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Agreement between microscopical diagnosis and indirect fluorescent-antibody tests in Plasmodium vivax and Plasmodium falciparum infections

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9. ABSTRACT

A description of a study that compared the results of identifying two species of malaria in infected humans by two different means. One means was the indirect fluorescent antibody (IFA) test. The other means was by microscopic diagnosis of blood films. The two methods were applied to three groups of humans infected by malaria. One group consisted of 120 persons infected with Plasmodium vivax. The second group consisted of 39 persons infected by Plasmodium falciparum. The third group consisted of 79 servicemen who had attacks of vivax or falciparum malaria after returning from Vietnam. The IFA test correctly identified the parasite species in 76.7% of the 159 persons in the first two groups. The basis for species identification was a fourfold or greater difference in titers. In application of the IFA test to the group of 79 servicemen, correct identification of malaria species was obtained in 81% of the cases when the test was made 0 to 14 days after the onset of symptoms, and in 96.2% of the cases when the test was made from 15 to 60 days after the onset of symptoms. The IFA test was unreliable for determining species when tests were made of serum samples taken six months after the onset of symptoms.

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AGREEMENT BETWEEN MICROSCOPICAL DIAGNOSIS AND INDIRECT FLUORESCENT-ANTIBODY TESTS IN *PLASMODIUM VIVAX* AND *PLASMODIUM FALCIPARUM* INFECTIONS*

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ABSTRACT: Results of tests by the indirect fluorescent-antibody (IFA) technique of serum samples were compared with results from microscopical diagnosis of blood films from two groups of patients with malaria: 159 persons infected with *Plasmodium vivax* (120 cases) or *Plasmodium falciparum* (39 cases); and from 79 servicemen who had attacks of vivax or falciparum malaria after returning from Vietnam. In the 159 persons, the parasite species was correctly identified by the IFA test in 76.7%, fourfold or greater differences in titer being used as a basis for speciation, as compared to identification of species by blood film examination. In the second group, the same criteria were used and correct identification was obtained in 81% of the cases from 0 to 14 days after the onset of symptoms, and in 96.2% from 15 to 60 days after the onset of symptoms. The IFA test was unreliable for speciation in tests of serum samples taken 6 months after the onset of symptoms.

(Accepted 3 June 1970)

Since the beginning of the war in Vietnam, the number of slides submitted to the National Communicable Disease Center for diagnosis or confirmation of malaria has increased markedly. In 1960, only 38 slides were received from 27 cases of suspected malaria; of these, 16 cases were positive. By 1967 the number of slides submitted had increased to about 5,000 from 2,252 cases, of which 2,130 were positive. As servicemen return from endemic areas and discontinue prophylaxis, recrudescence and relapse occur. Often, when therapy is begun before the infecting parasite has been identified, blood films taken later in the course of the disease may prove impossible to speciate because medication causes marked morphological changes in the parasites or reduces the parasite level below detection. In addition, chloroquine-resistant *Plasmodium falciparum* has been reported from different areas of the world, and blood transfusions have increased the incidence of malaria during the past 2 or 3 years. A method for detecting the species of malaria in donors by a serological test would be particularly useful since blood-film examinations are usually very time consuming due to the scarcity or absence of parasites in healthy carriers. Although slide examination remains the method of choice in the identification of malaria, dependable serodiagnostic tests may often be a valuable aid.

Many workers have used fluorescent antibody (FA) techniques to study the rise and fall of antibody to malaria,[2-5] cross-reactions between species,[6-8] effects of therapy on antibody levels,[9] and determinations of antibody levels in populations of endemic areas.[9-14] Because of the sharp rise in requests for diagnostic assistance in the identification of species of *Plasmodium*, the use of fluorescent antibody tests is of particular interest in our laboratories.

With the establishment of *P. falciparum* and *Plasmodium vivax* infections in *Aotus* monkeys, specific antigens became available for studies on the sensitivity, specificity, and reproducibility of fluorescent antibody tests. We report here the results of a study of slide-positive malaria cases in relation to identification of the species of the infecting parasite by the IFA test.

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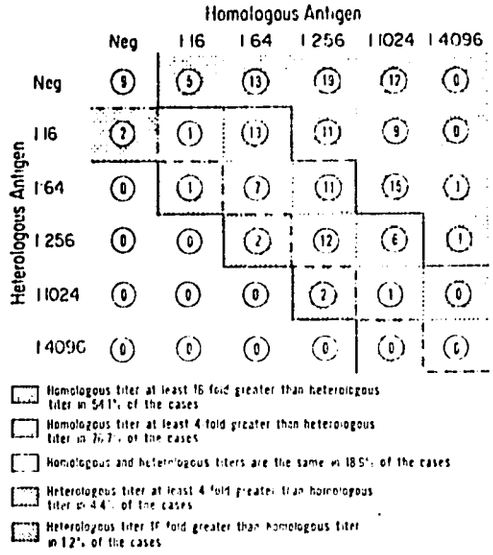

FIGURE 1. Diagram of levels of titer of indirect fluorescent antibody in single specimens of serum from 39 cases of infection with *P. falciparum* and 120 cases of infection with *P. vivax*, identified microscopically.

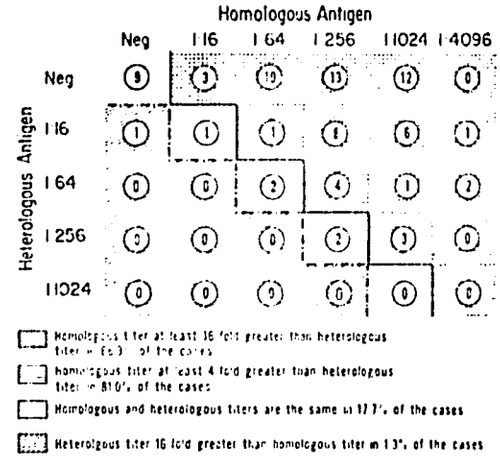

FIGURE 2. Diagram of levels of titer of indirect fluorescent antibody in multiple specimens of serum from 70 cases of infection with *P. vivax* and 9 cases of infection with *P. falciparum*, identified microscopically. Serum specimens were drawn 0 to 14 days after onset of clinical symptoms.

MATERIALS AND METHODS

Two groups of patients with slide-proven malaria were evaluated: 1) 159 patients (mostly military personnel) from whom single samples of serum were collected, and 2) 79 servicemen at Fort Bragg, North Carolina, from whom thick and thin films and two or more samples of serum were received. In the latter group, samples were collected on the day of admission to hospital, the day of discharge, and, when possible, about 6 months after onset of the disease.

Blood films from each patient were submitted to the National Malaria Repository where species identification was made. Only thin films were obtained from about 50% of the first group. Poorly stained or unstained films were stained with the standard Giemsa technique.

The washed-cell, thick-smear indirect fluorescent antibody (IFA) technique used in this study was described by Sulzer and Wilson in 1967[12] and further evaluated by Sulzer et al.[13] in 1969. The use of washed-cell, thick-smear antigens of *P. vivax* and *P. falciparum* with 15 or more parasites per field, and the omission of the acetone and hydrochloric acid wash before the application of the serum dilution to the antigen slide, are departures from previously described reports. (For details of the current test, the reader is referred to either of the cited papers.) Each serum sample was tested with homologous and heterologous antigens containing an appreciable percentage of schizonts, and the optimum difference in titer level was determined.

RESULTS

Figure 1 shows the comparison of titers with homologous and heterologous antigens among the group of men from whom single serum samples were received. As determined by slide examination, 120 of the patients were infected with *P. vivax* and 39 with *P. falciparum*. Nine serum samples were negative, and 21 (18.9%) of the samples reacted at the same titer levels with both antigens. Using a 16-fold, or greater, titer difference between homologous and heterologous antigens as the criterion for species identification, 65.1% of the cases were correctly diagnosed, and 1.2% were misdiagnosed, i.e., the heterologous antigen gave a higher titer. However, using a fourfold, or greater, titer difference between antigens, 76.6% of the cases were correctly identified and only 4.4% were misdiagnosed.

Figures 2 and 3 present the IFA titers of serum samples from 79 servicemen who had been stationed in Vietnam for a year. Therapy was started

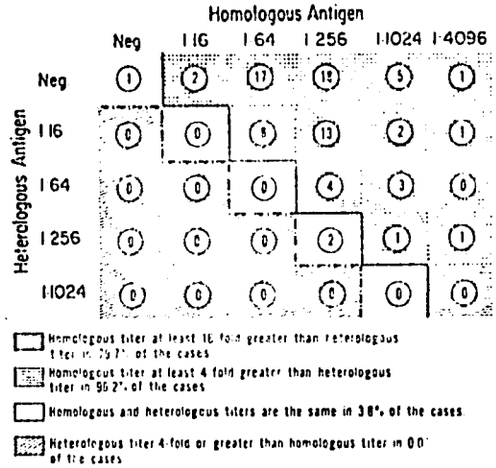

FIGURE 3. Diagram of levels of titer of indirect fluorescent antibody in multiple specimens of serum from 70 cases of infection with *P. vivax* and 9 cases of infection with *P. falciparum*, identified microscopically. Serum specimens were drawn 15 to 60 days after onset of clinical symptoms.

immediately at onset of symptoms in this group from whom two or more serum samples were obtained over a period of 0 to 230 days. Examination of blood films indicated 70 cases of *P. vivax* and 9 cases of *P. falciparum*.

Figure 2 shows the results when serum samples drawn 0 to 14 days (mean 3.7) after onset of symptoms were tested with both homologous and heterologous antigens. Although there is 81% agreement with the results of slide diagnosis at the difference of fourfold titer levels, no increase in the number of erroneous determinations over the 16-fold titer level is noted. Again, in about 18% of the samples, the species could not be determined by IFA tests.

The best serological agreement between results of slide diagnosis and titers of serum samples occurred with serum drawn 15 to 60 days (mean 22) after onset of symptoms (Fig. 3). In only 3.8% (3 serum samples) could the infecting species not be determined. Titers with the homologous antigen were at least fourfold greater than those with the heterologous antigen in 96.2% of the cases, with no erroneous determination.

Serum samples from 52 of these men were drawn 6 months after onset of symptoms and again tested. No detectable antibody was found in 52%, and the remaining samples reacted only at low-titer levels. At the fourfold titer-level difference between homologous and heterologous antigens, only 40.4% of the cases could be speciated by IFA tests, and 3.8% were misdiagnosed.

DISCUSSION

The data presented above indicate that determination of the infecting *Plasmodium* species by the indirect fluorescent-antibody test is feasible. A fourfold difference in titer levels between homologous and heterologous antigens accurately indicated the infecting species in at least 80% of the serum samples tested, whereas less than 5% of the samples are misdiagnosed. Best results, as shown in Figures 2 and 3, were found when samples were obtained about 3 weeks after onset of clinical symptoms.

With a small group of volunteers infected with vivax malaria, Lunn et al.[3] have shown that if parasitemia is completely suppressed by chemoprophylaxis at the beginning of the infection, malaria antibody cannot be detected. However, after treatment is stopped and the parasitemia is allowed to rise, antibody becomes detectable within a few days. Other workers have also observed the time lapse between onset of parasitemia and antibody detection.[2,4]

This finding may account for the negative results observed in the serum samples drawn 0 to 14 days after onset of symptoms. (Figure 2, as compared with those drawn 15 to 60 days after onset shown in Figure 3.)

In some instances, the titer level of the heterologous antigen was the same or greater than that of the homologous antigen. These may be accounted for by residual effects of previous attacks of another species since about half of the samples were from military personnel with histories of previous malaria illness while in Vietnam.

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