



U.S. AGENCY FOR  
INTERNATIONAL  
DEVELOPMENT

PD-ABL-146  
94873

AUG 23 1993

Dr. Ann R. Stevens  
Associate Vice President for Research  
Emory University  
1462 Clifton Road N.E.  
Atlanta, Georgia 30322

RECEIVED

OCT 1 1993

Subject: Cooperative Agreement No. HRN-6001(A)-001-3018-00

Dear Dr. Stevens:

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." or "Grantor") hereby provides to Emory University (hereinafter referred to as "University" or "Recipient") the sum set forth in Section 1C.2. of Attachment 1 of this Cooperative Agreement to provide financial support for the program described in Attachment 2 of this Cooperative Agreement entitled "Program Description."

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

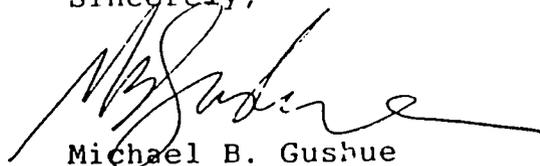
The total estimated amount of this Cooperative Agreement is the amount set forth in Section 1C.1. of Attachment 1, of which the amount set forth in Section 1C.2. is hereby obligated. A.I.D. shall not be liable for reimbursing the Recipient for any costs in excess of the obligated amount. However, subject 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 Cooperative Agreement.

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

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

We are pleased to provide this grant in support of your program.

Sincerely,



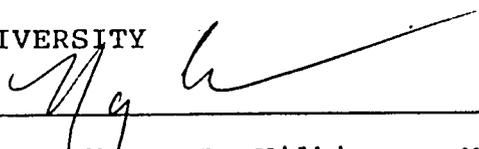
Michael B. Gushue  
Agreement Officer  
Division A, HRN Branch  
Office of Procurement

Attachments:

1. Schedule
2. ~~Program Description~~
3. ~~Standard Provisions~~
4. Program Reporting Requirements

ACKNOWLEDGED:

EMORY UNIVERSITY

BY: 

TYPED NAME: Nancy L. Wilkinson, MPH

TITLE: Associate Director for Research

DATE: 9-20-93

FISCAL DATA

. A. GENERAL

A.1. Total Estimated A.I.D. Amount: \$622,392  
A.2. Total Obligated A.I.D. Amount: \$196,588  
A.3. Cost-Sharing Amount (Non-Federal): \$ -0-  
A.4. Other Contributions (Federal): \$ -0-  
A.5. Project No.: 936-6001  
A.6. A.I.D. Project Office: R&D/H/CD  
A.7. Funding Source: A.I.D./W  
A.8. Tax I.D. No.: 58-0566256  
A.9. CEC No.: 066469933  
A.10. LOC No.: 72-00-1646

B. SPECIFIC

B.1.(a) PIO/T No.: 936-6001-3692373  
B.1.(b) Project No.: 936-6001.39  
B.1.(c) Appropriation: 72-1131021.1  
B.1.(d) Allotment: 341-36-099-00-20-31  
B.1.(e) BPC: DDVA-93-16900-KG11  
B.1.(f) Amount: \$58,976

B.1.(a) PIO/T No.: 936-6001-3692372  
B.1.(b) Project No.: 936-6001.39  
B.1.(c) Appropriation: 72-1131021.1  
B.1.(d) Allotment: 341-36-099-04-20-31  
B.1.(e) BPC: DDVA-93-16900-CG11  
B.1.(f) Amount: \$137,612

ATTACHMENT 1

SCHEDULE

1A. PURPOSE OF COOPERATIVE AGREEMENT

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

1B. PERIOD OF COOPERATIVE AGREEMENT

1B.1. The effective date of this Cooperative Agreement is the date of the Cover Letter and the estimated completion date is August 31, 1996. Funds obligated hereunder (see Section 1C.2. below) shall be used to reimburse the Recipient for allowable program expenditures incurred by the Recipient in pursuit of program objectives at any time during the period beginning on September 1, 1993 of this Cooperative Agreement and ending on the estimated completion date.

1B.2. However, because this Cooperative Agreement 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 COOPERATIVE AGREEMENT AND PAYMENT

1C.1. The total estimated amount of this Cooperative Agreement for its full period, as set forth in Section 1B.1. above, is \$622,392.

1C.2. A.I.D. hereby obligates the amount of \$196,588 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 Recipient for any costs in excess of the obligated amount, except as specified in paragraph (f) of the Standard Provision of this Cooperative Agreement entitled "Revision of Grant Budget" (see also Section 1C.4. below).

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

1C.4. As indicated in Section 1C.2. above, this Cooperative Agreement 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 Cooperative Agreement, additional increments of funds may be obligated by A.I.D. under this Cooperative Agreement (by a Cooperative Agreement 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 Cooperative Agreement entitled "Revision of Grant Budget".

1D. COOPERATIVE AGREEMENT BUDGET

1D.1. The following is the Budget for the total estimated amount of this Cooperative Agreement (see Section 1C.1. above) for its full period (see Section 1B. above). The Recipient may not exceed the total estimated amount or the obligated amount of this Cooperative Agreement, whichever is less (see Sections 1C.1. and 1C.2., respectively, above). Except as specified in the Standard Provision of this Cooperative Agreement entitled "Revision of Grant Budget," as shown in Attachment 3, the Recipient 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 entitled "Revision of Grant Budget."

1D.2. Budget

<u>Cost Element</u>	<u>Three Years</u>
	FR: 09/01/93
	TO: 08/31/96
Salaries :	\$303,427
Equipment	9,720
Supplies	149,397
Travel (Nat'l/Int'l	37,000
Indirect Costs	<u>122,849</u>
Total	\$622,393

1D.3. Inclusion of any cost in the budget of this Cooperative Agreement does not obviate the requirement for prior approval by the Agreement Officer of cost items designated as requiring prior approval by the applicable cost principles (see the Standard Provision of this Cooperative Agreement set forth in Attachment 3 entitled "Allowable Costs") and other terms and conditions of this Cooperative Agreement.

1E. REPORTING1E.1. Financial Reporting

1E.1.(a) Financial reporting requirements shall be in accordance with the Standard Provision of this Cooperative Agreement entitled "Payment - Letter of Credit," as shown in Attachment 3.

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.

1E.2 Program Reporting

The program reporting requirements are contained in Attachment 4.

1F SUBSTANTIAL INVOLVEMENT UNDERSTANDINGS

A.I.D. will be involved in two reviews during the period of this Agreement. This will include an in-depth analysis of the achievements of the Recipient to date. The Midterm Review will be conducted by an external technical peer group after approximately 18 months. The Recipient will provide a Midterm Report in an appropriate format. A final report will occur near the completion of the Agreement and will require submission of an End-of-Project Report in an appropriate format.

1G. INDIRECT COST RATES

1G.1. Pursuant to the Standard Provisions of this Cooperative Agreement 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 Recipient's accounting periods which apply to this Cooperative Agreement. Pending establishment of predetermined indirect cost rates for the initial period, provisional payments on account of allowable indirect costs shall be made on the basis of the following negotiated provisional rate(s) applied to the base(s) which is (are) set forth below:

Provisional Off-campus, Instruction (A) 40%  
 Period: 09/01/93 until Amended

Base of Application: Modified Total Direct Costs

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

**1H. SPECIAL PROVISIONS****1H.1. Limitations on Reimbursement of Costs of Compensation for Personal Services and Professional Service Costs****1H.1. Employee Salaries**

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

**1H.2. Publications**

1H.2(a) The Recipient agrees to provide one copy of the manuscript of any proposed publication to the A.I.D. Project Officer not later than submission to the publisher, and to give serious consideration to any comments received from the A.I.D. Project Officer.

1H.2(b) In the case of publication of any of the reports described in Section 1E.2. of this Cooperative Agreement, A.I.D. reserves the right to disclaim endorsement of the opinions expressed. For other publications, A.I.D. reserves the right to dissociate itself from sponsorship or publication. In both cases, the Recipient will consult with the A.I.D. Project Officer as to the nature and extent of any A.I.D. disclaimer of endorsement or dissociation from sponsorship or publication.

1H.2(c) If A.I.D. does not choose to disclaim endorsement or disassociate itself from sponsorship or publication, the Recipient shall, in accordance with the Standard Provision of this Cooperative Agreement entitled "Publications," acknowledge A.I.D. support as follows:

"This publication was made possible through support provided by the Office of Research, Bureau of Research and Development, U.S. Agency for International Development, under Cooperative Agreement No. HRN-6001-A-00-3018-00."

1H.2(d) In addition to providing one copy of all published works and lists of other written work produced under this Cooperative Agreement to the A.I.D. Project Officer, as required by paragraph (b) of the Standard Provision of this Cooperative Agreement entitled "Publications," the Recipient shall also provide two copies of such publications and lists to A.I.D., POL/CDIE/DI, Washington, D.C. 20523-1802.

### 1H.3. Equipment and Other Capital Expenditures

#### 1H.3.(a) Requirement for Prior Approval

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

1H.3.(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;

1H.3.(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

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

#### 1H.3.(b) Approvals

In furtherance of the foregoing, the Agreement 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 Cooperative Agreement, whichever is less (see Section 1C. above): Table Top Centrifuge, Dry Shipper (2), Slot Blot Apparatus..

#### 1H.3.(c) Exception for Automation Equipment

Any approval for the purchase of automation equipment which may be provided in Section 1I.3.(b) above or subsequently provided by the Agreement 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 Recipient must, under such circumstances, obtain the approval of the Agreement Officer for the total planned system of any automation equipment, software, or related services.

#### 1H.3.(d) Compliance with A.I.D. Eligibility Rules

Any approvals provided above or subsequently provided by the Agreement Officer shall not serve to waive the

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A.I.D. eligibility rules described in this Cooperative Agreement, unless specifically stated.

1H.4. Restricted Goods

See the Standard Provision in Attachment 3 entitled AID Eligibility Rules for Goods and Services.

1H.4.(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 Agreement Officer. 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 advance written approval of the Agreement 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.

1H.4.(b) Motor Vehicles

Motor vehicles, if approved for purchase above or subsequently approved by the Agreement Officer, must be of U.S. manufacture and must be of at least 51% U.S. componentry. Motor vehicles are defined as self-propelled vehicles with passenger carriage capacity, such as highway trucks, passenger cars and

(see next page)

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.

**1H.4.(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; (3) the pharmaceuticals must be of at least 51% U.S. componentry; (4) the pharmaceuticals must be purchased from a supplier whose nationality is in the U.S. (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 Recipient and the Standard Provision of this Cooperative Agreement entitled "Procurement of Goods and Services."

**1H.4.(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 desiccant.

**1H.4.(e)            Rubber Compounding Chemicals and Plasticizers**

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

**1H.4.(f) Used Equipment**

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

**1H.4.(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 Agreement Officer.

**1H.5. Limitation on Use of Funds**

1H.5.(a) The Recipient 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.

1H.5.(b) The reports 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.

1H.5.(c) The Recipient agrees to refund to A.I.D. upon request an amount equal to any United States funds used for the purposes prohibited by the provisions of this grant.

1H.5.(d) No funds provided by A.I.D. under this Cooperative Agreement 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 Cooperative Agreement entitled "Ineligible Countries").

**1H.6. Defense Base Act (DBA) and/or Medical Evacuation Insurance**

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 Recipient is authorized to purchase DBA and/or medical evacuation insurance under this Cooperative Agreement.

## 11. STANDARD PROVISIONS

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

### 11.1. Mandatory Standard Provisions For U.S., Nongovernmental Recipients

- ( 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)

### 11.2. Additional Standard Provisions For U.S., Nongovernmental Recipients

- ( 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 (November 1985)

- ( X ) AID Eligibility Rules for Goods and Services (June 1993)
- ( 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)
- ( X ) 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)
- ( X ) 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)
- ( ) Cost Sharing (Matching) (August 1992)
- ( X ) Use of Pouch Facilities (August 1992)
- ( X ) Conversion of United States Dollars to Local Currency (November 1985)
- ( X ) Public Notices (August 1992)
- ( X ) Rights in Data (August 1992)

(end of Schedule)

ATTACHMENT 2

PROGRAM DESCRIPTION

The Recipient's proposal entitled "Epitope Polymorphism in P. falciparum Vaccine Antigens" and dated September 29, 1992 is incorporated herein and made a part of this Cooperative Agreement.

(see next page)



level of a population of parasites in one host and on the level of the populations of parasites in host populations. Thus, the information obtained on the variation of the immunodominant B- and T- cell determinants, and the distribution and prevalence of polymorphs in individuals of different immune status in two malarious regions of different endemicity, will be valuable in malaria vaccine development.

2. BACKGROUND AND SIGNIFICANCE. Malaria, caused by protozoa of the genus *Plasmodium*, is presently estimated to affect 300-500 million people world wide, with an estimated annual death toll at 1-2.5 million per year (1). Four *Plasmodium* species that are known to infect human and cause malaria are: *P. falciparum* which causes practically all malaria related deaths; *P. vivax*, also widespread, causes considerable morbidity. The less prevalent species are *P. ovale* and *P. malariae*.

In the case of *P. falciparum*, out of several malaria candidate vaccine antigens that have been characterized, three antigens have emerged as leading vaccine candidates. These are, the circumsporozoite (CS) protein, the merozoite surface protein-1 (MSP-1), and the 25 kd ookinete antigen (Pfs25). In addition, recent monkey vaccine trials with the blood-stage antigens of *P. falciparum*, the apical membrane antigen-1 (AMA-1) and the rhoptry associated protein-1 (RAP-1), have been very encouraging. Presently, the vaccine developers are faced with two major problems, first, failure or inconsistent outcome of immunization, and second, genetic and antigenic polymorphism of candidate vaccine antigens.

2A. Molecular biologic and immunologic studies of *P. falciparum* vaccine antigen. In this section of the proposal, following a general discussion on the molecular basis of parasite's antigenic variability, we will present background information on polymorphism in the three *P. falciparum* vaccine antigens that are the focus of this proposal.

2A.I Molecular basis of parasite's antigenic variability: There is an ongoing debate on the origin, selection, and maintenance of polymorphism in the B and T-cell immunodominant regions of malarial protein (2-4). One view supports that the human immune response is important in selection of variation, and the other view doubts the existence of selectively maintained polymorphism in the regions of the protein that interface with host immune cells.

From the vaccine developers perspective, however, information about the nature and extent of epitope variation is crucial, regardless of how polymorphism in the B-and T-cell sites arose. Whereas, a variant protective B-epitope bearing parasite will escape vaccine induced immunity, variation in the T-cell determinants can result in direct loss of memory and/or protection, depending on the epitope (T-helper, T-proliferation or T-cytotoxic) involved.

A surface protein determinant(s) first obligation is to ensure successful host-parasite interaction. Nonsilent mutations in the regions of the protein that nullify the parasites interaction with host cellular receptors will be rapidly selected against, and the representative parasites would disappear from the population of parasites. This would imply that the nonpolymorphic regions of the malaria antigen involved in host-parasite interaction may harbor the biologically sensitive targets of the protein (5).

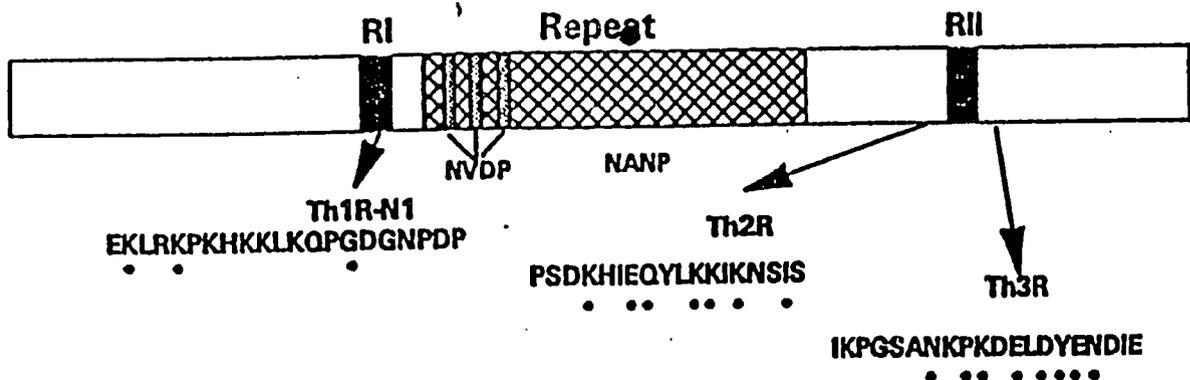
From immunologic considerations, however, parasite protein sequences and/ or conformations predicate the immune dominance or immunogenicity of a region, and the epitopes which interface with the host immune system would preferentially accumulate changes to escape the pressures of immunity. In situations where biologically and immunologically sensitive regions of the protein coexist, accumulation of any nonsilent mutations would have to first pass through the biologic filter before being tested at the immunologic level. In the case of purely immunodominant determinants, however, nonsilent changes can be positively selected, particularly if the mutation results in an amino acid change that helps the parasite evade host immune pressures.

Parasite protein polymorphism also needs to be examined from epidemiologic and entomologic perspectives. It remains to be determined whether the low malaria endemicity of a region is due to the presence of fewer polymorphic parasites, or due to other human host and

vector associated factors. Examination of the molecular structure of parasite protein polymorphism in low and high malaria-endemic regions would increase our ability to make intelligent guesses in this matter. Indeed, results from our studies suggest that in low endemic areas the polymorphism is less prevalent than in the high endemic areas.

**2A.II The circumsporozoite protein:** The CS proteins are the most extensively studied of the malaria parasite's antigens (6). In vitro and in vivo studies have shown strong vaccine potential of this sporozoite surface antigen (7-11). The genes encoding the CS proteins have been cloned and sequenced from rodent, monkey, chimpanzee, and human malaria parasites. These CS proteins generally contain a central repetitive B-cell epitope flanked by genus-conserved regions, referred to as Region I and Region II. In the human malaria parasite *P. falciparum*, the central repeat region of the CS protein is composed of NANP (major) and NVDP (minor) repeat sequence (12). The remainder of the nonrepeat region of the CS protein contains polymorphic T-cell determinants, and putative anchor and signal sequences. The identified T-cell sites of the CS protein are: 1) Th1R, a T-proliferation epitope that overlaps with the putative hepatocyte binding site (N-1) and is referred to hereafter as Th1R-N1; 2) Th2R, a T-helper epitope that resides carboxyl to Region II, and is part of a cytotoxic T-cell (CTL) epitope recognized by human CD4<sup>+</sup> T cells in a class II restricted manner; and 3) Th3R, a T-proliferation site that overlaps the other identified MHC class I restricted (CTL) epitope of the CS protein (Fig. 1).

Sequence determination and hybridization of the CS protein from field-derived parasites have shown varying degrees of sequence polymorphism outside the repeat regions of the protein (13-18). Immunologic experiments have shown that while some variant amino acids nullify the immune reactivity of the determinants, others did not affect the immune functions (19-21). The repeat region and the CTL determinants of the CS protein are important from vaccine development consideration. While other two determinants (Th1R and Th2R) play an accessory role, antibodies against the NANP motif and the CTL induced against the Th3R overlapping determinant have been correlated with protection. Presently, malaria vaccine developers are faced with a challenge to develop practical means to induce protective immune responses using vaccines that contain the repeat and/or the CTL determinants of the CS protein.



**Figure 1. Map of the CS protein gene:** The B-cell and T-cell determinant sequences of the 7G8 clone of *P. falciparum* are shown. The (\*) indicates polymorphic positions in these T-cell determinants.

**2A.III Merozoite surface antigen-1 (MSP-1):** Several studies suggests that the MSP-1 is immunogenic and the target of protective immunity (22-32). Results of antibody probes and peptide mapping studies suggest that the MSP-1 molecule contains both conserved and variable determinants (Fig. 2 A). A comparison of the deduced sequence for the protein from different laboratory strains has clarified the results of variable and conserved regions as deduced by peptide mapping and Southern blot analysis (Fig. 2). The repeat falls into two groups, although there is much degeneracy in both repeat number and sequence (Fig. 2A). In all strains analyzed, the region immediately flanking the tripeptide repeat also falls into one of two groups (Fig. 2C).

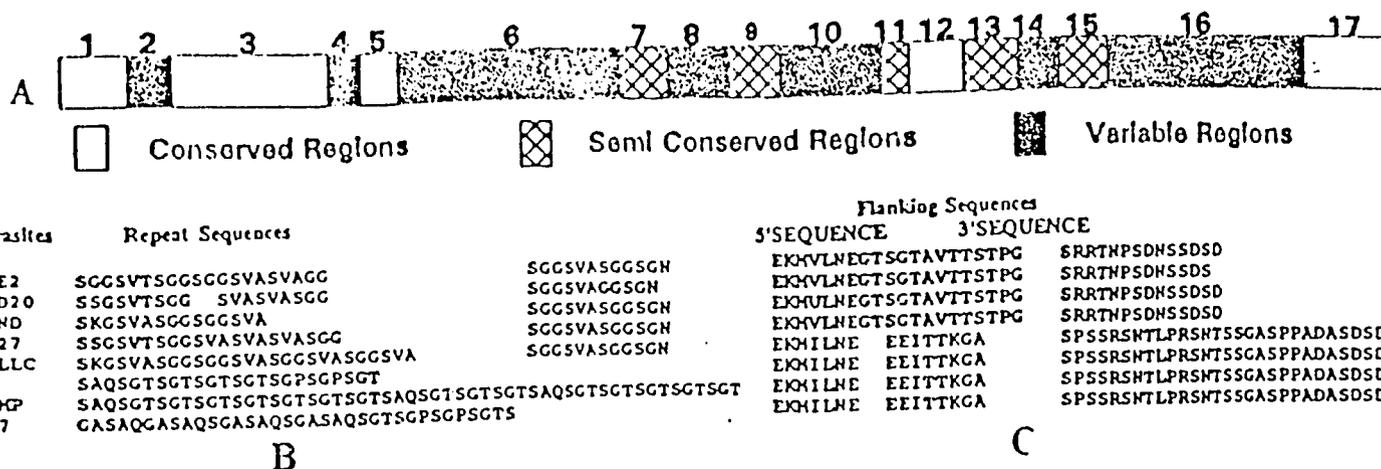


Figure 2. Map of the MSP-1 protein gene of (A). *P. falciparum*: The position of the conserved, semiconserved and the variable domains is marked; (B). shows the comparison of the repeat sequences; and (C) shows the comparison of the sequence flanking the repeat sequences in the MSP-1 gene of laboratory cultured *P. falciparum*.

The amino acid sequence comparison of the MSP-1 molecule outside of the repeat regions reveals that the Wellcome (West African) and K1 (Thailand) strains differ in only 19 of 1,523 amino acids (33). Comparison of these sequences with the 5' sequence of the CAMP (Malaysia) strain and the entire MAD-20 (Papua New Guinea) sequence reveals a pattern of homology. There are blocks of conserved (>87% homology), extensively divergent (10% homology) and semi-conserved sequences.

This comparison gives us an opportunity to analyze distribution of polymorphic parasites. For instance, strains SGE2, MAD20, WELLCOME, and HONDURAS I are from Zaire, Papua New Guinea, West Africa, and Honduras, respectively, but share the sequences flanking the repeats. Kimura et. al. have recently studied diversity of the MSP-1 gene of parasites from infected individuals (34). They have found multiple infections of genetically distinct parasites within an infected malaria patient. Sequence analysis and oligonucleotide hybridization of the PCR products demonstrated the prevalence of a third polymorphic form of MSP-1 (34).

MSP-1 domains recognized by human T and B cells have been localized to the conserved regions towards the amino terminus of the protein (31,35). Additional B-cell epitopes distributed throughout the molecule have been demonstrated with mouse monoclonal antibodies, although these epitopes have not been mapped to particular amino acid sequences (36,37). The immune response to MSP-1 has been examined in congenic mouse strains differing in H-2 haplotype (38). All strains were capable of producing MSP-1 specific antibodies. Some strains recognized a few unique fragments or displayed more intensive reactivity with a specific processed fragment. The results indicate that while mice of diverse MHC are capable of recognizing MSP-1, recognition of specific T and B epitopes may be under the control of the MHC.

Riley et. al. recently investigated the pattern of acquired immune responses to the MSP-1 protein of *P. falciparum* in a malaria endemic population in west Africa (39). The prevalence and concentration of antibodies to all regions of the molecule increased with age with the highest prevalence of antibodies being detected against the regions of the molecule that are highly conserved between parasite isolates. In these studies, significant associations were found between antibody and lympho-proliferative responses to protein from the C terminus of

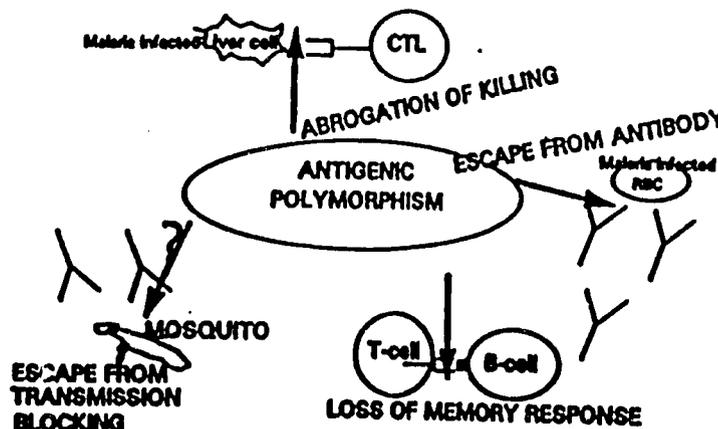
the molecule and resistance episodes of fever associated with high parasitemia in partially immune children. In another recent elegant study, Chang et. al. has confirmed that conformation dependent antibodies against the conserved C-terminal domain (region 17, Fig. 2) are important for protection (29).

**2A.IV Pfs25 kDa zygote/ookinete antigen:** Pfs25 is expressed predominantly by zygotes, and in contrast to other targets of transmission blocking immunity, is immunogenic and induces transmission blocking antibodies in different congenic mouse strains and in monkeys. Pfs25 also was found to lack significant antigenic diversity (40). The absence of genetic restriction in mice and the lack of antibodies to Pfs25 in people living in endemic areas (since this protein is expressed only in the mosquito stage of parasite's life cycle) suggest that Pfs25 has not been under strong immune pressure. We have also found limited polymorphism in this antigen in parasites from PNG (41). However, we consider it is important to analyze the gene of this protein for polymorphism utilizing large parasite sample size; fully realizing that this antigen may not be as polymorphic as compared to the sporozoite-stage and the blood-stage antigens of malaria parasite.

Since natural malaria infection will not present this protein to the immune system, a vaccinee may not be able to rely on the natural boosting after primary immunization with a transmission-blocking vaccine. The use of novel adjuvants, carrier proteins, and slow-release vaccine formulations may overcome this problem. Even though, protective epitopes of this antigen have not been identified, it is clear that conformation dependent epitopes mediate protection against the Pfs25 kDa antigen.

**2A.V Immunologic implications of parasite variability:** Proteins which contain many epitopes will elicit a response in many or most members of a given species. A sequence variation in a single T-epitope would probably not have much effect in this situation. Some proteins, however, contain few T-epitopes. Indeed, the CS protein of *P. falciparum* appears to contain limited T-cell determinants, as defined by studies in mice and humans. Variation in the T-epitopes in molecules that contain only one or two sites may significantly affect the potential to boost the immune response from prior immunization. Polymorphism in the T-cell determinants has been significant in some cases, but of no immunologic consequence in others in studies of immune response to antigens (42-45).

How can natural amino acid variation affect immune reactivity? The key components of a desired vaccine are T and B cell epitopes that the host immune system recognizes and keeps in memory during the generation of the immune response. The T cell epitopes that typically consists of about 9-12 amino acids in a linear sequence, should have binding motifs for HLA antigens (agretope), as well as for T cell receptors (epitope). One or more amino acid substitutions in these short stretches of polypeptides can result in loss of binding to either HLA antigen or T cell receptor, and consequently the epitope may become immunologically inert.



**Figure 3. Possible effects of polymorphism on immune response:** The diagram shows the developmental stages in the life cycle of malaria parasite, arms of the host immune system that can play a role in controlling the infectivity of parasite, and the effect of polymorphism on immune reactivity.

Evidence for natural variation-induced loss of immunologic reactivity: This has been demonstrated in the CS protein of *P. falciparum*, where natural amino acid substitutions in a T helper and T-proliferative epitope has been shown to abrogate the helper function of this epitope (19,20). Recently, Moreno et al. have identified a CD4<sup>+</sup> CTL determinant in the CS protein that overlaps with the TH2R region of the protein (46). It remains to be tested how polymorphism in this region affects CTL response in the malaria endemic region.

We have observed that some natural amino acid changes in the CD8<sup>+</sup> CTL epitope of CS protein of *P. falciparum* from Brazil and PNG abrogates the CTL reactivity (please see preliminary results section for more details). Since these experiments have been performed in a single genetic background, it will be interesting to see how polymorphic changes influence immune response in different genetic backgrounds. It will also be important to extend these studies to field settings so that we can determine how the immune response is affected in the natural host population as a result of antigenic polymorphism.

Recent studies have shown that both human and mice recognize the same CTL epitope of the CS protein of *P. falciparum* (47). Malaria exposed individuals in Kenya have been shown to contain CS protein specific CD8<sup>+</sup> CTL (48). This study used the sequence of the CTL determinant of *P. falciparum* (7G8) from Brazil. Our preliminary data on the Kenyan *P. falciparum* population suggests that about 28% of the parasite population in the study site has this genotype (CTL sequence similar to 7G8 CS protein). There are at least seven other genotypes present in the study site (Table 1). Therefore, it will be crucial to determine the cross reactivity of CTL epitopes of local parasite population. A similar study should be conducted in Kenya and elsewhere, but using peptides that are representative of the natural populations of the region.

**Significance of testing the immunologic cross-reactivity between the variant sequences and vaccine trials:** Irrespective whether a malaria vaccine is composed of antigens that are expressed in single stage, or contain components from several stages of malaria parasites, it is imperative that the molecular and immunologic mechanisms associated with protein polymorphism of natural populations of malaria parasite be studied using clinically, epidemiologically, and immunologically well characterized human hosts.

The testing of any malaria vaccine will best be carried out in populations where the impact of disease manifestations of malaria, the biology of infection, and the polymorphic nature of the vaccine antigens in the target population are well characterized. This information will allow a greater understanding of the relationship between antigenic variability, infection, disease, and vaccine efficacy.

**Future studies in antigenic diversity are clearly needed to address the following questions with respect to antigenic polymorphism in the CS, Pfs25, and MSP-1 proteins, in preparation of a field vaccine trial.**

- 1. What is the extent of natural polymorphism in the Pfs25, the CS, and the protective epitope(s) of the MSP-1 vaccine candidate antigen.**
- 2. Investigate immunologic implication of polymorphism in the CTL epitopes of the CS protein of natural *P. falciparum* populations.**
- 3. Where are the T-proliferative and T-helper sites located in the MSP-1 protective domain, and does polymorphism affects the cell mediated and humoral arm of the host immune system.**
- 4. Compare the nature and extent of polymorphism in three vaccine antigens from parasites of high endemic and comparatively low endemic region.**

Program Reports

1. The Recipient shall submit five copies of a periodic progress report and a final report to the Cognizant Technical Officer specified in the Agreement letter, which briefly presents the following information in addition to the data set forth in the Recipient's proposal dated September 30, 1992. These reports shall be submitted within 30 days following the end of the reporting period (as requested by the Cognizant Technical Officer); within 90 days for submission of the final report.
  - a. A comparison of actual accomplishments with the goals established for the period, the findings of the study or both.
  - b. Reasons why established goals were not met.
  - c. Other pertinent information including, when appropriate, an analysis and explanation of cost overruns or high unit costs.
2. Between the required performance reporting dates, events may occur that have significant impact upon the program. In such instances, the Recipient shall inform the AID Agreement Officer as soon as the following types of conditions become known:
  - a. 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 project work units by established time periods. This disclosure shall be accompanied by a statement of the action taken, or contemplated, and any AID assistance needed to resolve the situation.
  - b. Favorable developments or events that enable time schedules to be met sooner than anticipated or more work units to be produced than originally projected.
3. If any performance review conducted by the Recipient discloses the need for change in the budget estimates in accordance with the criteria established in the standard provision entitled "Revision of Grant Budget", the Recipient shall submit a request for a budget revision.

- 2
4. The following specific reports shall be submitted to the Cognizant Technical Officer; four copies each:
- a. Technical progress reports submitted 9 months and 27 months following award, or at other times as required by the Cognizant Technical Officer. These reports will conform to a format provided by the Cognizant Technical Officer or his/her designee, and will include technical progress towards each objective and sufficient data to permit independent assessment of overall progress and accomplishment. Distribution of the report will be restricted to AID and designees involved in the evaluation process. In addition, an abstract of technical progress should be included which may be circulated to other AID funded investigators; preliminary, proprietary or other data included in the restricted distributions should not be included in the abstract. Preprints and reprints of all publications should be submitted as part of the progress report.
  - b. In addition, Mid Project and End of Project reports shall be submitted prior to the mid project evaluation and prior to the completion of the agreement period as specified by the Cognizant Technical Officer. These reports will also include progress on technical objectives, as well as preprints and reprints of all publications and other information as specified by the Cognizant Technical Officer or his/her designee.
  - c. Trip Reports shall be submitted for AID supported travel within thirty days after completion of the travel. These reports will include the purpose of the trip, description of activities, outcomes of the trips, and the total cost. Reports may be waived or additional information requested at the time written approval for such travel is obtained from AID.
  - d. Reports will be submitted within 30 days of the conclusion of the clinical phases of any experiments/trials involving the use of nonhuman primates and/or human subjects.
5. One copy of the final report shall be submitted to the AID Agreement Officer, FA/OP/A/HRN, Room 1532, SA-14, Washington, D.C. 20523-1427.