A longitudinal study of the role of T cell subset, Th1/Th2 cytokines and antiplasmodial antibodies in uncomplicated malaria in a village population chronically exposed tp *Plasmodium falciparum* malaria.

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ABSTRACT

Immune individuals living in malaria endemic areas commonly show low-grade parasitemia without clinical manifestation. In those areas, unprotected children suffer repeated and sometimes severe attacks that become milder with age. Malaria infection results in the production of multiple immune mechanisms We hypothesized that T cell subsets ratio, Th1 and/or Th2 Cytokines; and IgG subclasses to crude malaria antigens contribute to the development of protective immunity and resistance to clinical effects of multiple infections.

This longitudinal study of the acquisition of naturally acquired immunity to malaria in Simbok village allows us to follow 108 villagers who presented with multiple episodes of *P. falciparum* malaria fever within the 45-month period of the study. Malaria in Simbok was qualified as perennial in transmission and meso to hyperendemic in character. Fever and anemia were reported as the main symptoms observed in our studied patients. Single infections with *P. ovale* were only observed in healthy subjects. The level of CD4+/CD8+ ratio increased with the episode of fever ($r = 0.48$). We observed that patients presented a decreased CD16+/CD3+ ratio as the number of episodes increased ($r = -0.27$).

We observed that the mean IL–5 was lower in anemic (6.4±8.4 Pg/ml) than in non-anemic patients (19.0±53.5 Pg/ml): P=0.001. There was a decrease in the plasma level of IL–5 as the number of clinical episode of fever increases ($r = -0.47$). We considered that individuals with multiple malaria attacks were not protected but that they had weak immunity to malaria. The overall profile of IFN–γ production is reflected by its correlation with the episode of clinical fever ($r=0.39$). Among patients, the mean plasma level of TNF – α in malaria positive slides was higher (77.8±68.6 Pg/ml) than that observed in slide negative (52.3±56.8 Pg/ml) (P=0.002). In contrast, such difference was not observed in the control group (P=0.08). As found in this study the low level of Th2 (IL – 5) and high levels of Th1 (TNF – α and IFN-γ) in association with malaria severity suggested that a Th1-like response may be more important in the primary immunity to malaria compared to a Th2-like response. The role of the Th1 response seems to reduce the parasite density at a low level that can be easily controlled by the Th2 immune response. From our data, Th1 and Th2 responses are required for antimalarial protection.

No matter the health status of the studied individuals, the plasma level of total IgG to crude malaria antigen was higher in slide negatives than in slide positives ($P<0.05$). In addition, a slight negative correlation was observed between the level of IgM and the level of total IgG to malaria ($r = -0.25$), verifying that during malaria infection, IgM formation starts first and as IgG levels begin to rise, IgM levels do reduce. Our results showed that except for IgG4 (22.3%), the prevalence of each IgG subclass was above 70% and that the level of total IgG correlated with those of all the four IgG subclasses. The level of IgG4 to crude malaria antigens positively correlated with the episode of clinical fever and negatively with the age of the study subjects whereas the levels of IgG1, IgG2 and IgG3 decreased following the episode of fever. From these results we suggest that antimalarial IgG1, IgG2 and IgG3 antibodies might be positively involved in building-up clinical acquired immunity to malaria while IgG4 might be negatively implied in the same phenomenon.

INTRODUCTION

Malaria is one of the vector-transmitted diseases that cause most of the childhood morbidity and mortality in developing countries. The neutralization of the parasite and its antigens has been demonstrated to depend on antibodies acting alone or, more commonly in conjunction with effector cells. T cells and the corresponding cytokines produced have been suggested to play important role (Tshuji et al., 1994) in immunity to malaria. Little is known about the changes in blood level of T-cell subset, cytokines and antimalarial antibodies in individuals continuously exposed to *Plasmodiun falciparum*. We hypothesised that T cell subsets ratio, Th1 and/or Th2 Cytokines; and levels of IgG subclasses contribute to the development of protective immunity and resistance to clinical effects of malaria. During this work, we attempt to carry out a longitudinal study in individuals repeatedly exposed to malaria in order to determining the role of T cell subset populations (CD3+, CD4+, CD8+ and CD16+ cells), Th1/Th2 cytokines (TNF–α, IFN– γ, IL-5) and IgG subclasses in asymptomatic and clinical malaria. By so doing, we intend to have a better understanding of how people repeatedly exposed to malaria develop natural immune response that reduce the rate and density of infection and how they acquire protective immunity to the disease.

MATERIALS AND METHODS

This longitudinal study was conducted in Simbok, a malaria endemic village located near Yaounde, the capital city of Cameroon. It is within the rainforest belt of Central Africa and is irrigated by two rivers (Biyeme and Mefou), that flow through a network of swamps. Permission to undertake field studies was obtained from the Cameroonian Ministry of Health. Ethical clearance to conduct the research was obtained from the Ethical Committee, Faculty of Medicine and Biomedical Sciences, University of Yaounde I and the Georgetown University Institutional Review Board. The project was covered by assurance N° S-9601-01. After reaching agreements with the authorities of the village, details of field and laboratory procedures were explained and participation in this project was voluntary.

The first part of this study consisted of recruitment of samples and was carried out within 45 months [December 1996 – August 2000] during which patients were allowed to come for medical consultation without constrains any time that they were sick. Within a month (28 days), eligible subject had only one opportunity to be considered as a sample. Nevertheless, a subject could be sampled many times within the period of study. Included in the study was any patient without age or gender discrimination who presented with fever and diagnosed by a medical doctor to be suffering from malaria. The following inclusion criteria were also taken into account: fever (axillary temperature + 0.5 ≥ 37.5 °C) or previous fever within the last 48 hours that preceded consultation (Ingegerd-Rooth et al., 1992; Schmitt, 1984; Trape at al., 1985; Muriel-Cornet, 1998); chill (axillary temperature + 0.5 ≤ 36.5 °C) or previous chill within the last 48 hours that preceded consultation, general body malaise, abdominal discomfort (vomiting, anorexia...etc.) and headache; absence of any other infection that could cause fever. A code number was assigned to each patient. Age, sex, axillary temperature and body weight of each patient were also recorded. For each patient who filled the inclusion criteria, 5ml venous blood was collected in EDTA tube for laboratory analysis. All the patients were provided with free medical consultation and essential health services. Our sample size was calculated according to Lorentz's formula (WHO, 1989). The theoretical number of subjects needed was 380. We effectively sampled 626 patients and that was in favor of the accuracy of our results.

The second part of this study was carried out in the laboratory of the Biotechnology Centre of the University of Yaounde I and in the Biology Department, Georgetown University, Washington DC. Parasitology for malaria parasite was done by Microscopy and by Polymerase Chain Reaction (PCR). For microscopy, thin and thick smears were made on the same slide, air-dried and stained using Field rapid stain (eosin and methylene blue). A slide was considered positive after the demonstration of malaria parasites in the blood. Morphological details of the parasites were determined on the thin film and parasite density on the thick film. Each slide was reviewed by two different microscopists. A blood film was considered negative if no parasite was detected in 200 oil immersion fields of the thick smear at a magnitude of X100. The parasite density per microliter (µl) of blood was calculated by assuming an average of 8,000 leucocytes per µl of blood. (Ingegerd-Rooth et al., 1992; Muriel-Cornet, 1998). We counted 200 leucocytes at the same time with malaria parasites and extrapolated to 8,000 leucocytes.

Anemia was assessed using the packed cell volume (PCV) determination in fresh blood. For each blood sample, a microcapillary tube was filled up to about three-quarter of its volume, then sealed with critoseal and spun in a microcapillary centrifuge (IEC Micro-MB centrifuge) for 5 minutes at 15,000 rpm. The packed cell volume was read using a PCV reader (WHO, 1980). Anemia and severe anemia were defined as PCV<32% and PCV≤22% respectively (Muriel-Cornet et al., 1998; Bojang et al., 1997).

For the assessment of T cell subset, immuno-alkaline phosphatase labeling procedures, applied to EDTA anticoagulated peripheral blood samples was used to determine CD3+, CD4+, CD8+, and CD16+ cells. The ZIMED kid was used and the steps were as earlier described by Erber et al in 1984 and by Wong et al in 1986, but with few modifications. Duplicates of test samples were sent to Kampala in Uganda for the analysis of T cell surface markers by flow cytometric procedures (methods not described in this work). The results of both methods were compared.

For quantitative determination of Cytokine levels in plasma, three commercial Enzyme Immunoassay (EIA) kits were used: IL – 5 EIA kit (N° 1983 – 96 tests), IFN – γ EIA kit (N° 1743 – 96 tests) and TNF– α EIA kit ((N° 1121 – 96 tests) from Immunotech. Each kit was performed according to the manufacturer instructions.

The Semi quantitative determination of antibodies to malaria antigens was done using the indirect ELISA procedure. The variation coefficient for inter-plate variation was also calculated with the critical value standing at 10%. The cut off point defined as the limit of negativity was calculated by adding two times standard deviation to the mean of the negative control value.

Pearson's χ^2 analysis was performed to test differences in distributions, z and Student's t tests to evaluate differences between means. The Spearman's rank correlation test was used to determine the relationship between two or more data sets. Statistical significance was designated as $P \leq 0.05$.

RESULTS

The following are the age specific rates of infection with *plasmodium* detected through three consecutive surveys in Simbok (1996 – 1998):

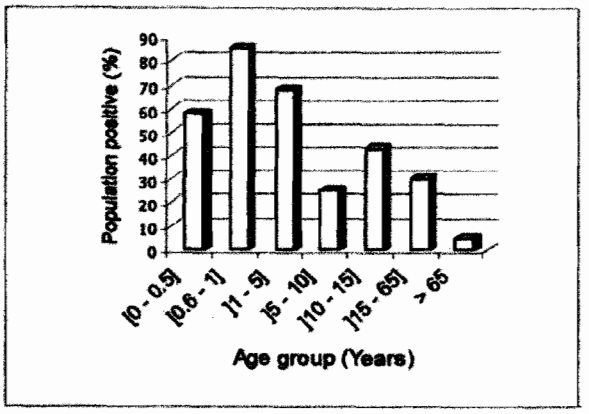

Figure 1: Malaria prevalence in Simbok (November 1996)

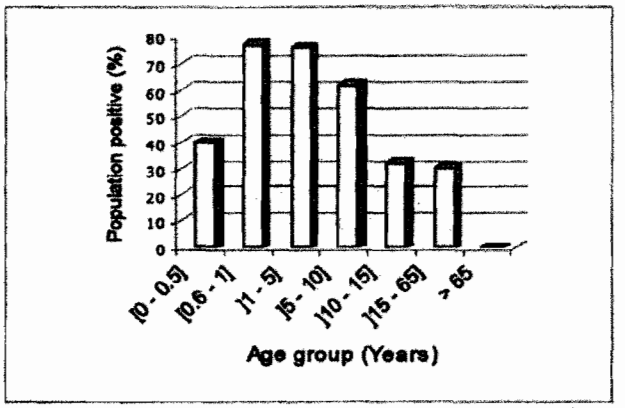

Figure 2 : Malaria prevalence in Simbok (November 1997

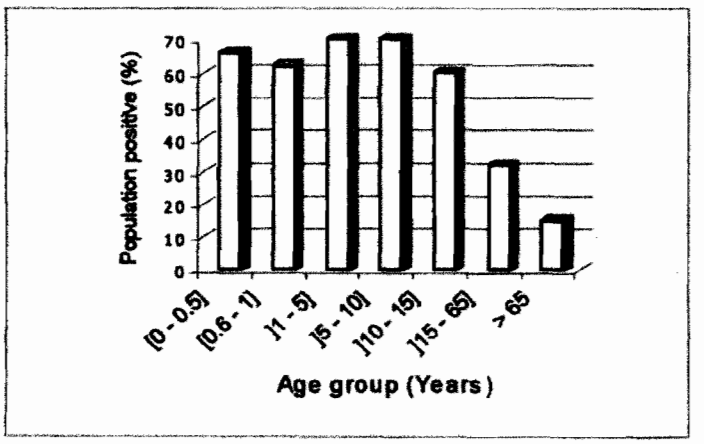

Figure 3 : Malaria prevalence in Simbok (November 1998)

The parasite rate, defined as the proportion of the survey population with patent *Plasmodium* parasitemia in the three surveys, in 1996, 1997 and 1998, was 41% (102/249), 45% (144/317) and 54 % (203/370) respectively (figure 1 – 3). Infections were predominantly *P. falciparum* (98%). Malaria in Simbok was qualified as perennial in transmission and meso to hyperendemic in character.

Of the 626 volunteers who took part in this study, 60% (377/626) were female and 40% (249/626) were male; 5.91% (37/626), 22.36% (140/626), 15.02% (94/626), 12.78% (80/626) and 43.93% (275/626) aged in the following ranges: [0 – 1],]1 – 5],]5 – 10],]10 – 16[and [16 – 67] respectively. The younger age was 2 months and the higher age was 67 years. The mean age was 19.43 ± 18.63 years.

During the study period, 108 patients attended the clinic more than once. Among them, 6.48% (7/108), 24.07% (26/108), 16.67% (18/108), 12.04% (13/108) and 40.74% (44/108) were babies ([0 – 1] year), infants (]1 – 5] years), children (]6–10[years), junior ([11 – 16] years) and adults (≥ 16 years) respectively. Of them, 59.26% (64/108) were female and 40.74% (44/108) were male. As controls, 55.56% (60/108) participated. Of controls, 50% (30/60) were female and 50% (30/60) were male. All the age groups were represented as shown in table 1.

Table 1: Frequency and age distribution of studied subjects

	Age group (years)											
	[0 – 1 [		]1 – 5]		]5 – 10]		]10 – 16[		]16 – 65]		Total	
Total number of subjects (Population)	37 (5.91%)		140 (22.36%)		94 (15.02%)		80 (12.78%)		275 (43.93%)		626 (100%)	
	F	M	F	M	F	M	F	M	F	M	F	M
	17	20	57	83	56	38	53	27	194	81	377	249
Total number of patients with multiple episodes of clinical fever (Samples)	7 (6.48%)		26 (24.07%)		18 (16.67%)		13 (12.04%)		44 (40.74%)		108 (100%)	
	F	M	F	M	F	M	F	M	F	M	F	M
	4	3	9	17	11	7	9	4	31	13	64	44
Controls (free of clinical fever)	3 (5 %)		18 (30 %)		11 (18.33%)		3 (5 %)		25 (41.67%)		60 (100 %)	
	F	M	F	M	F	M	F	M	F	M	F	M
	2	1	5	13	6	5	2	1	15	10	30	30

Legend:
F = Female
M = Male

The incidence, defined as the number of new recruited cases per year (WHO, 1989) was reported in table 2.

Table 2: The incidence of clinical fever in Simbok

Age groups (Years)	1997	1998	1999	2000 (8 months)	Mean ±SD
[0 – 1 [	10	5	7	5	6.90 ± 2.10
]1 – 5]	29	29	28	15	26.20 ± 5.40
]5 – 10]	31	11	7	13	15.73 ± 9.69
]10 – 16[	15	10	9	21	13.10 ± 4.47
[16 – 67]	76	51	40	44	53.55 ± 14.51
Total	161	106	91	98	115.45 ± 28.76

Table 3: Number of studied subjects per episode of fever and in each age group.

		Episode				
		1st	2nd	3rd	4th	5th
Age groups (years)	[0 – 1 [	37	7	2	2	0
	]1 – 5]	140	26	12	1	0
	]5 – 10]	94	18	6	4	3
	]10 – 16[	80	13	6	2	2
	[16 – 67]	275	44	14	6	1
	Total	626	108	40	15	6

The number of subjects decreases as the number of episode increase ($r = -0.8$).

The mean temperature of anemic patients was higher (38.3±1.1) than that recorded in non-anemic (37.9±0.9) (P=0.001). The mean age of patients who presented with high temperature (16.2±17.4) was lower than that of those without (26.9±19.5): $P=4.6\times10^{-6}$ (figure 4).

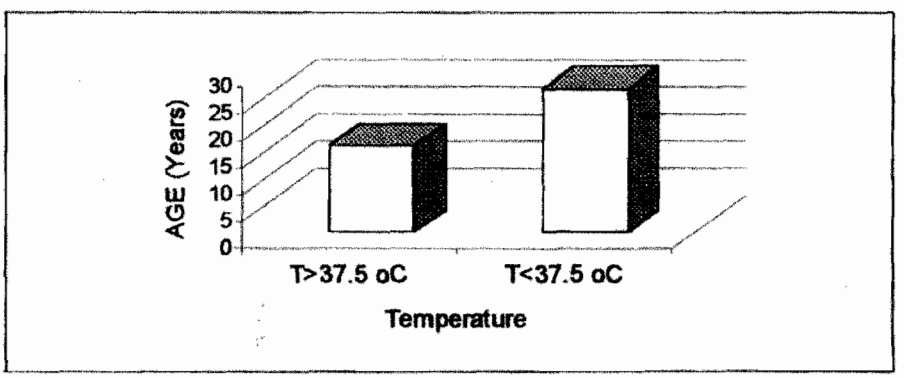

Figure 4: Relationship between the body temperature and the age of patients

Most of the samples (86.6%) presented with high temperature (T ≥ 37.5 °C) while 11.3% and 2.1% had normal temperature (36.5 ≤ T<37.5 °C) and low temperature (T < 36.5 °C) respectively. We also recorded that the mean packed cell volume (PCV) of patients with high temperature (T≥37.5 °C) was low (33.9±5.5%) compare to that of patients without (36.3±5.2%): P=0.0006 (figure 5).

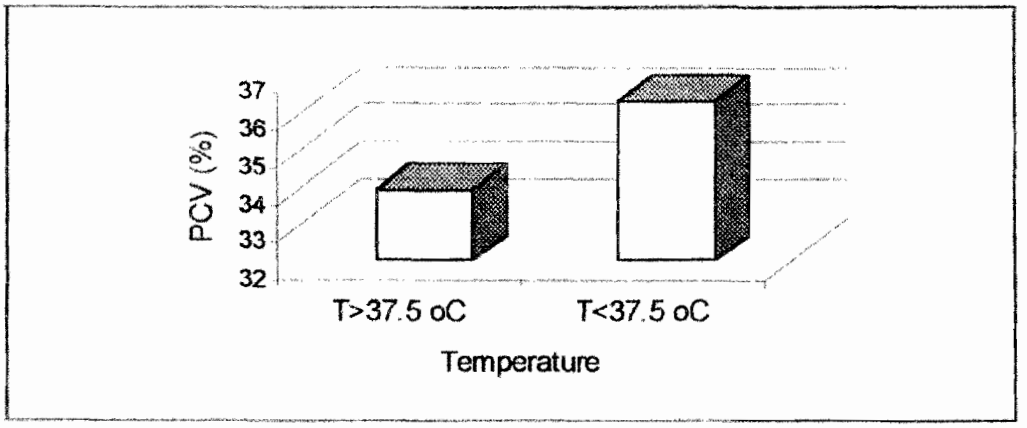

Figure 5: Relationship between PCV and temperature

In the studied subjects, the prevalence of anemia (25% < PCV < 32%) and severe anemia (PCV ≤ 25%) were 22.6% (54/239) and 3.8% (9/239) respectively; while 13.3% (8/60) and 1.7% (1/60) of the control group presented with anemia and severe anemia respectively. The mean age from which a Simbok baby presented with malaria parasites was 7.3 ± 4.0 months.

Table 4: Results of the malaria parasite speciation by PCR

	Patients	Healthy samples (Controls)
P. falciparum	99.4% (158/159)	81.3% (26/32)
P. malariae	9.4% (15/159)	40.6% (13/32)
P. ovale	3.1% (5/159)	12.5% (4/32)
P. vivax	0% (0/159)	0% (0/32)
P. falciparum + *P. malariae*	8.8% (14/159)	25% (8/32)
P. falciparum + *P. ovale*	3.1% (5/159)	9.4% (3/32)
P. malariae + *P. ovale*	0% (0/159)	6.3% (2/32)
P. falciparum + *P. malariae* + *P. ovale*	0% (0/159)	6.3% (2/32)
P. malariae alone	0.6% (1/159)	15.6% (5/32)
P. ovale alone	0% (0/159)	3.1% (1/32)

In patients, all the *P. ovale* infections were associated with *P. falciparum* infections. Single infections with *P. ovale* were only observed in healthy subjects.

The mean parasite density was high in anemic (19406±68538) compare to non-anemic patients (6957±69201). P=0.05 (figure 6).

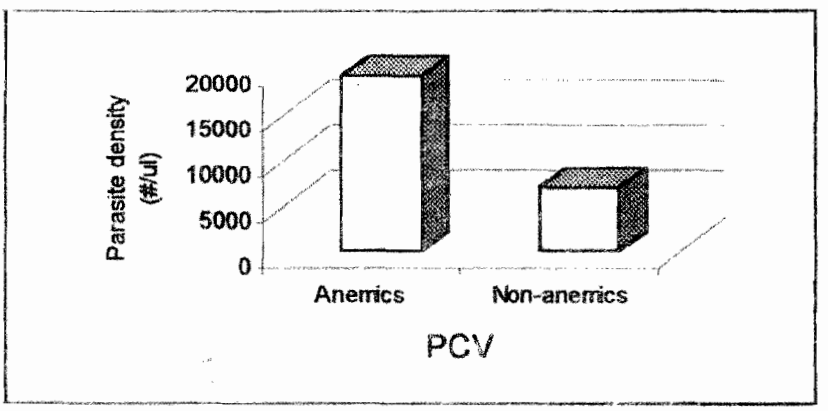

Figure 6: relationship between the parasite density and the PCV in patients

The difference between the results obtained by immunoenzymatic method and those obtained by Flow Cytometric method were not statistically significant (t = 1; P=0.36). The difference between CD4+/CD8+ ratio (respectively CD16+/CD3+ ratio) in slide positive patients did not differ from that in slide negative patients: P=0.2 and P=0.16 respectively. In addition, the level of CD4+/CD8+ ratio increased with the episode of fever (r = 0.48). We observed that patients presented a decreased CD16+/CD3+ ratio as the number of episodes increased (r= -0.27).

One of the specific objectives was to determine the role of Th1/Th2 cytokines (IL-5, IFN- γ and TNF-α,) in clinical malaria and protective immunity. We observed that among patients, the mean plasma level of IL-5 in slide positives was lower than that in the slide negatives (P= 0.04). Similarly, we observed among healthy subjects that the mean IL-5 levels in slide positives was low compared to that in slide negatives (Figure 48). In addition, we observed that the mean IL-5 was lower in anemic (6.4±8.4 Pg/ml) than in non-anemic patients (19.0±53.5 Pg/ml): P=0.001 (Figure 7). Moreover, there was a decrease in the plasma level of IL-5 as the number of clinical episode of fever increases (r= -0.47).

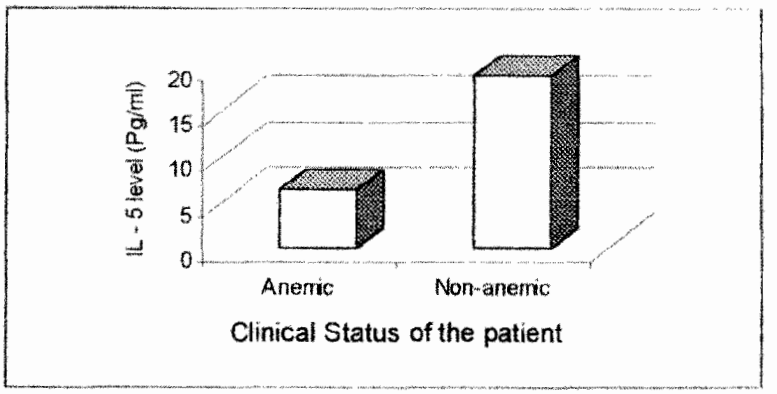

Figure 7: Relationship between the blood level and the clinical status of patients

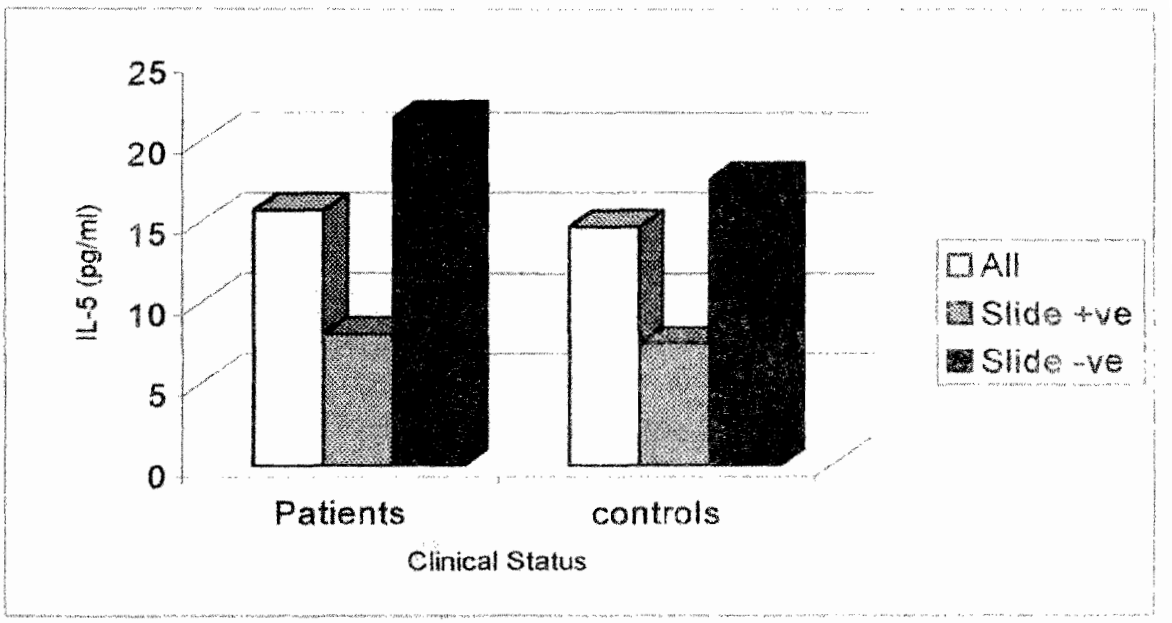

Figure 8: Relationship between the mean plasma level of IL-5 and the clinical status

The overall profile of IFN-γ production was reflected by its correlation with the episode of clinical fever (r=0.39). Among patients, the mean plasma level of TNF – α in malaria positive slides was higher (77.8±68.6 Pg/ml) than that observed in slide negative (52.3±56.8 Pg/ml) (P=0.002) (Figure 9). In contrast, such difference was not observed in the control group (P=0.08). In addition, a high level of TNF – α was recorded in anemic (77.2±90.6 Pg/ml) compare to non-anemic patients (58.7± 52.7 Pg/ml): P=0.049 (figure 10).

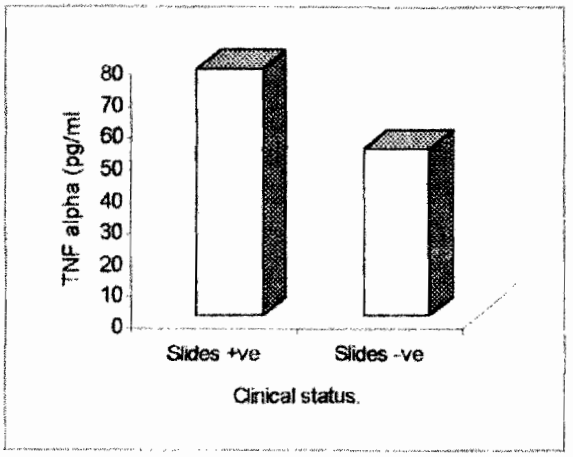

Figure 9: Relationship between the mean plasma level of TNF – α and the clinical status of patients

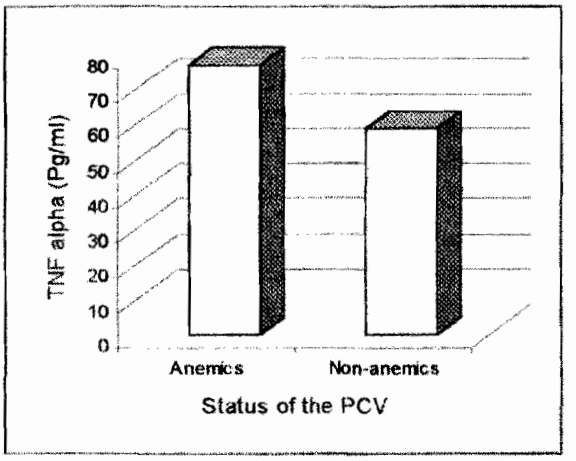

Figure 10: Relationship between the mean plasma level of TNF – α and the PCV of patients

97.6% (279/286) and 91.3% (73/80) of the patients were positive for total IgG and IgM respectively while 96.6% (56/58) and 100% (19/19) of the control samples were respectively positive for the same antibodies. No matter the health status of the studied individuals, the plasma level of total IgG to crude malaria antigen was higher in slide negatives [OD = 0.84 ± 0.25 for patients and 0.84 ± 0.21 for healthy subjects (controls)] than in slide positives (OD = 0.78 ± 0.26 for patients and 0.68 ± 0.25 for controls respectively) (P=0.015 for patients and P= 0.006 for healthy subjects) (Figure 11). In addition, the level of antimalarial IgG was higher in slide positive patients than in healthy subjects with malaria positive slides (P=0.03).

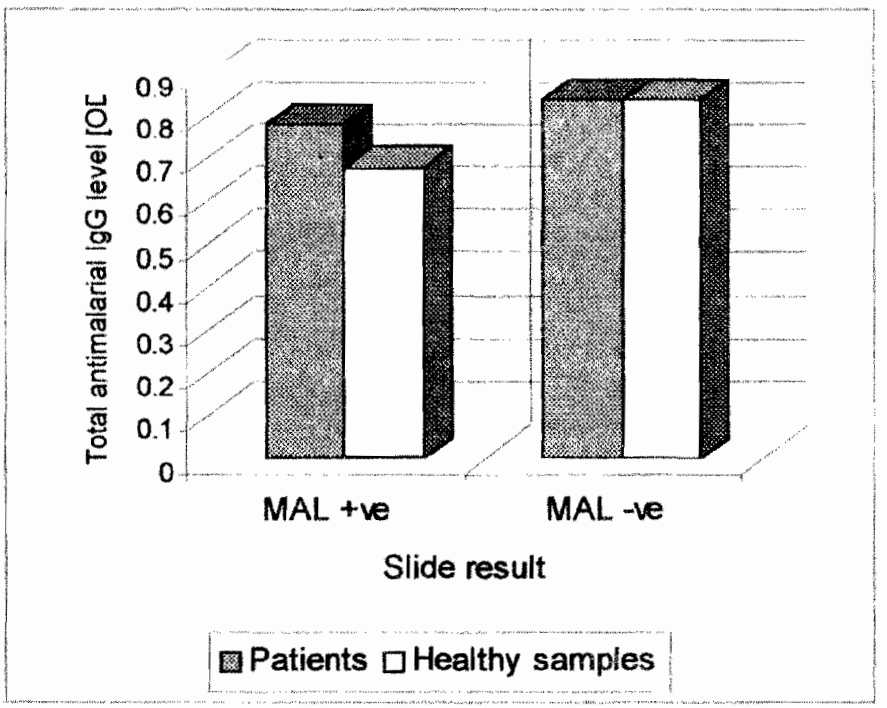

Figure 11: Relationship between the plasma level of total IgG and the health status

A slight negative correlation was observed between the level of IgM and the level of total IgG to malaria (r= – 0.25).

83% (229/276) and 72.8% (198/272) of the patients were positive for IgG1 and IgG2 respectively while 81% (47/58) and 77.6% (45/58) of the control samples were respectively positive for the same antibodies. In addition, 93.4% (254/272) and 22.3% (51/273) of the patients were positive for IgG3 and IgG4 respectively while 100% (58/58) and 19.6% (11/58) of the control samples were respectively positive for the same antibodies

Table 5: Relationship between the plasma level of antimalarial IgG subclasses and the episode of fever in the studied patients

	Coefficient of correlation (r)		
	All the patients	Slide +ve	Slide -ve
Total IgG	**0.93**	0.30	0.92
IgG1	**-0.32**	-0.29	-0.73
IgG2	**-0.55**	-0.32	-0.38
IgG3	**-0.40**	-0.79	0.22
IgG4	**0.91**	0.57	0.73

Table 38 illustrated that the plasma level of antimalarial total IgG, IgG1, IgG2 and IgG3 subclasses to crude malaria antigens correlated with the age of the studied subjects. In contrast the IgG4 subclass negatively correlated with age.

Table 6: Relationship between the IgG subclasses and the age of the studied subjects

	Coefficient of correlation (r)					
	Patients			Controls (Healthy subjects)		
	All the patients	Slide +ve	Slide -ve	All the subjects	Slide +ve	Slide -ve
TIgG	**0.24**	0.06	0.30	**0.30**	-0.30	0.51
IgG1	**0.14**	0.09	0.14	**0.06**	0.12	0.09
IgG2	**0.35**	0.40	0.31	**0.30**	0.37	0.26
IgG3	**0.42**	0.29	0.45	**0.31**	0.04	0.39
IgG4	**-0.12**	-0.05	-0.10	**-0.12**	-0.22	-0.1

DISCUSSION

Of the 626 subjects who participated in this study, special attention was drown to 108 patients (17.3%) who had fever at least twice during the course of the study. As controls, 55.6% (60/108) of them accepted to participate after recovery. High temperature was recorded in 86.6% of cases and 13.4% reported intermittent fever. Many patients thus probably came to the consultation in a non-febrile phase of an intermittent fever. The mean age of patients who presented with high temperature and/or anemia was lower than that of those without This result was in accordance with the observation of Taylor et al., 1998 stating that protective immunity to malaria increases with age in residents of endemic area. In fact, malaria is one of the major factors of anemia in endemic areas and the mechanism appears to involve mostly hemolysis caused directly by the parasite and dyserythropoiesis. Our patients presented with most of the malaria infections (99.4%) due either to *P. falciparum* alone or concomitantly with *P malariae* or *P ovale*. Similarly to the results obtained in Congo by Trape and collaborators in 1985, we observed that *P. malariae* or *P. ovale* alone caused none of the clinical attacks.

One of our specific aims was to examine the role of T cell subset populations (CD3+, CD4+, CD8+, CD16+ cells) in asymptomatic and clinical malaria in population with and without clinical malaria and look for possible correlation with disease and protection. We hypothesis that T cell subsets ratio contributes to the development of protective immunity and resistance to clinical effects of multiple infections. According to our results, the difference between CD4+/CD8+ ratio (respectively CD16+/CD3+ ratio) in slide positive patients did not differ from that in slide negative patients: P=0.2 and P=0.16 respectively. It resulted from this observation that the parasite density may not play a significant role in protective immunity to malaria due either to either CD4+ or to CD8+. We observed that patients presented a decreased CD16+/CD3+ ratio as the number of episodes increased (r= –0.27). These results imply that lymphocytes that mostly display CD16+ molecules (K and NK cells) may be more involved in the primary than in the secondary immune response to malaria as earlier mentioned by Winkler et al., 1999a in a hyperendemic region of Gabon. In fact, NK cells are increasingly recognised as important components of the early innate immune response against murine and human plasmodium spp. and have been shown to be considerable producers of cytokines such as IFN-γ (Winkler et al., 1999b).

One of the specific objectives was to determine the role of Th1/Th2 cytokines (IL-5, IFN– γ and TNF–α,) in clinical malaria and protective immunity. Our results showed that the mean plasma level of IL–5 in slide positives was lower than that in the slide negatives (P= 0.04). Similarly, we also observed that among healthy subjects the mean IL–5 levels in slide positives was low compared to that in slide negatives. In addition, we observed that the mean IL–5 was lower in anemic than in non-anemic patients (P=0.001). From these observations, we suggested that malaria infection correlates with low IL–5 levels, no matter the clinical status. Low IL–5 levels also correlates with the severity of anemia. It is clear from our data, that IL-5 expression is down-regulated in acute *P. falciparum* malaria. From our results, a

decrease in the level of IL – 5 coincided with the vulnerability to malaria fever. Then, IL – 5 coincided with protective immunity to malaria.

The overall profile of IFN–γ production is reflected by its correlation with the episode of clinical fever (r=0.39). We then postulate that despite de dichotomic role of IFN – γ, it seems to be more implied in pathogenesis than in protective immunity to malaria.

Among patients, the mean plasma level of TNF – α in malaria positive slides was higher than that observed in slide negative (P=0.002). In contrast, such difference was not observed in the control group (P=0.08) In addition, a high level of TNF – α was recorded in anemic compare to non-anemic patients (P=0.049). Those observations gave right to suspect high levels of TNF – α in patients with fever who live in malaria endemic region not only as an index of malaria, but also as an index of disease severity. These results were not unexpected, since it has been shown that IFN-γ and TNF-α suppress hematopoiesis and that TNF-α may contribute to severe malaria as a result of up-regulation of intercellular adhesion molecule-1 (ICAM-1) in cerebral blood vessel endothelium (Miller et al., 1994).

As found in this study the low level of IL – 5 and high levels of TNF – α and IFN-γ in association with malaria severity suggested that a Th 1-like response may be more important in the first line of immunity to malaria compared to a Th2-like response. In fact, the beneficial and harmful role of Th1 cytokines (IFN-γ and TNF – α) during acute malaria has been reported (Cruz-Cubas et al., 1993). At low concentration, they beneficially contribute in the immune response and at high concentration, they reflect difficulties of controlling parasite growth.

Our results showed that almost all our patients and controls were positive (>91%) for IgM and total IgG to crude malaria antigen. We also observed that no matter the health status of the studied individuals, the plasma level of total IgG to crude malaria antigen was higher in slide negatives than in slide positives. This looks reasonable if we consider that in slide positives, the implication of IgG in neutralization of the parasites has contributed to reduce the concentration of free and circulating IgG. This also implies that high IgG response coincides with recovery from malaria. The slight negative correlation observed between the level of IgM and the level of total IgG to malaria (r= –0.25) verify the well-documented statement reporting that during malaria infection, IgM formation starts first and as IgG levels begin to rise, IgM levels do reduce.

Part of our interest in this work was to study if there is any combinations of IgG subclasses (IgG 1, 2, 3, 4) that may be responsible for protective immunity. Our results showed that except for IgG4 (22.3%), the prevalence of each IgG subclass was above 70% and that the level of total IgG correlated with those of all the four IgG subclasses. We considered that individuals with multiple malaria attacks were not protected but that they had weak immunity to malaria. The level of IgG4 to crude malaria antigens positively correlated with the episode of clinical fever and negatively with the age of the study subjects whereas the levels of IgG1, IgG2 and IgG3 decreased following the episode of fever. These results mean that antimalarial IgG1, IgG2 and IgG3 antibodies might be positively involved in building-up clinical acquired immunity while IgG4 might be negatively implied in the same phenomenon. Similarly, Chumpitazi et al., 1996 also reported a negative role of IgG4 and a positive role of IgG1 and IgG3 in the acquisition of natural immunity to malaria during a longitudinal study in a meso-endemic region of Madagascar.

CONCLUSION

We suspected from this study that in addition to the protective role of CD8+ cells, their high level may play a role in pathogenesis of malaria while CD4+ cells appear to be actively implied in reducing the clinical manifestations, thus in protective immunity to malaria. The parasite density seems not to play a significant role in protective immunity due either to CD4+ or to CD8+. This work demonstrates NK cells as important components of the early immune response to malaria.

It is clear from our data that type 2 cytokines (IL – 5) coincide with protective immunity to malaria whereas type1 cytokines (IFN – γ and TNF – α) participate in both pathogenesis and protective immunity to malaria. A balance level of Th1/Th2 cytokines is required for and optimal immune response to malaria. The challenge is to determine and induce a protective balance of Th1-Th2 response in the group at risk of malaria.

This study illustrates that IgG and IgM contribute in the acquisition of immunity to malaria and that IgG1, IgG2 and IgG3 antibodies are likely to be positively involved in building-up clinical acquired immunity to malaria.

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