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Indirect fluorescent and haemagglutinating antibodies to malaria in Nigerian students resident in Washington D.C., U.S.A.

PETER O. FASAN, A. J. SULZER,* H. LOBEL* AND I. B. KAGAN*

*Department of Preventive and Social Medicine, University of Ibadan, Ibadan, Nigeria and *Parasitology Section, National Centre Disease Control, U.S. Department of Health, Education and Welfare, Atlanta, Georgia 30333, U.S.A.*

Summary

Fluorescent and haemagglutinating antibody tests were performed on sera collected from fifty-two adult African students from a holoendemic region of malaria in Nigeria. Period of continuous residence in the U.S.A. among members of the group varied from 2 days to nearly 10 years. None has been diagnosed as having malaria since resident in the U.S.A. and none had taken any form of anti-malaria drug prophylaxis since departure from Nigeria. Both tests indicated that all the students had probably had a past infection with falciparum malaria. All gave a positive reaction to fluorescent antibody (FA) test and in 70% indirect haemagglutination (IHA) titres of 1:16 and above were demonstrated. Twenty-three per cent had FA titres of 1:4096. The malarial antibody titre levels decreased with increase in the interval since last exposure. Generally, the IHA titres were lower than the FA. No malaria parasite was seen in the blood of the students on the one occasion they were examined. The high titre values recorded in those who have been away from the endemic region for over three years suggests that falciparum parasites may persist in the body longer than the usually accepted limit of 3 years.

Résumé

Des épreuves d'immunofluorescence indirecte (IFI) et d'hémagglutination (HAI) ont été effectuées sur le sérum de 52 étidiants africains adultes résidant aux Etats-Unis d'Amérique avaient séjourne d'une façon ininterrompue aux Etats-Unis pendant les périodes allant de deux jours à près de dix ans. Aucun d'entre eux n'avait été reconnu paludéen depuis son arrivée aux Etats-Unis et aucun n'avait pris de traitement antipaludéen quelconque à titre prophylactique depuis son départ du Nigéria. Les deux examens ont montré que tous ces étudiants avaient probablement souffert dans le passé d'une infection à *Plasmodium falciparum*. Tous avaient une réaction positive à l'IFI, et chez 70% d'entre eux on a pu mettre en évidence un titre d'HAI de 1:16 et plus. La MGIT d'IFI de 700 et, dans 23% des cas, le titre d'IFI était de 1:4096. A mesure que passait le temps depuis la dernière exposition à la maladie au Nigéria, les titres d'anticorps diminuaient. Le plus souvent, le titre d'HAI était inférieur à celui d'IFI. Lors du seul examen qu'aient subi ces étudiants, aucun parasite n'a été trouvé dans leur sang. Les valeurs élevées d'anticorps signalées chez ceux qui s'étaient trouvés absents de la région d'endémie depuis plus de trois ans donnent à penser que *P. falciparum* peut persister dans l'organisme au-delà de la limite de trois ans généralement admise.

Correspondence: Dr P.O. Fassan, PMB 12003, Lagos, Nigeria.

Introduction

Information is required on the persistence of antibodies as detected by the indirect fluorescent antibody (IFA) test and other serological tests in immune individuals in the absence of re-exposure, and on the rate of decline of malarial antibodies in immigrants from hyperendemic areas. Such information is necessary to interpret IFA and other serological titre values of specimens from highly endemic areas. It may also provide some explanation for the recorded observation that previously immune individuals develop severe malaria infection on re-exposure in the hyperendemic areas after a period of absence in a non-malarious region

(Colbourne, 1955). In addition, with the renewed interest in the immunology of malaria and searches for effective methods of immunization, these findings may forecast expectations should vaccine production become possible.

Several studies have been done on the persistence patterns of malaria antibodies in induced malaria infections on non-immunes. These infections were usually of limited duration and often interrupted by chemotherapy after one or few episodes of patent parasitaemia (Sadun *et al.*, 1969).

Materials and methods

The subjects of our study are in a different category having been drawn from a semi-immune population. They are all Africans who have been continuously exposed to malaria in the holoendemic setting of their homes from infancy to a day or sometimes the hour prior to their departure from Nigeria for the United States of America for further studies. They were all healthy, ambulatory subjects from a homogeneous racial group in South Western Nigeria. Several workers have reported the prevalence of malaria in Nigeria (Bruce-Chwatt, 1951), and in Nigerians after early childhood (Fasan, 1969). It has been estimated that the average West African has about two fresh malaria infections per annum when resident in the holoendemic areas of the country (Miller, 1958; Bruce-Chwatt, 1963). The malaria experiences of the students can be regarded as uniform with regard to exposure and the species of infecting parasites.

Altogether fifty-two students—all adults—were interviewed and examined between 25 November 1970 and 5 January 1971. They have been resident in the United States of America for lengths of time varying from just 2 days (new arrivals) to 10 years (graduate students and an assistant professor in Howard University). All were Yorubas[1] and apart from two from the Kwara State, all the rest came from the Ondo Province of Western State. In both areas malaria is holoendemic. Following personal interviews and the answers provided to the questionnaires, it was concluded that none of them had reported ill with malaria since arrival in the United States of America and none had taken any form of antimalaria prophylaxis since leaving Nigeria. Only a few of them stated that they were on antimalarial prophylaxis while in Nigeria, but none took the drugs in a regular fashion. Filter paper blood smears were collected from a finger-prick for each subject and a thick smear was made on a microscope glass slide for examination for malaria parasites. The blood smears were punched out from a marked area of the filter papers and reconstituted in 1 ml of phosphate buffered saline at pH 7·2. Four-fold dilutions of the sera were prepared, starting at 1:64. Frozen *Plasmodium falciparum* slide antigens from Nigeria, were used, as this is the most prevalent plasmodium in West Africa. The conjugation was done with fluorescent isothiocyanate-labelled goat antihuman gamma-globulin produced at the Centre for Disease Control, Atlanta, Georgia. Examination was done as outlined by Sulzer, Wilson & Hall (1969). The indirect haemagglutination test (IHA) was performed with a lysate of *Plasmodium knowlesi*, as described by Rogers, Fried & Kagan (1968). The thick blood smears on the glass slides were stained with 4% Giemsa for 30 min before examination under the oil immersion objective and ×8 eyepieces.

Results

All the subjects gave a positive IFA reaction to *P.*

[1] The Yoruba tribal group is a homogeneous racial group of 12 million people resident in the South West region of Nigeria.

TABLE 1. Indirect fluorescent antibodies (FA) to malaria in Nigerian students in Washington D.C.

FA Titres	Absence from exposure (months)					All groups
	≤12	12–23	24–35	36–47	≥48	
≥4096	6	3	1	1	1	12
1024	5	5	5	4	0	19
256	3	2	0	4	7	16
≤64	1	2	1	0	1	5
Total number examined	15	12	7	9	9	52

TABLE 2. Indirect haemagglutination antibodies (IHA) to malaria in Nigerian students in Washington D.C.

IHA titres	Absence from exposure (months)					All groups
	≤ 12	12–23	24–35	36–37	≥48	
≥32768	0	0	0	0	1	1
16384	0	0	0	0	0	0
8192	2	0	0	0	0	2
4096	0	0	0	0	0	0
2048	0	0	1	1	0	2
1024	1	1	2	0	0	4
512	0	1	0	0	1	2
256	4	1	0	0	0	5
128	1	2	0	0	2	5
64	2	1	1	1	1	6
32	1	1	0	2	1	5
16	1	1	1	1	1	5
≤2	3	4	2	4	2	15
Total number examined	15	12	7	9	9	52

falciparum antigen with the titres varying from 1:64 to 1:4096 (Table 1) and an overall geometrical mean reciprocal titre (GMRT) of 704. The GMRT for those who have been away for under 1 year was 1120, whereas after 4 or more years since last exposure the GMRT was 300. The difference noted between the GMR titre of those absent for less than 2 years and those over 3 years was statistically significant, $\chi^2 = 8{\cdot}34$ on 3 d.f.—$0{\cdot}05 > P > 0{\cdot}025$.

The IHA test gave results that also indicated that there is a steady, though slow fall in the haemagglutination titres as the period of absence from a malarious area lengthened. The GMRT fell from 97 in those who were most recently exposed to 69 in those whose last exposure was 4 years and over. Forty per cent of the students gave negative or doubtful reactions to the IHA at a dilution of 1:16 and below (Table 2). Malaria parasite was not detected in any of the thick blood smears obtained from the students. Two subjects had moderate infection of *microfilaria loa loa*.

Discussion

It has long been recognized that changes occur in the serum protein patterns of West Africans when they leave the shores of their home to reside in the more developed temperate climates where there is freedom from exposure to the innumerable parasitic diseases endemic in West Africa. Thus Schofield (1957) investigating the serum protein patterns of West 'Africans' resident in Britain found that the typical African pattern with low serum albumin and high gamma-globulin, changes steadily toward a typical 'European' pattern with a marked fall in the gammaglobulin fraction, as the African settles down in the new environment. He concluded that these changes reflected a recovery from pathological effects induced by the previous African environment of malaria and malnutrition. Evidence that high gamma-globulin levels in serum bear correlation to malaria antibodies has been provided by Kuvin *et al.* (1962). Kuvin & Voller (1963) found that malaria antibody titres in West Africans resident in Britain are low, obtaining a maximum titre of 1:200 in one of the twenty-six subjects that they examined. These workers therefore concluded that malarial antibody production is reduced on leaving endemic malarial zones. In this study, we have obtained a much higher IFA titre of 1:4096 in a number of our subjects and an overall GMRT of 700. It should be pointed out, however, that a standard technique with standardized reagents is not yet available for the IFA test. Figures from one laboratory cannot therefore be justifiably compared with those from another. There is much subjectiveness in the interpretation of fluorescence, and workers show preferences in the choice of filters, microscopes and plasmodial antigens. Secondly, the study of the West Africans in Britain by Kuvin & Voller (1963)

was done on a heterogenous group of twenty-six hospital patients whereas our fifty-two subjects were healthy Western Nigerian Yorubas, a more homogenous group with a more uniform malaria experience.

The level of IFA titres obtained in our subjects indicate that the malarial antibodies fall steadily but slowly over many years though still detectable even after 10 years from last exposure. This finding is of interest in view of the recorded observations that adult Africans returning home after a period of absence in non-malarious areas are highly susceptible to and develop severe malaria on re-exposure.

The mere presence of these antibodies in the serum after more than 5 years since last exposure is an interesting and important observation, since the view that is widely held concerning *falciparum* infection is that most of the parasites disappear in about a year from the body in the absence of re-exposure and probably all in under 3 years. Secondary exoerythrocytic forms of the parasite are not known. Moreover, it seems unlikely that molecules of gamma-globulin should be detectable in a subject 6 or more years after their synthesis. It is therefore reasonable to suggest that a memory retaining mechanism is responsible for the continued production of the malarial antibodies, or that *P. falciparum* parasites survive in the body much longer than hitherto suggested. A prepatent *falciparum* infection would explain the persistence of the antibodies in these semi-immune individuals.

The differences noted in the levels of IFA and IHA titres in the Nigerian students also deserve some comment. The two tests differ significantly both in the materials required and the technique used for their performance. The IHA test measures precipitating antibody. The results observed are at variance with the findings of Wilson *et al.* (1971), based on an investigation of malarial antibodies in sixty-eight presumably non-immune United States Army Soldiers, from Vietnam. In the semi-immune subjects in the present study it is the IFA test that appears to detect antibody for longer periods than the IHA test (Table 3). Titre levels obtained in the subjects who were positive to both tests cannot be validly compared since it is uncertain if the two tests are measuring the same antibody. Sadun *et al.* (1969) have observed that antibody titres in *P. falciparum* infections in volunteers were usually higher and persisted longer than those in *P. vivax* infections. But they also noted that the antibody curves with the two tests followed almost parallel lines. The IHA titres observed in our semi-immune subjects were lower but the values yielded a curve that is similar to that of the IFA titres; both showed a decline from a high level close to recent exposure to a low level some years from last exposure. It is to be noted, however, that whereas only 8% of the subjects showed low but positive IFA titres, this proportion was slightly higher in respect of IHA.

It is likely, therefore, that these two tests measure antibodies that, are differently produced, possess different functions and biological properties, though both are specific for malaria.

In order that we might know whether these antibodies are protective, our subjects or similar persons will have to be closely observed and re-examined on return to the endemic area with a view to marking any changes in the IFA and IHA on re-exposure to malaria.

TABLE 3. Comparison between antimalarial positive and negative IFA and IHA tests in sera of Nigerians after leaving the Malaria endemic region

Mean interval since the last exposure (months)	IFA / IHA	+ / +	− / −	+ / −	− / +	No. of sera examined
4		11 (73%)	0 (0)	4 (27%)	0 (0)	15
16		7 (58%)	0 (0)	5 (42%)	0 (0)	12
28		4 (57%)	0 (0)	3 (43%)	0 (0)	7
41		4 (44%)	0 (0)	5 (56%)	0 (0)	9
71		6 (67%)	0 (0)	3 (33%)	0 (0)	9

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