AGENCY FOR INTERNATIONAL DEVELOPMENT

PROJECT DATA SHEET

1. TRANSACTION CODE: A = Add, C = Change, D = Delete — Amendment Number

DOCUMENT CODE: 3

2. COUNTRY/ENTITY: S&T/Interregional

3. PROJECT NUMBER: 931-0453.17

4. BUREAU/OFFICE: S&T/H/CD — 10

5. PROJECT TITLE (maximum 40 characters): Malaria Immunology - Scripps

6. PROJECT ASSISTANCE COMPLETION DATE (PACD): MM 10 DD 14 YY 87

7. ESTIMATED DATE OF OBLIGATION (Under 'B.' below, enter 1, 2, 3, or 4)
A. Initial FY 84 B. Quarter 3 C. Final FY 86

8. COSTS ($000 OR EQUIVALENT $1 =)

A. FUNDING SOURCE	FIRST FY 84 B. FX	C. L/C	D. Total	LIFE OF PROJECT E. FX	F. L/C	G. Total
AID Appropriated Total	~~1095~~		1095	3266		3266
(Grant)	(1095)	( )	(1095)	(3266)	( )	(3266)
(Loan)	( )	( )	( )	( )	( )	( )
Other U.S. 1.						
Other U.S. 2.						
Host Country						
Other Donor(s)						
TOTALS	~~1095~~	1095		3266		3266

9. SCHEDULE OF AID FUNDING ($000)

A. APPROPRIATION	B. PRIMARY PURPOSE CODE	C. PRIMARY TECH. CODE 1. Grant	2. Loan	D. OBLIGATIONS TO DATE 1. Grant	2. Loan	E. AMOUNT APPROVED THIS ACTION 1. Grant	2. Loan	F. LIFE OF PROJECT 1. Grant	2. Loan
(1) S&T/H	540	542				3266			
(2)									
(3)									
(4)									
TOTALS						3266			

10. SECONDARY TECHNICAL CODES (maximum 6 codes of 3 positions each)

11. SECONDARY PURPOSE CODE

12. SPECIAL CONCERNS CODES (maximum 7 codes of 4 positions each)
A. Code
B. Amount

13. PROJECT PURPOSE (maximum 480 characters)

The purpose of this project is to identify, isolate, and begin chemical characterization of some of the macromolecular components of the asexual erythrocytic forms of human malaria parasites which can be used to induce immunity in primates.

14. SCHEDULED EVALUATIONS
Interim MM 03 YY 85 MM 06 YY 86 Final MM 10 YY 87

15. SOURCE/ORIGIN OF GOODS AND SERVICES
☒ 000 ☐ 941 ☐ Local ☐ Other (Specify)

16. AMENDMENTS/NATURE OF CHANGE PROPOSED (This is page 1 of a ____ page PP Amendment.)

17. APPROVED BY
Signature: Dr. George Curlin, M.D.
Title: Director, Office of Health
Date Signed: MM 03 DD 28 YY 84

18. DATE DOCUMENT RECEIVED IN AID/W, OR FOR AID/W DOCUMENTS, DATE OF DISTRIBUTION
MM DD YY

AID 1330-4 (8-79)

UNITED STATES INTERNATIONAL DEVELOPMENT COOPERATION AGENCY
AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON D C 20523

PROJECT AUTHORIZATION

Name of Entity: Interregional

Project Title: Malaria Immunity and Vaccination Research

Project No.: 931-0453.17

Grantee: Scripps Clinic and Research Foundatior

1. Pursuant to Seciton 104 of the Foreign Assistance Act of 1961, as amended, I hereby authorize the research project entitled "Antigens of Plasmodium falciparum" involving not to exceed $3,266,000 of S&T Bureau grant funds over a three year period from the date of authorization subject to the availability of funds in accordance with the A.I.D. OYB/Allotment process to help in financing the costs of the project.

2. The project will conduct research on red blood cell antigens of the human malarias through the use of monoclonal antibodies and genetic engineering techniques.

Clearances:

A. ST/H: G. Curlin Date 3/28/84
B. ST/PO: G. Eaton Date 4/3/84

S&T/HP, J. Sarn, M.D.
Agency Director for
Health and Population

4-5-84
Date

UNITED STATES INTERNATIONAL DEVELOPMENT COOPERATION AGENCY
AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON D C 20523

March 26, 1984

ACTION MEMORANDUM FOR THE AGENCY DIRECTOR
FOR HEALTH AND POPULATION

FROM: S&T/HP, George Curlin, M.D. GCurlin MD

Action: Your approval is requested for a grant of $3,266,000 from Section 104 of the Foreign assistance Act of 1961 as amended for project 931-0453.17, Malaria Immunity and Vaccination Research, Scripps Clinic and Research Foundation.

Discussion: This project is designed to further identify, isolate, and begin chemical characterization of some of the macromolecular components of the asexual erythrocytic forms of human malaria parasites which can be used to induce immunity in primates, including man.

This project and its proposed scope of work mesh well with the current thrust of the A.I.D. acceleration program for the development of human malaria vaccines. The thrust of this project will focus on the use of monoclonal and polyclonal antibodies from monkeys and humans to isolate potential antigens. These same antibodies will be used to identitfy recombinant bacterial clones containing *Plasmodium* cDNA which are producing parasite antigens of interest. The primary peptide structures will be determined from both cDNA and genomic DNA sequences. Primary structural analysis will be performed to predict those portions of the molecule which are likely to protrude into the aqueous environment and thus become potential simple protective antigens, basic to vaccine production.

Justification to the Congress: Project Funding is included in the FY 1984 Congressional Presentation, Annex V, Centrally funded Programs, page 53.

Clearances Obtained: This project was approved by the AID external expert panel on the basis of scientific methodology, qualifications and experience of the proposed investigators, and the institutional capability in accordance to FPR 1-4.909.

Recommendations: 1. That you sign the attached authorization.
2. That you sign the attached justifications for non-competitive procurement.

Attachments:
1. Project Authorization
2. Non competitive Justifications for non-competitive procurement
3. Project Paper

Clearance:
S&T/PO, G. Eaton KMP Date 4/2/84

UNITED STATES INTERNATIONAL DEVELOPMENT COOPERATION AGENCY
AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON D C 20523

March 26, 1984

MEMORANDUM

TO: OM/COD/PE, Ms. Johni Pittnger

FROM: S&T/HP, James E. Sarn, M.D.

SUBJECT: Justification for non-competitive procurement of unsolicited proposal from the Scripps Clinic and Research Foundation entitled "Antigens of Plasmodium falciparum."

The subject research proposal is an unsolicited proposal. The substance of the proposal is not available to the Government without restriction from another source, nor does it resemble any pending competitive solicitation. The substance is sufficiently unique to justify acceptance as an unsolicited proposal.

The project officer certification with reference to A.I.D. PR Notice 78-4 follows:

> I certify that neither I nor, to the best of my knowledge and belief, any other A.I.D. employee solicited the proposal or had any prior contact with the proposing institution, other than to convey interest in the field of malaria immunity and vaccination relative to the efforts described in the unsolicited proposal.

James M Erickson

Dr. James M. Erickson, Project Manager

I request that you award this contract on a non-competitive basis to the Scripps Clinic and Research Foundation without consideration of other sources.

Clearances:
S&T/PO, G. Eaton Date 4/3/84
S&T/H, G. Curlin Date 3/28/84

IV

UNITED STATES INTERNATIONAL DEVELOPMENT COOPERATION AGENCY
AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON D C 20523

APR 5 1984

MEMORANDUM

TO: SER/CM, Johni Pittenger

FROM: S&T/HP, James E. Sarn, M.D.

SUBJECT: Justification for Non-Competitive Procurement under Project No. 931-0453.17, Malaria Immunity and Vaccination Research, the Scripps Clinic and Research Foundation.

I request that you negotiate only with the Scripps Clinic and Research Foundation for the malaria vaccine researched in the PAF and PIO/T. The following justifies non-competitive procurement for this unsolicited proposal under FPR 1-4.910 (b) and (c).

1. Need Which This Procurement is Intended to Satisfy:

The latest estimates of the world wide incidence of malaria range, by WHO projection, is at between 300-400 million cases with between 2-4 million deaths due to malaria, primarily malaria caused by *Plasmodium falciparum*. The Agency's malaria vaccine program is accelerating its pace to develop, test, and market a safe, effective malaria vaccine against the several forms of human malaria. Although significant progress has been made against the mosquito stage (=sporozoite) and several prototypes are being tested, much work continues to be done to isolate "protective" antigens from the red blood cell phases (=RBC) of malaria parasites. The main reasons for the difficulties involved in research on RBC parasite forms lies in part with difficulties in "purity" involved with blood-based *in-vitro* cultures and, as well, the fact that during the RBC stages, the malaria parasites are intraerythrocytic and are therefore very difficult to attack with antibodies. This area, the RBS antigens, was specifically identified as an area for program acceleration in the Administrator's recent approval of a greatly expanded malaria vaccine program.

2. Scripps Clinic and Research Institute Qualifications to Meet the Need:

The unsolicited research proposal submitted by the Scripps Clinic and Research Foundation, San Diego, California, covers the research area to be addressed to isolate, identify, and chemically characterize the macromolecular components of the asexual erythrocytic forms of human malaria parasites which can be used to induce immunity in primates. The proposal has been reviewed and approved by A.I.D.'s external panel of expert consultants. The panel, which is composed of leading scientists in the field of malaria immunology, parasite biochemistry, vaccine development in-vitro cultivation and primate biology has endorsed (in accordance with FPR 1-4.909) the scientific methodology, the exceptional experience and qualifications of Dr. Reese and his staff, and the institutional capacity of the Scripps Clinic and Research Foundation to conduct the proposed scope of work. The panel concluded that the Scripps Clinic and Research Foundation is the only institute in the United States dealing with synthesized peptides from the cDNA library constructed from the poly A+ fraction of the RNA isolated from trophozoite/schizont stage Honduras I isolates of *Plasmodium falciparum*.

3. Prohibition Against Conducting a Market Search for this Procurement per FPR 1-4.911:

Any advertisement to find other sources to meet the objectives of this procurement would require disclosure of the research methodology and experimental design which was proposed by Scripps Clinic and Research Foundation, including details of specific isolated candidate protective antigens as well as the genetic engineering plans including the specific insect vectors, insect sites (especially plasmids pUC8, pUC9), and the details of the preparation of the DNA and RNA maps. These components of the research are fundamental to the research program, are extremely proprietary, and cannot be obtained by A.I.D. from any other source other than Scripps Clinic and Research Foundation. This exclusive availibility was confined by the expert review panel mentioned in paragraph 2 above.

Clearance:

S&T/H: G. Curlin Date 3/20/84
S&T/PO: K. Milow Date 4/3/84

A. Title Page

1. Organization and Address: Department of Immunology
   Scripps Clinic and Research Foundation
   10666 North Torrey Pines Road
   La Jolla, CA 92037

2. Title: Antigens of *Plasmodium falciparum*

3. Investigator and Rank: Robert T. Reese, Associate Member

4. Submitted: December 1983

5. Proposed Starting Date: 15 September 1984 to extend through 14 September 1988

6. Principal Investigator Administrator

7. The human experimentation plan (use of human blood components to culture the parasite) has been reviewed by the Institution's Human Research Committee. Approved 14 February 1983. Human Research protocols are reviewed yearly. When a new approval has been received, a copy will be forwarded.

8. The proposal has not been submitted to any other agency to obtain support.

9. This application involves recombinant DNA.

B. Relevance of Proposed Work to AID

1. Malaria is currently the most prevalent death-threatening infectious disease in the world. The objective of this work is to develop a method for obtaining components of the human malaria parasite, P. falciparum, which can be used to stimulate protective immunity to this organism.

2. Malaria is predominantly a problem in developing nations; thus, if a vaccine could be produced, it would be likely to have dramatic effects on the general health and productivity of these people.

3. Most of the work will be done in the continental United States because much of the necessary technology is not readily available in many areas of the world. As potential immunogens are characterized, it is likely that use of them in large scale immunization experiments can best be conducted at some other site either in the United States or some country such as Columbia, where the appropriate monkeys are readily available.

4. Although accurate statistics are not readily available, there are likely to be 300-500 million cases of malaria per year. Approximately 1% result in death, the rest (297-495 million) have varying degrees of illness which on the average claim one or more weeks of effective work time, even among the educated who are properly diagnosed and treated. Thus, the morbidity due to this disease is even more devastating to the economy of these countries than is the mortality. If we assume the cost in productivity, physician and epidemiologist time, hospital, pharmaceutical, and funeral expenses is even as small as $10 per case, then the cost to these countries is billions of dollars per year. Because immunity to this disease would have such a dramatic impact in so many societies, it is impossible to predict in any

realistic fashion what the effects on the world would be. It would clearly improve the general health of all of the individuals in these countries, increase the productivity and reduce the number of childhood deaths significantly. In doing so, it might initially contribute to a further increase in the rate of population growth. As the number of childhood deaths decreases and the general productivity of each individual increases due to better health, then the necessity for larger families should be reduced. Historically, higher living standards have led to smaller families. Reduced family size will decrease population growth and decrease hunger and starvation by increasing the ratio of food to the number of people.

C. Scientific Aspects of Proposed Work

1. Objectives

During this project we will attempt to further identify, isolate, and begin chemical characterization of some of the macromolecular components of the asexual erythrocytic forms of human malaria parasites which can be used to induce immunity in primates.

2. Rationale

Data have been accumulated from our laboratory as well as from others which demonstrate that it is possible to induce immunity to malaria which is not strain specific (1). Thus, immunization with antigens from a single strain of *P. falciparum* should confer immunity to all strains. More important, data from several laboratories suggest that serum or purified globulin from an immune animal can cure an animal infected with *P. falciparum*, and thus, that the antibodies from immune animals should be able to bind selectively with the parasite components which stimulate immunologic protection (1). With this premise, we will use the antisera obtained from immune monkeys, immune humans (polyclonal), as well as monospecific monoclonal and polyclonal antibodies, to identify and then to help in the isolation of the desired macromolecular structures. These same antibodies will be used to identify recombinant bacterial clones containing *Plasmodium* cDNA which are producing parasite antigens of interest. The primary peptide structures will be determined from both the cDNA and genomic DNA sequences. We will try to use primary structural analysis of the purified proteins to rigorously determine the amino terminus of the molecules. Computer analysis of the primary structures will be used to predict those portions of the molecule which are likely to protrude into the aqueous environment and thus are potentially antigens (2). Judging from results obtained with a variety

of antigens which have been studied in this way, such analysis should identify the maximum number of regions which might be useful immunologically. This information will be put together with information gained by mutagenizing the cloned structures to identify the human and monkey antibody binding sites. I expect the hydrophilicity analysis to be particularly useful in predicting peptides which might serve as important immunogens. The ability of a peptide structure to stimulate antibody in a particular individual is a unique characteristic controlled by the genetics of the individual challenged. It would probably require a pool containing a large number of immune sera to define the entire set of potential epitopes from any single molecular species. Thus, based on hydrophilicity analysis and mutagenicity studies, peptides will be synthesized, covalently attached to carriers, and used to immunize first mice and then monkeys. The above approach is applicable regardless of the complexity of the molecules being analyzed. If we find that the specific molecules being studied contain large regions of repeating sequence (as found for the two parasite proteins for which sequence data are available) then prediction of regions potentially useful as immunogens will be greatly simplified.

PROGRESS REPORT and BACKGROUND INFORMATION

In our initial proposal we provided a detailed review of the literature covering immunity to malarial parasites. Since then a large number of new reports have appeared. To simplify the review and reduce the length of the proposal we have omitted this previous documentation as well as any information learned more recently which is extraneous. We have included a relatively detailed report of our progress, which provides the main foundation for our future studies, and have added supplemental and supporting information gained from other laboratories where pertinent.

Approximately 2.5 years ago we moved our laboratory from Rockefeller University in New York to the Research Institute of Scripps Clinic in La Jolla, California. This provided us with a significant increase in space and facilities so that now we can use a broad immunochemical-molecular biological approach. During the first period after our move ($\sim$ 6-8 months) our progress was very slow. Nevertheless, it is clear from the manuscripts included that we are not only productive, but are now in an excellent position to do the work outlined in this proposal.

In addition to the work directed at identifying merozoite components which might play a role in immunity, several side issues were addressed because of their potential importance in understanding the disease. At the time the award was made we had found that parasites maintained in continuous culture for up to a year contained a subpopulation of organisms which no longer induced "knobs" in the host erythrocyte surface. These we had called K^- (3). K^- variants of three different strains had been isolated and examined. In each case the K^- variant was found to grow better under culture conditions than its K^+ parent (4) and it did not appear to bind antibodies found in immune owl monkey serum to its surface when examined by

electron microscopy (5). Recently we have cloned a K^+ isolate, and after a limited time in culture, cloned a K^- from the K^+ progeny. This demonstrates that K^- arise from K^+ and that the K^- are not simply a minor subpopulation which overgrows because of their greater ability to grow under culture conditions (6). Extrapolating from the length of the growth period which was necessary before the K^- was apparent, it is likely that K^- arise continuously at a relatively low rate ($\sim 10^{-6}$). The K^- clearly have a selective advantage in culture and, although they probably also arise *in vivo*, there is no information available on their role in the disease. (Manuscript #8). This work has not been taken any further in response to suggestions made by a 1983 Site Visit Team.

During our studies on malaria we have developed several *in vitro* assays which allow us to detect the interaction of antibodies with the surface of the parasitized erythrocyte and the merozoite. Each assay appears to detect a separate population of antigens all of which may be useful in providing protection against the disease.

Several years ago, we demonstrated by immunoelectron microscopy that antibodies in immune owl monkey serum could bind to the surface of red cells infected with schizont-stage parasites and with the surface of merozoites (5). At least some of these antigens were shared among different strains since cross reactivity among parasite strains from widely different areas of the world was observed (1,5). In order to assess the potential importance of various antigens, we developed alternative assays.

After the demonstration (1,7) that immune owl monkey sera could inhibit *in vitro* growth of the parasite, immune sera were tested for their ability to block replication of K^+ and K^- strains which had been synchronized with Physiogel or sorbitol (8). When tested in typical Trager-Jensen cultures

(9); inhibition always occurred at the point of schizont release, thus the antibody was judged to block by inhibiting merozoite invasion of new RBCs. Although there was some variance of the ability of sera to inhibit different parasite strains, highly inhibitory sera appeared to inhibit the growth of all parasites efficiently even if only 1% immune serum was used in the assay (1). A number of controls were used to demonstrate that these inhibitory antibodies were present in both human and monkey sera and that the effect was not due to antimalarial drugs which might have been taken by the humans (1).

As we were leaving New York, we found that serum from one of the rabbits we had hyperimmunized with parasite material could inhibit the uptake of radiolabeled amino acids by both K^+ and K^- parasites even at the ring stage. Because the serum was lost during the move to Scripps and we were unable to reproduce the effect with other sera, the work was never published. At Scripps we found that by maintaining the parasitized cells in suspension during the assay, all of the cells were exposed to the antibodies in the medium which made inhibition apparent during intracellular growth of the parasite. However, unlike the experiments with the rabbit serum, inhibition by immune monkey or human serum did not occur until trophozoites began appearing in the cultures; in addition, inhibition was only demonstrable with K^+ strains. When we tried to compare intracellular inhibition with fluorescence titers determined on fixed parasitized cells, sera which had the lowest immunofluorescence titers inhibited as well or better than those with the highest demonstrating that the antibody populations against the surface of the cells were distinct from those against antigens on the inside of the cells (10). The details of this study are contained in manuscript #4.

In parallel with these studies we began to examine the ability of parasitized cells to activate the cascade of serum enzymes referred to as complement. The objective was 2-fold. If complement was activated in the presence or absence of antibody, then we had a well defined link with a series of very potent biological mediators of immunity including several aspects of the cellular immune response. In addition to providing an independent assay for biological significance, activation of complement might serve as a highly sensitive assay for important antigens on the parasitized erythrocyte surface and eventually lead to a way of isolating the components of these structures. In manuscript #6 we have included the first part of this study. Here we demonstrate (11), using a series of independent but confirmatory assays, that red blood cells (RBCs) parasitized with K^+ falciparum schizonts can activate the human complement system by the alternative pathway. Binding of antibody to the parasitized RBC surface was found to augment activation by the alternative pathway, and to lead to significant deposition of C3b by the classical pathway. Because sera which were highly inhibitory by the direct inhibition assay [suspended culture (10)] do not necessarily activate complement well and vice versa, either they detect different functional subpopulations of antibody or different antigens on the parasitized erythrocyte surface. As recommended by the 1983 Site Visit Team, this work is not being continued as part of the AID sponsored program.

Armed with a series of assays which detect surface antigens present on either the schizont or merozoite, and which were thus likely to be useful in stimulating immunologic protection against the parasite, we then sought to identify the antigens with which these various antibody populations bind. Initially we used Iodogen to ^{125}I label various stages of the parasite

including schizonts and purified merozoites (1). Paper #1 presents some of these data. When ^{125}I-labeled proteins from detergent solubilized schizonts are immunoprecipitated with either immune owl monkey or human sera, and then separated by SDS-PAGE, many of the same (based on electrophoretic mobililty) 20-25 proteins are detected. This helps to confirm the usefulness of the Aotus as a model for human falciparum malaria. When sera are selected which cause high or low in vitro inhibition of parasite growth (anti-merozoite assay) and used for immunoprecipitations of schizont proteins, comparison of the profiles suggests that molecules of approximately 45,000; 78-85,000; and 200,000 are likely to be important in immunity (1). Immunoprecipitation of ^{125}I-labeled purified merozoites with the same types of immune sera provides a much simpler picture than with labeled schizonts. The ability to bind a 46K protein is common among all of the highly inhibitory sera.

More recently, an alternative approach has been taken. It is based on the assumption that during the processing steps through which most membrane proteins go, at certain points sugars may be attached. With this in mind, we have tried to define what glycoproteins are made during the asexual erythrocytic cycle and detect those which are associated with the merozoite and are immunoprecipitable with immune owl monkey sera. Although 13-15 glycosylated structures can sometimes be detected in schizonts, too little glycosylation has been observed during ring-stage development to be convincing (12,13). Of those observed at the schizont stage, only 185 K, 88 K, 56 K, 46 K, 41 K, and 34 K appear to be reproducible. The 185 K, 88 K, 46 K, and 41 K structures can be labeled to varying degrees with ^{35}S-methionine; thus they are believed to be glycoproteins. Co-migration of ^{3}H-glucosamine labeled and ^{35}S-methionine labeled structures on 2-dimensional electrofocusing SDS-PAGE gels has also suggested that the molecules at

185 K, 88 K, 46 K, and 41 K are glycoproteins. The 56 K and 34 K molecules do not label well with ^{35}S-methionine but molecules of the appropriate M_r on SDS-PAGE are observed in samples labeled with either a group of 6 ^{3}H-amino acids or ^{3}H-glucosamine. In addition, treatment of all of these ^{3}H-glucosamine-labeled structures with proteases such as pronase reduces them to relatively small carbohydrates. Immunoprecipitation with monoclonal antibodies has demonstrated that the glucosamine labeled 185 K and 56 K molecules are the same as the 185 K and 56 K proteins labeled with ^{3}H-amino acids and that Gp56 may contain a very small amount of methionine.

By ^{3}H-glucosamine or ^{35}S-methionine labeling of synchronized schizonts, incubating them in unlabeled medium, transfering the purified schizonts to unlabeled red cells, and following the radiolabeled merozoite proteins as they are carried into the ring stage, we have been able to get a minimal picture of the glycoproteins associated with the merozoite (12,13). Such a picture has been very elusive until now because merozoites autolyzse so rapidly. During attempts to purify merozoites, extensive degradation occurs so that a realistic picture is difficult to obtain. Reports in which viability has been used as a criterion of merozoite integrity after purification have been difficult to interpret because the preparations were acknowledged to contain more than enough schizonts to account for all of the viable merozoites in the preparations (14). Because of this, merozoites were said to contain no glycoproteins. Our studies clearly demonstrate that a group of glycoproteins of M_r 185 K, 88 K, 56 K, 46 K, 41 K, and 34 K which are all made during the schizont stage become part of the merozoite and are carried into the newly infected red cell as the ring is formed. This, taken together with immunoprecipitation data obtained with ^{125}I-labeled schizonts, suggests that Gp185, 88, 46, 41, and 34 are merozoite glycoproteins which

may all be important in stimulating immunity. Gp56 was apparently not efficiently labeled when the schizonts were labeled with ^{125}I. Nevertheless, immune sera which effectively inhibit in vitro growth of the parasite contain considerable levels of antibody to all of these antigens; thus, they are major parasite glycoproteins which are immunogenic in both monkeys and man. The details of these studies are contained in manuscripts #5,7,9-11.

Having identified glycoproteins which were likely to be of immunologic importance in man, we have begun obtaining monoclonal and polyclonal sera which specifically bind them. Monoclonal antibodies have now been produced against Gp185, Gp56, and p82 of the merozoite (9,10). Monospecific, polyclonal rabbit immune sera have been produced against Gp185. By indirect immunofluorescence, the monoclonal antibodies to Gp185 and Gp56 outline the merozoite; the anti p82 create a high intensity dot at the apical end of the merozoites and are likely to be binding to the rhoptries or the organelle at the apex of the organism. Preliminary immunoelectron microscopic studies suggest the anti-Gp185 and anti-Gp56 both specifically bind to the merozoite surface; the anti-p82 may not (9,10). The anti-p82 appears to bind a series of smaller polypeptides (70 K and 41 K) which may be proteolytic products of each other or parts of a single parasite structure held together by protein-protein interactions which are not disrupted by the detergents used from immunoprecipitation. When reacted with parasite samples from widely different areas of the world, homologues of Gp185 are bound which vary slightly in M_r. The structural basis for this molecular heterogeneity is being sought.

All of the monoclonal antibodies against Gp56 examined so far have been isolate specific. What appear to be homologues of Gp56 found in different

isolates of P. falciparum vary considerably in M_r. Whether exposed epitopes can be identified which are shared will require further study. When examined by immunoelectron microscopy, both the anti-Gp185 and anti-Gp56 monoclonal antibodies bind to antigens on the merozoite surface which on a molecular level are likely to be widely separated from each other. Polyclonal antibodies to these antigens might be expected to have the capacity to cross-link merozoites, thus agglutinating them and interfering with their capacity to invade red blood cells even if a monoclonal would not.

We are particularly interested in producing antibodies specific for the other merozoite proteins with the idea of finding those which react with what we believe to be the specific receptor and penetration apparatus. These organelles appear to be located at the apical end of the organism and antibodies to them might sterically interfere with interactions necessary to allow penetration to procede. By immunofluorescence such a monoclonal would be expected to produce a dot pattern similar to that seen with the anti-p82.

Now that at least 2 merozoite surface glycoproteins have been identified which may be useful in stimulating immunity to P. falciparum, two separate but complementary approaches are being taken to characterize these molecules. Polyadenylated RNA has been isolated from trophozoite- through schizont-stage parasites, cDNA prepared, Eco R1 linkers attached and the cDNA cloned into λ gt 10. Twice, the cloning efficiency above background was below 10%, We, therefore, changed our approach. Rather than continue with this phage, we decided to use the pUC8 - pUC9 expression vectors. cDNA was prepared containing EcoR1 linkers on one end and Sal 1 linkers on the other. The linkers were attached in both orientations so that regardless of which DNA strand is read by the parasite we will capture the information

assuming we have the proper reading frame. We are also tailing and trying to clone into the Pst-1 site. All of these sites potentially have the capacity to express the cloned material as part of β -galactosidase (15). Using the high efficiency transformation procedure of Hanahan (16), which approximates the efficiency given by packaging of λ (5×10^7 colonies/ μg DNA), we appear to have generated a substantial library (>30,000 clones) having inserts ranging from ∿ 200 bp to ∿1000 bp. These colonies will be screened with immune serum obtained from Aotus monkeys for those which express cloned cDNA from the parasites. If expressed proteins are found, the monoclonal and monospecific polyclonal antibodies will be used to identify clones coding for the particular antigens of interest.

As yet we do not know if any of the parasite proteins we seek will be expressed by these vectors in E. coli. Thus, in parallel with the cloning work, we are attempting to obtain primary structural information by sequential Edman degradation of the purified polypeptides. Such primary structural work will be necessary to establish the amino terminus of the molecules and what the processed structure really is. Relatively large amounts of anti-Gp56, Gp185, and p82 have been produced from hybridomas in culture, isolated, and then covalently conjugated to beads. These are being used to purify specific parasite proteins. After elution of the proteins from the affinity columns the absorbance at 260, 280, and 550 is measured and aliquots of the preparations are being electrophoresed and silver stained as a means of evaluating their homogeneity. We are in the process of purifying proteins for both primary structural work and immunization studies.

Work Supported by Previous Award

1. R.T. Reese, M.R. Motyl and R.N. Hofer-Warbinek. Reaction of immune sera with components of the human malarial parasite, Plasmodium falciparum. Am. J. Trop. Med . Hyg. 30: 1168 (1981).

2. C.A. Gritzmacher and R.T. Reese. Translation in vitro of RNA from the human malarial parasite Plasmodium falciparum. Bioscience Reports 2: 667 (1982).

3. M.R. Motyl and R.T. Reese. Plasmodium falciparum: Comparison of in vitro growth of knobby and knobless isolates. Am. J. Trop. Med. Hyg.32(2): 226 (1983).

4. H.A. Stanley and R.T. Reese. In vitro inhibition of intracellular growth of Plasmodium falciparum by immune sera. Am. J. Trop. Med. Hyg., in press.

5. R.F. Howard and R.T. Reese. Synthesis of merozoite proteins and glycoproteins during the schizogony of Plasmodium falciparum. Molec. Biochem. Parasit., in press.

6. H.A. Stanley, J.T. Mayes, N.R. Cooper and R.T. Reese. Complement activation by the surface of Plasmodium falciparum infected erythrocytes. Molec. Imm., in press.

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Outline of Proposed Work

I. Production of monospecific probes:

A) Mouse monoclonal antibodies

B) Monkey-mouse hybrids

C) Immunization with semipurified proteins as haptens - from parasite; from clones which express parasite antigens

D) Affinity purification of monkey and human antibodies

II. Demonstration of the specificity of monospecific probes

A) Immunoprecipitation - metabolic labels; ^{35}S-methionine, ^{3}H-glucosamine, with schizont and schizont-labeled ring stage, analysis on one- and two-dimensional gels

B) Western blots to assess 1. proteolysis, 2. non-specific binding, 3. protein-protein interactions among chains of molecules, or parts of organelles

C) analysis by indirect fluorescence

D) immunoelectron microscopy

III. Purification of antigens potentially useful for stimulating protective immunity in primates

A) Monoclonal antibodies

B) Monospecific polyclonal antibodies

C) Immune human or monkey sera

IV. Primary structural analysis

A) Protein chemistry

B) Molecular biology

V. Immunization with purified antigens or peptides of known sequence

A) Digests of purified proteins coupled to carriers to convert them to haptens

B) Synthetic peptides

Proposed Work

I. Production of Monospecific Probes:

A) Mouse monoclonal antibodies:

Immunization of Balb C and Ajax mice with semipurified merozoite preparations has allowed us to obtain hybridoma cell lines secreting anti-merozoite antibodies specific for Gp185, Gp56, and p82. Using similar immunization protocols, other laboratories have apparently been able to obtain antibodies specific for several of these same structures (17-21). This suggests that these molecules are the most immunogenic in the mouse and that unless the antigens are presented in a considerably different form, the same results are likely to be observed as products of future fusions. To counter this, future immunizations will be done with semipurified or purified protein preparations which have been fragmented with either trypsin and/or chemical reagents such as CNBr in an attempt to generate a family of peptides of approximately 20-30 residues in length. These peptides will be connected as haptens by chemically conjugating them to either keyhole limpet haemocyanin or tetanus toxoid. They will be injected into mice with MDP (N-acetyl-muramyl-L-alanyl-D-isoglutamine) as an adjuvant. By converting them to haptens we can expect to reduce the amount of antigen necessary by 10-100 fold based on studies with dinitrophenol (22), and can also anticipate their being stronger immunogens because they are being presented on strongly immunogenic carriers. Such an immunization protocol should stimulate antibodies to the entire polypeptide structure thus providing us with probes by which we should be able to isolate individual fragments and therefore map each protein.

B) Primate-rodent hybrids:

Using suggestions made by 2 individuals at Hybritech, we have begun trying to make monkey monoclonal antibodies by monkey-mouse fusions. We have generated a number of fusion partners some of which appear to be producing anti-parasite antibodies. Although they grow exceedingly slowly, we hope by January 1984 we will know if such fusions are likely to be stable and if this is a reasonable route to obtain the monospecific probes we need. We are particularly interested in this system because immune Aotus are capable of controlling the disease and are likely to produce antibodies against most of the proteins which we expect to be of use in immunizing humans.

C) Immunization with parasite-specific polypeptides from recombinant clones:

If recombinant clones of microorganisms can be isolated which are synthesizing parasite specific polypeptides, they will be solubilized, and the polypeptides isolated using human or monkey polyclonal antibodies conjugated to beads (23). The polypeptides will be converted to haptens as in I-A and used for immunization. This approach will depend on the availability of the appropriate recombinant clones.

D) Affinity purification of monkey and human antibodies:

Parasite-specific proteins are expected to be isolated in a series of ways using as a source either parasitized erythrocytes of recombinant clones of microorganisms which are expressing these structures. Once parasite antigens are available in appropriate amounts, they will be conjugated to insoluble supports and used to isolate antibodies from immune human or monkey serum. This procedure will be used as an option but will not be applicable in many cases. Procedures A & B were presented first because they are expected to be more widely applicable.

II. Demonstration of Specificity of Probes

A) Immunoprecipitation of metabolically labeled samples:

Parasite material metabolically labeled with ^{35}S-methionine, ^{3}H-glucosamine, or a series of other ^{3}H-amino acids will be immunoprecipitated to determine the antigens with which they bind. The schizont, schizont-labeled ring stage, and if necessary, purified merozoites will be examined. Both one-dimensional SDS-PAGE and two-dimensional electrofocusing-SDS-PAGE will be used to determine the isoelectric points and molecular weights of the molecular species bound.

B) Western Blots:

There are at least 3 things which can confuse interpretation of immunoprecipitation data obtained on parasites solubilized in non-ionic detergents:

1. Proteolysis: As part of processing, or simply due to hydrolysis which occurs following the release of the proteases contained within the cell, polypeptides of varying molecular size can be produced. Whether this degradation is programmed by the cell or simply occurs as an experimental artifact can be approached by solubilizing in 3% SDS with reducing agents for Western blots.

2. Non-specific binding: A major problem with interpreting immunoprecipitation data using mouse monoclonal antibodies is caused by non-specific binding of parasite proteins. In most cases this creates a background of at least 4-5 bands on a fluorograph. Western blots tend to minimize this.

3. Protein:protein interactions: A basic premise of any immunoprecipitation system is that the detergents used to solubilize the sample will not appreciably interrupt high energy protein:protein

interactions. If the detergents do, then the antibodies themselves will not be able to react. Both the antibody, and many, if not most of the antigens are proteins. This can obviously create problems in trying to analyse the molecular components of an organism. Organelles of the parasite as well as many cytoskeletal and membrane structures are not likely to be fully dissociated in any non-ionic detergent system which allows immunoprecipitation. Antibody interaction with a single component of such a complex would be expected to precipitate the entire complex. The complex could also be a multichained molecule composed of non-identical chains. Whatever the case, a series of different polypeptides could be immunoprecipitated even though the antibody reacts with only one. Western blots would help to untangle this complexity.

C) Indirect fluorescence:

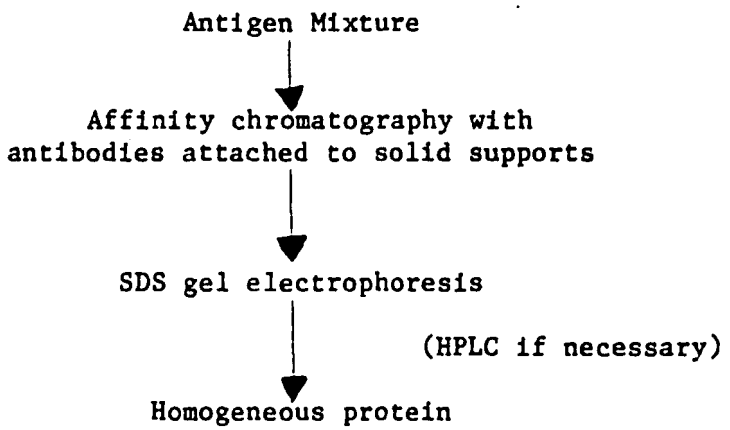
Although a series of procedures have been tried, indirect fluorescence still appears to provide the best initial screening procedure for monospecific antibodies. It provides some idea of the type of structure the antibody reacts with as well as its prevalence in the cell.

D) Immunoelectron microscopy:

To establish the molecular structure, or organelle of the parasite with which a particular monospecific antibody interacts, electron microscopy will be necessary. At present we are using both gold- and ferritin-labeled antibodies and 2 levels of sensitivity, scanning and transmission. The Institute has just acquired a new transmission electron microscope containing computer enhanced graphics capabilities. We are led to believe this instrument may allow us to view molecular structure. Such equipment will be used to demonstrate visually the components of the cell with which each monospecific antibody population binds.

III. Purification of antigens potentially useful for stimulating protective immunity

A single general protocol will be used to purify antigens potentially useful for stimulating immunity:



The source of antigen will either be parasite cultures or recombinant clones expressing parasite polypeptides. As a first step, antibodies will be used in affinity chromatography. Monospecific polyclonal, monoclonal, or polyspecific polyclonal (immune human or monkey) antibodies will be used depending on availability. Antigens will be eluted from the affinity columns and then electrophoresed on SDS gels. The electrophoresis step appears to be essential because virtually all of the affinity systems we have used, bind some other proteins non-specifically. Although the second step clearly reduces the yield, at present it appears necessary to assure the purified material is homogeneous. The SDS-PAGE is likely to be particularly useful if the monoclonal antibody precipitates a multichained structure or binds the same epitope on polypeptides of different molecular sizes which are perhaps proteolytic products of each other. From our current work purifying Gp185 we believe that sufficient amounts of homogeneous material can be isolated to do the studies outlined.

IV. Primary structural analysis

A) Protein chemistry: Together with Varian Instruments, we have developed a system which separates all 20 amino acids in a relatively short period of time by HPLC; a hybrid CN-C_{18} column is being used. In addition, using the protocol listed under III above, we have been able to isolate in a homogeneous form approximately 400 ng of Gp185. Assuming the 185,000 is an accurate molecular weight, then this would represent approximately 2 pM of polypeptide. The actual polypeptide size may be considerably smaller since glycosylation creates either a much greater Stokes radius or allows binding of much less SDS than the equivalent mass of polypeptide. This causes glycoproteins to travel less rapidly in SDS gels making them have an artificially large M_r. Artificially large molecular weights translate into estimates of molar concentration which are proportionally small. The 2 pM estimate is therefore likely to be conservative.

We believe we will be able to spectrophotometrically detect 5-10 pM of a PTH-amino acid after HPLC separation. Since we should be able to scale our purification up 30 fold without too great a difficulty, at least the first groups of residues can be identified spectrophotometrically as well as by radioactivity. The gas-phase sequencer is quite capable of sequencing these amounts of polypeptides; unequivocal identification of the residues as they are cleaved is the problem.

Initially, our objective is to obtain whatever information we can on the amino terminus of the molecule. Such information will be useful in itself as well as providing a basis for construction of oligonucleotide probes (IV-B). Regardless of what information can be obtained from sequencing the DNA, modifications which occur as part of processing cannot be predicted as yet, thus an accurate knowledge of structure still requires

protein sequencing. In the case of the sporozoite antigen of P. knowlesi, sequencing of the genomic DNA did not necessarily generate an accurate structure because no information was available on sites of processing, the exact location of the signal sequence, and the amino terminus of the processed protein (24). Since signal sequences (25) do not always come just before the amino terminus of a molecule (they may be internal as with ovalbumin), and since signal sequences do not generally have a unique well-prescribed sequence (26), it may be difficult to tell what the exact boundaries of the expressed protein really are. This may well have happened with the published structure for the sporozoite antigen thus accounting for the major discrepancies between the size predicted by the DNA sequence (36,792) and that predicted by SDS gel electrophoresis (52,000).

Depending on the rate at which information is obtained by molecular biological studies, we will also fragment the molecules using a series of chemical means (CNBr, NCS) and enzymes (i.e. trypsin, chymotrypsin), separate the peptides generated, and systematically sequence them (27) so that a more complete structure can be generated for each molecule.

B) Molecular Biology: Due to the numerous internal poly A stretches which plasmodial RNA and DNA contain, much of the technology for cDNA cloning does not work efficiently on this parasite. For this reason, it has taken us, as well as others, unusually long times to produce libraries which are likely to be useful (28,29). We have a cDNA library constructed from the poly A^+ fraction of RNA isolated from trophozoite- through schizont-stage Honduras I isolate Plasmodium falciparum parasites. That portion of cDNA which was inserted into the Sal I, Eco RI sites of pUC8 contains inserts ranging from $\sim$200 to $\sim$ 1000 bp. Approximately, 30-40 thousand clones have been harvested for the pUC8

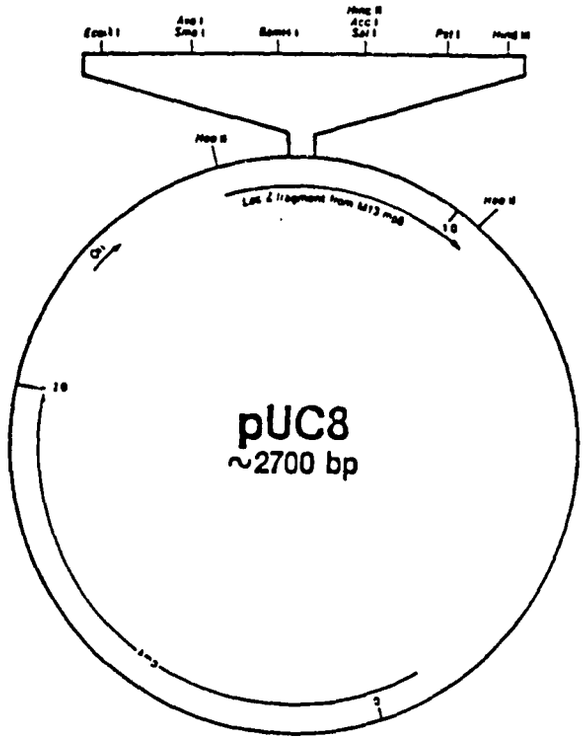
portion of the library (see Figure I). At this point, before harvesting the complete library, we are beginning to work on an immunologic screening procedure in the hope that some expression will be detected. Using immunoabsorbents composed of E. coli, non-immune and immune monkey serum have been absorbed to reduce the antibacterial antibodies which create high backgrounds in these procedures. If clones which express parasite antigens can be identified, monoclonal antibodies will be used as pools of antibodies having a specificity for the same parasite protein. As monospecific polyclonal antibodies become available they will be employed because of the greater probability of their detecting the fragments of unmodified plasmodial polypeptide antigens likely to be produced by the recombinant clones.

The DNA coding for these epitopes will be sequenced directly (30). They will also be used to probe a genomic library constructed by shearing plasmodial DNA, methylating it, attaching Eco R1 linkers, and then inserting the DNA into λ L41 or L47 (31). An estimate of the entire protein structure will be deduced from the genomic DNA using the computer programs of Stadin (32). Potential regions of antigenicity will be determined in two ways: 1) Using mutagenesis (33), those regions which are responsible for antibody binding will be identified. Families of overlapping synthetic peptides will allow precise characterization of these epitopes (15 residue peptides can be constructed within 1.5-2 weeks at Scripps) which will simplify this procedure. 2) Computer generated hydrophilicity plots (2) will be used to predict potentially antigenic sites. This should predict a large set of possible antigens of which genetically different individuals should respond to a series of overlapping subsets. Analysis of a pool of immune human and monkey sera with families of peptides should allow us to define the members of this inclusion set.

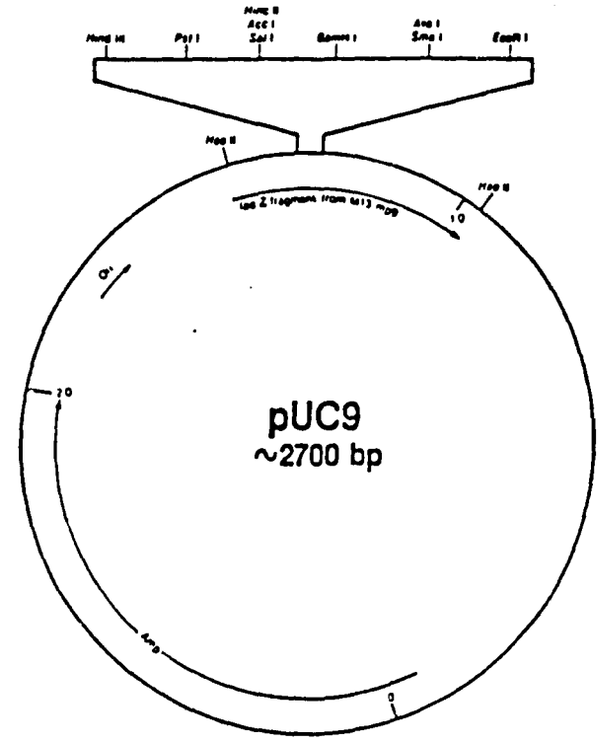
Figure I

pUC 8 and pUC 9 are complementary plasmid vectors which contain a "multiple cloning site" in the gene which codes for β-galactosidase. The multiple cloning site can be seen from the figure to have an exactly opposite orientation in the two vectors. To make optimal use of these complementary vectors the cDNA sample is split in half after the second strand is completed. One type of linkers (i.e. EcoRI) is attached to one pool; a different set (i.e. SalI) to the second pool. The "hairpin" turn which connects the two cDNA strands is then removed and the opposite set of linkers attached to the second end. The cDNA now has polarity and regardless of which strand of the original DNA is the coding strand in the parasite, 50% of the cDNA clones should contain at least a partial copy of it.

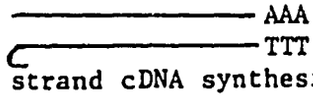
Restriction Map of pUC8



Restriction Map of pUC9

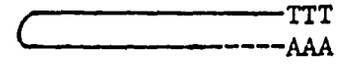


1) mRNA



1st strand cDNA synthesis by reverse transcriptase

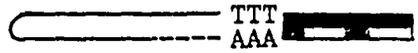
2)



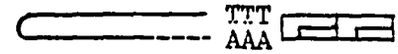
2nd strand cDNA synthesis by polI large fragment

divide double-stranded cDNA into two pools

3) Linker addition by blunt-end ligation

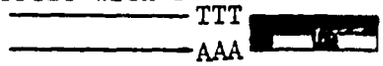


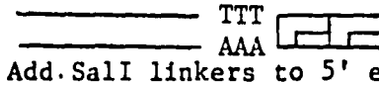
Add SalI linkers to 3' end



Add EcoRI linkers to 3' end

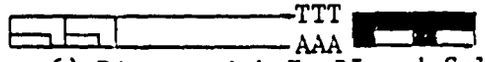
4) Treat with S1 nuclease

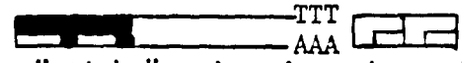




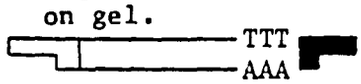
5) Add EcoRI linkers to 5' end

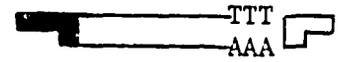
Add SalI linkers to 5' end





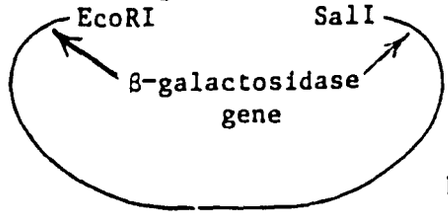
6) Digest with EcoRI and SalI to get "sticky" ends, then size select on gel.

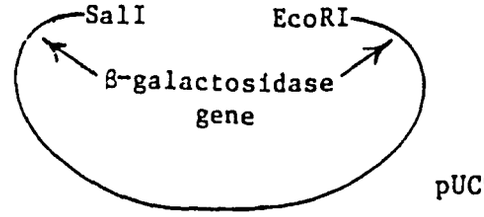




7) Ligate into pUC8 cut by EcoRI and SalI - sticky end ligation

Ligate into pUC9 cut by EcoRI and SalI





Alternative Approaches

Because it has been possible to get expression in *E. coli* of both a *P. knowlesi* sporozoite surface antigen (33) and several *P. falciparum* antigens (29), it is probable that the expression system we are using will also allow this straight forward approach. There may, however, be molecules of interest, which for a variety of reasons are not detectable by such a screen. With this in mind, we must have alternative approaches. One such approach will be to screen with RNA fractionated into 4-5 size ranges by sedimentation in sucrose. Clones hybridizing with RNA of the proper sizes will then be examined in groups by hybrid selection (34). Antibody precipitation of the translate with human or monkey polyclonal antibodies will provide an estimate of the size of the translate and whether it is recognized by these sera (is immunologically important). As monospecific polyclonal antibodies become available (1A), this step will be all that is necessary to recognize clones of interest. It will be more cumbersome to detect clones for molecules for which no monospecific antiserum exists. A procedure being considered is as follows:

Possible detection system:

To monoclonal antibody covalently linked to beads or adsorbed to a plate, add parasite material solubilized in precipitation buffer (1), allow binding and wash, add polyclonal monkey or human serum, incubate, and wash; (it may be necessary to covalently link this antigen as well) add radiolabeled translate from hybridized RNA, wash, count to detect binding.

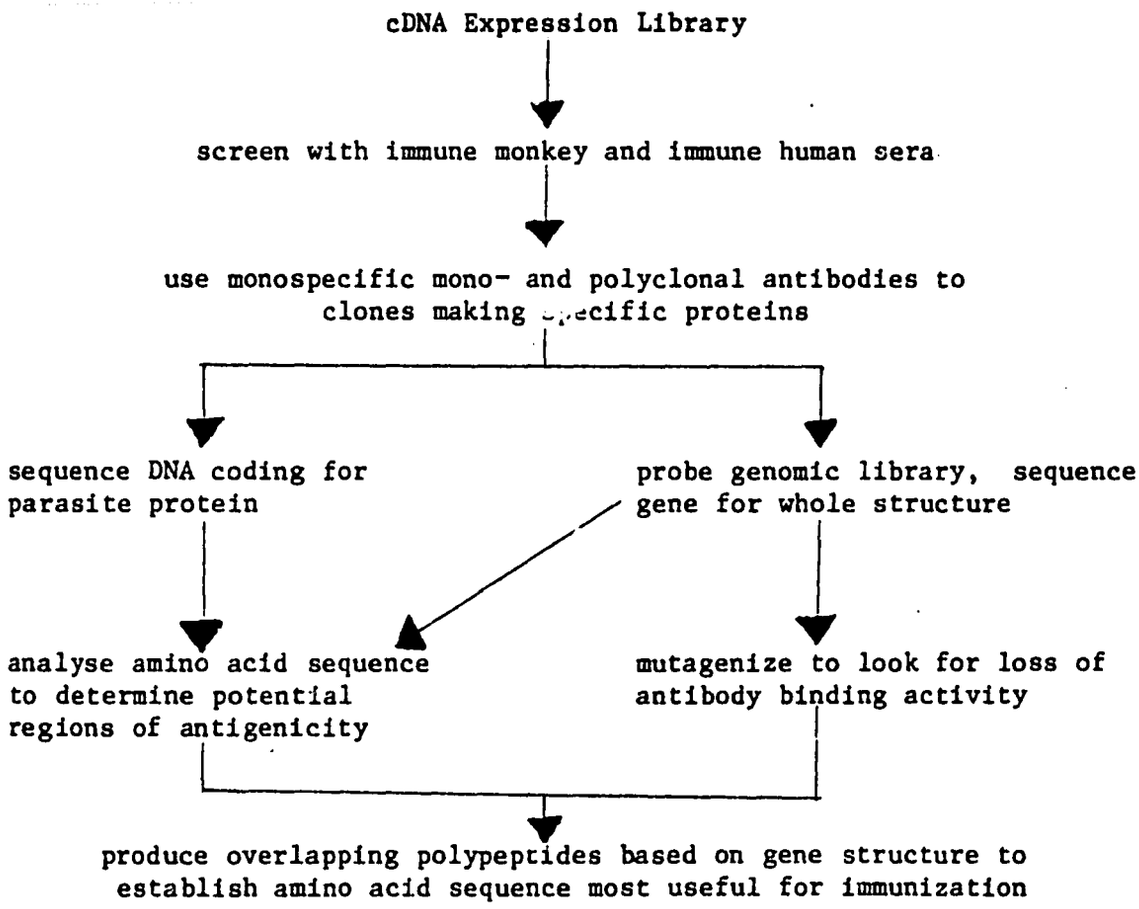
This could also be converted to an ELISA to allow large scale screening.

We believe, however, that under most circumstances such procedures will not be necessary. Currently, we have at least 5 different monoclonals and one polyclonal rabbit antiserum against the Gp185 merozoite surface glycoprotein. We are actively trying to produce a family of monospecific polyclonal sera against 4 merozoite proteins of interest. Since our molecular biology group is too small to examine more than one or two of these molecules simultaneously, we believe the antisera for screening will become available as they are needed.

Another alternative will arise as primary structural information becomes available from protein sequencing. Once 12-15 residues of amino acid sequence are available for a particular molecule then corresponding oligonucleotide probes will be constructed. Because of the degeneracy of the amino acid code (of *E. coli*), this will require a family of probes which increases in size rapidly with the length of the amino acid sequence to be coded. Machines are now available which can efficiently do these syntheses. However, until it becomes clear that such a purchase is essential, we will not request such an item.

Scheme for Cloning of Genes for

Parasite Antigens of Interest



V. Immunization with purified antigens or polypeptides of known sequence

A) Digests of purified proteins coupled to carriers like haptens:

Immunization protocols will be tested in mice and then used in monkeys once they have been shown to effectively stimulate antibody. Before any *in vivo* tests are made on the monkeys, the titers of the antibodies will be tested as well as their molecular specificity (immunoprecipitation and Western Blots). Immune sera against different surface structures of the parasite will be examined independently and in mixtures to determine their capacity to inhibit growth *in vitro* (7,10). Based on predictions from *in vitro* assays, monkeys will be challenged with parasites only after they are likely to have substantial immunity. This may require immunization with antigens from a variety of parasite molecular structures.

B) Synthetic peptides:

If protective monospecific antibodies can be obtained as well as the primary structures of the corresponding antigens, then by using increasingly small synthetic peptides it is possible to deduce the minimal essential size for a particular antigen. It is likely, however, that a somewhat longer polypeptide will be necessary to stimulate the appropriate antibody *in vivo*. Thus, once primary structural information is available from whatever source, computer analysis will be used to predict potential antigens based on hydrophilicity (2). Families of over-lapping synthetic peptides will be constructed based on the hydrophilicity plot and the ability of these peptides to block protective antibodies tested. These structures will be coupled to carriers (I-A) and tested as immunogens (V-A). The ability of peptides synthesized from DNA structures to protect monkeys against a challenge with parasites will be evaluated only after substantial antibody levels are being produced.

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32. R. Staden. A new computer method for storage and manipulation of DNA gel reading data. Nucl. Acids Res. 8: 3673 (1980).

33. J.R. Lupski, L.S. Ozaki, J. Ellis and G.N. Godson. Localization of a Plasmodium surface antigen epitope by Tn5 Mutagenesis of a Recombinant cDNA clone, Science 220: 1285 (1983).

34. J. Ellis, L.S. Ozaki, R.W. Gwadz, A.H. Cochrane, V.Nussenzweig, R.S. Nussenzweig and G.N. Godson. Cloning and expression in E. coli of the malarial sporozoite surface antigen gene from Plasmodium knowlesi. Nature 302: 536 (1983).

35. T. Maniotis, E.F. Fritsch and J. Sambrook. Molecular Cloning (1982).

Ethical Aspects of Proposed Research

Human subjects

a.Subjects will be normal, healthy laboratory personnel.
b.Units of blood will be collected from selected, paid donors to provide human serum and human red cells.
c.Blood will be obtained from the General Clinical Research Center whose personnel will recruit donors from among the Institute's employees. Standard consent forms will be employed to obtain consent for the drawing by venepuncture of up to 450 ml of blood. Subjects will receive a copy of the "Experimental Subjects Bill of Rights" form.
d.No substantial risks are to be expected. The drawing of blood is a routine procedure.
e.Subjects will be screened to verify that they have no medical history predisposing the drawing of blood, and that they have a hematocrit within normal limits at the time blood is to be collected. To eliminate any possibility of injury (due to fainting), subjects will be asked to recline on a cot during the drawing of blood. Information solicited from potential donors in the form of a short medical history will be considered confidential, as will the records of any studies with the donor's blood.
f.While the risks to the blood donors are minimal, their blood components will be used to obtain information contributing to the efforts to produce immunologic agents against human malarial parasites.

Vertebrate animals

Owl monkeys will be used in this study because they currently provide the best model for human malaria caused by *P. falciparum*. Other than human patients, these animals provide the only reliable source of blood containing relatively large numbers of parasites. They will be housed in our new vivarium facilities, caged in stainless steel cages ($\sim$16 ft^3), and fed a special diet developed specifically for them. We will provide them with care identical to that which we provide our Columbian owl monkey breeding colony. This care easily fulfills Federal requirements. When animals are bled, they will be sedated with ketamine. Mice and rabbits are housed in quarters developed specifically for their care. These quarters conform to Federal requirements.

Facilities Available

The laboratory is housed in ~ 2100 square feet of space in which are contained offices and a cold room as well as the following equipment:

3 biological flow hoods
temperature-regulated incubators
media-making facilities
standard, inverted, and fluorescence/phase-contrast light microscopes
cylindrical and slab polyacrylamide and agarose gel electrophoresis apparatus
Varian high performance liquid chromatograph and chart recorder
IBM personal computer
Altex pH meter
Gilford scanning spectrophotometer
Sorvall ultracentrifuge and high speed centrifuge
3 table-top centrifuges (two refrigerated)
3 high-speed microcentrifuges
chromatography columns
peristaltic pumps
LKB fraction collector with UV detector
lyophilizer
analytical balances
water baths
4 liquid N_2 freezers
4 -20° freezers
Kelvinator -85° freezer
Packard β counter
Packard γ counter
analytical balances
Napco vacuum oven

BUDGET

Reese, Ph.D. Robert T.	Principal Investigator	

Ramasamy, Ph.D. Ranjan	Visiting Professor	Immunochemist

Primary structural analysis of purified parasite proteins

Technician III

Purification of proteins and glycoproteins for primary structural analysis

Stanley, Ph.D. Harold A.	Assistant Member	Immunoparasitologist

Primate monoclonals, immunoelectron microscopy for antibody localization - 50% time

Somers, Cynthia	Technician III

Production and screening of monoclonals - work with Dr. Stanley

Flint, Ph.D. Janette, E.	Postdoctoral Fellow	Immunoparasitologist- Molecular Biologist

Mouse monoclonals, polyclonal antibodies, molecular biology (DNA sequencing)

Technician II

Large scale culture and purification of proteins for immunization studies

Saint, Ph.D. Rob	Assistant Member	Molecular Biologist

Technician III

Work with Dr. Saint

Howard, Ph.D. Randall F.	Research Associate	Cell Biologist- Biochemist

Identification and characterization of parasite components important in immunity

Lyszczasz Diane M.	Technician II

Work with Dr. Howard on antigen identification and characterization

Ardeshir, Ph.D. Feroza	Research Associate	Molecular Biologist
	Technician III	

Work with Dr. Ardeshir

Gritzmacher, Ph.D. Christine A.	Research Associate	Molecular Biologist
Coukoulis, Helen	Technician II	

Work with Dr. Gritzmacher

Castillo, Rosario	Technician II

Maintenance of stock cultures, production of media, large scale parasite production

	Year 1	Year 2	Year 3
Reese (70%)	49,749	53,729	58,027
Ramasamy	44,982	48,581	52,467
Stanley (50%)	22,491	24,290	26,234
Saint	44,982	48,581	52,467
Howard	25,846	27,914	30,147
Ardeshir	26,717	28,854	31,163
Gritzmacher	26,717	28,854	31,163
Flint	18,240	19,699	21,275
Technician III	23,538	25,421	27,455
Somers	23,538	25,421	27,455
Technician III	23,538	25,421	27,455
Technician III	23,538	25,421	27,455
Technician II	21,349	23,057	24,901
Lyszczasz	21,349	23,057	24,901
Coukoulis	21,349	23,057	24,901
Castillo	21,349	23,057	24,901
Total	439,272	474,414	512,367

Year 01

Personnel		$439,272
Supplies		$118,093
Animals		
Monkeys (Board, Breeding, Bleedings, etc.)	$ 32,499	
Rabbits and Mice	$ 5,198	
Total Animal Costs	$ 37,697	$ 37,697
Travel - 8 professionals, 1 meeting per year		$ 10,000
Publication costs, graphics, etc.		$ 8,000
Equipment		
Monkey cages 10 @ 400 each	$ 4,000	
VAX computer system:		
VAX 11/730 System Kernel	$ 23,650	
204MB Removable Disk	$ 24,200	
1600 BPI Tape 25/100 IPS	$ 10,890	
Multi-function 8-async/1 sync lpdr - peripheral control	$ 4,395	
Inst. & Warr. Service	$ 8,096	
Graphics Terminal	$ 8,580	
Versatec V-80 laser printer-plotter	$ 9,845	
Software:		
VAX-Fortran package	$ 9,570	
VAX-Basic package	$ 10,120	
Intelligenetics software for molecular biology	$ 11,000	
University of Wisconsin DNA package	$ 2,200	
Western Electric UNIX System III multi-user operating system	$ 1,650	
UNIX System III 5 manual set	$ 138	
Berkeley UNIX upgrade	$ 193	
UCSF DNA Sequence Analysis package	$ 550	
Total	$125,077	
Total Equipment		$129,077
Total Direct Costs		$742,139

	Year 01	Year 02	Year 03
Personnel	439,272	474,414	512,367
Supplies	118,093	127,540	137,743
Animals	37,697	40,713	43,970
Travel	10,000	10,800	11,664
Publication Costs, Graphics	8,000	8,640	9,331
Subtotal	613,062	662,107	715,075
Equipment	129,077	0	0
Total Direct Costs	742,139	662,107	715,075
Indirect Costs	353,124	381,374	411,883
Total Costs	1,095,263	1,043,481	1,126,958

A VAX 11/730 with tape drive, laser printer-plotter, video terminal with graphics upgrade kit, Fortran and Basic languages, as well as software for molecular biological work are being sought. We will use the equipment to help obtain and then to analyse the data generated on various molecular components of human malarial parasites. Available software makes it possible to enter the information from DNA sequencing gels directly into the computer and generate a primary amino acid sequence. Programming will be acquired which will allow us to analyse such sequences and then to predict regions which are likely to be antigens. Graphics programs will be obtained (see enclosed manuscript) which allow analysis of autoradiographs and fluorographs which permit objective evaluation of such data even when the images are of limited intensity.

CURRICULUM VITAE

Robert Trafton Reese

Education:

Institution	Field of Study	Attended/ Degree
Virginia Polytechnic Institute Blacksburg, VA	Biology/ Chemistry	1960-64 B.S.
George Washington University Washington, DC	Microbiology/ Bacteriology	1964-66
Yale University New Haven, CT	Microbiology/ Immunology	1966-71 Ph.D.

Research and Professional Experience:

Laboratory Instructor in Microbiology, George Washington University

Microbiology Departmental Fellowship, George Washington University

Laboratory Instructor in Microbiology, Yale University

NIH Predoctoral Fellowship, Yale University

Postdoctoral Fellow, Johns Hopkins University, 1971-74

Research Associate, Rockefeller University, July - September 1974

Assistant Professor, Laboratory of Immunology and Hypersensitivity, Rockefeller University, October 1974 - November 1976

Assistant Professor, Laboratory of Parasitology, Rockefeller University November 1976 - March 1981.

Associate Member, Scripps Clinic and Research Foundation, Department of Immunology, April 1981 - present.

Special Consultant to World Health Organization on Immunobiology of Malaria

Visiting Professor, University of Ibadan, Ibadan, Nigeria - taught WHO sponsored course on *in vitro* cultivation and immunobiology of malaria, March - June 1978

Professional Societies:

Sigma Xi

American Association of Immunologists

New York Academy of Sciences

American Society for Microbiology

American Society of Tropical Medicine and Hygiene

New York Society of Tropical Medicine

Southern California Branch American Society for Microbiology

Review for the following journals:

Journal of Immunology
The American Journal of Tropical Medicine and Hygiene
Archives of Pathology and Laboratory Medicine

Principal Investigator on Grants from:

National Institutes of Health
Agency for International Development

Bibliography

1. R. Hugh and R. Reese. Designation of the type strain for Bacterium anitratum Schaub and Hauber, 1948. Intl. J. Syst. Bact. 17: 245 (1967).

2. R. Hugh and R. Reese. A comparison of 120 strains of Bacterium anitratum Schaub and Hauber with the type strain of the species. Intl. J. Syst. Bact. 18: 207 (1968).

3. R. Hugh and R. Reese. The susceptibility of Bacterium anitratum to antimicrobial agents. Jour. Pub. Health. Dir. 27: 174 (1969).

4. R.T. Reese and H.P. Treffers. Rapid polymer-phase assay of antibodies to enzymes: applications to the E. coli alkaline phosphatase system. Fed. Proc. 28: 308 (1969).

5. R.T. Reese and H.P. Treffers. The effects of specific metals on the antigenic structure of alkaline phosphatase from Escherichia coli. J. Immunol. 110: 10 (1973).

6. R.T. Reese and H.P. Treffers. Use of an aqueous polymer phase system as an assay for antibodies to Escherichia coli alkaline phosphatase. J. Immunol. 111: 624 (1973).

7. R.T. Reese. Examination of specific humoral antibodies from strain 13 guinea pigs which have no detectable delayed hypersensitivity to dinitrochlorobenzene. Fed. Proc. 32: 4411 (1973).

8. R.T. Reese and J.J. Cebra. Anti-dinitrophenyl antibody production in strain 13 guinea pigs fed or sensitized with dinitrochlorobenzene. J. Immunol. 114: 863 (1975).

9. R.T. Reese and J.J. Cebra. Comparison of antibody produced by DNCB fed, sensitized and conventionally immunized strain 13 guinea pigs. Immunochem. 13; 103 (1976).

10. R.T. Reese. Antibody production by strain 2 and strain 13 guinea pigs. I. Use of a monomeric guinea pig albumin carrier to demonstrate immune response gene control. Cellular Immunol. 33: 245 (1977).

11. R.T. Reese. Differential regulation and immune responsiveness to two antigens on the same molecule. Fed. Proc. 36: 1199 (1977).

12. S.G. Langreth, R.T. Reese, J.B. Jensen and W. Trager. Development of human malaria parasites in continuous culture. J. Cell Biol. 75: 94a (1977).

13. C.K. Miller, D.A. Miller, O.J. Miller, R. Tantravahi and R.T. Reese. Banded chromosomes of the owl monkey, Aotus trivirgatus. Cytogenetics and Cell Genetics, 19: 215 (1977).

14. R.T. Reese, M.W. Chase and G.P. Naegel. Control of antibody heterogeneity in strain 2 guinea pigs. Proc. Natl. Acad. Sci. USA 75: 959 (1978).

15. R.T. Reese. A highly restricted homocytotropic antibody produced by strain 13 guinea pigs stimulated with a hapten-conjugated self protein. Cellular Immunol. 40: 303 (1978).

16. R.T. Reese, W. Trager, J.B. Jensen, D.A. Miller and R. Tantravahi. Immunization against malaria with antigen from in vitro cultivated Plasmodium falciparum. Proc. Natl. Acad. Sci. USA 75: 5665 (1978).

17. S.G. Langreth, J.B. Jensen, R.T. Reese and W. Trager. The fine structure of Plasmodium falciparum in vitro. J. Protozool. 25: 443 (1978).

18. S.G. Langreth and R.T. Reese. Antigenicity of the infected erythrocyte surface in falciparum malaria. J. Cell. Biol. 79: 89 (1978).

19. M.R. Motyl and R.T. Reese. Immunologic inhibition of the in vitro growth of Plasmodium falciparum. J. Cell. Biol. 79: 80 (1978).

20. R.T. Reese and M.R. Motyl. Inhibition of the in vitro growth of Plasmodium falciparum. 1. The effects of immune serum and purified immunoglobulin from Aotus monkeys. J. Immunol. 123: 1894 (1979).

21. S.G. Langreth, R.T. Reese, M.R. Motyl and W. Trager. Plasmodium falciparum: Loss of knobs on the infected erythrocyte surface after long-term cultivation. Exp. Parasitol. 48: 213 (1979).

22. S.G. Langreth and R.T. Reese. Antigenicity of the infected erythrocyte and merozoite surfaces in falciparum malaria. J. Exp. Med. 150: 1241 (1979).

23. R.T. Reese, S.G. Langreth and W. Trager. Isolation of stages of the human parasite Plasmodium falciparum from culture and from animal blood. Bull. WHO 57(Suppl. 1): 53 (1979).

24. R.T. Reese, M.R. Motyl and R.N. Hofer-Warbinek. Reaction of immune sera with components of the human malarial parasite, Plasmodium falciparum. Am. J. Trop. Med. Hyg. 30: 1168 (1981)

25. H.A. Stanley, S.G. Langreth, R.T. Reese, and W. Trager. Plasmodium falciparum merozoites — isolation by density gradient centrifugation using Percoll and antigenic analysis. J. Parasitol. 68(6): 1059 (1982).

26. C.A. Gritzmacher and R.T. Reese. Translation in vitro of RNA from the human malarial parasite Plasmodium falciparum. Biosci. Rpts. 2: 667 (1982).

27. M.R. Motyl and R.T. Reese. Plasmodium falciparum: Comparison of in vitro growth of knobby and knobless isolates. Am. J. Trop. Med. Hyg. 32(2): 226 (1983).

28. H.A. Stanley and R.T. Reese. In vitro inhibition of intracellular growth of Plasmodium falciparum by immune sera. Am. J. Trop. Med Hyg. in press.

29. R.F. Howard and R.T. Reese. Synthesis of merozoite proteins and glycoproteins during the schizogony of Plasmodium falciparum. Molec. Biochem. Parasitol. in press.

30. H.A. Stanley, J.T. Mayes, N.R. Cooper, and R.T. Reese. Complement activation by the surface of Plasmodium falciparum infected erythrocytes. Molec. Immunol. in press.

31. R.F. Howard, A. Varki, and R.T. Reese. Merozoite proteins synthesized in P. falciparum schizonts. In Malaria and the Red Cell Workshop, ed. G.J. Brewer, Alan R. Liss, Inc., New York, NY. in press.

CURRICULUM VITAE

Harold Austin Stanley

Education:

Institution	Field of Study	Attended/ Degree
University of Vermont Burlington, VT	Zoology	1968-72 B.A.
University of Massachusetts Amherst, MA	Parasitology	1972-76 M.S.
University of Massachusetts Amherst, MA	Parasitology	1976-78 Ph.D.

Research and Professional Experience:

Teaching Assistant, Department of Zoology, University of Massachusetts, September 1972 - May 1974.

Research Assistant, Department of Zoology, University of Massachusetts, June 1974 - August 1974 and July 1977 - October 1977.

NIH Predoctoral Fellowship, Department of Zoology, University of Massachusetts, September 1974 - June 1977 and October 1977 - June 1978.

NIH Postdoctoral Fellowship, Department of Zoology, University of Massachusetts, October 1978 - June 1979.

Research Associate, Laboratory of Parasitology, Rockefeller University, September 1979 - June 1980.

Assistant Professor, Laboratory of Parasitology, Rockefeller University, July 1980 - August 1981.

Research Associate, Department of Immunology, Scripps Clinic and Research Foundation, September 1981 - present.

Professional Societies

Sigma Xi

American Association for the Advancement of Science

American Society for Microbiology

American Society of Parasitologists

American Society of Tropical Medicine and Hygiene

Publications

1. Honigberg, B.M., Cunningham, I., Stanley, H.A., Su-Lin, K.E. and Luckins, A.G. 1976. Trypanosoma brucei: Antigenic analysis of bloodstream, vector, and culture stages by quantitative fluorescent antibody methods. Exp. Parasitol. 39:496-522.

2. Stanley, H.A., Honigberg, B.M., and Cunningham, I. 1978. Analysis of the antigenic composition of Trypanosoma brucei brucei bloodstream and culture forms by the quantitative direct fluorescent antibody methods. J. Protozool. 25:245-252.

3. Stanley, H.A., Honigberg, B.M. and Cunningham, I. 1979. Antigenic analysis, by agglutination and indirect fluorescent antibody methods, of Trypanosoma brucei parasitemia initiated with metacyclics produced in vitro. Z. Parasitenkd. 58:141-149.

4. Trager, W., Tershakovec, M., Lyandvert, L., Stanley, H.A., Lanners, N. and Gubert, E. 1981. Clones of the malaria parasite Plasmodium falciparum obtained by microscopic selection: Their characterization with regard to knobs, chloroquine sensitivity and formation of gametocytes. PNAS 78:6527-6530.

5. Trager, W., Stanley, H.A., Allen, R.D. and Allen, N.A. 1982. Knobs on the surface of erythrocytes infected with Plasmodium falciparum: Visualization by video-enhanced differential interference contrast microscopy. J. Parasitol. 68(2)332-333.

6. Stanley, H.A., Langreth, S.G., Reese, R.T. and Trager, W. 1982. Plasmodium falciparum merozoites — isolation by density gradient centrifugation using Percoll and antigenic analysis. J. Parasitol. 68(6):1059-1067.

7. Trager, W., Lanners, H.N., Stanley, H.A. and Langreth, S.G. 1983. Immunization of owl monkeys to Plasmodium falciparum with merozoites from cultures of a knobless clone. Parasite Immunol. 5(3):225-236.

8. Stanley, H.A. and Reese, R.T. In vitro inhibition of intracellular growth of Plasmodium falciparum by immune sera. Am. J. Trop. Med. Hyg. in press.

9. Stanley, H.A., Mayes, J.T., Cooper, N.R. and Reese, R.T. Complement activation by the surface of Plasmodium falciparum infected erythrocytes. Molec. Immunol. in press.

CURRICULUM VITAE

Ranjan Ramasamy

Education

Institution	Field of Study	Attended/ Degree
University of Cambridge Cambridge, England	Biochemistry/ Natural Sciences	1968-71 B.A.
University of Cambridge Cambridge England	Immunology	1971-74 Ph.D.

Research and Professional Experience:

Scientist, Department of Immunology, ARC Institute of Animal Physiology, Babraham, Cambridge, England, 1974 - 1976.

Assistant Professor, Departments of Biochemistry and Biology, College of Medicine, King Faisal University, Dammam, Saudi Arabia, 1976 - 1979.

Senior Lecturer, Department of Biochemistry, University of Nairobi, Kenya, 1979 - 1982.

Professor and Head, Department of Biochemistry, Faculty of Medicine, University of Jaffna, Sri Lanka, 1982 - 1983.

Visiting Investigator, Department of Immunology, Scripps Clinic and Research Foundation, September 1983 - present.

PUBLICATIONS

1. Ramasamy, R. and Munro, A.J. Surface T and B cell markers on murine lymphomas and plasmacytomas. Immunology 26: 563-570 (1974).

2. Ramasamy, R., Munro, A.J., and Milstein, C. A possible role for the Fc receptor on B lymphocytes. Nature 249: 573-574 (1974).

3. Ramasamy, R. A fluorescent stain for viable rosette forming cells. Journal of Immunological Methods 5: 305-306 (1974).

4. Ramasamy, R. and Lawson, Y. Independent movement of surface immunoglobulin from Fc receptors on lymphocyte membranes. Immunology 28: 301-304 (1975).

5. Ramasamy, R. and Williams, H. C3 receptors on direct plaque forming cells. Immunology 28: 577-580 (1975).

6. Ramasamy, R., Secher, D.S. and Adetugbo, K. C_H3 domain of IgG as the binding site to Fc receptor on mouse lymphocytes. Nature 253: 656 (1975).

7. Ramasamy, R. Role of membrane structures in B lymphocyte activation. The effect of binding anti-immunoglobulin, aggregated IgG and immune complexes. Immunology 30: 559-564 (1976).

8. Ramasamy, R., Richardson, N.E. and Feinstein, A. The specificity of the Fc receptor on murine lymphocytes for immunoglobulins of IgG and IgM classes. Immunology 30: 851-858 (1976).

9. Ramasamy, R. The attachment of the antigen receptor to the B cell membrane. Immunochemistry 13: 705-708 (1976).

10. Ramasamy, R. Surface proteins on cercariae and schistosomula of *Schistosoma mansoni*. International Journal for Parasitology 9: 491-493 (1979).

11. Shah, J. and Ramasamy, R. Common antigens on cercariae and adult worm teguments of *Schistosoma mansoni* detected by immunodiffusion. East African Medical Journal 55: 330-333 (1981).

12. Ramasamy, R., Jamnadas, H. and Mutinga, M.J. Proteins and surface proteins on *Leishmania* promastigotes and their possible relevance to the characterisation of strains. International Journal for Parasitology 11: 387-390 (1981).

13. Ramasamy, R. Surface antigens on haemoparasites and their relevance to protective immunity. Biochemical Society Transactions 9: 535-536 (1981).

14. Khauka, G and Ramasamy, R. Haemolytic activity of trypanosomes. East African Medical Journal 58(12): 907-911 (1981).

15. Ramasamy, R. Effect of inhibitors on the respiration of Leishmania donovani promastigotes from culture. East African Medical Journal 59(6): 379-382 (1982).

16. Shah, J. and Ramasamy, R. Surface antigens on cercariae, schistosomula and adult worms of Schistosoma mansoni. International Journal of Parasitology 12: 451-461 (1982).

17. Shitakha, V.M., Nantulya, V.M., Musoke, A.J., Ramasamy, R. and Buscher, G. Complement activation and fibrinolysis during infection with Theileria parva (East Coast Fever). Veterinary Immunology and Immunopathology 4:361-373 (1983).

18. Ramasamy, R., Kar, S.K. and Jamnadas, H. Cross-reacting surface antigens on Leishmania promastigotes. International Journal for Parasitology, 13(4): 337-342 (1983).

19. Jamnadas, H., Ramasamy, R., Shah, J., Mutinga, M.J. and Kar, S. Surface antigens on a Kenyan strain of Leishmania donovani. East African Medical Journal, 60 283 (1983).

20. Kar, S.K. and Ramasamy, R. Monoclonal antibodies against Leishmania surface antigens. Journal of Immunological Methods, in press.

21. Sturrock, R.F., Butterworth, A.E., Houba, V., Cottrell, B.J., Kimani, R., Joseph, M., Capron, A., Ramasamy, R. and Shah, J. Attempts to induce resistance to schistosomiasis in Kenyan baboons (Papio anubis). 1. Manipulation of specific responses to Schistosoma mansoni, submitted for publication.

Recent Conference Proceedings

In "Recent Developments in Medical Research in Eastern Africa" - proceedings of the First Annual Medical Scientific Conference of the Kenya Medical Research Institute, edited by A.R. Njogu, P.M. Tukei and J.M.D. Roberts (1980).

1. General approaches to the identification of protective antigens in parasitic diseases. Ramasamy, R. p 320-324.
2. Surface molecules on Leishmania. Sharda, H., Ramasamy, R. and Mutinga, M.J. p 325-326.
3. Surface antigens of Schistosoma mansoni. Shah, J. and Ramasamy, R. p 327-328.

In "Trends in Research on Diseases of the Tropics with special emphasis on Leishmaniasis" - proceedings of the Second Annual Medical Scientific Conference of the Kenya Medical Research Institute, edited by A.R. Njogu, P.M. Tukei and J.M.D. Roberts (1981).

4. Sub-cellular localisation of haemolytic activity derived from Trypanosoma evansi. Khauka, G. and Ramasamy, R.
5. Identification and the characterisation of the variant surface antigen on cultured forms of Trypanosoma brucei derived from the salivary glands of Glossina morsitans morsitans. Alemu, P., Shah, J., Ramasamy, R. and Nyindo, M.
6. Surface antigens on cercariae, schistosomula and adult worms of Schistosoma mansoni and their relevance to immunity in schistosomiasis. Shah, J. and Ramasamy, R.
7. Proteins and surface proteins of Leishmania promastigotes and their possible relevance to immunity and the characterisation of strains. Ramasamy, R, Jamnadas, H. and Mutinga, M.J.

In "Current Medical Research in Eastern Africa with Emphasis on Zoonoses and Waterborne Diseases". Proceedings of the Third Annual Medical Scientific Conference of the Kenya Medical Research Institute (1982).

8. Studies on possible biological variations on Schistosoma surface antigens. Shah, J., Ramasamy, R. and Kinoti, G.K.

CURRICULUM VITAE

Randall Forster Howard

Education:

Institution	Field of Study	Attended/ Degree
Vanderbilt University Nashville, TN	Molecular Biology	1969-73 B.A.
University of Minnesota Minneapolis/St. Paul, MN	Cell Biology	1973-78 Ph.D.

Research and Professional Experience

Postdoctoral Fellow, Institutes of Pharmacology and Virology, University of Giessen, West Germany, 1978-81.

Research Associate, Department of Immunology, Scripps Clinic and Research Foundation, August 1981 - present.

Publications

1. Marz, R., J.M. Zylka, P.G.W. Plagemann, J. Erbe, R.F. Howard, and J.R. Sheppard. (1977) G_2 + M Arrest of Cultured Mammalian Cells After Incorporation of Tritium-labeled Nucleotides. J. Cell Physiol. 90: 1-8.

2. Howard, R.F. and J.R. Sheppard. (1981) Cell Cycle Changes in the Adenylate Cyclase of C6 Glioma Cells. J. Cell Biology 90: 169-175.

3. Foulkes, J.G., R.F. Howard, and A. Ziemiecki. (1981) Detection of a Novel Mammalian Protein Phosphatase with Activity for Phosphotyrosine. FEBS Letters 130: 197-200.

4. Howard, R.F., R.R. Friis, J.R. Sheppard, and H. Glossmann. (1982) Increase in β-adrenergic Stimulated Adenylate Cyclase Activity Following Rous Sarcoma Virus Transformation. J. Cell Physiol. 111: 295-302.

5. Howard, R.F., H. Glossmann, R.R. Friis, and J.R. Sheppard. (1982) pp60src-dependent Increase in Adenosine-stimulated Adenylate Cyclase Activity. Exptl. Cell Res. 139: 436-9.

6. Howard, R.F. and R.T. Reese. Synthesis of Merozoite Proteins and Glycoproteins During the Schizogony of *Plasmodium falciparum*. Molec. Biochem. Parasitol., in press.

7. Howard, R.F., A. Varki, and R.T. Reese. Merozoite Proteins Synthesized in *P. falciparum* Schizonts. In *Malaria and the Red Cell Workshop* ed., G.J. Brewer. Alan R. Liss, Inc., in press.

CURRICULUM VITAE

Christine Arlene Gritzmacher

Education:

Institution	Field of Study	Attended/ Degree
University of Wisconsin Eau Claire, WI	Biology/ Chemistry	1968-72 B.S.
Purdue University Lafayette, IN	Bacterial Genetics	1972-77 Ph.D.

Research and Professional Experience

Teaching Laboratory Assistant, Department of Chemistry, University of Wisconsin - Eau Claire, Summer 1970

Research Assistant, Department of Biology, University of Wisconsin - Eau Claire, December 1969 - June 1972

Teaching Assistant, Department of Biological Sciences, Purdue University, August 1972 - December 1972

Research Associate, Department of Biological Sciences, Purdue University, August 1977 - October 1977

NIH Staff Fellow, Laboratory of Molecular Genetics, NICHD, National Institutes of Health, 1977 - 1981

Research Associate, Department of Immunology, Scripps Clinic and Research Foundation, July 1981 - present

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4. Gritzmacher, C.A. and M. Levinthal. Instability of a small genomic duplication (pi-his) in Salmonella typhimurium: Evidence for further genetic rearrangements. (in preparation).

5. Gritzmacher, C.A. and R.A. Weisberg. Intramolecular site-specific recombination promotes deletion formation in an xin mutant of bacteriophage lambda. (in preparation).

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7. Gritzmacher, C.A. and R.T. Reese. Evidence for phenotypic change of a cloned strain of Plasmodium falciparum during growth in culture. (in preparation).

CURRICULUM VITAE

Feroza Ardeshir

Education:

Institution	Field of Study	Attended/ Degree
Indian Institute of Technology Kharagpur, India	Chemistry	1969-72 BSc
Indian Institute of Technology Kanpur, India	Chemistry	1972-74 MSc
Ohio State University Columbus, OH	Biochemistry	1974-75
University of California Berkeley, CA	Biochemistry	1975-81 Ph.D.

Research and Professional Experience

Graduate Research Assistant, Biochemistry Department, Ohio State University, Columbus, September 1974 - March 1975 and June 1975 - September 1975.

Teaching Assistant, Undergraduate Biochemistry Laboratory, Ohio State University, Columbus, March 1975 - June 1975.

Teaching Assistant, Undergraduate Biochemistry Laboratory, University of California, Berkeley, September 1975 - December 1975 and March 1976 - June 1976.

Graduate Research Assistant, Department of Biochemistry, University of California, Berkeley, January 1976 - March 1976 and July 1976 - February 1981.

Postdoctoral Fellow, Department of Biochemistry, Stanford University, February 1981 - July 1982.

Research Associate, Department of Immunology, Scripps Clinic and Research Foundation, July 1982 - the present.

PUBLICATIONS

A. In Research Journals

1. D. Balasubramanian, P. Chopra, and F. Ardeshir. Cycloinopeptide, an Antamanide Analogue. (1976) FEBS Letters, 65: 69.

2. G. Ferro-Luzzi Ames, K.D. Noel, H. Taber, E. Negri-Spudich, K. Nikaido, J. Afong, and F. Ardeshir. Fine Structure Map of the Histidine Transport Genes in Salmonella typhimurium. (1977) J. Bacteriology, 129: 1289.

3. F. Ardeshir and G. Ferro-Luzzi Ames. Cloning of the Histidine Transport Genes of Salmonella typhimurium and Characterization of an Analogous Transport System in Escherichia coli. (1980) J. Supramol. Structure, 13: 117.

4. S.M. LeVine, F. Ardeshir, and G. Ferro-Luzzi Ames. Isolation and Characterization of Acetate Kinase and Phosphotransacetylase mutants in E. coli and S. typhimurium. (1980) J. Bacteriology, 143: 1801.

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8. F. Ardeshir, E. Giulotto, J. Zieg, O. Brison, W.S.L. Liao and G.R. Stark. Structure of Amplified DNA in Different Syrian Hamster Cell Lines Resistant to N-(Phosphonacetyl)-L-Aspartate. Molec. and Cellular Biol. 3: 2076 (1983).

9. J. Zieg, C.E. Clayton, F. Ardeshir, E. Giulotto, E.A. Swyryd and C.R. Stark. Properties of Single-Step Mutants of Syrian Hamster Cell Lines Resistant to N-(Phosphonacetyl)-L-Aspartate. Molec. and Cellular Biol. 3: 2089 (1983).

B. Other

10. C.F. Higgins, F. Ardeshir, and G.F.-L. Ames. Active Transport of Amino Acids in Bacteria: Advances Resulting from Genetic and Recombinant DNA Techniques. In Membranes and Transport, a Critical Review, 1982. Ed. A.N. Martonosi, Plenum Press, New York.

11. G.R. Stark, O. Brison, F. Ardeshir, and J. Zieg. A General Method for Cloning Amplified DNA; Properties of Cloned Amplified Fragments near the CAD gene. In Gene Amplification (1982) Ed. R.T. Schimke, Cold Spring Harbor Laboratories, Cold Spring Harbor, New York.