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Immunity in malaria: depression of delayed hypersensitivity reaction in acute *Plasmodium falciparum* infection

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Summary

The effect of acute *Plasmodium falciparum* malaria infection on the cell-mediated immune response of 30 Nigerian children attending the General Out-patient (GOP) Clinic of the University College Hospital, Ibadan, Nigeria, was assessed in a controlled study. Delayed-type hypersensitivity skin reaction to five tuberculin units (5 TU) of purified protein derivative (PPD) was used as an indicator of cell-mediated immunity. The results showed marked depression of delayed hypersensitivity reaction to PPD in 27 (90%) of the malarious children, compared with four (16%) of the 25 control healthy subjects ($P < 0.0005$). This depression was observed despite evidence of previous BCG vaccination in 38 (69.1%) of the 55 children in the study. The possible clinical significance of these observations in tropical paediatric practice, and the immunopathological implications, are discussed.

Résumé

L'effet du paludisme aigu — due au *Plasmodium falciparum* — sur la réponse d'immunité cellulaire de 30 enfants nigérians presentant au service de consultations polycliniques de l'University College Hospital, Ibadan, Nigéria est examiné. L'intradermoréaction à la tuberculine (5 unité de PPD) est utilisée comme l'indicateur d'allergie cellulaire. Les résultats ont démontré une dépression significative de l'hypersensitivité retardée envers la PPD chez 27 (90%) des enfants atteints du paludisme tandis que quatre (16%) des 25 contrôle ont démontrée une dépression ($P < 0.0005$). Cette dépression est realisée malgré l'évidence de vaccination — avec le bacille Calmette Gúerin (BCG) — dans 69% de tous les enfants éxaminés. L'importance de ces observations dans les consultations pédiatriques tropicales et les implications immunopathologiques sont discutées dans cet article.

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Introduction

Particulate and soluble malarial antigens have variously been described in experimental animals and human infections. These include the erythrocyte membrane and the parasite within the red cell [1] as well as malarial soluble serum antigens (MSSA) [2,3]. These antigens will singly or collectively stimulate cellular, humoral, or both, immune responses in the host. Elevated levels of immunoglobulins in prolonged exposure to malarial antigens have been described [4], while malarial antibodies have also been detected in studies on Gambian children [5], but the role played by different components of cellular immunity in malarial infections has not been clearly defined.

T-cells have been implicated in the maintenance of protective immunity against malaria, especially in monkeys [6], but comparable information on man was not possible because the technique involved was not suitable for humans. However, spontaneous blastoid transformation of lymphocytes was reported in unstimulated cultures of peripheral leucocytes from Nigerian children infected with *Plasmodium falciparum* while depressed lymphocyte response to stimulation by phytohaemaggluti-

nin (PHA) was also observed [7]. The exact role of cell-mediated immunity in malaria infection became more paradoxical in the light of reports of depleted circulating T-lymphocytes during acute malaria infection [8,9], although increased activity of natural killer cells has now been demonstrated in *P. falciparum* infection [10]. The present study was designed to investigate the in-vivo effect of acute malaria infection on the widely used delayed hypersensitivity skin reaction to purified protein derivative (PPD).

Patients and methods

Selection of subjects

The subjects of the present study comprised children aged 6 months to 12 years who were recruited during the months of June–October (rainy season) in 1981 from the General Outpatient (GOP) Clinic of the University College Hospital (UCH), Ibadan. The criteria for selection into the study were:

(i) attendance at the GOP for the first time with fever;
(ii) weight-for-age (WFA) of between 3rd and 97th percentile using Janes' data [11,12];
(iii) moderately heavy parasitaemia of *P. falciparum* trophozoites, demonstrated on slides made from capillary blood obtained by sterile finger prick.

Excluded from the study were those children who showed features of measles infection and protein–energy malnutrition, even though parasitaemia was demonstrated in the peripheral blood films of some of them. Also excluded were children whose illness had started for more than 3 days before presentation at the clinic and/or had been given any anti-malarial drugs in the preceding 3 months.

With the above criteria, 30 consecutive children were selected for the study group. Twenty-five control subjects, who were free of fever and parasitaemia, were also recruited from the Institute of Child Health (ICH), UCH, Ibadan.

Methods

All the study and control subjects were registered and seen at the daily general paediatric clinics run at the Institute of Child Health. Information obtained from the subjects' parents or guardians included the correct birth date of the subjects, history of illness and previous history of vaccination with Bacille Calmette-Guérin (BCG). Such vaccinations were confirmed either from a record card or by the identification of an appropriate scar on the left forearm or on the left deltoid area (the two common sites for BCG vaccinations in Ibadan, Nigeria). A general clinical examination, as well as assessment of nutritional status, was then carried out in all the subjects. Tuberculin skin test was performed on all children before each malarious child was treated using the WHO Standard Field Test dosage [13] of 25 mg chloroquine base per kg body weight over 3 days. This 3-day course of chloroquine has been found to be adequate for clearing parasitaemia in Nigerian children suffering from *P. falciparum* malaria [14,15].

Tuberculin skin test

An intradermal tuberculin skin test was performed on the first day using five tuberculin units (5 TU) of PPD (Swiss Serum and Vaccine Institute, Berne, Switzerland) on the right forearm of all selected children and before any form of treatment, for the malarious cases, was instituted. Each mother was requested to bring back her child 3 days later for recording the delayed skin hypersensitivity reaction. This second visit, the importance of which was explained to the mother, enabled us to review the clinical state of the malarious children and to eliminate those who might have been incubating measles earlier. Koplik's spots and morbiliform measles rash were again looked for. Two separate diameters, at right angles to each other, were recorded for each tuberculin reaction, and the mean diameter was taken for each child. We regarded only those children in whom conspicuous mean induration of at least 5 mm diameter was seen, as having positive results. Indurations were usually not seen clearly below this diameter in the dark skin, and those falling below this size were recorded as negative tuberculin reactions.

Levels of parasitaemia

Thin and thick blood films were made from capillary blood to quantify the degree of para-

sitaemia, using a standard technique as applied in a previous study [10]. The levels and range of parasitaemia were classified as ± (< 2%), + (3–5%), ++ (6–10%), +++ (11–15%) and ++++ (> 15%). For the purpose of the present study, all levels below 6% were regarded as scanty (and excluded) while levels between 6% and 10% were regarded as moderately heavy, 11–15% as heavy and 15% as very heavy parasitaemia.

The Chi-square (χ^2) test and Student's *t*-test were used to analyse the results.

Results

At presentation, the body temperature of the healthy subjects (controls) ranged from 36.6 to 37.5°C (mean 37.0 ± 0.5°C s.e.), whereas the malarious children had temperatures ranging from 37.8 to 40.8°C (mean 39.4 ± 0.16°C s.e.). Eighteen of the 30 malarious children had parasitaemia of between 6% and 10%, while nine and three others had parasitaemia of 11–15% and above 15%, respectively.

Table 1 shows the summary of BCG vaccination among the malarious and healthy control subjects. Thirty-eight of the 55 (69.1%) children, comprising 19 malarious and 19 healthy subjects, had been adequately vaccinated with BCG.

The mean induration diameter was 9.5 ± 1.0 mm (s.e.) (range 0–17) among the healthy subjects, with 17 of them (68%) having diameters of ≥ 10 mm, and four (16%) with diameters between 5 mm and 9 mm; four reactions (16%) were negative. Mean diameter for the malarious children was 1.17 ± 0.7 mm (s.e.) and was significantly lower than normal ($P < 0.0005$).

Table 1. BCG vaccination in malarious and healthy children

	BCG vaccination		
	Yes	No	Total
Malarious	19	11	30
Healthy	19	6	25
Total	38	17	55

$\chi^2 = 1.02$, df: 1, $0.5 > P > 0.3$.

Table 2 shows the frequency distribution and mean tuberculin reaction diameters (TRD) of the malarious and healthy children in different age groups, as well as showing an initial decline in mean TRD with age amongst the healthy children. The lowest diameter was recorded in the 5–9 year age group, rising thereafter to attain the overall mean TRD of 9.5 mm at about the age of 10 years. A summary of the proportion of positive and negative reactivity in all the children is shown in Table 3, where 27 out of 30 (90%) malarious children had a negative tuberculin reaction in contrast to four of the 25 (16%) healthy children. It is of interest to note that out of the 19 malarious subjects who had BCG vaccination, the tuberculin reaction was negative in 16 (84.2%) of them. Conversely, 21 of the 25 (84%) healthy subjects, as distinct from three of the 30 (10%) malarious children, had positive tuberculin reactions.

Discussion

The present study has shown a strong association ($P < 0.0005$) between *P. falciparum* infection and tuberculin reaction, which is a recognized in-vivo test for cell-mediated immunity. The observation that 19 out of 30 (63.3%) of the malarious children had BCG vaccination (Table 1) is interesting since one would normally expect a positive tuberculin reaction in such children. Unexpectedly, a further analysis of the 19 malarious subjects with BCG vaccination shows that the tuberculin reaction was negative in 16 (84.2%) of them. The most likely explanation for the observation is the effect of *P. falciparum* infection, since other known common factors that will depress delayed hypersensitivity reactions (like protein–energy malnutrition and measles infection) had been eliminated earlier. Moreover, the Chi-square test for any possible influence of BCG vaccination on malaria, in our selection, was negative ($0.5 > P > 0.3$). This would also mean that our analysis does not put the 36.6% malarious children who were not previously vaccinated at a disadvantage when compared with the 24% healthy control children who were similarly not vaccinated.

The possibility of suspected BCG failures [16–18] cannot be ruled out from the present study, the result of which could either mean

Table 2. Mean tuberculin reaction diameter (TRD) of malarious (M) and healthy (H) children in different age groups

Age (months)	6–11		12–23		24–59		60–119		>120		All ages	
	M	H	M	H	M	H	M	H	M	H	M	H
Mean TRD (in millimetres)	0	17	0.6	12	1.3	7	4	6.6	0	10	1.17	9.5
s.d.	—	—	2.5	3.7	3.5	5.7	8.1	6.3	—	2.9	3.8	5.0
s.e.	—	—	1.1	2.6	0.9	1.8	3.3	2.4	—	1.3	0.7	1.0
n	3	1	5	2	15	10	6	7	1	5	30	25
t value	—		4.04		2.84		0.245		7.69		6.83	
degree of freedom (df)	—		5		23		11		4		53	
Significance	—		$P < 0.005$		$P < 0.005$		$P < 0.10$		$P < 0.001$		$P < 0.0005$	

Table 3. Tuberculin reactions in both malarious and healthy children

	Tuberculin reaction		
	Positive	Negative	Total
Malarious	3	27	30
Healthy	21	4	25
Total	24	31	55

$\chi^2 = 27.42$, df: 1, $P < 0.0005$.

that adequate cellular immunity to the tubercle bacillus has never developed or that an initial immunity has been lost over the years. The latter might be the case with the four children among our control subjects who gave a negative response to the delayed hypersensitivity reaction, despite previous BCG vaccination. Epidemiological, environmental, immunological and genetic factors have been suggested to be responsible for this phenomenon. There is, therefore, the need to define, in future studies, the individual or combined role(s) of these factors. Although the mothers were also questioned about the frequency of fevers likely to be malaria, it is also acknowledged that some of them might have given wrong information on this subject. It is not unexpected to note that six (all healthy children) of the 24 (25%) total tuberculin-positive children had not had BCG vaccination, yet they developed positive reactions. It is possible that these children might have been sensitized by a previous exposure to the tubercle bacillus, for example, during a probable subclinical primary tuberculosis, in an environment like Ibadan where tuberculosis is still not uncommonly seen [19]. The possibility of a previous sensitization by atypical mycobacteria, which may also be common in tropical populations, cannot be entirely ruled out. This is also expected to give a certain degree of protection against tuberculosis, although weaker than that induced by a potent BCG vaccine. Any sensitization would lead to the production of competent T-lymphocytes that would be able to take part in delayed hypersensitivity reactions, following an intradermal injection of a related antigen.

However, our patients also included three acutely ill malarious infants above the age of 6 months. This is at a period in childhood when the level of transplacentally transferred malarial antibodies is expected to be undetectable and other factors (humoral and/or cell-mediated) may dominate immune responses. Two of these infants had parasitaemia of 10% each and the third showed 15% parasitaemia. The third also had evidence of BCG vaccination but the tuberculin reaction was negative at the time of examination. This is unexpected because tuberculin conversion is normally observed after about 6 weeks of a successful BCG vaccination in the infant (unpublished observations); in fact, the 10-month-old healthy control in this study had BCG vaccination and

tuberculin converted to 17 mm diameter (Table 2). The other two malarious infants, however, had no evidence of BCG vaccination, and the tuberculin reaction was negative in both. Although this is expected it may also be due to a poorly developed cellular immunity to subclinical mycobacterial infections (including atypical) at this age.

Previous workers [8,9] had observed a marked fall in the differential and absolute counts of T-cells during acute malaria. It is, therefore, conceivable that while T-cells are actively engaged in the malarial antigen elimination, the remaining uncommitted cells may not be adequate for other cellular functions such as delayed-type hypersensitivity reactions. It has also been shown that immunosuppression to unrelated antigens occurs in malaria infection [20,21]. Our study lends support to this observation and further highlights possible clinical cellular immunodeficiencies that may follow *P. falciparum* infections. The titres of malarial antibodies in our series (to be published) did not demonstrate any appreciable level of humoral immunity in most of the malarious children, again suggesting a possible humoral immunosuppression in acute *P. falciparum* malaria. Brasseur *et al.* [22] and Druilhe *et al.* [23] recently demonstrated negative delayed cutaneous reactions in the severe forms of *P. falciparum* malaria. Repeated clinical malarial infections may have a cumulative depressive effect on cellular immunity and thereby render the body immunologically incompetent in combating infections known to attack the cellular components of the immune system. Such infections include measles and the incubation period of primary tuberculosis [24], as well as the Epstein–Barr virus, which has been suggested in the aetiology of Burkitt's lymphoma in malarious areas [25–27].

It is hoped that further studies on the duration of the depression of cellular immunity will help to establish how soon a normal delayed hypersensitivity reaction would occur after an acute attack of *falciparum* malaria.

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