost Sharing (Matching) (August 1992)
- (X) Use of Pouch Facilities (August 1992)
- (X) Conversion of United States Dollars to Local

- (X) Currency (November 1985)
- (X) Public Notices (August 1992)
- (X) Rights in Data (August 1992)

1L. COST SHARING AND OTHER CONTRIBUTIONS

1L.1. The Grantee agrees to expend an amount not less than (a) the amount shown in the budget of this Grant for financing by the Recipient and/or others from non-federal funds (see Sections 1D. and/or 1H.), and (b) the amount shown in the budget of this Grant for financing by the Recipient and/or others from other federal funds.

1L.2. The Standard Provision of this Grant entitled "Cost Sharing (Matching)" makes reference to project costs. "Project Costs" are defined in Attachment E of OMB Circular A-110 as all allowable costs (as set forth in the applicable cost principles [see the Standard Provision of this Grant entitled "Allowable Costs"]) incurred by a Grantee and the value of in-kind contributions made by the Grantee or third parties in accomplishing the objectives of this Grant during the program period.

1L.3. The restrictions on the use of A.I.D. funds provided hereunder, as set forth in this Grant, do not apply to cost-sharing (matching) or other contributions unless such restrictions are stated in the applicable federal cost principles and/or imposed by the source of such cost-sharing (matching) funds or other contributions.

ATTACHMENT 2

PROGRAM DESCRIPTION

The Grantee's proposal number 13.411 entitled "Prepatancy in Children with Onchocerciasis" and dated March 15, 1993 (Principal Investigator: Dr. Alan Scott) is attached hereto as the Program Description (Attachment 2) and is made a part of this Grant.

ATTACHMENT 3

STANDARD PROVISIONS

Note: Only those Standard Provisions indicated in Section 1K. of this Grant apply to this Grant.

4) It was determined that, when compared to a crude parasite extract or to recombinant antigens used separately, a cocktail of the recombinant *O. volvulus* antigens OV11, OV27 and OV29 functioned as an efficient, specific and sensitive indicator of the anti-*O. volvulus* IgG response in children. We are in the process of extending the evaluation of the recombinant cocktail to the analysis of IgG subclass responses and to IgE.

5) As reported by Lobos et al. (1991), the IgG response against OV16 has potential to be a valuable indicator of the prepatent condition. We tested 70 children for their response to OV16. We found a group of microfilaria-negative children who had highly elevated responses to OV16. By comparison, the responses of children harboring patent infections tended to be lower. Lobos et al. (1991) concluded that the IgG response to OV16 is at its highest during the latter part of the prepatent period. After the on-set of patency, the anti-OV16 response waned. It may be that microfilaria-negative/OV16-positive children found in our preliminary study were in the late stages of prepatency.

The cross sectional nature of the preliminary studies places a large number of restrictions on the interpretation of data. Many of the important and interesting issues concerning the diagnostic and predicative power of the responses against recombinant antigens and the relevance of quantitative and qualitative changes in antibody to the immunobiology of the disease could be explored more effectively by monitoring the immune responses and parasitological status of individuals over time. In this proposed study, we anticipate that we will be able to follow children as they make the transition from prepatency to patency so that we can address some of the issues that are important for developing accurate diagnostic tools and for understanding the infection.

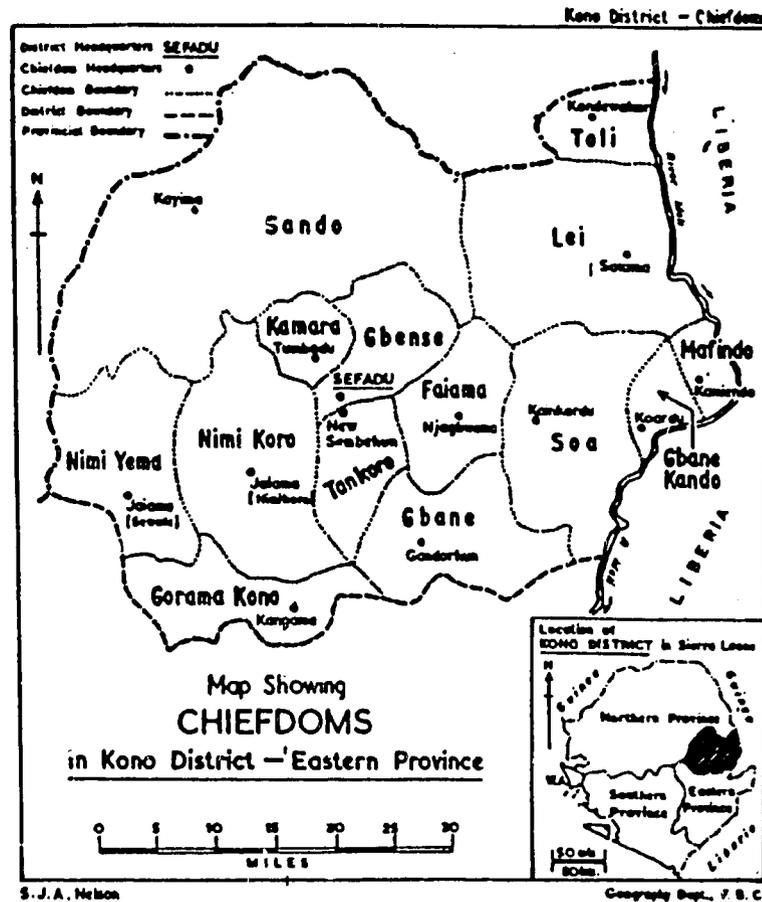
While none of the proposed technical approaches for the analysis of the humoral and cellular responses are new, their application using recombinant antigens in a large-scale longitudinal study of West African children is unique. One of the potential gains to be made through this project is a validation of the utility of using recombinant antigens for in a rapid, accurate and cost-effective serodiagnostic test. The development of a test could be invaluable in efforts to prevent the consequences of the disease, since these pathological manifestations are known to be directly related to the intensity of infection and the reinfection rate in endemic areas (WHO 1987). In addition, with the success of the OCP vector control program in interrupting transmission and the mass distribution of ivermectin campaign, the need for a sensitive and specific rapid immunological test will be more pressing because the lower the microfilaria density in the skin, the less effective skin snips are in detecting infection. Furthermore, because this proposed research will utilize four different recombinant antigens individually and as a cocktail; it will overcome the broad cross-reactivity among filarial and other helminths that has plagued serological tests for onchocerciasis in the past. The test would assist in truly discriminating between prepatency and low-level infections with *O. volvulus* from those not infected at all or infected with other filarial parasites of man.

In this proposed study, we will evaluate the changes in the cellular and humoral immune responses in children during prepatency. We feel that a systematic and coordinated investigation of both the humoral and the cellular arms of the immune response during this pivotal time in the infection will afford us unique insights to the immunological responses that are attendant to the onset of the chronic stage of infection. These data may be important for understanding the pathogenesis of onchocerciasis and for the development of vaccine strategies.

C. TECHNICAL WORK PLAN

Study Area

The study will be carried out in the Gorama Chiefdom in the Kono District, Eastern Province of Sierra Leone. The Kono District (Fig. 1) lies between latitude 8 20' N and 9 05' N of the equator and longitudes 10 28' W and 11 20' W of the Greenwich Meridian.



Gorama Chiefdom is one of 14 chiefdoms constituting the Kono District. The chiefdom has a population of 6,147 persons, with a population density of about 38 persons per square Km, according to the 1985 National Census Survey. Onchocerciasis is hyperendemic in the Gorama Chiefdom with a prevalent rate of over 80.0 % (Gbakima et al. 1986; 1992, In Press). The chiefdom falls within the interior plateau and mountain regions of Sierra Leone, and the entire area is composed of hilly region drained by several large rivers with numerous breeding sites for blackflies, i.e., Sewa, Bafin, Bagbe rivers, etc. While there has been a change of government in Sierra Leone recently through a military coup, the political climate is relatively calm and stable. We will deal directly with the Paramount Chief, the natural leader and the administrator of the chiefdom. He will be able to authorize our activities with his section chiefs, town chiefs and village chiefs, who, in turn, will assist us in maximizing community participation and compliance. Dr. Gbakima has extensive experience working on onchocerciasis in this area. The parents of the children who will be participating in this study are well informed about the dangers of onchocerciasis. Seven villages in all - including families living in the areas surrounding the seven villages - will be included in the study. The seven villages are: Kangama, Bunabu, Njagbwema, Matama, Moimandu, Topkumbu and Saowa.

The Gorama Chiefdom was also used in the preliminary study.

Subjects

The children recruited for this study will be 1 to 10 years of age. We will attempt to recruit 100 children in each of the following age groups: 1-3 years, 4-6 years and 7-10 years. Consent will be obtained from the child's parent or guardian to participate in the study. A large percentage of the children in the 1 to 4 year old range should be serologically

and parasitologically negative at the beginning of the study. We expect that most of the older children will have serological or serological plus parasitological evidence of infection with *O. volvulus*. It is anticipated that over the three years of the study the immunological and parasitological status of each child will alter allowing us to prospectively identify responses that are associated with the onset of infection and prepatency.

Children will be excluded from initial or subsequent participation in the study if they are found to be obviously malnourished, anemic or ill. They will be immediately referred to a clinic for appropriate treatment. Children that have been treated with ivermectin will also be excluded from participation in the study. Those children participating in the study who convert to a microfilaria positive status will, if they meet the criteria, be treated with ivermectin and dropped from the roles of the study.

At the time they enter the study, the children will be given an identification number. To facilitate proper identification of children during follow-up visits, we will take polaroid pictures of each child with their identification number and their parent or guardian. The pictures will be useful for locating the children even if they should move to another village.

Samples for Immunological Analyses

A syringe will be used to draw 2-5ml of venous blood from each child at the beginning of the study and at each follow-up. The heparinized blood will be placed in a tube and centrifuged to obtain the plasma. The plasma will be aliquoted, labeled with the date and the identification number and stored at -20C.

Peripheral blood mononuclear cells (PBMC) will be separated using density gradient centrifugation and cryopreserved in liquid nitrogen using standard methodology (Strong et al., 1975; Semba et al., 1992). We will use a modification of the standard Ficoll-Hypaque density gradient centrifugation technique. After removing the plasma, the cells will be diluted 1:3 with RPMI 1640. We have found that diluting the cells in RPMI 1640 increases the yielded of viable PBMC. Cryoperserved human lymphocytes retain similar function and cell surface markers as fresh lymphocytes (Strong et al., 1975; Semba et al., 1992). Based on our experience in Indonesia, we expect to obtain to obtain $2-4 \times 10^6$ lymphocytes/ml of blood from the younger children and $1-2 \times 10^6$ lymphocytes/ml of blood from the older children.

At each visit a smear will be taken to conduct a differential blood count.

Samples for Parasitological Analyses

The "gold standard" for the diagnosis of onchocerciasis is still the skin snip. Two biopsies of approximately 5mg will be taken at each sample time using a corneoscleral punch. After cleaning the site with alcohol, one skin snip will be taken from the shoulder region and the other from the iliac crest. An antibiotic cream will be applied to the skin snip site and an adhesive plaster will be applied to protect against bacterial infection. When performed by a skilled worker, the process of taking a skin snip is essentially painless. In this study, the biopsies will be performed by physicians and technicians who have extensive experience in taking skin snip samples from adults in children under the auspices of the WHO-sponsored Onchocerciasis Control Program.

The samples will be placed in a dish containing RPMI 1640 and examined after 24 hours of incubation for the presence of *O. volvulus* microfilariae in the medium. After being blotted to remove excess medium, the wet weight will be recorded for each snip so that microfilaridemia can be expressed on a per mg basis.

Examinations will also be carried out to determine if the children are harboring other parasites that could influence the immunological results. A thin and thick peripheral blood smears will be made for the diagnosis of blood filarial parasites in the children. Stool and urine specimens will be obtained at each visit and examined for the presence of intestinal and urogenital parasites. If the children are found to harbor parasites, they will be referred to a

local clinic for the appropriate treatment. The drugs most commonly use to treat these parasites will not influence the *O. volvulus* status of the patient.

Anti-*O. volvulus* Antibodies

Antigens - Each plasma sample will be tested for the IgE and the IgG subclass responses against the epitopes contained in a crude extract of *O. volvulus* adult male and female parasites.

Although lacking specificity, the responses directed against crude antigen will provide a useful indicator of the general immune status of the patient. Adult worms will be obtained by digesting nodules with collagenase and a soluble antigen extract prepared as described in Gbakima et al. (1992).

The results of a preliminary cross-sectional study on children from an *O. volvulus* endemic area (see above), indicated that a cocktail of recombinant *O. volvulus* proteins provides a sensitive and apparently specific set of epitopes for the assessment of the immune response during the early stages of onchocerciasis. The cocktail will be comprised of equal molar concentrations of three recombinant peptides: OV11, OV27 and OV29. All three are produced as fusion products with the maltose binding protein and will be provided by Dr Jan Bradely (Imperial College, London).

A fourth recombinant antigen that will be used is OV16 that will be provided by Dr. Thomas Nutman (National Institutes of Health). As noted in the discussion of the results of the cross-sectional study, OV16 appears to hold great potential for identifying individuals during the prepatent stages of infection.

For the recombinant antigens listed above, we have data that indicate that they will be useful in the identification of responses associated with prepatency. There are several other antigens that are available through the consortiums put together by the Edna McConnell Clark Foundation and the MacArthur Foundation (Dr. Scott is a member of both consortiums) that may be useful in defining these early immune responses. Several of these cloned antigens (such as L3-specific and male-specific antigens) will be screened and added to the analyses if they appear to have promise.

ELISA - Plasma will be used in ELISA assays to determine the IgG subclass responses. Wells of polyvinyl microtiter plates (Costar, Cambridge, MA) will be coated with either 100ng of the crude adult extract or 100ng of the recombinant antigen cocktail (a mixture of 33ng each of OV11, OV27 and OV29) using a published procedure (Gbakima et al. 1992). The plates will be washed five times with PBS containing 0.5% Tween20 (PBS-T20) and blocked for one hour at 37°C with PBS-T20 plus 2% normal goat serum (NGS). All sera will be run in duplicate at 1:100 dilution and incubated at 37°C for two hours. For assays of total IgG, FC-specific, horseradish peroxidase-conjugated goat anti-human IgG (Cappel, Durham, NC). For IgG subclass assays, murine monoclonal antibodies specific for IgG1 (Zymed Laboratories, San Francisco, CA) or murine monoclonal antibodies that specifically recognize human IgG2, IgG3 and IgG4 (Zymed). Since all of the recombinant antigens are fused to MBP, an MBP control will be run each time a specimen is evaluated to determine the background level of anti-MBP reactivity. The anti-MBP reactivity will be subtracted prior to the calculation of the anti-*O. volvulus* response. An estimate of the antigen-specific total IgG and IgG subclasses will be obtained by comparing the optical densities of known amounts of standard preparations of human IgG (Sigma), IgG1, IgG2, IgG3 or IgG4 (The Binding Site, Birmingham, UK), respectively.

Anti-*O. volvulus* T cell Responses

Determination of the lymphocyte proliferation response to a crude mix of *O. volvulus* antigens will be carried out according to standard thymidine incorporation methodology (Gallin et al. 1988; Ward et al. 1988; Soboslay et al. 1991). PBMC will be incubated in 96-well plates at a concentration of $1-2 \times 10^5$ /ml in RPMI with 10% autologous plasma and

antibiotics for 5 days in the presence of *O. volvulus* antigens. One mCi of ^3H -thymidine will be added to each well for the last 24 hours of incubation. The cells will be harvested onto glass fiber filters and ^3H -thymidine incorporation determined in a liquid scintillation counter. Autologous plasma has shown to improve the consistency and strength of response (Semba et al. 1992).

The crude *O. volvulus* adult antigen will be titrated to determine the optimum concentration to use in the stimulation assays. The recombinant antigens will be used initially as a cocktail to test if any of the recombinant proteins contain important T cell epitopes. If a cell sample responds to the cocktail (i.e. significantly higher than the no antigen and MBP controls), the cells will be assayed again using the individual recombinant antigens to determine where the epitope(s) may reside.

Incubation of PBMC with the mitogens phytohemagglutinin (PHA) and *Mycobacterium tuberculosis* (PPD) for 72 hours will be used as a gross indicator of the ability of the cells to proliferate to a nonspecific stimulus. Labeling of cells with ^3H -thymidine will be carried out as outlined above. Proliferation assays will be carried out at Johns Hopkins University by Dr. Gbakima.

T cell proliferation is not the only valid measure of the response to an antigen. Cytokine production is also an important indicator of cellular activation. Even though we consider cytokine production important, the cost of assaying multiple cytokines produced by even a portion of the estimated 1,800 cell samples that will be collected in this study places conventional analysis far beyond the economic scope of this proposal. We propose to use an alternate method to profile the changes in cytokine production during prepatency -- the ELISPOT. The ELISPOT has proven to be a simple and efficient method for the determining the number of cells secreting a given cytokine or antibody.

We will use a modification of the ELISPOT assay described by van der Meide et al. (1991) to measure the changes in the number of cells in the peripheral blood that are producing IL-2, IL-4, IL-5, IL-10 and IFN-g. Antibodies against a specific cytokine will be used to coat the bottom of a 96-well plate (Falcon 3070; Becton Dickinson, Lincoln Park, NJ). After blocking non-specific binding sites with PBS-T20 containing 4% BSA, mitogen-stimulated lymphocytes will be plated into the wells in triplicate at 1×10^3 and 5×10^3 per well in RPMI 1640 containing 5% FCS, 0.05mM 2-mercaptoethanol, 2mM glutamine, 100IU/ml penicillin, 60mg/ml streptomycin and 5mg/ml PHA. After a 6 hour incubation at 37°C, the cells will be lysed by hypotonic shock and the wells washed several times with PBS-T20. The cytokines that are bound to the plate via the capture antibody will be detected with cytokine-specific enzyme-labeled antibodies and a precipitating substrate. The number of cytokine-producing cells will be enumerated using a microscope to count the number of discrete areas on which cytokines were deposited by the stimulated lymphocytes. The cytokine "footprint" left by each stimulated lymphocyte will appear as an accumulation of substrate on the bottom of the well. The results of the ELISPOT assay will be used to calculate the number of cytokine-producing cells per 10^6 lymphocytes.

With the exception of anti-human IL-10 all of the antibodies needed for the ELISPOT cytokine assays are commercially available. Anti-human IL-10 is available in Dr. Scott's laboratory.

D. REFERENCES

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