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Serologic Diagnosis of Malaria

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During the past year, research in two areas of malaria serology was carried out:

1. Evaluation of the indirect hemagglutination (IHA) test under the guidance of Dr. Wallace A. Rogers, Jr.
2. Evaluation of the indirect fluorescent antibody test (IFA) under the guidance of Dr. Alexander J. Sulzer.

Accomplishments:

- a) Studies on enhancing the sensitivity and specificity of the IHA test for malaria:

The reproducibility and sensitivity of the IHA test was improved by the use of homologous red cells in tests with primate and human serum. Thus, substituting simian and human cells for sheep red cells improved the sensitivity of the test.

Specificity of the test was also increased by use of 5% normal rabbit serum to saturate the antigenic sites on the red cell after sensitization with antigen and before use in the test.

A manuscript, "Evaluation of the Indirect Microhemagglutination Test for Malaria", was accepted for publication in The American Journal of Tropical Medicine and Hygiene. A copy of the manuscript is appended (#1).

b) Evaluation of serologic methods for field studies:

To facilitate collecting serum samples under field conditions, various grades of filter paper were tested. The paper found to be most practical for IHA studies was produced by the Rochester Paper Company (#1023.038). Pieces of paper were cut into 1x3-inch strips with 2 circles 14 mm in diameter, scribed on the paper. Finger bloods were allowed to soak up through the paper until the circles were filled. The blood-soaked circles were punched out with a 13/32-inch metal punch and the blood eluted in 0.2 ml of phosphate buffered saline. Studies to date indicate that the eluted plasma corresponds to a dilution of approximately 1:16 (varying between 1:11-1:22) which is useful for screening purposes. Studies on filter paper collections sent to us from Nepal and Panama suggest that, if properly packed in dry plasticene bags, dry, blood-soaked filter papers can be sent to Atlanta for titration.

c) Evaluation of malarial antibody in military recruit populations:

Seroepidemiologic studies on sera of the approximately 12,000 military recruits from the United States, Brazil, Colombia, and Argentina, were tested for malaria antibody with the IHA test. Results of these studies were presented in a working paper prepared for the Scientific Group on Parasitology of Malaria, Teheran, Iran, September 16-23, 1968. A copy of this working paper is appended (#2).

In summary, seroepidemiologic studies carried out in four countries in the Western Hemisphere indicate that the technique may have some merit for survey purposes.

Current Program Goals:

The current program in the Malaria Serology Laboratory is to continue field studies to evaluate the usefulness of the IHA test in the study of malaria. In Nepal, a study to determine if antibody can be detected in individuals living above 4,000 feet is in progress. Titration curves obtained in serologic studies from Nepal, Panama, and South Vietnam are being analyzed to determine if the shapes of the curves shed light on the transmission of malaria in the area sampled.

A second program goal is to produce a better antigen for use in the IHA test. Stability of the antigen needs to be further studied and improved. Experiments to study the factors which cause antigen decay are in progress.

Fluorescent Antibody Laboratory

a) Indirect fluorescent antibody (IFA) test evaluation:

Studies are in progress to evaluate the IFA test for malaria in terms of sensitivity, specificity, and the value of the thick-smear antigen for diagnosis. The thick-smear antigen has proven useful and perhaps superior to the usual thin-smear preparation containing a few parasites. A research note on the preparation of the thick-smear antigen was published and is appended (#3).

Evaluation of the IFA test suggests that the lowest significant titer in malaria IFA is 1:16. Specificity is 99% and sensitivity, 95%. Reproducibility on a test-to-test basis has been found to be plus or minus one 4-fold dilution. One can speciate with homologous antigen between Plasmodium vivax and P. falciparum with an 87% certainty of being correct. The rate of misdiagnosis is only 1%, and in 12% the evaluation cannot be made. A paper detailing the above study has been accepted for publication, and a copy of the manuscript is appended (#4).

b) Antigen preparation:

The need for homologous antigen has led to a program of providing a source of antigen for the human species from simian infections. Both P. vivax and P. falciparum have been established in the South American night monkey, Aotus trivirgatus. We were able to obtain these infections through the generosity of Dr. Quentin Geiman of Stanford Medical Center. Dr. Geiman is working on establishing P. malariae in the night monkey, and we hope to have this third species in our laboratory in the near future. Three separate attempts in our laboratory to establish P. ovale in this species of monkey were unsuccessful.

c) Automation of the IFA test:

Working with the scientists in the Space Division of Aerojet-General Corporation; preliminary studies on adapting the IFA test for malaria to an automatic prototype machine have been made. Preliminary study indicates that performance of the IFA test by machine may be feasible. Further studies are planned.

Current Program Goals:

Current studies are to evaluate the duration of the IFA response in returning Vietnam volunteers infected and cured of their malaria and in individuals who have, until coming to the United States for studies, lived in holoendemic areas all of their lives. Two projects to follow students from West Africa at the University of Pittsburg and Harvard University are in progress.

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