ship to chronic myelosclerosis is probably similar to that of acute granulocytic leukaemia to chronic granulocytic leukaemia, and it may provide a link between reticulum-cell sarcoma and the myeloproliferative disorders.

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MALARIAL ANTIBODY TITRES OF WEST AFRICANS IN BRITAIN

BY

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In the past it has been almost impossible to know the status or duration of immunity to malaria in endemic malaria zones, owing to the difficulty in not being able to differentiate a relapse from reinfection, and owing to the fact that, where malaria is prevalent, sometimes two, if not three, of the human-malaria parasite species may be present, appearing consecutively in the same individual (Coggeshall, 1943). Acquired immunity to malaria is manifested only gradually in some malarial infections but more suddenly in others, and frequently results in the suppression and elimination of the infection and in refractiveness to super-infection and reinfection (Boyd, 1949).

Little is known about malarial immunity in individuals previously immune who have left their environment of repeated exposure to malaria for one where none exists. Coggeshall (1938) and Maier and Coggeshall (1944) found that immunity to reinfection with *Plasmodium knowlesi* lasts from three to fourteen months after complete sterilization of the blood with sulpha drugs. Boyd *et al.* (1936, 1939) found immunity to the homologous strain of *P. vivax* three and seven years after the primary attack of malaria. Coggeshall (1943) has demonstrated that latent malaria in experimental animals can be destroyed by chemotherapy and that the duration of immunity is brief, usually a matter of months, while the eradication of an acute infection in its earlier stages leaves the host with no immunity whatsoever. More recently, Kuvin *et al.* (1962a, 1962b) followed the course of malarial antibody production in normal volunteers, utilizing the indirect method of immunofluorescence. They found detectable malarial antibody for as long as 335 days after sporozoite infection. In addition, all the infected volunteers demonstrated an increase in their serum gamma-globulin levels.

Schofield (1957) investigated the serum protein patterns of West Africans resident in Britain. He demonstrated by electrophoretic methods that when healthy adult Africans enter a European environment the serum proteins change steadily but very slowly from a typical "African" pattern with low serum albumin and high gamma-globulin values, toward a typical "European" pattern, with a marked fall in the gamma-globulin fraction. He concluded that these changes in gamma-globulin levels reflected a recovery from pathological effects induced by the previous African environment of malaria and malnutrition. The belief that *protective* malarial antibodies were present at some time after infection was given support when Coggeshall and Kumm (1937) demonstrated that the serum of rhesus monkeys with chronic *P. knowlesi* infections conferred passive immunity upon monkeys with acute infections with this parasite. Cohen *et al.* (1961) clinically correlated the high serum gamma-globulin levels in Gambian Africans infected with malaria with the production of protective antibodies.

Previously, attempts have been made to follow the course of malarial-antibody production with specific complement fixation and precipitation reactions with sera from patients infected with malaria, but these efforts have met with only limited success (Coggeshall and Eaton, 1938; Lippincott *et al.*, 1945; Mayer and Heidelberger, 1946). The fluorescent antibody technique has been used in the specific staining of malaria parasites (Ingram *et al.*, 1961; Tobie and Coatney, 1961; Voller, 1962), and previous investigations employed the indirect method of immunofluorescence to follow the course of antibody production in man (Kuvin *et al.*, 1962a, 1962b; Voller and Bray, 1962; Tobie *et al.*, 1962). These studies suggested that the fluorescent antibody technique provides a specific and sensitive method for titrating malarial-antibody production in man.

The object of the present investigation was to determine whether malarial antibody production and serum gamma-globulin levels were altered in individuals from endemic malarial areas by residence outside these zones. We were also interested in evaluating the suitability of the simian parasite *P. cynomolgi bastianellii* as a diagnostic and investigative antigen for use in studying the immune response in human malaria, utilizing the fluorescent antibody technique. This parasite can be readily maintained in rhesus monkeys as a laboratory infection, whereas human-malaria parasites cannot be grown in culture, will not readily infect animals, and are obtained only from patients with clinical malaria.

Material and Methods

Serum was obtained from 26 West Africans between the ages of 19 and 46 years admitted to the Hospital for Tropical Diseases. All were of the African race and native to West Africa where *P. falciparum* is endemic. They left West Africa to reside in Britain for reasons of employment or study. In addition to taking a malarial history, physical examination was performed, special attention being paid

to palpation of the spleen. Thick smears of peripheral blood were stained for the presence of malaria parasites. A definite history of malaria was difficult to obtain in most patients, but undiagnosed "fever" had occurred in all patients several times during their childhood or adult life. Only one patient (Case 17) took prophylactic antimalarial chemotherapy before leaving Africa (for two years).

The indirect method of immunofluorescence (Weller and Coons, 1954) was used and the sera were allowed to react with homologous and heterologous species of parasites. Thin blood films of the simian parasite *P.c. bastianellii* were prepared from rhesus monkeys experimentally infected with this parasite. Thin blood films of *P. falciparum* were obtained from patients with falciparum malaria in East Africa. The preparation of the blood films and the performance of the fluorescent antibody test was the same as that described by Kuvin *et al.* (1962b). The preparations were examined by fluorescence microscopy by means of a Reichert zetopan microscope with the "binolux" twin lamp unit, using a wavelength of 4,100Å. Fluorescence was graded 1+ to 4+, and a reading of 3+ or greater was regarded as positive. Control sera were tested from four normal volunteers. The total proteins were estimated by the colorimetric method of Johnston and Gibson (1938). Paper electrophoresis was carried out by the method of Flynn and DeMayo (1951) and stained with azocarmine.

Results

The malarial-antibody titres and serum gamma-globulin levels found in 26 West Africans resident in Britain from three months to seven years are shown in the Table. Eighteen lived in Britain less than two years, five between two and four years, and three for more than four years. None of these patients had parasites demonstrated in their peripheral blood by thick-smear technique, and only two (Cases 2 and 3) exhibited palpable splenomegaly. Malarial antibody was demonstrated in all patients with the highest titre 1:200 (Case 8). There was no significant difference in titre shown when the sera were tested using the homologous antigen *P. falciparum*, or the heterologous simian antigen *P.c. bastianellii*. The relationship between antibody production and gamma-globulin levels in each patient is shown in the Table. All patients had elevated serum gamma-globulin levels, with a mean of 2.31 g./100 ml.

Fluorescent Antibody Titres and Serum Gamma-globulin Values of West Africans Residing in Britain

Case No.	Length of Time in Britain (Months)	Malarial Antibody Titres		Serum Gamma-globulin (g./100 ml. (Normal 0·438–1·042)
		P. falciparum	*P.c. bastianellii*	
1	2	1:50	1:20	2·19
2	3	1:1	1:10	2·88
3	4	1:200	1:50	2·01
4	4	1:100	1:200	3·07
5	7	1:20	1:20	1·54
6	8	1:10	1:20	2·66
7	9	1:20	1:20	1·97
8	9	1:200	1:200	2·40
9	9	1:1	1:10	2·11
10	11	1:100	1:20	2·28
11	11	1:1	1:1	3·02
12	12	—	1:20	2·20
13	12	1:20	1:10	2·61
14	13	1:20	1:1	2·29
15	15	1:50	1:20	1·56
16	18	1:100	1:100	2·45
17	20	1:50	1:50	2·63
18	22	1:20	1:50	2·65
19	27	1:10	1:10	2·11
20	27	1:20	—	2·30
21	30	1:50	1:20	3·19
22	42	1:10	1:20	2·27
23	46	1:100	1:50	2·75
24	62	1:50	1:20	2·05
25	68	1:100	1:10	1·90
26	84	1:50	1:10	2·37

Discussion

It is well known that repeated malarial infections confer a degree of immunity on the host after the acute attack has subsided. However, the humoral and cellular mechanisms responsible for converting an acute attack of malaria into a chronic one, the character and location of the immune responses, and the duration of immunity to any particular strain or species of malaria parasite are all questions of considerable controversy.

Many clinicians believe that the departure of an immune individual from an endemic malarial zone, where he is subjected to repeated infection and antigenic stimulation, to an area where no malaria exists results in a lessening of the individual's immunological resistance to infection with the malaria parasite. Until recently there has been no way of measuring malarial immunological activity, because no reliable serological technique was available for the investigation of antibody production in this disease.

The results of our present study demonstrate that malarial antibody titres in West Africans residing in Britain are relatively low, with maximum titres of 1:200 (see Table). All the patients demonstrated some malarial antibody in their serum, detectable by the antigen-antibody reaction using *P. falciparum* and *P.c. bastianellii*, for as long as seven years after leaving endemic malarial areas. Kuvin *et al.* (1962b) found titres as high as 1:5,120 in volunteers infected with *P. vivax* and *P.c. bastianellii* 25 to 48 days after infection. However, antibody was detectable in titres only as high as 1:40 in the vivax-infected group after 150 days, and 1:80 in the bastianellii-infected group after 335 days. Voller and Bray (1963) have also demonstrated malaria-antibody titres as high as 1:6,400 using the fluorescent antibody technique in studying the sera of populations residing in endemic malarial zones in Liberia. Our results suggest that malarial-antibody production is reduced on leaving endemic malarial areas. This reinforces the clinical opinion that repeated infections with the homologous species of malaria parasite maintaining continuous antigenic stimulation produce adequate antibody levels protective against malaria. However, it must be emphasized that, although our investigations demonstrate that a specific malarial antibody is being produced, we are unable from these studies to say whether this antibody is of the protective type.

Immunological similarities between different species of malaria have been shown in cross-reaction experiments using the complement-fixation test (Kingsbury, 1927; Eaton and Coggeshall, 1939) and precipitation test (Taliaferro *et al.*, 1927). More recently, Voller (1962) and Tobie *et al.* (1962) have shown considerable cross-reactivity between human and simian parasites utilizing the fluorescent antibody technique. Our results have demonstrated considerable cross-reactivity between *P. falciparum* and the simian parasite *P.c. bastianellii*, with essentially the same antibody titres. The ease with which *P.c. bastianellii* can be maintained in laboratory monkeys and the high degree of cross-reactivity it exhibits with the human-malaria parasites suggest that this simian parasite may be suitable as a diagnostic and investigative antigen for use in following antibody production in human malaria, utilizing the fluorescent antibody technique. The human-malaria parasites *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale* can be obtained from no other source than clinical cases of human malaria.

The diagnosis of acute malaria is usually not a problem in clinical medicine, since the parasites are almost always

demonstrable in the peripheral blood by the usual staining methods some time during the acute attack. We do not consider the fluorescent antibody test a suitable diagnostic alternative to these conventional methods in this phase of the disease. However, there is considerable difficulty in diagnosing chronic or latent malaria where the patient may be without symptoms and with few or no parasites to be found in his peripheral blood by ordinary methods of staining. The fluorescent antibody technique using the simian parasite *P.c. bastianellii* as a test antigen appears to be useful in diagnosing *past* infection with human malaria, and may prove to be an even more useful tool in establishing a diagnosis of latent or chronic malaria after further studies determine what are significant malarial antibody titres, or rises in titre, in this form of the disease. Induced malaria, by blood transfusion, is also a problem in warm climates, and this fluorescent antibody test may also prove to be a useful aid in screening blood donors for the presence of past or present infection.

Several reports in the literature suggest that infection with the malaria parasite stimulates the production of gamma-globulin by inducing the formation of specific malarial antibodies (Holmes *et al.*, 1955; McGregor *et al.*, 1956; Edozien, 1957; Edozien *et al.*, 1960, 1962; Cohen *et al.*, 1961). McGregor *et al.* (1963) have shown that the gamma-globulin from West Africans is active against East African malaria, thus indicating antigenic similarities between the East and West African *P. falciparum* parasites. Kuvin *et al.* (1956b) correlated the raised gamma-globulin levels found in malarial infections in normal volunteers with the production of malaria antibodies as determined by the fluorescent antibody technique, and concluded that the humoral element of malarial immunity is only a part of the excess gamma-globulin produced.

In our present investigation we found raised levels of serum gamma-globulin in all the patients studied. These serum gamma-globulin values were similar to those found by Schofield (1957) in his study of Africans resident in Britain and were of the "African" pattern. The high gamma-globulin values which we found are probably related to the relatively short time most of these Africans have been resident in Britain. Eighteen lived in Britain less than two years, five between two and four years, and three for more than four years. Schofield (1957) states that after more than six years' residence outside Africa the protein pattern of Africans approaches that of Europeans. Our findings of low malarial antibody titres and high serum gamma-globulin values in West Africans resident in Britain suggest that malarial antibody is responsible for only a small fraction of the excess gamma-globulin produced in tropical populations.

Summary

Sera from 26 West Africans resident in Britain from three months to seven years were examined for malarial antibody by the fluorescent antibody technique. All the sera contained malarial antibody in low titre, suggesting that antibody production is reduced on leaving endemic malarial areas. This reinforces the clinical opinion that repeated infection with the malaria parasite is necessary to maintain adequate protective levels of malarial antibody.

The simian parasite *P.c. bastianellii* demonstrates considerable cross-reactivity with human-malaria parasites and appears to be useful as an antigen which can be maintained in laboratory monkeys and used in diagnosing past malarial infection utilizing the fluorescent antibody technique. This method may prove to be an even more valuable tool in the future in establishing a diagnosis of chronic or latent malaria and in the screening of blood donors for the presence of past or present infection with this disease.

The combination of low malaria-antibody titres and high serum gamma-globulin levels in West Africans resident in Britain suggests that malarial antibody is only a small fraction of the excess gamma-globulin produced in tropical populations.

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The Minister of Health, Mr. Enoch Powell, speaking at the annual conference of the Royal National Institute for the Blind in London on July 18, said that in the last few years the proportion of registered blind to the population had been falling. "In the four years to the end of 1961," he said, "the blind under 65 fell by nearly 6% in absolute numbers and by 8½% as a proportion of the total; and though the numbers of blind over 65 rose by nearly 3%, they too fell—though only by 2%—as a proportion of the total population over 65. These facts are more than statistical in origin, reflecting merely a shift of classification between blind and partially sighted; they are in part, at least, the fruit of improved and widened hospital treatment of diseases of the eye. There is good reason to think, and to plan on the assumption, that the downward trend in the proportion of blind to the population will continue, at least for a number of years to come. This is borne out by the 1962 figures, which continue the same general trend I have indicated for the years 1957–61."