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The γ/δ T-Cell Response to *Plasmodium falciparum* Malaria in a Population in Which Malaria Is Endemic

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Frequencies and absolute numbers of peripheral γ/δ T cells have been reported to increase after episodes of *Plasmodium falciparum* malaria in adults with limited or no previous malaria exposure. In contrast, little is known about the γ/δ T-cell response to malaria in children from areas where malaria is endemic, who bear the burden of malaria-related morbidity and mortality. We investigated the γ/δ T-cell response in 19 Ghanaian children from an area of hyperendemic, seasonal malaria transmission. The children presented with cerebral malaria ($n = 7$), severe malarial anemia ($n = 5$), or uncomplicated malaria ($n = 7$) and were monitored from admission until 4 weeks later. We found no evidence of increased frequencies of γ/δ T cells in any of the patient groups, whereas one adult expatriate studied in Ghana and three adults admitted to the hospital in Copenhagen, Denmark, all with uncomplicated, primary *P. falciparum* malaria, showed increased γ/δ T-cell frequencies similar to those previously reported. All patients had lowered absolute numbers of peripheral γ/δ T cells at admission, changing to increased numbers by days 7 to 14 and then returning to normal levels. The study raises questions regarding age and degree of previous exposure as determinants of malaria-induced γ/δ T-cell responses.

γ/δ T cells have been implicated in the immune response to malaria (17). A number of studies have shown in vitro recognition of malaria antigens by γ/δ cells (1, 7, 21), and others have reported increases in frequencies and absolute numbers of peripheral γ/δ T cells following clinical episodes (4, 11, 12, 22). Although the bulk of malaria-associated morbidity and mortality is borne by African children, most of the above data have been derived from studies of adults with little or no previous exposure to malaria. The relevance of the above findings to the naturally acquired immune response in a setting in which malaria is endemic is thus largely unknown.

To investigate this issue, we have examined frequencies and absolute numbers of peripheral γ/δ T cells following episodes of *Plasmodium falciparum* malaria in 19 children from an area of hyperendemic malaria transmission in Ghana and in four adults without previous history of malaria. All the children were febrile (≥37.5°C) at admission to the Department of Child Health, Korle-Bu Teaching Hospital, Accra, Ghana, and were diagnosed as having cerebral malaria ($n = 7$), severe malarial anemia ($n = 5$), or uncomplicated malaria ($n = 7$). Diagnostic criteria for cerebral malaria were a Blantyre coma score of <3 (19), lasting ≥30 min and not responding to intravenous glucose, and exclusion of meningitis, encephalitis, head trauma, diabetes, and a history of neurological disorder. The criteria for severe malarial anemia were as follows: hemoglobin, ≤5 g/dl; asexual parasitemia, ≥10,000/μl; full consciousness; no recent severe bleeding or other known cause of anemia; and an absence of other febrile disease. For uncomplicated malaria, the criteria were as follows: hemoglobin, ≥8 g/dl; asexual parasitemia, ≥10,000/μl; full consciousness; and exclusion of other febrile illness. All children were treated with a standard regimen of chloroquine (in cerebral malaria cases by nasogastric tube) (20), and all children recovered fully. Patients with sickle-cell hemoglobin in either heterozygous or homozygous form were excluded from the study. One European adult with uncomplicated *P. falciparum* malaria but without any history of previous malaria episodes was also studied in Ghana. Finally, three patients admitted to the Department of Infectious Diseases of the Rigshospitalet in Copenhagen, Denmark, with primary attacks of uncomplicated *P. falciparum* malaria were included in the study.

Samples of heparinized, peripheral blood (250 to 400 μl) were collected from the patients at admission. Additional samples were obtained 1, 2, and 4 weeks later. The study was approved by local Ghanaian and Danish ethical committees. Following automated hematological analysis, samples were centrifuged and the plasma was removed. After resuspension of cells in phosphate-buffered saline (PBS), 50-μl cell aliquots were labelled (20 min, room temperature) with directly fluorochrome-conjugated monoclonal antibodies to CD3 (clone SK7) and the γ/δ form of T-cell receptor (TCR-γ/δ) (clone 11F2) or with nonspecific isotype control antibodies. Erythrocytes were subsequently lysed with fluorescence-activated cell sorter lysing solution, washed twice in PBS–3% fetal calf serum, and analyzed by flow cytometry (using a Becton Dickinson FacScan cytometer in Ghana and a Coulter EPICS-XL cytometer in Denmark). All reagents were from Becton Dickinson, San Jose, Calif. Samples were live gated by forward and side scatter on lymphocytes, and 10,000 events were collected. Flow cytometric data are presented as means and 95% confidence intervals (children) or as individual data (adults).

Comparison of the three pediatric groups at particular time points was done by one-way analysis of variance (F), and that of patient data at different time-points was done by one-way

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TABLE 1. Clinical characteristics of Ghanaian donors at admission

Donor illness	*n*	Mean ± SD			Parasitemia (parasitized erythrocytes/μl)[a]
		Age (yr)	Temp (°C)	Symptoms (days)	
Cerebral malaria	7	6.0 ± 2.2	39.1 ± 1.0	3.6 ± 1.6	73,114
Severe anemia	5	6.8 ± 1.8	38.4 ± 0.8	4.4 ± 1.7	92,470
Uncomplicated malaria	7	6.3 ± 3.8	38.9 ± 1.1	3.7 ± 2.8	58,333

[a] Geometric mean.

repeated-measure analysis of variance (F), supplemented with the Student-Newman-Keuls test (q).

SigmaStat 1.02 software (Jandel Scientific, San Rafael, Calif.) was used for the statistical analysis.

Donor characteristics. Apart from the coma score and hemoglobin-hematocrit (which were used as defining criteria for clinical characterization), clinical characteristics such as age, axillary temperature, symptom duration prior to admission, and parasitemia were similar in the three pediatric clinical groups ($0.52 < P < 0.89$ in all cases) (Table 1).

γ/δ T-cell frequencies. The data on γ/δ T-cell frequencies are summarized in Fig. 1, expressed as the percentage of $CD3^+$ cells that were TCR-γ/δ^+. There were no significant differences between the mean frequencies in either cerebral malaria (Fig. 1A), severe malarial anemia (not shown), or uncomplicated malaria (Fig. 1B) at any particular time point ($0.38 < P[F] < 0.89$ at all times). Similarly, we could not detect any significant differences between frequencies at different time points in any of the pediatric clinical groups ($0.42 < P[F] < 0.64$) (data not shown). Patient day 30 γ/δ T-cell frequencies were similar to those measured in nine age-matched, healthy children from a nearby community (Student's t test, $P[t] = 0.21$).

In contrast to the findings in the Ghanaian children, γ/δ T-cell frequencies increased about twofold from days 0 to 7 in all four adult patients (Student's t test, $P[t] = 0.018$) (Fig. 1C). By day 28, γ/δ T-cell frequencies remained elevated in only one of these patients.

Absolute numbers of γ/δ T cells. The data on absolute numbers of γ/δ T cells are summarized in Fig. 2. In the Ghanaian children, absolute γ/δ T-cell numbers varied significantly with time ($P[F] < 0.001$) (Fig. 2A). Supplementary analysis revealed that numbers were significantly depressed at day 0 compared with those at days 7, 14, and 30 ($P[q] < 0.05$ in all cases) and significantly increased at day 7 compared with those at day 30 ($P[q] < 0.05$). There were no detectable differences between pediatric clinical groups (not shown). Absolute γ/δ T-cell numbers at day 30 were similar to those measured in nine age-matched, healthy children from a nearby community (Student's t test, $P[t] = 0.29$).

A pattern similar to that observed in the children was seen in the adult patients (Fig. 2B).

T cells express either of two distinct forms of the TCR, i.e., TCR-α/β or TCR-γ/δ (2). The former is by far the dominant in the peripheral blood of healthy adult Caucasians, in which only about 5% of $CD3^+$ cells express TCR-γ/δ (10). However, the limited data available (reference 8 and the present report) suggest that somewhat higher frequencies are typical of children in areas in Africa where malaria is endemic.

Several lines of evidence suggest a role for γ/δ T cells in the immune response to malaria as well as to other infectious diseases (17). A number of studies have shown marked responses of unprimed, human peripheral γ/δ T cells after stimulation by *P. falciparum* antigens in vitro (1, 7, 21), although limited γ/δ T-cell responses have been found in other studies (5, 16, 24). In addition, persistent increases in frequencies and absolute numbers of TCR-γ/δ^+ cells following *P. falciparum* malaria episodes are a general finding (3, 11, 21, 22). In common in all these studies is the fact that adult patients with little or no previous malaria exposure, and suffering from uncomplicated disease episodes, were studied. Little is known regard-

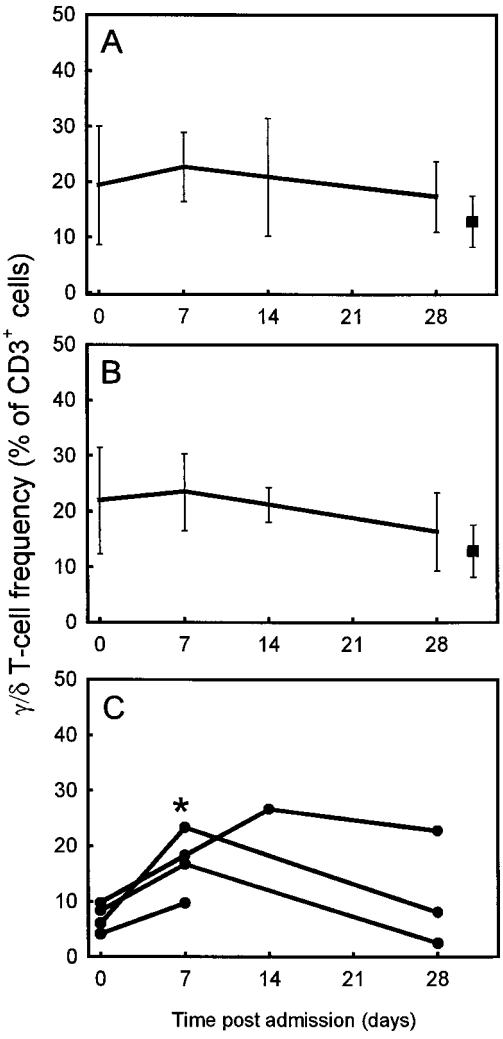

FIG. 1. Kinetics of peripheral γ/δ T-cell frequencies following episodes of *P. falciparum* malaria in seven Ghanaian children with cerebral malaria (A), seven Ghanaian children with uncomplicated malaria (B), and four adults without previous malaria episodes (C) (A and B) means and 95% confidence intervals for means; (C) individual data. The asterisk indicates mean values statistically different ($P < 0.05$) from corresponding day 0 values (see text for details).

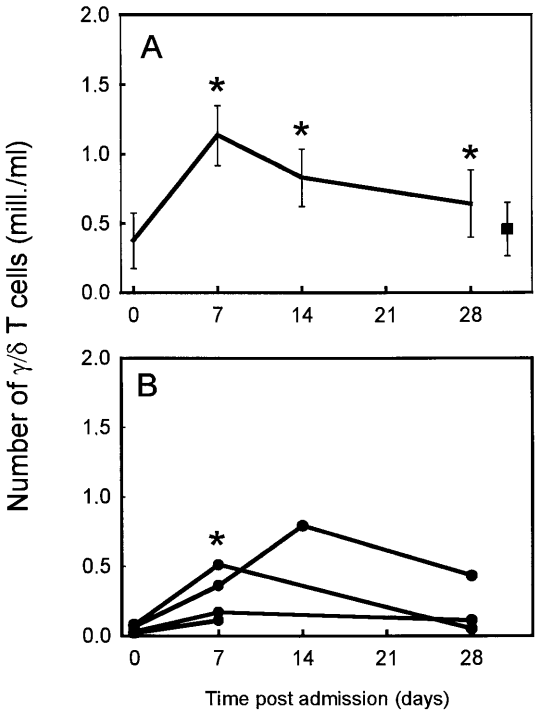

FIG. 2. Kinetics of absolute numbers of γ/δ T cells in peripheral venous blood following episodes of *P. falciparum* malaria in 19 Ghanaian children (A) and in four adults without previous malaria episodes (B). Data presentation as in Fig. 1.

ing the γ/δ T-cell response to malaria in areas of high malaria endemicity, in particular in children, who carry the vast majority of malaria-related morbidity and mortality in such areas.

In the present study of children from an area where malaria is hyperendemic (coastal Ghana), we found no evidence of increased frequencies of peripheral γ/δ T cells following episodes of *P. falciparum* malaria. In contrast, γ/δ T-cell frequencies in an adult expatriate monitored after a primary attack of uncomplicated *P. falciparum* malaria in Ghana, and in another three similar patients studied in Copenhagen, showed increases similar to those seen in previous studies.

There are several possible explanations for the observed difference between the patients from the area where malaria is endemic and the previously unexposed patients. While the frequency of γ/δ T cells appears to increase following *P. falciparum* malaria in individuals with little previous exposure, it may reach a plateau in a setting where the disease is endemic, after which little further change occurs. Alternatively, γ/δ T-cell responses may be different in children and in adults. Finally, it is possible that changes too transient to be detected with the present or previous approaches do occur. We are currently investigating this last possibility.

Lymphopenia (4, 9, 18) and endothelial inflammation (6, 14, 15) are general findings in acute *P. falciparum* malaria. In accord with this, we found that absolute numbers of γ/δ T cells were lower than normal at admission, probably reflecting disease-induced T-cell reallocation (6, 13). That malaria-induced reallocation of γ/δ T cells does occur is supported by a recent paper by Rzepczyk et al. (23), in which numbers of peripheral γ/δ T cells dropped at the onset of erythrocytic parasitemia in two patients experimentally infected with *P. falciparum*. In the present study, the absolute numbers of γ/δ T cells were above normal on day 7 postadmission, presumably indicating release of sequestered cells with the submission of disease-induced inflammation.

γ/δ T cells have been implicated in both the pathogenesis of and protection against malaria (17). Not much evidence exists for the former hypothesis, and most evidence for the latter is based on in vitro experimentation and/or animal models, the relevance of which to human infection is uncertain. In any case, we were unable to detect any differences in the γ/δ T-cell responses between different well-defined clinical groups of children in the present study.

The overall conclusion must be that much remains to be learned about γ/δ T cells in relation to malaria, before conclusions on their role can be attempted. To this end, studies of those most vulnerable to morbidity and mortality in areas where malaria is endemic would appear to be the most informative.

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