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9. ABSTRACT

The blood serum of 67 soldiers returned from Vietnam with vivax malaria was examined for up to one year after onset of clinical symptoms of the disease. The test used was the indirect fluorescence test for malaria antibodies (IFA). Over the entire period, the antibody response was similar in patients with and without a history of previous malaria attacks. Among those with a previous history of attacks, the antibody response was not altered by the number of previous attacks. Nor was it altered by the time interval since their occurrence. Among the small number of patients who later had relapse, the post-treatment, pre-relapse titers of antibodies were not significantly different from those of radically cured patients. In vivax malaria, the IFA titer against Plasmodium vivax antigen usually exceeds that against Plasmodium falciparum. However, the reverse was true early in the clinical course of one patient with a history of two previous attacks of falciparum malaria.

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FLUORESCENT-ANTIBODY PATTERNS IN NATURALLY
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FLUORESCENT-ANTIBODY PATTERNS IN NATURALLY ACQUIRED VIVAX MALARIA*

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ABSTRACT: The serum of soldiers returned from Vietnam with vivax malaria was examined for up to 1 year after clinical onset by the indirect fluorescence test for malaria antibodies (IFA). Over the entire period, the antibody response was similar in patients with and without a history of previous malaria attacks, and among the former was not altered by the number of previous attacks nor by the time interval since their occurrence. Among the small number of patients who later had relapse, the post-treatment, pre-relapse titers of antibodies were not significantly different from those of radically cured patients. In vivax malaria, the IFA titer against *Plasmodium vivax* antigen usually exceeded that against *Plasmodium falciparum*; however, the reverse was true early in the clinical course of one patient with a history of two previous attacks of falciparum malaria.

(Accepted 11 July 1969)

The detection of malaria antibodies by the indirect fluorescence technique, with a thick-smear antigen, has been developed by Sulzer and his co-workers¹ and shown to be highly acceptable in terms of specificity, sensitivity, and reproducibility. Gleason and others have shown that the indirect fluorescent antibody (IFA) technique is effective in determining the species of *Plasmodium* causing clinical malaria.² Wilson *et al.* have also demonstrated that in soldiers returned from Vietnam with malaria caused by *Plasmodium vivax*, IFA titers generally reach their highest level within 60 days after the onset of illness and decline thereafter, so that at 12 months after cure, the serum of 59% of patients is negative, of 33% positive at the lowest titer only (1:16), and that of the remaining 8% is positive at 1:64.³

In an attempt to determine if an analysis of clinical data from soldiers returned from Vietnam with vivax malaria would identify subgroups with differing IFA responses, we designed a study to answer the following questions: 1) Is the IFA response of vivax patients who have had previous malaria attacks (any species) different from the IFA response of patients with vivax malaria who have not had previous attacks? 2) In patients with vivax malaria who have a history of previous malaria attacks, is the IFA response influenced by

the number of previous attacks or by the time interval since the last attack? 3) In patients who have received radical-cure treatment for vivax malaria, is the IFA test helpful in distinguishing the cured patients from those destined to relapse? and 4) Is determination of vivax malaria with the IFA technique influenced by the patient's malaria history?

MATERIALS AND METHODS

Sixty-seven patients were included in this study; all were American soldiers hospitalized at Womack Army Hospital, Ft. Bragg, North Carolina, for treatment of vivax malaria acquired in South Vietnam. The diagnosis in each case was based upon the identification of *P. vivax* parasites in peripheral-blood films; all positive films were verified by the National Malaria Repository, NCDC. At the time of hospitalization, each patient was interviewed, and his available health records were reviewed to determine the date of onset of illness and previous malaria experience, *i.e.*, the number of previous malaria attacks for which he had been hospitalized and treated, the dates of these attacks, and, when known, the causative *Plasmodium* species. Serum samples were obtained from each patient at the time of hospitalization and when possible, at the time of discharge from hospital, and at intervals ranging up to 1 year thereafter. Patients were discharged from the hospital after treatment with chloroquine (1.5 g of base administered over a

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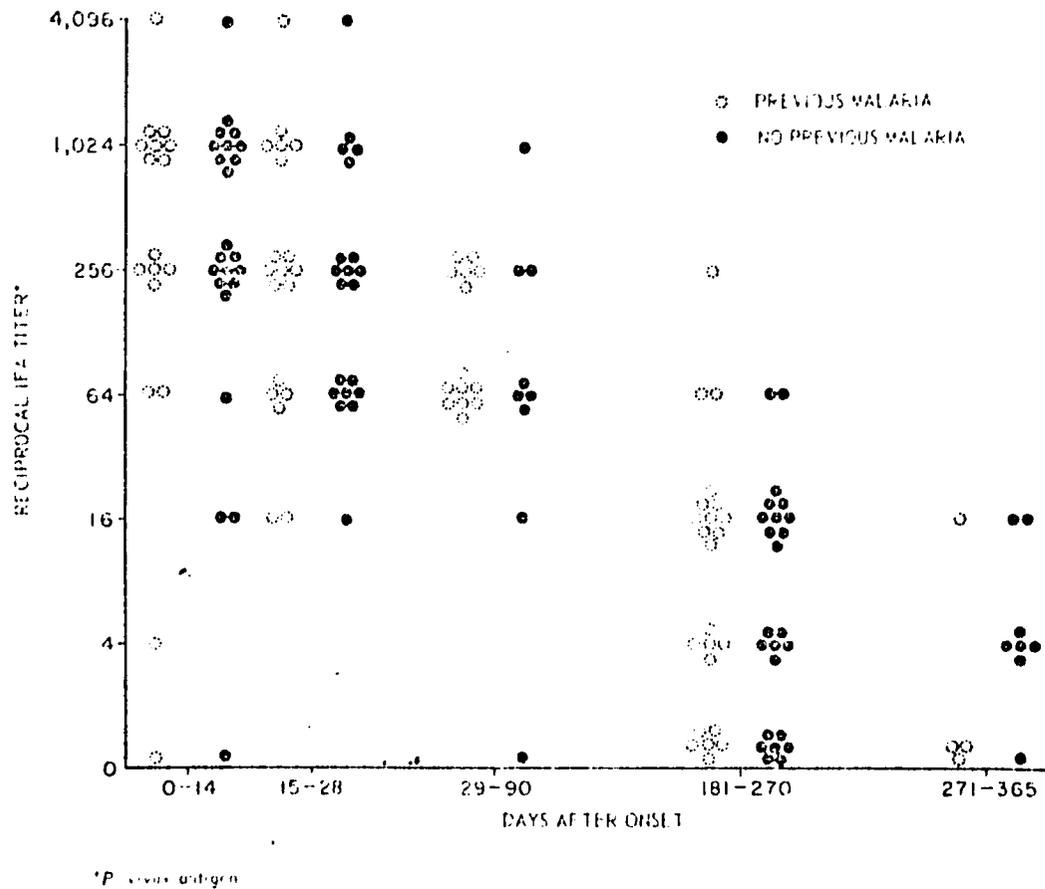


FIGURE 1. Schema of IFA response in 47 cases of vivax malaria followed for 6 or more months by previous malaria experience and time after onset of illness at Ft. Bragg.

3-day period) and primaquine (15 mg of base daily for 14 days).

After discharge from the hospital, all patients were followed by the Malaria Surveillance Unit, NCDC, to identify those who experienced post-treatment attacks of malaria; this clinical follow-up period ranged from 9 to 15 months; patients who did not experience malaria attacks during this period were considered cured.

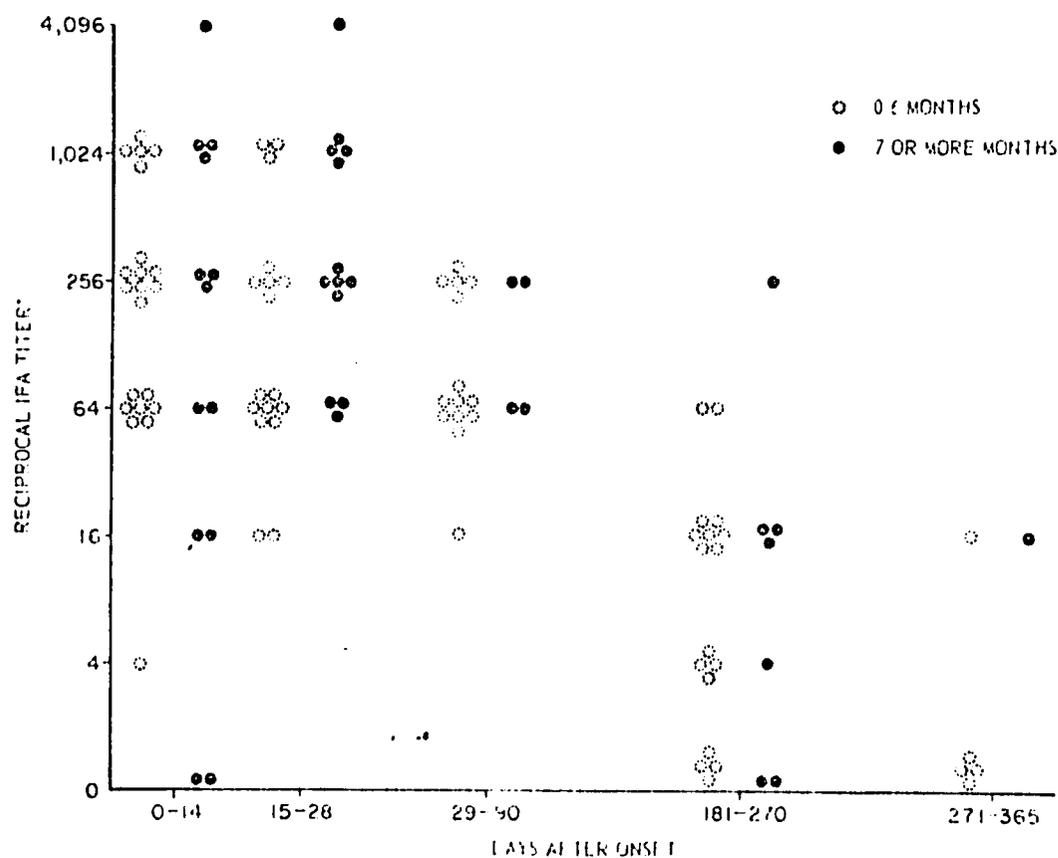
All serum specimens were analyzed for malarial antibodies by the indirect fluorescence technique of Sulzer *et al.*¹ *P. vivax* and *Plasmodium falciparum* antigens being used. With this test, a titer of 1:16 or greater is considered positive.

RESULTS

Of the 67 patients studied, 47 were tested serologically for 6 to 12 months after the onset of their attack of vivax malaria at Ft. Bragg.

Twenty-three of the 47 gave a history of previous malaria attacks, and 24 did not. All 47 were cured of malaria. Over the entire course of serologic observation, the distribution of IFA titers in serum obtained from the 23 patients who had experienced previous attacks was not significantly different from the distribution of titers in serum obtained from the 24 patients who had not experienced previous attacks (Fig. 1).

In addition to the 23 patients who had previously had malaria and were tested serologically for 6 or more months, there were 14 patients who had previously had malaria and were tested serologically for less than 6 months. Four of these 14 patients experienced two attacks of vivax malaria at Ft. Bragg, and a fifth patient experienced three attacks; all six of these relapses were studied serologically. Thus, a total of 43 cases of vivax malaria with a history of previous



**P. vivax* antigen

FIGURE 2.—IFA response in patients with vivax malaria who had had previous malaria, plotted by interval since last attack and time after onset of illness at Ft. Bragg.

malaria were followed with the IFA malaria antibody test. Of the 43, the 26 who had experienced a malaria attack within the preceding 6 months had IFA responses similar to those of the 17 whose previous attacks had occurred 7 or more months previously (Fig. 2). In addition, the 31 who had experienced one or two previous malaria attacks had IFA responses similar to those of the 12 with three or four previous attacks (Fig. 3).

Of the 67 patients studied, nine experienced a relapse of vivax malaria during the 9- to 15-month period of clinical follow-up. Eleven post-treatment, pre-relapse serum samples had been obtained from these nine patients an average of 12.5 weeks before relapse (range 2 to 30 weeks), and their IFA titers did not differ substantially from the titers observed in the 58 cured patients (Fig. 4).

Fourteen of the patients had experienced previous malaria attacks due to only one *Plasmodium* species; in nine, the previous species was *P. vivax* and in five, *Plasmodium falciparum*. After these 14 patients had had an attack of vivax malaria at Ft. Bragg, their IFA titer against *P. vivax* was greater than or equal to their titer against *P. falciparum* in 32 of 33 samples tested over a period of serologic follow-up ranging from 0 to 365 days (Table 1). The only exception was a patient who had experienced attacks of falciparum malaria 4 months and 6 months before his vivax attack at Ft. Bragg; serum obtained from this man 3 days after onset had an IFA titer that was greater against *P. falciparum* than against *P. vivax* (1:16 vs. 1:4). However, 14 days later, titers to both had risen to 1:256.

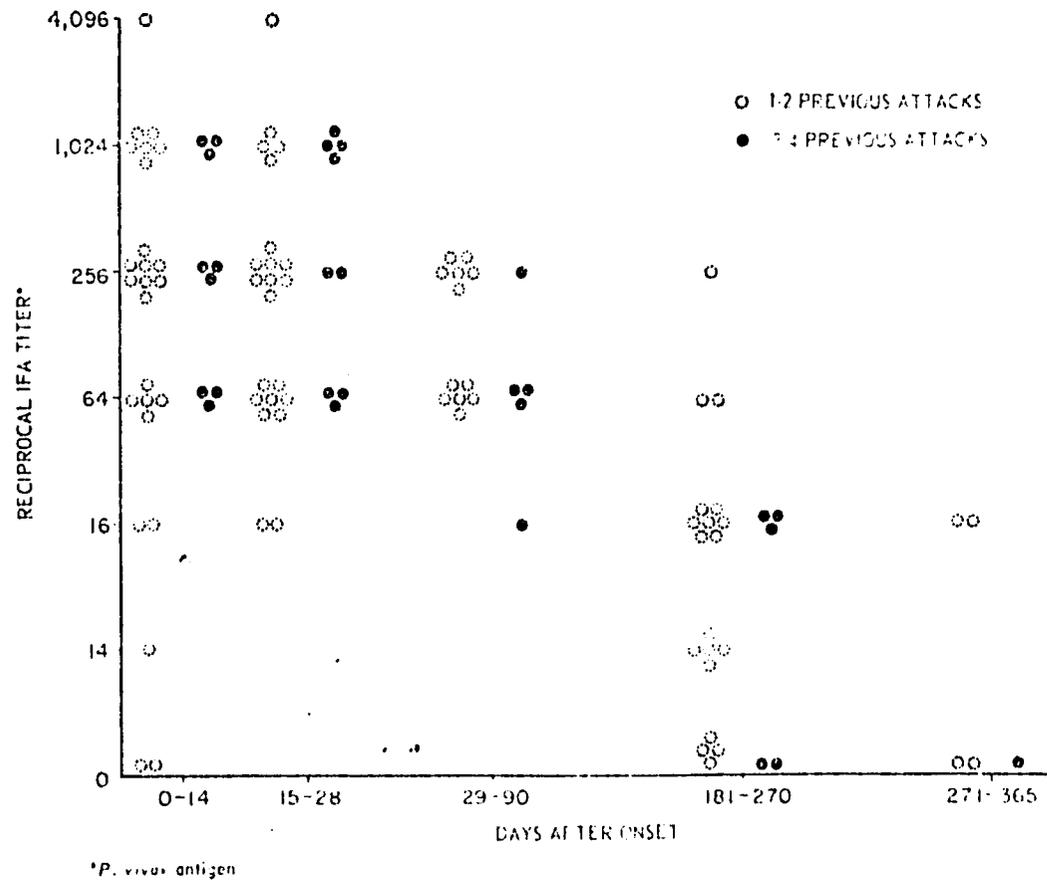


FIGURE 3. IFA response in patients with vivax malaria who had had previous malaria, plotted by number of previous attacks and time after onset of illness at Ft. Bragg.

DISCUSSION

The patients included in our study probably had not acquired significant protective immunity against malaria, since their exposure in the endemic area was of limited duration (generally 12 months), and the possibility of repeated or prolonged episodes of parasitemia was reduced both by the widespread use of suppressive medication and by the prompt administration of therapy when clinical attacks did occur. Our data are thus obtained from a relatively nonimmune population and, as demonstrated by Collins and his co-workers,¹ may not be applicable to patients with significant acquired immunity, e.g. persons who have experienced repeated malaria attacks with prolonged episodes of parasitemia.

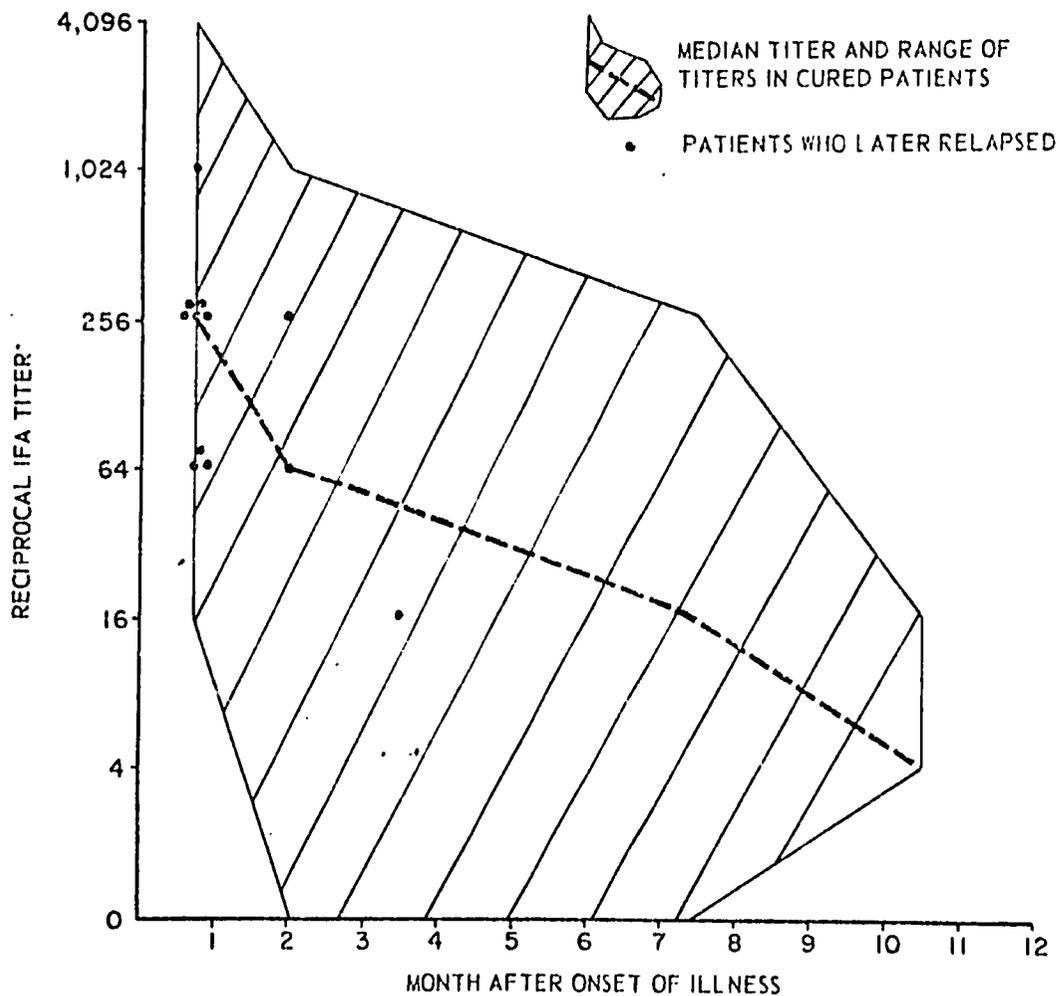
Our data indicate that the IFA response of soldiers returning from Vietnam who have clinical

vivax malaria is not influenced by the patient's previous malaria experience, either in absolute or relative terms. It thus appears that use of the IFA test as a clinical and epidemiologic tool will

TABLE 1
Accuracy of determination of species with IFA in 14 proved cases of vivax malaria

Time after onset (days)	<i>Plasmodium</i> species of previous attacks					
	<i>P. vivax</i> only			<i>P. falciparum</i> only		
	≥ 1	< 1	> 1	≥ 1	< 1	> 1
0-3	2*	1	-	2	-	1
4-14	4	-	-	3	-	-
15-28	5	-	-	2	1	-
29-180	5	-	-	1	-	-
>180	4	-	-	2	-	-

* Number of patients with IFA titer greater against *P. vivax* than against *P. falciparum*; thirty-three serum samples from 14 patients were examined.



**P. vivax* antigen

FIGURE 4. Post-treatment IFA titers in patients cured of vivax malaria compared with post-treatment IFA titers in patients who later had relapse.

not require knowledge of the patient's malaria history, provided the population under study is relatively nonimmune.

A serologic test capable of separating patients who have had vivax malaria and have been radically cured from those who are destined to relapse would find significant clinical use. Our preliminary observations suggest that this distinction cannot be made with the IFA technique when the prerelapse serum samples are obtained shortly after treatment of the initial attack, or many weeks before relapse.

The IFA technique promises to be useful in

the diagnosis and determination of the species of *Plasmodium* causing clinical malaria in patients who receive schizonticidal therapy before blood films have been obtained. Our limited data suggest that determination of vivax malaria may be difficult with serum obtained from acutely ill patients who have experienced previous malaria attacks caused by other *Plasmodium* species.

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