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Quarterly Report

Oct. 1, 1969-Dec. 31, 1969
Jan. 1, 1970-Mar. 31, 1970

Submitted by

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SEROLOGIC DIAGNOSIS OF MALARIA

PASA Control No. RA(HA) 5-68

PIO/T 931-17-511-485-72-3106759
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Purpose and Scope of Project

The project is designed to develop simple, rapid, accurate methods for the serologic diagnosis of malaria which would be applicable in the worldwide malaria eradication program.

Toward this end, this laboratory has concentrated its efforts on the evaluation and standardization of the indirect hemagglutination technique (IHA) for the detection of malaria antibody for epidemiological studies and the indirect fluorescent antibody test (IFA) for diagnostic and serologic speciation of malaria.

Studies on the indirect hemagglutination (IHA) test

I. Studies on the epidemiology of malaria in Haiti

Dr. Irving G. Fagan visited with Dr. Hans Lobel, Chief Malaria Advisor, Malaria Eradication Program, on October 30-November ?, 1969, in Port-au-Prince, Haiti. A protocol was made to collect sera from above 500 meters, in an area with a current outbreak of malaria and in the area near the Hospital Albert Schweitzer. In the latter area, emphasis will be made on collecting sera from young children since malaria is believed to be under control. If this is true, sera from young people born and reared in this area should be negative or of low titers in comparison to other areas in Haiti. Dr. Henry Mathews is scheduled to depart for a two-week collecting trip April 5-18, 1970 to initiate this study.

II. Studies on the use of stable, sensitized cells for seroepidemiology

Investigation has continued on the production of a stable, sensitized human group "O" red cell as an antigen carrier. Modifications in published procedures for the preparation of gluteraldehyde-treated cells have made this type cell almost as sensitive as fresh, tanned red cells. Studies are now under way to determine the stability of these cells. Modified gluteraldehyde-treated cells now appear to be superior to other types of preservation, i.e., formalin or pyruvic aldehyde treatment.

Alternative methods to tannic acid for coupling antigens to cells have been investigated. The use of barium chloride and chromium chloride as coupling agents were attempted without success. Bis-diazotized-benzidine (BDB) in preliminary studies on gluteraldehyde and formalin-treated cells appears to couple antigens well enough to warrant further evaluation. These studies are being implemented.

III. Studies on the stabilization of antigen for the IHA test

Malaria antigens isolated from various infected organs of human origin (Williams & McFarlane, 1968; McGregor et al., 1966) prompted a search for similar antigens in organs of infected rhesus monkeys. Brain and spleen tissue from infected monkeys were disrupted and antigen extractions attempted. The product obtained produced non-specific agglutination which was not reduced by the addition of stabilizers such as normal rabbit serum, bovine albumin, or poly-vinylpyrrolidone. Future studies on antigens of this type will be held in abeyance pending outcome of more promising methods of extraction.

Following the methods of Wilde et al. (Military Medicine 134: 1284, 1969) attempts have been made to isolate antigen from lysates of red cells infected with Plasmodium knowlesi and P. falciparum. Antigen fractions obtained from ammonium sulfate and rivanol precipitation in conjunction with column chromatography on DEAE and gel filtration with Sephadex G-200 are being evaluated. These studies, with the collaboration of Dr. Shirley E. Maddison, have produced antigens with improved stability but reduced sensitivity. Fractionation of the crude antigen material results in loss of sensitivity, probably due to thermolability of the antigenic material. At present the equipment necessary for maintaining low temperatures during fractionation is not available. The promising results obtained from the efforts to date warrant continued emphasis on these methods. The purchase of equipment to fractionate lysates in the cold has been budgeted for the next fiscal year.

IV. Seroepidemiologic studies

The following studies have been completed:

New Guinea: 205 sera - part of continuing study with Dr. J. J. Saave

Afghanistan - 1115 sera - in collaboration with Drs. Anderson and Buck, Johns Hopkins University

Pakistan - 266 filter paper samples - study of urban malaria in Karachi

Foreign students - 170 sera - study of antibody prevalence and persistence in foreign students in the U.S.A. - with Drs. Sulzer and Dover

Uganda - 300 sera - malaria antibody prevalence in persons with Burkett's lymphoma - Dr. Feorino - NCDC

Ethiopia - a) 235 filter paper specimens - part of continuing study of malaria distribution

b) 514 filter paper specimens: Malaria antibody distribution in Gambela, Ethiopia, with Dr. Armstrong, NAMRU 3.

Haiti - 97 filter paper specimens as part of pilot project

Miscellaneous - 58 Ateles sp. monkey sera - study of antibody persistance in this experimental host.

V. New studies being planned

1. Brazil - Preliminary discussions are under way to determine the feasibility of collaborating with the staff of the Central America Malaria Research Station in a series of studies to be undertaken in Brazil.

2. East Pakistan - A change in assignment for Dr. M. Rahman from Karachi to Dacca has required modification of the study of urban malaria. The distribution of malaria in the western regions of East Pakistan will be studied in collaboration with Dr. Rahman and his staff.

VI. Publications

Accepted for publication:

Kagan, I. G., H. M. Mathews, W. A. Rogers, and J. Fried. Seroepidemiologic studies by indirect hemagglutination tests for malaria. 1. Military recruit collections from the United States, Brazil, Colombia, and Argentina

Bull. W. H. O.

Mathews, Henry M., George U. Fisher and Irving G. Kagan. The Persistence of malaria antibody in Tobago, West Indies, following eradication as measured by the indirect hemagglutination test

American Journal of Tropical Medicine and Hygiene.

Submitted for NCDC clearance:

Mathews, H. M., J. A. Fried, and I. G. Kagan

A seroepidemiologic study of malaria in the Republic of the Philippines by the indirect hemagglutination test.

Fluorescent Antibody Laboratory

I. Evaluation and development of the indirect fluorescent antibody (IFA) test for malaria

a) At the present time, all human malaria except Plasmodium ovale can be maintained in a simian host. The only simian host for P. ovale is the chimpanzee. As soon as facilities are available to house the chimpanzee, inoculation of human strains of P. ovale will be made. Funds to establish such a facility are being requested in next years budget.

b) The significance of the IFA titer has been evaluated in young military returnees who have received curative treatment. In this group the titer diminishes to less than 1:256 in six months. An individual with a titer of 1:256 or greater six months after treatment is believed to be a treatment failure. Many students resident in the U.S. with a history of lifetime residence in endemic areas for malaria have a persistent high malaria antibody titer with no evidence of infection. We believe such individuals pose a threat as blood donors in transfusion cases and as a source of infection to mosquitoes in control and non-endemic areas because they may have cryptic or occult malaria. To study this question, blood from 168 foreign students in five United States universities representing African and Asiatic origins have been tested for malaria antibody. Thirty individuals in three colleges with malaria antibody titers have been given curative treatment for malaria and their serologic titers will be followed for at least one year at three-month intervals. Individuals in two colleges will not be treated and will serve as a control. If the treated individuals do indeed have cryptic malaria, a fall in serologic titer would be expected with no diminishing of titer in the control group.

c) Antisera obtained from a human with a Babesia infection was found to react with both Babesia and P. falciparum antigen in the IFA test. To further explore cross reactions of the two genera of blood parasites, antigens of four species of Babesia have been prepared. Initial tests indicate that cross reactions

between the two genera may be as great as cross reactions within each of the genera. This would be the first reported instance of such an intergeneric cross reaction in the malaria IFA tests if evaluation of this question substantiates early pilot studies.

d) Liver shunt therapy has been recently introduced for cases of hepatic coma. The baboon is often utilized as the donor animal in this clinical procedure. Since about one-fourth of wild baboons are known to have infections with Hepatocystis, a genus of blood parasites closely related to malaria, persons so treated may be exposed to this parasite. A Hepatocystis antigen was prepared, and sera of 15 baboons with Hepatocystis infection and four with no infection were tested. Eleven of the 15 animals gave positive reactions; the uninfected ones were negative. When these same sera were tested with malaria antigens, all were positive (both those infected and those not infected) with P. falciparum; all were negative with P. vivax and some were positive with P. malariae. A test for Hepatocystis infection is available if needed and the cross reactions with malaria antigens is under investigation.

II. New studies being planned

1. Significance of antibody titer in U. S. nationals with long residence in areas endemic for malaria will be assessed. A battery of 100 sera from U. S. missionaries living in endemic areas from Dr. Frame, a medical missionary physician in New York, has been acquired. Persons found to have positive serologic reactions will be given curative treatment and followed to determine if antibody titers diminish after treatment.

2. Cross reactions in the malaria IFA test will be investigated further. A guest researcher, sponsored by the Australian Meat Institute, will spend three months in the FA laboratory during the summer of 1970 for the purpose of exploring the Babesia-Plasmodium cross reaction system and its significance in diagnostic serology.

3. Antisera from a primitive tribe of Bolivian Indians have been found to give strong reactions to P. vivax and P. malariae antigens but with very weak or no reactions to P. falciparum. A similar serology response has been observed with sera from Africans who give positive responses with some malaria species antigens but not with P. vivax. It has been postulated that infection with one or more species of human malaria will produce

cross reacting antibodies to all other species in a population. Studies will be designed to explore this question further.

III. Papers presented at meetings

1. Three papers were presented at the Joint Meetings of the American Society of Tropical Medicine and Hygiene and The American Society of Parasitology held in Washington, D. C., November 1969.

   a) A long-term, high-level peripheral parasitemia due to Plasmodium brasilianum in the spider monkey, Ateles geoffroyi. A. J. Sulzer

   b) Serological relationships of Plasmodium species in malaria IFA tests. A. J. Sulzer

   c) Comparison of the indirect fluorescent antibody and indirect hemagglutination tests for malarial antibody. Marianna Wilson.

IV. Manuscripts being cleared for publication

Two manuscripts, "Comparison of the indirect fluorescent antibody and indirect hemagglutination tests for malaria antibody" by M. Wilson, A. J. Sulzer, W. A. Rogers, H. M. Mathews, and J. Fried, and "Agreement of slide diagnosis and IFA serology in Plasmodium vivax and P. falciparum infections" by Neva Gleason, A. J. Sulzer, M. Wilson, and K. Runcik, have been prepared and are now in process of being cleared for publication.