FLUORESCENT ANTIBODY PATTERNS IN NATURALLY-ACQUIRED VIVAX MALARIA

by

George U. Fisher, Alexander J. Sulzer, Marianna Wilson and Karel Runcik

1. Introduction

The detection of malaria antibodies by the indirect fluorescent technique, using a thick smear antigen, has been developed by Sulzer and his co-workers (1969) and shown to be highly acceptable in terms of specificity, sensitivity and reproducibility. Cleason et al. (1969) have shown that the indirect fluorescent antibody (IFA) technique is effective in the speciation of clinical malaria. Wilson et al. (1969) have also demonstrated that in expatriates returning from Viet-Nam with malaria caused by *Plasmodium vivax*, IFA titres generally reach their highest level within 60 days after the onset of illness and decline thereafter, so that at 12 months after cure, 59% of patients are serologically negative, 33% are positive at the lowest titre only (1:16) and the remaining 8% are positive at 1:64. In an attempt to determine if an analysis of clinical data from Viet-Nam returnees with vivax malaria would identify sub-groups with differing IFA responses, we designed a study to answer the following questions:

1. Is the IFA response of patients with vivax malaria 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?
4. Is speciation of vivax malaria with the IFA technique influenced by the patient’s malaria history?

2. Materials and methods

Sixty-seven patients were included in this study; all were American military personnel hospitalized at Womack Army Hospital, Ft Bragg, N.C., for treatment of vivax malaria acquired in the Republic of Viet-Nam. The diagnosis in each case was based upon the identification...
of *P. vivax* parasites in peripheral blood smears; all positive smears 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 his illness and his 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 hospital discharge and at intervals ranging up to one year thereafter. Patients were discharged from the hospital after treatment with chloroquine (1500 mg of base administered over a three-day period) and primaquine (15 mg base daily for 14 days).

Following 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 nine to 15 months; patients who did not experience malaria attacks during this period were considered cured.

All sera were analysed for malaria antibodies by the indirect fluorescent technique of Sulzer et al. (1969) using *P. vivax* and *P. falciparum* antigens. With this test, a titre of 1:16 or greater is considered positive.

3. Results

Of the 67 patients studied, 47 were followed serologically for six to 12 months after the onset of their vivax attack at Ft Bragg. Twenty-three of the 47 gave a history of previous malaria attacks and 24 did not. All 47 were cured of their malaria. Over the entire course of serological observation, the distribution of IFA titres in sera obtained from the 23 patients who had experienced previous attacks was not significantly different from the titre distribution in sera obtained from the 24 patients who had not experienced previous attacks (Fig. 1).

Similarly, the 26 patients who had experienced a malaria attack within the preceding six months had IFA responses similar to those of the 17 patients whose previous attacks had occurred seven or more months previously (Fig. 2). Finally, the 31 patients who had experienced one or two previous malaria attacks had IFA responses similar to those of the 12 patients with three or four previous attacks (Fig. 3).

Of the 67 patients studied, nine experienced a relapse of their vivax malaria during the nine to 15 month period of clinical follow-up. The post-treatment, pre-relapse IFA titres of these nine patients were obtained an average of 12.5 weeks before relapse (range two to 30 weeks) and did not differ substantially from the titres 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 *P. falciparum*. After these 14 individuals had their vivax attack at Ft Bragg, their IFA titre against *P. vivax* was greater than or equal to their titre against *P. falciparum* in 32 of 33 samples tested over a period of serological follow-up ranging from 0-365 days (Table 1). The only exception was an individual who had experienced attacks of falciparum malaria four months and six months before his vivax attack at Ft Bragg; serum obtained from this man three days after onset had an IFA titre which was greater against *P. falciparum* than against *P. vivax* (1:16 against 1:4). However, 14 days later, titres to both had risen to 1:256

4. 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 parasitaemia
was minimized both by the widespread use of suppressive medication and the prompt administration of therapy when clinical attacks did occur. Our data are thus obtained from a relatively "non-immune" population and, as demonstrated by Collins and his co-workers (1964), may not be applicable to patients with significant acquired immunity, e.g. individuals who have experienced repeated malaria attacks with prolonged episodes of parasitaemia.

Our data indicate that the IFA response of Viet-Nam returnees 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 epidemiological tool will not require knowledge of the patient's malaria history, provided the population under study is relatively "non-immune".

A serological test capable of separating vivax patients who 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 pre-relapse serum samples are obtained shortly after treatment of the initial attack and/or many weeks before relapse.

The IFA technique promises to be useful in the diagnosis and speciation of clinical malaria in patients who receive schizonticidal therapy before blood smears have been obtained. Our limited data suggest that speciation of vivax malaria may be difficult with acute sera obtained from patients who have experienced previous malaria attacks caused by other Plasmodium species.

<table>
<thead>
<tr>
<th>TABLE 1. ACCURACY OF SPECIATION WITH IFA IN 14 SLIDE-PROVEN VIVAX CASES, BY DAYS AFTER ONSET OF ILLNESS AND SPECIES OF PREVIOUS MALARIA ATTACKS</th>
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<tr>
<td>Days after onset</td>
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<td>0-3</td>
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<td>15-28</td>
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* Number of patients with IFA titre greater against P. vivax than against P. falciparum. Thirty-three serum samples examined in 14 patients.

ACKNOWLEDGEMENTS

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Résumé

Des militaires américains de retour du Viet-Nam où ils avaient été infectés par *P. vivax* ont été soumis, pendant des périodes allant jusqu'à un an après l'apparition clinique de la maladie, à une surveillance sérologique fondée sur la technique d'immunofluorescence indirecte (IFA). Pendant toute la période d'observation, la réponse en anticorps était la même chez les sujets avec ou sans antécédents d'atteinte paludéenne; chez les premiers, elle était indépendante aussi bien du nombre des atteintes antérieures que du temps s'étant écoulé depuis leur apparition. Parmi le petit nombre de malades ayant ensuite rechuté, les titres en anticorps (IFA) après le traitement et avant la rechute ne différaient pas notablement de ceux qui étaient observés chez les sujets radicalement guéris. Dans le paludisme à *P. vivax*, le titre d'anticorps (IFA) anti-*Plasmodium vivax* est habituellement supérieur au titre d'anticorps anti-*P. falciparum*. On a toutefois constaté le phénomène inverse au début de l'évolution clinique chez un sujet dont les antécédents comportaient deux atteintes de paludisme à *P. falciparum*.

REFERENCES


Gleason, N. N. et al. (1969) Serological speciation of *Plasmodium vivax* and *P. falciparum* infections by the malaria IFA test (In manuscript)


Wilson, M., Sulzer, A. J. & Runcik, K. (1969) The relationship of malaria antibody titer to parasitemia as measured by the indirect fluorescent antibody test (In manuscript)
FIGURE 1

IFA RESPONSE IN 47 VIVAX MALARIA CASES FOLLOWED FOR 6 OR MORE MONTHS, BY TIME AFTER ONSET OF ILLNESS AND PREVIOUS MALARIA EXPERIENCE

*P. vivax antigen
FIGURE 2

IFA RESPONSE IN VIVAX PATIENTS WITH MALARIA, BY TIME AFTER ONSET OF ILLNESS AND INTERVAL SINCE LAST ATTACK

RECRIPROCAL IFA TITRE*

0-6 MONTHS
7 OR MORE MONTHS

DAYS AFTER ONSET

0
1-14
15-28
29-90
181-270
271-365

*P. vivax antigen
FIGURE 3
IFA RESPONSE IN VIVAX PATIENTS WITH PREVIOUS MALARIA, BY TIME AFTER ONSET AND NUMBER OF PREVIOUS ATTACKS

*P. vivax antigen
FIGURE 4

EFFECT OF RELAPSE ON IFA RESPONSE IN VIVAX PATIENTS

MEDIAN TITRE AND RANGE OF TITRES IN CURED PATIENTS

- PATIENTS WHO LATER RELAPSED

*P. vivax antigen
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