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THE RELATIONSHIP OF MALARIA ANTIBODY TITRE TO PARASITAEMIA
AS MEASURED BY THE INDIRECT FLUORESCENT ANTIBODY TEST¹

by

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The indirect fluorescent antibody test has proven to be very efficient in detecting the presence of malarial antibody. However, a literature search has not revealed an evaluation of the relationship of antibody titre to parasitaemia in clinical diagnosis.

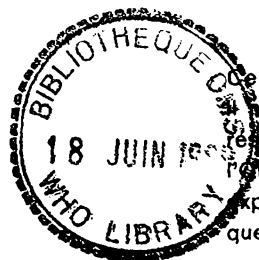
Several workers have investigated the rise and persistence of malarial antibody in Plasmodium vivax and P. falciparum infections. Kuvin et al. (1962) found maximum antibody titres to P. vivax 25-42 days after infection of non-immune volunteers. Positive titres were retained by two P. vivax patients for 150 days. Lunn et al. (1966) working with volunteers infected with P. vivax and P. falciparum found positive titres persisting 90-243 days after the end of patent parasitaemia. Luby et al. (1967) found a low level of antibody remaining in non-immune individuals that had been infected 13 years previously by natural transmission with P. vivax. Collins et al. (1964) reported that with P. falciparum, Colombian strain, antibody in non-immune volunteers persisted up to 20 months after infection with only slight decreases in serum dilution endpoints between 12 and 20 months. They also reported (1968) that, in general, the median antibody response decreased sharply three years after termination of infection and continued to decrease thereafter.

The above studies were performed on sera from non-immune and semi-immune volunteers under carefully controlled experimental conditions and natural transmission in the United States of America.

The majority of specimens submitted to NCDC for serological diagnosis of malaria are from American citizens who acquired malaria by natural transmission in endemic areas. Interpretation of positive reactions has been difficult due to lack of knowledge about the relationship of titre levels to patent parasitaemia and after chemotherapeutic cure. This knowledge is important in cases of suspected malaria when there is no detectable parasitaemia, i.e. in patients with fevers of unknown origin who have been exposed to malaria. It may also be used to detect the infected blood donor in transfusion induced malaria (Lupascu et al., 1967) and index cases of natural transmissions in the United States of America. A study to determine the pattern and persistence of malarial antibody in non-immune and semi-immune individuals was devised to evaluate these parameters for use in routine diagnosis of malaria by the indirect fluorescent antibody test.

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MATERIALS AND METHODS

Serum specimens were collected from 69 United States Army personnel who had served in Vietnam. After returning to Fort Bragg, North Carolina, these men experienced clinical attacks of malaria. The infecting species was identified as *P. vivax* (66 cases) and *P. falciparum* (three cases) by stained blood smear examination at Womack Army Hospital, Fort Bragg, and confirmed at the National Malaria Repository, NCDC, Atlanta, Georgia. Serum samples were drawn from the patients on four occasions: (1) 0-14 days after onset of symptoms; (2) 15-60 days after onset of symptoms; (3) approximately six months after onset of symptoms; and (4) approximately one year following onset of symptoms. For 34 patients, this was the first experience with malaria; 35 had had one or more previous attacks. All patients received supervised curative treatment and none have relapsed. Therapy regimens for *P. vivax* cases consisted of 1.5 g chloroquine base administered over the first three days and 15 mg primaquine base administered daily for 14 days. Patients with *P. falciparum* received 650 mg quinine sulfate upon admission three times a day for 12 days, and 25 mg phrimethamine three times a day for three days and 25 mg dapsone (DDS) daily, beginning on the seventh through the thirty-fourth day of treatment.

The antigen employed in the IFA test consisted of washed-cell, thick-smear slides (Sulzer et al., 1967) of *P. vivax* and *P. falciparum* parasitized erythrocytes from human patients and experimentally infected *Aotus* monkeys. Antigen slides were stored at -70°C . All sera were tested with both antigen species as follows: antigen slides were removed from the freezer and lysed in distilled water for 10 min. A fourfold serum dilution, 1:4-1:4096, was placed on each mount. The slides were incubated in a moist chamber at 37°C for 30 min, and then washed for 15 min in physiological phosphate buffered saline (PBS pH 7.2). The appropriate dilution of anti-human globulin conjugate with Evans blue counterstain (0.2 per cent.) was placed on each mount and incubated 30 min then washed 15 min in PBS. The slides were mounted under a cover slip in buffered glycerol (pH 8.0) for examination.

Fluorescence was read on either a Leitz Ortholux¹ or an American Optical¹ fluorescence microscope equipped with BG-12 and UG-2 exciter filters and an OG-1 secondary filter. Fluorescence was graded 0-4+ with 2+ the lowest reaction considered positive and reproducible. To insure objectivity in evaluation of the reactions, all slides including controls were assorted and coded before examination.

RESULTS

The results of 231 sera, each tested with its homologous antigen in the malaria IFA test, are shown in Table 1. In the first two categories, sera drawn 0-60 days after onset of symptoms, reactions ranged from seven sera giving no response to 78 sera having titres of 1:256-1:4096. Sera drawn six months later generally showed a sixteenfold decrease in titre range; only one serum had a titre of 1:256. One year after clinical symptoms, the highest titre observed was 1:64.

The data was analysed in terms of non-immune (no history of previous attack) and semi-immune (history of one or more previous attacks) individuals. Comparisons by chi-square analysis of titres of the two groups showed no significant differences, although there appeared to be a slight tendency for semi-immune patients to have higher titres. The geometric mean titre for specimens drawn 0-14 and 15-60 days after onset of symptoms were 1:311 and 1:217 for the 35 semi-immune men and 1:143 and 1:127 respectively for the 34 non-immune men. During the observation period of 422 days, no difference in persistence of titres was detected between non-immune and semi-immune patients.

¹ Use of trade names is for identification only and does not constitute endorsement by the Public Health Service, the United States Department of Health, Education and Welfare, or the Department of the Army.

The results of the 231 sera when tested with the heterologous antigens are shown in Table 2. Titres ranged from negative to 1:256 in the 0-60 day categories.

DISCUSSION

Our previous work with the washed-cell, thick-smear antigen (Sulzer et al., 1969) showed that titres of 1:16 or above indicate the presence of specific malarial antibody; a titre of 1:4 was considered negative.

Data for the present study indicated that antibody levels peak within two months of onset of parasitaemia and radical cure, thus agreeing with earlier findings (Kuvin et al. (1962), Coudert et al. (1965) and Lunn et al. (1966)). Six months after clinical symptoms, antibody levels had fallen to low levels of reactivity (1:16 and 1:64). One year after onset of symptoms, antibody levels had decreased only slightly from those at six months.

Other workers (Kuvin et al. (1963) and Collins et al. (1964)) have suggested that the presence of parasites at patent or subpatent levels is essential to the maintenance of high-antibody titre. Our results substantiate this theory. Following radical treatment and presumable cure, malarial antibody in a non-immune and semi-immune United States population declined within six months from relatively high to minimal levels.

When the serum specimens in the 0-60 day categories were tested with their homologous antigens, seven were negative. Six of these sera, from six men, were drawn 0-7 days after onset of symptoms. The second specimen from these men, drawn two weeks later, gave positive reactions of 1:16 (one man), 1:64 (four men), and 1:256 (one man). The seventh negative serum, drawn 39 days after onset of symptoms, was from a patient who never developed detectable antibody during the year's observation period. Examination of this patient's medical history, serum electrophoreses and quantitative immunoglobulin assay of his serum revealed no abnormalities. This patient had positive blood smears on only one day. Perhaps the short duration of parasitaemia in this case did not furnish enough antigenic stimulation to produce a detectable amount of antibody.

Several conclusions which can be drawn from the data presented in Table 1 may be very useful in the serological diagnosis of malaria in American citizens. When tested with the washed-cell, thick-smear antigen, individuals with titres of 1:256-1:4096 can be said to have had a patent parasitaemia within the past two months 98.7 per cent. of the time. A titre of 1:64 may be indicative of recent infection. If the patient with a titre of 1:256 or greater has not been treated for malaria within the past six months, careful slide examination may detect low patent parasitaemia. If there is no history of recent treatment, titres of 1:256 or greater may indicate need of antimalarial treatment, even in the absence of demonstrable parasites. Titres of 1:16 can be interpreted only as evidence of past or present experience with malaria.

Collins et al. (1964) reported that titres in semi-immune volunteers reinfected with P. falciparum rose to levels much higher than had been reported for non-immune patients. Also, in 1968, they reported observing a slight tendency for increased persistence of malarial antibody in patients infected with both P. falciparum and P. malariae. Analysis of our data showed no statistically significant reinforcement of titres in semi-immune patients, although there was a slight tendency for the semi-immune patients to have higher titres than the non-immune patients. The observation period of one year in this study was not sufficient to detect any increased persistence of antibody due to multiple attacks. Serum samples will be collected from these individuals 18 and 24 months after clinical symptoms to obtain more information on antibody duration in this population.

The pattern of cross-reacting antibody was not as dramatic as the specific reactions. Titres were generally higher in the 0-60 day categories than in the other two categories, but maximum peaks were sixteenfold lower than were the serum reactions with homologous antigens.

The necessity of testing sera with all four human species is emphasized by the lack of detection of antibody in 69 of the 114 sera in the 0-60 day categories. Determining the infecting species by the IFA test is the subject of another paper and will not be dealt with here (Gleason et al. (In press)).

TABLE 1. REACTIONS WITH HOMOLOGOUS ANTIGENS OF PLASMODIUM VIVAX AND P. FALCIPARUM ANTISERUM FROM 69 MEN DRAWN 0-443 DAYS AFTER ONSET OF SYMPTOMS

Interval since onset of symptoms	Number of serum with IFA endpoint titres of:						Total no. of sera	Geometric mean titres
	Neg.	1:16	1:64	1:256	1:1024	1:4096		
0-14 days	6	3	10	18	18	3	59	1:184
15-60 days	1	4	21	26	11	2	65	1:178
182-271 days	36	26	5	1	0	0	68	1:9
305-443 days	23	13	3	0	0	0	39	1:8

TABLE 2. REACTIONS WITH HETEROLOGOUS ANTIGENS OF PLASMODIUM VIVAX AND P. FALCIPARUM ANTISERUM FROM 69 MEN DRAWN 0-443 DAYS AFTER ONSET OF SYMPTOMS

Interval since onset of symptoms	Number of serum with IFA endpoint titres of:						Total no. of sera	Geometric mean titres
	Neg.	1:16	1:64	1:256	1:1024	1:4096		
0-14 days	32	18	7	2	0	0	59	1:10
15-60 days	37	20	6	2	0	0	65	1:9
182-271 days	60	8	0	0	0	0	68	1:5
305-443 days	37	2	0	0	0	0	39	1:4

SUMMARY

Two hundred and thirty-one sera from United States Army personnel who served in an endemic malaria area for approximately one year were tested by the washed-cell, thick-smear malaria IFA test. These men developed clinical episodes of P. vivax and P. falciparum malaria after returning to the United States of America. After onset of illness, sera were drawn four times during the year. Antibody titres to both homologous and heterologous antigens rose rapidly to maximum peaks of 1:64-1:4096 by 60 days after onset of symptoms. Six months after clinical symptoms, antibody levels had fallen to low levels of reactivity (1:16 and 1:64); at the end of one year, titres had declined only slightly more. Infections with circulating parasitaemia were differentiated from cured infections by titre level. No reinforcement of titres by multiple infections was evident.

RESUME

Les sérums de 69 sujets infectés par P. vivax (66) et P. falciparum (3) ont été soumis à l'épreuve indirecte des anticorps fluorescents, et cela à 4 reprises après l'apparition des premiers symptômes, soit respectivement entre les jours 0 et 15, entre les jours 15 et 60, le sixième mois environ et, enfin, le douzième mois; 231 sérums au total ont été examinés avec des antigènes homologues et hétérologues. Les sujets étudiés avaient présenté des symptômes cliniques de paludisme à leur retour aux Etats-Unis d'Amérique, après un séjour au Viet-Nam. Tous ont suivi un traitement radical selon une posologie adaptée à l'espèce de plasmodium responsable.

Les résultats de l'étude montrent que les titres d'anticorps, mesurés par l'épreuve des anticorps fluorescents, ont rapidement augmenté aussi bien avec les antigènes homologues qu'avec les antigènes hétérologues, pour atteindre des maximums compris entre 1:64 et 1:4096 dans les 60 jours suivant les premiers symptômes. Les sérums prélevés et examinés le sixième mois ont faiblement réagi (titres compris entre 1:16 et 1:64); au douzième mois, la réaction était un peu plus faible encore. Les infections accompagnées d'une parasitémie circulante se différenciaient des infections guéries par le niveau du titre positif limite. On peut ainsi affirmer que, dans 98,7 % des cas, les sujets dont les titres se situaient entre 1:256 et 1:4096 avaient eu une parasitémie patente dans les deux mois précédents. Dans de cas d'un malade dont le titre est égal ou supérieur à 1:256 et qui n'a pas reçu de traitement contre le paludisme dans les six mois qui précèdent, un examen attentif des lames de sang peut permettre de déceler une faible parasitémie. Si des sujets à réaction positive à des titres égaux ou supérieurs à 1:256 n'ont pas été soignés récemment, un traitement antipaludique peut s'avérer nécessaire, même en l'absence de parasitémie à l'examen microscopique. Des titres de l'ordre de 1:64 peuvent révéler une infection récente, mais un titre de 1:16 dénote très certainement une atteinte ancienne ou un début de maladie. Les résultats numériques de l'étude sont présentés dans deux tableaux.

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