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Wilson, Marianna; Sulzer, A.J.; Rogers, W.A.; Fried, J.A.; Mathews, H.M.

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Titers of 226 serum specimens tested by indirect-flourescent antibody (IFA) and indirect-hemagglutination (IHA) tests for malaria were compared. Sixty-eight U.S. Army veterans who experienced clinical attacks of Plasmodium vivax malaria at Ft. Bragg, North Carolina, were promptly given radical curative treatment. Serum specimens were drawn from these men on four occasions: at diagnosis and onset of treatment, and at 3 weeks, 7 months, and 12 months after the onset of symptoms. For the first two time categories, the IFA and IHA titers were similar for 79.3% and 92.1% of the sera, respectively. In the 7-month and 12-month categories, however, the IHA titers were higher and present for longer periods than the IFA titers. IHA titers from men who had experienced previous attacks of malaria were significantly higher than those from men who had had no previous attacks. The IFA-test titers did not show this difference.

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MARIANNA WILSON, A. J. SULZER, W. A. ROGERS, JR., J. A. FRIED, AND H. M. MATHEWS

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COMPARISON OF THE INDIRECT-FLUORESCENT ANTIBODY AND INDIRECT-HEMAGGLUTINATION TESTS FOR MALARIAL ANTIBODY*

MARIANNA WILSON,† A. J. SULZER,† W. A. ROGERS, JR.,‡
J. A. FRIED,† AND H. M. MATHEWS†

Parasitology Section, National Communicable Disease Center, Health Services and Mental Health Administration, Public Health Service, U.S. Department of Health, Education, and Welfare, Atlanta, Georgia 30333

ABSTRACT: Titers of 226 serum specimens tested by indirect-fluorescent antibody (IFA) and indirect-hemagglutination (IHA) tests for malaria were compared. Sixty-eight U.S. Army veterans who experienced clinical attacks of *Plasmodium vivax* malaria at Ft. Bragg, North Carolina, were promptly given radical curative treatment. Serum specimens were drawn from these men on four occasions: at diagnosis and onset of treatment, and at 3 weeks, 7 months, and 12 months after the onset of symptoms. For the first two time categories, the IFA and IHA titers were similar for 79.3% and 92.1% of the sera, respectively. In the 7-month and 12-month categories, however, the IHA titers were higher and present for longer periods than the IFA titers. IHA titers from men who had experienced previous attacks of malaria were significantly higher than those from men who had had no previous attacks. The IFA-test titers did not show this difference.

(Accepted 6 July 1970)

The indirect-fluorescent antibody (IFA) and the indirect-hemagglutination (IHA) tests have been excellent for detecting malaria antibody in human serum.[1-7] Wellde et al. tested the time-course development of antibodies in rats infected with *Plasmodium berghei* by IHA, IFA, and the soluble-antigen fluorescent-antibody test (SAFA).[8] They reported that IFA and SAFA titers were closely parallel, but that titers of serum from four rats tested by IHA followed a bimodal curve. Sadun et al., using a *Plasmodium falciparum* lysate antigen, compared the results of IHA and SAFA tests on serum from human volunteers with induced infections of *Plasmodium vivax* or *P. falciparum*.[9] They found that SAFA and IHA antibody curves followed almost parallel lines.

On numerous occasions, serodiagnosis of malaria has been performed with both the IFA and IHA methods in our laboratories. Generally, the results were comparable. However, some differences were also observed. We therefore planned a comparison of the IHA and IFA tests to define more definitely their relative diagnostic efficiency.

MATERIALS AND METHODS

A battery of 226 serum specimens was collected from 68 U.S. Army soldiers, veterans from Vietnam. After reporting to Ft. Bragg, North Carolina, these men experienced clinical attacks of malaria. After examination of thick and thin blood films at Womack Army Hospital, Ft. Bragg, the infecting species was identified as *P. vivax*, and the identification was confirmed by the National Malaria Repository, Center for Disease Control, Atlanta, Georgia. Serum samples were obtained from the patients on four occasions: 1) 0 to 14 days (mean, 4 days) after onset of symptoms; 2) 15 to 60 days (mean, 22 days) after onset of symptoms; 3) about 7 months after onset of symptoms; and 4) about 1 year after onset of symptoms. For 33 patients, this was the first experience with malaria; 35 had had one or more previous attacks in Vietnam. All patients received supervised, radical, curative therapy, begun the day the first serum sample was drawn. None had a clinical relapse during the year after onset of symptoms.

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† Parasitology Section, National Communicable Disease Center, 1600 Clifton Road, Atlanta, Georgia 30333.

‡ Department of Pathology and Oncology, College of Medicine, University of Vermont, Medical Alumni Building, Burlington, Vermont 05401.

TABLE 1

Comparison between antimalarial positive and negative IFA and IHA tests in sera after the onset of symptoms of Plasmodium vivax *infection*[a]

Interval since onset of symptoms	IFA + IHA +	IFA − IHA −	IFA + IHA −	IFA − IHA +	Sera (No.)
0–14 days	46 (79.3)	4 (6.9)	6 (10.3)	2 (3.5)	58
15–60 days	58 (92.1)	1 (1.6)	4 (6.3)	0 (0)	63
7 months	27 (40.3)	8 (11.9)	3 (4.5)	29 (43.3)	67
12 months	12 (31.6)	9 (23.7)	0 (0)	17 (44.7)	38

[a] Percents are shown in parentheses.

The indirect fluorescent-antibody test procedure, using thick-smear antigens of *P. vivax*, was that of Sulzer et al.[6] The indirect-hemagglutination test was performed with a lysate of *Plasmodium knowlesi*, as described by Rogers et al.[7]

RESULTS

Table 1 shows the detection rates of the IFA and IHA tests for malarial antibody. Agreement was best in the first two groups, reaching 93.7%, between 15 to 60 days (3 weeks) after onset of illness. The titers of serum drawn 7 and 12 months after onset show some disagreement particularly with serum specimens that were IHA positive but IFA negative. Antibody is apparently detected for a longer time by the IHA test than by the IFA test.

Titer levels for the two tests ranged as high as 1:4,096 in the first two categories and decreased progressively thereafter (Table 2). The IFA test detected antibody slightly more efficiently than the IHA test during the initial 2 weeks after onset of symptoms, as shown by the geometric mean reciprocal titers (GMRT). However, the IHA test began to detect antibody at somewhat higher levels than the IFA test 3 weeks or more after onset. This trend becomes more apparent at 7 months and continues through the observation period of 1 year.

To determine whether antibody levels are reinforced by multiple attacks of malaria, we divided the men into two subgroups, those who had experienced one or more attacks of malaria previously, and those who had never experienced a clinical episode before the present one. Figure 1 shows the GMRT results. In patients who had never had a symptomatic malaria attack, the IFA test is more efficient for detecting the initial antibody response. However, the IHA test detects significantly higher antibody levels (χ^2 test, $P < .05$) in patients who have had previous experience with malaria; this trend continues throughout the 1-year observation period. This difference was not observed in the IFA test.

TABLE 2

Comparison between antimalarial titer levels of the IFA and IHA tests in 226 serum specimens from 68 Vietnam returnees after onset of symptoms of P. vivax *infection*

Interval since onset of symptoms	Test performed	Number of sera with endpoint titers of: Neg.	1:16	1:32	1:64	1:128	1:256	1:512	1:1,024	1:2,048	1:4,096	Total	GMRT
0–14 days	IFA	6	3		10		20		16		3	58	191
	IHA	10	1	2	5	4	6	12	11	6	1	58	163
15–60 days	IFA	1	3		21		27		9		2	63	176
	IHA	5	1	2	8	6	14	11	5	7	4	63	232
7 months	IFA	37	25		4		1		0		0	67	8.5
	IHA	11	7	11	16	11	7	3	1	0		67	41
12 months	IFA	30	8		3		0					41	6.4
	IHA	9	3	10	9	4	1	0				36	22

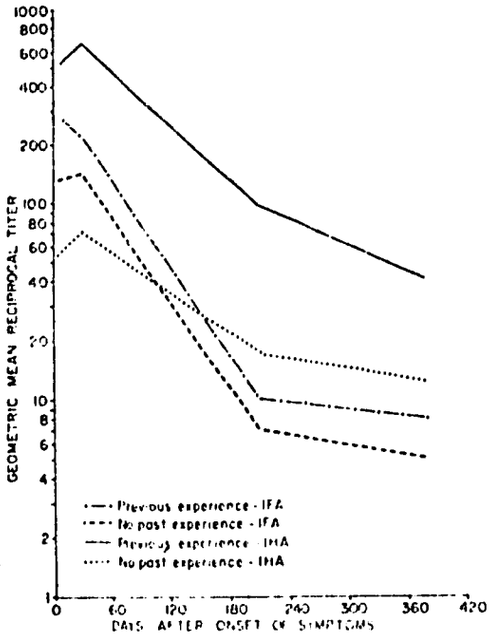

FIGURE 1 Geometric mean reciprocal titers for IFA and IHA tests for malarial antibody in serum from 68 Vietnam veterans after the onset of symptoms of *P. vivax* infections.

DISCUSSION

In the first 2 months after acute infection and radical cure, the IFA and IHA tests generally detect malaria antibody at equivalent rates. Although the IFA test detects antibody in a few more sera, the small percentage of positive reactions not detected by the IHA is compensated by the ease and rapidity with which one can test hundreds of samples by IHA in mass surveys. For diagnosing individual cases in the first 2 months, however, the IFA test is preferred. In a few instances, neither test will detect malaria antibody from patients with slide-diagnosed malaria. The four serum specimens in the 0- to 14-day category, undetected by both tests, may have been drawn too early in the attack, before antibody had reached a detectable level. A serum sample in the 15- to 60-day category undetected by both tests, was from a patient treated immediately after onset of parasitemia, perhaps before antibody had reached a detectable level.

Differences between the tests become apparent several months after the last clinical attack and treatment. The IHA test is more efficient than the IFA test in detecting malarial antibody 7 months or more after an acute episode. If the patients have had previous clinical attacks, the IHA test detects antibody at much higher levels and for longer periods than the IFA. In contrast, however, if the patient does not report previous experience with malaria, the IFA test is a more reliable indicator of recent attack because it detects antibody at higher levels and earlier in the disease than the IHA test in such cases.

Although the reasons for the differences observed in both tests are unknown, one can speculate on the causes.

The IFA test occasionally tends to detect antibody earlier in the disease than the IHA. Gleason et al. have reported that the initial antibody measured by the IFA test is highly specific for the current infecting species of malaria parasite with little cross-reaction with the other species.[10] One would expect the IFA test to detect the specific antibody more readily than the IHA test because the antigen is derived from the homologous *P. vivax* species in the IFA test, but from a soluble lysate of the heterologous *P. knowlesi* in the IHA test. This tendency could also be due, in part, to the type of antibody. Perhaps nonprecipitating antibody is produced initially, which could be detected by IFA but not by IHA.[11] Nonprecipitating antibody might also participate in the IHA test, in the presence of precipitating antibody, to inhibit or block the reaction.[12] Detection of different antibodies by the two tests may also account for enhancement of IHA test-titer levels in patients with previous experience with malaria. The antibodies most active in the IFA test are probably directed toward antigenic sites on the exterior of the intact, particulate parasite. Those most active in the IHA test may be directed toward soluble interior and exterior antigens of the parasite or its metabolic products. If autoantibody to erythrocytes or erythrocyte-malaria complexes exists, as suggested by several authors,[13-15] it might also play a part in the enhancement of titers seen in the IHA test. This antibody, if developed during previous attacks of malaria, might be detected in the IHA test because of its soluble-antigen human erythrocyte-carrier complex, but not to the same extent in the IFA test. We have occasionally seen fluorescence of the erythrocyte stroma, but it is easily differentiated from the specific parasite fluorescence.

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