

The Complement Fixation Reaction as a Diagnostic Aid in Malaria

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The need for an improved method of diagnosis is recognized as one of the outstanding essentials in malaria. There is no disease more readily diagnosed than malaria during the acute stage when there are circulating parasites, but unfortunately this stage lasts a very short time. The chief difficulty is encountered in the chronic stage of the disease when the clinical symptoms are vague and when it is practically impossible to detect the parasites in the blood smears. The belief that chronic infections may persist for many years has been substantiated repeatedly by the transmission of malaria following blood transfusions from donors who had their initial attack of malaria as long as 25 years previously. Other than the recognition of the parasite in the blood smear there is no accurate diagnostic procedure, and the negative blood smear has little value in this disease.

The complement fixation reaction as used in many diseases has been tried in malaria with little success. The chief difficulty was the lack of a satisfactory source of parasites for an antigen. Since it is not possible artificially to cultivate the malaria parasite, early investigators had to depend upon infected whole blood or placentas from infected mothers for their supply of parasites. Their results demonstrated important possibilities for the test, but the lack of a standard satisfactory antigen prohibited its trial as a diagnostic aid.

In our laboratories it has been discovered that a malaria infection highly virulent for the rhesus monkey furnishes abundant quantities of antigen that binds complement in the serum of patients with chronic infections of either vivax or falciparum malaria (1, 2). The test is specific, and the technique is essentially the same as that of the ordinary Wassermann test.

Antigen. The antigen was made from parasitized red cells obtained from rhesus monkeys after infection with *Plasmodium knowlesi*, a natural parasite of cynomolgus monkeys which has a similar morphological appearance to some of the human strains of malaria. This parasite produces an overwhelming infection, and the blood is obtained shortly before the expected death of the animal when approximately 50 per cent of the red cells are infected. The red cells are then washed free from the serum and preserved by freezing and drying in 5 cc. amounts.

Method of Performing the Test. The stored antigen was rehydrated and diluted 1:100 in normal saline. This was well beyond the anti-complementary range and yet highly antigenic. The tests were set up like the ordinary Wassermann test, employing 2 units of complement with

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a sheep cell system. The experimental sera in this study were obtained from patients with induced vivax and falciparum malaria induced for therapeutic purposes, and the controls were sera from normal individuals, from patients with strongly positive Wassermann reactions, and a large number of sera from patients with different infectious diseases as lobar pneumonia, yellow fever, lymphocytic choriomeningitis, etc. Antigen controls were normal monkey red cells.

Specificity of the Test. It was found that the serum of patients with vivax and falciparum malaria would fix complement with the monkey parasite antigen in approximately the same dilutions as the serum from patients with *P. knowlesi* malaria, which was administered for the treatment of general paresis. This was an indication that the antigen was broad in its antigenic power, a useful characteristic because it is readily obtained and easily prepared yet just as capable of reacting with heterologous as with homologous malaria serum. That the positive reactions were specific and caused by the malaria infections alone was proved by the fact that negative tests before the acute attack became positive during convalescence. The serum of individuals with positive Wassermann tests was negative in the absence of malaria and positive only when a malaria infection was present. The sera obtained from patients with acute infectious diseases or from animals with blood stream protozoal infections such as trypanosomiasis or piroplasmosis gave negative tests.

Relationship of the Complement Fixation Test to Presence of Circulating Parasites. As mentioned earlier, the usefulness of this test as a diagnostic procedure depended entirely upon its being positive when it was not possible to detect parasites in stained thick or thin blood smears. Accordingly, serum was obtained from a group of twelve patients with general paresis, and they were inoculated with vivax malaria. They were then bled at 10-day intervals, and in addition their blood was examined daily for parasites. After parasites were no longer found serum specimens were obtained until the malaria complement fixation test became negative. The results of this investigation revealed that complement-fixing antibodies appeared in the serum shortly before the peak of the malaria infection or about 2 weeks after the onset of clinical symptoms and persisted for approximately 5 months after the disappearance of circulating parasites. This finding is given added significance when it is realized that the particular strain of malaria plasmodium employed in this study does not produce a long, drawn out chronic infection as is the characteristic behavior of many other strains of the same organism. Presumably serum obtained from individuals in areas of endemic malaria where chronic infections are prevalent should yield positive results for even longer periods of time than was obtained in the case of the mild therapeutic infections.

Summary

A complement fixation test has been devised as an aid to the diagnosis of chronic malaria infections. The antigen for this test is prepared from red cells infected with *Plasmodium knowlesi*, a monkey malaria

parasite which will fix complement in human malaria serum. The test is specific for malaria and has not been found to be influenced by the presence of luetic complement-fixing antibodies. In induced therapeutic malaria infections the test becomes positive about the second week of infection and persists for approximately 5 months after it is no longer possible to detect parasites in the blood smears. The final evaluation must come from studies in the field where malaria is endemic, although the experimental studies thus far suggest that the test may have considerable merit.

Bibliography

1. Coggeshall, L. T., and Eaton, Monroe D., 1938. *J. Exp. Med.*, **67**:871.
2. Eaton, Monroe D., and Coggeshall, L. T., 1939. *J. Exp. Med.*, **69**:379.