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Isotypic analysis of maternally transmitted *Plasmodium falciparum*-specific antibodies in Cameroon, and relationship with risk of *P. falciparum* infection

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SUMMARY

In malaria-endemic areas, infants are relatively protected against malaria infection. Such protection is though to be related principally to the transplacental transfer of maternal antibodies. We measured total and *Plasmodium falciparum*-specific IgG (including subclasses), IgM, and IgE antibodies in 154 paired maternal–cord serum samples from an area of meso- to hyperendemic malaria in South Cameroon. Among peripheral mother blood samples, total IgG and IgM were detected in all samples, IgE in all but two. *Plasmodium falciparum*-specific IgG were detected in all serum samples, IgM and IgE in >75% of samples. The prevalence rates of anti-*P. falciparum* IgG subclasses varied from 75% to 97%. With the exception of *P. falciparum*-specific IgG, all antibody class and subclass levels were lower in cord blood than in peripheral mother blood. *Plasmodium falciparum*-specific IgG1 and IgG3 isotypes were transferred to the offspring more often and more efficiently than IgG2 and IgG4. The detection of total and *P. falciparum*-specific IgM and IgE in some cord serum samples demonstrated that fetuses can mount humoral response against malaria parasites. We also determined whether transplacentally acquired antibodies protect against malaria infection by relating the antibody levels at birth to the risk of acquiring *P. falciparum* infection during the first 6 months of life. Among various classes and subclasses of *P. falciparum*-specific antibodies, only IgG2 were related to a decrease in the risk of acquiring a *P. falciparum* peripheral blood infection from birth to 6 months of age.

Keywords malaria *Plasmodium falciparum* placenta antibody isotype

INTRODUCTION

In malaria-endemic areas, although children under 5 years old are usually considered a high risk group for malaria-related morbidity and mortality, infants appear to be relatively protected against malaria infection [1]. Indeed, *Plasmodium falciparum* parasitaemia is less frequently observed during the first few months of life than later, and children usually do not suffer from severe malaria before their first birthday [2–4]. Such innate protection is though to be related to several factors, including the inability of fetal haemoglobin to support plasmodial development, a deficiency in *p*-aminobenzoic acid in breast-fed children [5], and may be a reduced aggressivity of *Anopheles* mosquitoes towards infants [6]. However, the major role in modifying susceptibility to malaria parasites is thought to be related to the transplacental transfer of maternal anti-*Plasmodium* antibodies [4].

Plasmodium falciparum-specific IgM, IgG1 to IgG4 subclasses, and IgE antibodies can be demonstrated in the serum of the immune populations from malaria-endemic areas, including pregnant women [7,8]. Of these immunoglobulins, only IgG are known to cross the normal placenta from the mother to the fetus, this passage involving an active process, the efficiency of which may differ between IgG subclasses [9]. Neither IgM nor IgE cross the placenta, but have been demonstrated in cord and neonatal sera [8], but only few studies compared *P. falciparum*-specific antibodies of each class and subclass in paired maternal and cord sera. Therefore, we measured *P. falciparum* IgG (including subclasses), IgM, and IgE antibodies in paired maternal–cord serum samples from the region of Ebolowa in South Cameroon, an area of meso- to hyperendemic malaria. In the same serum samples, we also investigated the distribution of antibodies to the Pf155/RESA antigen of *P. falciparum* [10]. Finally, we determined whether

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transplacentally acquired antibodies protect against infection by relating the antibody levels at birth to the risk of acquiring *P. falciparum* infection during the first 6 months of life.

SUBJECTS AND METHODS

Subjects

We enrolled 150 women of varying parity (see Results) sequentially presenting between May 1992 and July 1993 for delivery at two hospital maternity units (Enongal and Ekombitié) in Ebolowa, a town of 35 000 inhabitants located 160 km south of Yaounde, Cameroon. In this rain forest area, *P. falciparum* malaria is hyperendemic with perennial transmission. With maternal informed consent, umbilical cord blood samples, as well as maternal peripheral and placental blood, were obtained for parasitologic examination. At delivery, a whole blood cell count and erythrocyte haemoglobin concentration was performed on mothers' blood. Malaria parasites were detected on Giemsa-stained thick smears from cord, mother and placenta blood and counted against 1000 leucocytes.

Infants born after November 1992 were enrolled in a cohort study. During the first 6 months of life, all children were visited at home every month and a thick blood smear was collected. Due to cultural and commercial reasons, Ebolowa inhabitants regularly travel between the town and the villages they originated from. During the course of the follow up, around 60% of children were seen during each monthly visit. Only children for whom three or more thick blood smears were collected during the 6-month follow up were included in this part of the study.

Plasmodium falciparum *antigen*

The parasites (Palo Alto strain) were cultured in erythrocytes, according to Trager & Jensen [11], and harvested when parasite density had reached 5–10%. After two washes in PBS pH 7·2, erythrocytes were suspended in PBS at a 10% haematocrit. Fractions (2 ml) were placed into conical tubes on top of 2·5 ml of 55% (v/v) Percoll (Pharmacia, Uppsala, Sweden). Centrifugation for 15 min at 1500 *g* gave a distinct band, containing 50–100% parasitized erythrocytes with late trophozoites and schizonts as well as free parasites. This band was washed twice in PBS. For final processing of sufficiently large antigen batches, Percoll band fractions were pooled, suspended in a small volume of PBS and sonicated. The material was then stored as aliquots at −80°C until use.

Reagents

Mouse MoAbs specific for human IgG subclasses were from the following clones: HP 6069 (anti-IgG1), HP 6014 (anti-IgG2), HP 6047 (anti-IgG3), and HP 6023 (anti-IgG4). All were purchased from Caltag Labs (San Francisco, CA), stored at −80°C in small aliquots, and used at 1 μg/ml (IgG1), 0·5 μg/ml (IgG3), or 0·25 μg/ml (IgG2 and IgG4). For total IgE, a MoAb anti-human IgE (clone GE-1; Sigma, St Louis, MO) was used for coating at 2 μg/ml.

Different polyclonal antibodies conjugated to alkaline phosphatase were used: a goat anti-mouse IgG (H + L) human absorbed (Caltag) diluted 1:4000 was used for IgG subclasses; an anti-human IgG (Fc-specific) (Sigma) diluted 1:10 000 was used for IgG; a goat anti-human IgM-specific μ (Biosys, Burlingame, CA) diluted 1:2000 for IgM. For IgE, a goat affinity-purified biotinylated anti-human IgE (Biosys) diluted 1:1000 was used followed by alkaline phosphatase avidin D diluted 1:200.

A positive control serum pool was obtained by pooling serum samples from 10 adults from Madagascar (immunofluorescent assay (IFA) > 1/1024). A negative control serum pool was obtained by pooling serum from 10 healthy Caucasian blood donors. Both serum pools were stored at −80°C in small aliquots.

Measurement of P. falciparum*-specific IgG and IgM*

The procedure for the ELISA was described by Wahlgren *et al.* [12–14], and Dubois *et al.* [15]. Optimal dilutions of the reagents were determined by checker board titrations with positive and negative control serum pools [16]. Microtitre plates for IgG (3915; Falcon, Becton Dickinson, Lincoln Park, NJ) and IgM (Immulon 1; Dynatech, Billingshurst, UK) measurement were coated with 50 μl of the *P. falciparum* antigen preparation (stock solution: 1·4 mg/ml) diluted 1:200 in 0·05 M carbonate buffer pH 9·6. The plates were incubated overnight at 4°C. Non-adsorbed antigen was removed and replaced by 200 μl PBS pH 7·2 (PBS) containing 2% bovine serum albumin (BSA) to prevent non-specific protein binding to the plastic. After incubation for 3 h at 25°C, plates were washed three times for 3 min with PBS containing 0·5% Tween 20 (PBS–T), drained, and shaken to remove excess fluid. Then, 50 μl of test sera diluted 1:200 with PBS–T containing 1% BSA were added to the wells, and the plates were incubated overnight at 4°C. The plates were then washed as above and 50 μl of the appropriate conjugate diluted in PBS–T were added to each well. Again the plates were incubated for 2 h at 25°C, then washed twice in PBS–T and once in enzyme substrate buffer (10% diethanolamine, 0·5 mM $MgCl_2$, pH 9·8). Last, 100 μl of nitrophenylphosphate (Sigma) diluted in enzyme substrate buffer were added to each well. The time of incubation at room temperature in dark was adapted on the basis of the reaction obtained with standard sera. The absorbance at 405 nm (A_{405}) of the contents of each well was determined in a spectrophotometer. All sera were tested in duplicates.

Measurement of P. falciparum *IgG subclasses and IgE*

The procedure was the same as above, with the following modifications: serum samples were diluted in PBS–T at 1:10 for *P. falciparum* IgE and 1:50 for IgG subclasses, and incubated overnight at room temperature (IgE) or at 4°C (IgG subclasses) in microtitre plates (Falcon 3915 for IgG subclasses, and Immulon 4 from Dynatech for IgE). After incubation with serum samples and washing, an additional step was done by adding to each well 50 μl of the appropriate dilution in PBS–T of either the MoAb against IgG subclasses or biotinylated anti-huIgE. Plates were incubated overnight at 4°C, washed, and the conjugate was added.

Measurement of total IgG, IgM, and IgE

Concentrations of total IgG and IgM were determined in nephelometry in the Bichat hospital biochemistry laboratory. Total IgE were measured by an ELISA as described for *P. falciparum* IgE, except that a MoAb anti-human IgE was used for coating and serum samples were diluted 1:500. For titration, human IgE (ICN, Costa Mesa, CA) was used (0·09–11·6 ng/ml).

Measurement of anti-Pf155/RESA antibody

Anti-Pf155/RESA antibody was measured by erythrocyte membrane immunofluorescent assay (EMIF) using glutaraldehyde-fixed and air-dried monolayers of *P. falciparum* ring-infected erythrocytes [10].

Presentation of antibody data

Total IgG, IgM and IgE were expressed in concentration. The threshold of sensitivity of the method was 3·4 mg/ml for IgG, 0·25 mg/ml for IgM, and 75 ng/ml for IgE. For all *P. falciparum*-specific ELISA assays, the positive and negative control serum pools were included in each plate, and results expressed in arbitrary units (AU) [17], calculated from the formula:

$$100 \times \frac{\text{Ln}(A_{405}\text{ test serum}) - \text{Ln}(A_{405}\text{ Pool}-)}{\text{Ln}(A_{405}\text{ Pool}+) - \text{Ln}(A_{405}\text{ Pool}-)}$$

Positivity was determined from the mean reactivities of a blood bank serum battery +2 s.d. The thresholds for positivity were 20 AU for IgG; 30 AU for IgG1, IgG3 and IgE; and 40 AU for IgG2, IgG4 and IgM. For EMIF, the threshold for positivity was a reciprocal titre of 1:4, as this dilution gave consistently negative results with non-exposed donors' serum samples. EMIF data are expressed as the natural logarithm of the reciprocal titre.

The efficiency of the transplacental passage of antibodies was calculated from the ratio of the concentrations in cord and mother peripheral blood, and expressed as a percentage. In this calculation, we only considered cord–mother pairs of samples in which a given antibody was detected in the mother's blood sample.

Statistical analysis

Differences in prevalence rates between groups (mother–cord blood; parity and placental infection status of the mother) were tested by the χ^2 test or the Fisher exact test. Differences in the level of antibodies according to characteristics of the mother were tested by the unpaired *t*-test. Relations between matched pairs of mother and cord samples were tested by paired *t*-test and by the Pearson correlation test. All tests were two-tailed; *P* values $\leq 0{\cdot}05$ were considered significant. Statview 4.5 (Abacus Concept, Berkeley, CA) was used for all calculations.

RESULTS

Characteristics of women at delivery

Paired maternal–cord blood samples from 146 single deliveries and four twin deliveries were obtained. Among the mothers, 70 were primigravidae, and 80 were multigravidae (all twins were born from multigravidae). Compared with multigravidae, primigravidae mothers were younger and presented more frequently with a *P. falciparum* infection in the peripheral blood as well as a placental infection (Table 1). No parasites were seen in the stained thick films made from cord blood.

Table 1. Characteristics of mothers at delivery, by parity status

	Primigravidae	Multigravidae	*P**
n	70	80	
Age (years)†	18·49 ± 0·37	26·45 ± 0·57	< 0·0001
Term (weeks)†	38·76 ± 0·43	38·76 ± 0·37	0·99
Birth weight (g)†	2947·19 ± 65·23	3139·32 ± 45·56	0·02
Placenta infection (% +)	40·3%	13·0%	0·0002
Blood infection (% +)	36·2%	7·7%	0·005

*P value of the unpaired *t*-test or χ^2 test.
†Mean ± s.e.m.

Prevalence rates and levels of antibodies in serum of mothers

Total IgG and IgM were detected in all serum samples, and IgE in all but two. *Plasmodium falciparum*-specific IgG were detected in all serum samples, IgM in 77·7%, and IgE in 75·2% of samples (Fig. 1). The prevalence rates of anti-*P. falciparum* IgG subclasses varied from 76·2% for IgG4, to 83·0% for IgG2 and 97·3% for IgG1 and IgG3. Anti-Pf155/RESA antibodies, as detected by EMIF, were positive for 94·6% of serum samples. The prevalence rates of anti-*P. falciparum* IgG4 were higher in multigravidae than in primigravidae (84·4% *versus* 67·1%, $P = 0{\cdot}02$). Otherwise, no prevalence rates differed according to the parity or placental infection status of the mother.

Mean antibody levels in positive samples are shown in Table 2. *Plasmodium falciparum*-specific IgM levels were higher in multigravidae than in primigravidae (75·4 ± 1·9 *versus* 65·5 ± 2·1 AU, $P = 0{\cdot}001$). Both total IgG and IgE levels were higher in women with a placental infection than in others (IgG, 24·9 ± 0·7 *versus* 22·4 ± 0·6 mg/ml, $P = 0{\cdot}03$; IgE, 5·3 ± 0·5 *versus* 8·3 ± 1·1 μg/ml, $P = 0{\cdot}005$). Otherwise, no antibody levels differed according to the parity or placental infection status of the mother.

Prevalence rates and levels of antibodies in cord blood

All cord blood samples were positive for total IgG and for anti-*P. falciparum* IgG (Fig. 1). Anti-*P. falciparum* IgG1 and IgG3 were detected in more than 90% of cords, while IgG2 and IgG4 were present in less than 40%. Anti-Pf155/RESA antibodies were positive for 86·0% of samples. Total IgM and IgE were detected in 21·1% and 34·4% of cords, respectively. Anti-*P. falciparum* IgM were present in only two cord blood samples, both also contained total IgM and both mothers presented with an infected placenta. Anti-*P. falciparum* IgE were observed in 12 (7·9%) samples, four of the mothers presenting with an infected placenta.

The prevalence rates of anti-*P. falciparum* IgG2 (41·5% *versus* 24·3%, $P = 0{\cdot}04$) and IgG4 (43·9% *versus* 28·6%, $P = 0{\cdot}06$) were higher in cords from multigravidae than in those from primigravidae. Conversely, among positive samples for a given IgG subclass,

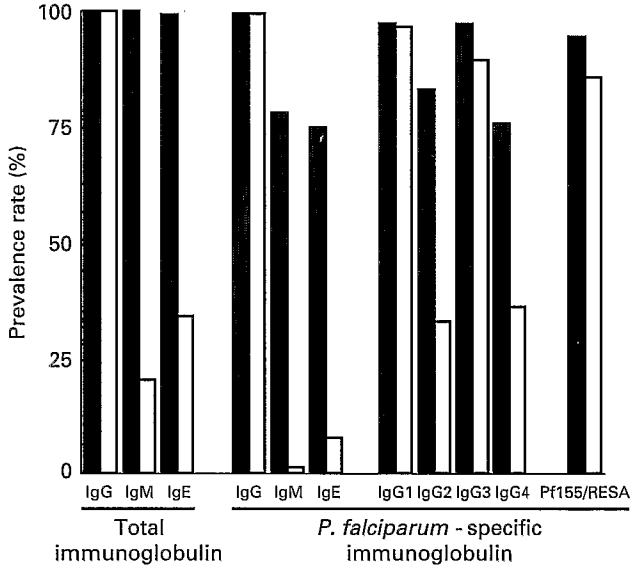

Fig. 1. Prevalence rates of total immunoglobulin classes and *Plasmodium falciparum* immunoglobulin classes and IgG isotypes in maternal (■) and cord (□) paired serum samples.

Table 2. Mean positive antibody levels in mother and cord blood samples

	Mother blood	Cord blood	*P**
Total IgG (mg/ml)	22·85 ± 0·47	18·15 ± 0·32	<0·001
Total IgM (mg/ml)	2·77 ± 0·13	0·73 ± 0·22	<0·001
Total IgE (μg/ml)	5·98 ± 0·4	0·55 ± 0·15	<0·001
Plasmodium falciparum IgG (AU)	84·24 ± 1·17	84·42 ± 1·16	0·73
P. falciparum IgM (AU)	70·95 ± 1·50	†	
P. falciparum IgE (AU)	77·78 ± 3·29	50·42 ± 6·55	<0·001
P. falciparum IgG1 (AU)	81·22 ± 1·40	70·93 ± 1·12	<0·001
P. falciparum IgG2 (AU)	80·20 ± 3·10	57·92 ± 1·97	<0·001
P. falciparum IgG3 (AU)	81·88 ± 2·13	66·28 ± 1·40	<0·001
P. falciparum IgG4 (AU)	80·73 ± 2·86	53·75 ± 1·72	<0·001
EMIF(Ln titre)	3·93 ± 0·14	3·01 ± 0·12	<0·001

Antibody levels are expressed as arithmetic means ± s.e.m.

*P value of the paired t-test.

†*Plasmodium falciparum*-specific IgM were detected in two cord blood samples at 50 and 80 arbitrary units (AU).

EMIF, Erythrocyte membrane immunofluorescent assay.

the mean levels of IgG1 (72·8 ± 1·6 *versus* 68·8 ± 1·5 AU, $P = 0·07$) and IgG3 (69·7 ± 1·9 *versus* 62·2 ± 1·9 AU, $P = 0·001$) were higher in cords from multigravidae than in those from primigravidae. Thus, cord blood samples from multigravidae mothers were more frequently positive for IgG2 and IgG4 anti-*P. falciparum* antibodies, and presented higher levels of IgG1 and IgG3. Conversely, the prevalence rate (3·6% *versus* 13·2%, $P = 0·04$) of anti-*P. falciparum* IgE was lower in multigravidae. None of the cord blood antibody levels differed according to malaria placental infection of the mother.

Transplacental passage of IgG antibodies

With the exception of specific IgG, all antibody class and subclass levels were lower in cord than in peripheral blood of donors (all $P < 0·001$, Table 2). Paired maternal–cord blood levels of total and specific IgG were highly correlated (both $P < 0·001$), as were those of antibodies to Pf155/RESA ($P < 0·0001$) (Table 3 and Fig. 2).

Table 3. Correlation analysis of IgG antibody levels in paired maternal cord serum samples

	Correlation of titres		Cord
	r	*P*	(%)
Total IgG	0·334	<0·001	83·0 ± 1·9
Plasmodium falciparum IgG	0·910	<0·0001	100·4 ± 1·1
P. falciparum IgG1	0·345	<0·0001	86·2 ± 2·1
P. falciparum IgG2	0·226	0·006	24·3 ± 3·1
P. falciparum IgG3	0·408	<0·001	83·6 ± 2·6
P. falciparum IgG4	0·187	0·03	30·8 ± 3·6
EMIF	0·717	<0·0001	52·7 ± 4·9

Variation of titres is calculated from the mean ratio between IgG level in cord/IgG level in paired mother serum sample and expressed as percentage.

EMIF, Erythrocyte membrane immunofluorescent assay.

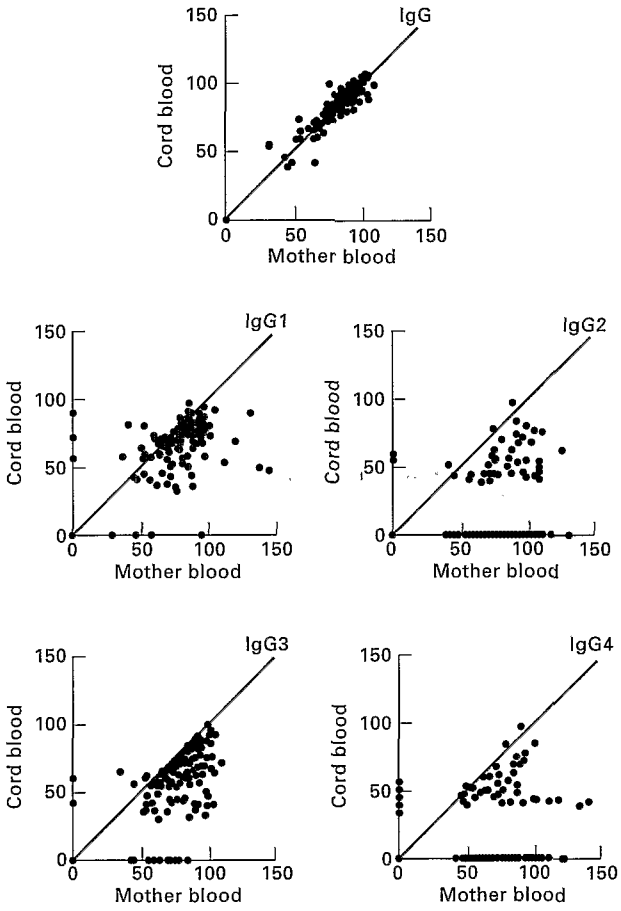

Fig. 2. Concentration in arbitrary units (AU) of *Plasmodium falciparum*-specific IgG and IgG isotypes in paired cord and maternal serum samples. 45° line is drawn.

This was also the case for all subclasses of anti-*P. falciparum* IgG (all $P<0{\cdot}03$); however, the cord–maternal blood ratios differed highly between IgG1 or IgG3 (>80%) and IgG2 or IgG4 (<30%). The reduced transplacental passage of IgG2 and IgG4 was related to both a reduction in the number of pairs in which a passage occurs, and to a reduction in the amounts of immunoglobulin that cross the placental barrier, when this passage occurs (see Table 2). Indeed, in 80/122 (65·6%) pairs in which anti-*P. falciparum* IgG2 were detected in mother blood, IgG2 were not present in cord blood (cord–maternal ratio = 0). Similar data were obtained in 65/115 (56·5%) pairs for IgG4, but were either not or rarely observed for IgG, IgG1 or IgG3 (0%, 2·7% and 9·6%, respectively). The rates of transplacental passage of both IgG2 and IgG4 were inversely related to the level of IgG1 in mother blood ($r=-0{\cdot}24$, $P=0{\cdot}007$ for IgG2; $r=-0{\cdot}27$, $P=0{\cdot}004$ for IgG4). No such relation was observed with any other IgG subclass. Although not significantly, IgG2 and IgG4, but not other subclasses, were less likely to cross the placenta in first than in subsequent pregnancies (IgG2, 58·2% *versus* 71·9%, $P=0{\cdot}13$; IgG4, 50·0% *versus* 68·1%, $P=0{\cdot}08$).

Conversely, and as expected, paired maternal–cord blood IgM and IgE levels were not correlated and the cord–maternal ratios were very low (<7%).

Relationship between levels of antibodies at birth and malaria morbidity during the first 6 months of life

We next investigated the relationship between the presence and level of the various *P. falciparum*-specific antibody classes and subclasses at birth and the occurrence of *P. falciparum* blood infection during the first 6 months of life. At least three thick blood smears were collected from 62 children. Only these 62 children were included in the analysis. Of these, 23 children presented at least one *P. falciparum* infection during the first 6 months of life (mean frequency of positive blood smears: $0{\cdot}45\pm0{\cdot}05$), while parasites were never detected in the other 39 children. The mean number of collected blood smears was similar in both groups of children. New-borns who had *P. falciparum*-specific IgG2 detected in cord blood were less likely to present with a *P. falciparum* infection during the first 6 months of life (17·4% *versus* 47·4%; $P=0{\cdot}03$); however, levels of *P. falciparum*-specific IgG2 were similar in both groups of samples ($P=0{\cdot}29$). All other antibody prevalence rates and antibody levels were similar in both groups of children (see Table 4).

Table 4. Prevalence rates of anti-*Plasmodium falciparum* IgG isotypes and anti-Pf155/RESA antibodies in cord blood samples from offspring that will present or not a *P. falciparum* infection during the first 6 months of life

	No infection ($n=39$) (%)	Infection ($n=23$) (%)	P
P. falciparum IgG1	94·7	95·7	0·99
P. falciparum IgG2	47·4	17·4	0·03
P. falciparum IgG3	92·1	87·0	0·66
P. falciparum IgG4	26·3	34·8	0·57
EMIF	97·4	91·3	0·55

EMIF, Erythrocyte membrane immunofluorescent assay.

DISCUSSION

In the present study, we confirm and extend previous reports demonstrating that *P. falciparum*-specific antibodies are transferred from mothers to new-borns, IgG1 and IgG3 isotypes being transferred more often and more efficiently than IgG2 and IgG4. The detection of total and *P. falciparum*-specific IgM and IgE in several cord serum samples indicates their possible fetal origin.

Plasmodium falciparum-specific IgG, IgM and IgE have been shown in immune individuals from malaria-endemic areas. Our observation of *P. falciparum*-specific IgE antibodies in maternal serum samples supports earlier observations of the occurrence of this class of immunoglobulin antibody in populations exposed to malaria. *Plasmodium falciparum*-specific IgE antibodies were reported in 60% of Liberian adults [10] and in 15–33% of adults from Papua New Guinea [8,18]. In the cord blood, while the occurrence of both IgM and IgE has been reported, there have been few studies on the specificities of these classes of immunoglobulin in paired maternal–cord serum samples. The presence of antigen-specific IgM in new-borns has been reported in several infections, including syphilis, toxoplasmosis, bancroftosis and malaria [19]. While the level of total IgE may be high in cord blood, and predictive of the risk of atopy during infancy, the presence of antigen-specific IgE has rarely been reported, except in the case of parasitic antigens, including filiaria [20] and malaria [8]. In Gabon, Chizzolini *et al.* [7] detected *P. falciparum*-specific IgM in 12% of cord serum samples. In Nigeria, Achidi *et al.* [21] detected IgM in all cord blood tested, while *P. falciparum*-specific IgM was present in 6% of cords. Lastly, in Papua New Guinea, *P. falciparum*-specific IgM were not detected in any of the 46 cord blood samples tested [8]. In the present study, 21·1% of cord serum samples had detectable levels of IgM, while only two (1·3%) samples had *P. falciparum*-specific IgM. These differences may reflect differences in malaria transmission or degree of placental parasitization and/or in the techniques used by the different investigators. In Papua New Guinea, *P. falciparum*-specific IgE antibodies were detected in 13·3% of cord blood serum samples [8]. In the present study, 34·4% of cord serum samples had detectable levels of IgE, and 7·9% had *P. falciparum*-specific IgE. The immunological role of anti-*P. falciparum* IgE is still unknown, and whether they are involved in mediating protection, as shown for *Schistosoma* [22] and *Necator* [23] infections, or allergy requires further studies. Similarly, the relationship between the presence of *P. falciparum* IgE in cord blood and the subsequent development of the infant remains to be established [24].

As neither IgM nor IgE cross the maternal–fetal barrier, these antibodies might be of fetal origin in response to intra-uterine antigenic stimulation. This is also suggested by the lack of correlation between maternal and cord levels of IgM or IgE, that also demonstrates that blood contamination was, at least, marginal. We previously reported that cord blood T cells from new-borns from the same area may proliferate and produce IL-2 and IL-4, following *in vitro* culture in the presence of *P. falciparum* antigens [25]. Whether these antibodies result from a fetal immune response triggered by soluble antigens that freely cross the placenta, or from the passage across the placenta of otherwise non-transferable molecules (malaria parasites and/or IgM and IgE) due to histopathological changes, remains to be established.

As IgG crosses the placenta, a correlation between maternal and cord IgG was expected. Our study confirms that the mean IgG cord level is lower than in paired maternal blood at delivery in

African populations, as opposed to most studies conducted in Caucasians [26,27]. The saturation of the active transport mechanism of IgG across the placenta, involving Fcγ receptors on syncytiotrophoblast cells [9], would explain that the feto–maternal IgG ratios depend on the levels of maternal IgG; this ratio being higher at low levels of maternal IgG. Although all *P. falciparum*-specific IgG subclasses were present in cords, IgG2 and IgG4 were observed much less frequently, and at lower levels, than IgG1 and IgG3. These findings agree with the lower efficiency of the transport of IgG2 and IgG4 across the placenta, especially when IgG1 are present at high levels in mother blood, suggesting, again, that transport of IgG2 and IgG4 is saturated [9]. Moreover, this is also in agreement with the lower prevalence and levels of anti-*P. falciparum* IgG2 and IgG4 than those of IgG1 and IgG3 in the adult immune population [7,15,28].

The relative non-susceptibility to malaria of young infants born in areas where the disease is highly endemic is thought to be related principally to the transplacental passage of specific antibodies. As passively transferred antibodies are expected to be present mainly during the first few months of life, we investigated the relationship between the presence and the level of these antibodies at birth and the probability of occurrence of blood infection during the first 6 months of life. Among various classes and subclasses of *P. falciparum*-specific antibodies, only IgG2 were related to a decrease in the risk of acquiring a *P. falciparum* peripheral blood infection from birth to 6 months of age. Such a relation was unexpected, as previous studies conducted in adults reported an association between protection and anti-malarial IgG3 and/or IgG1 [28–31]. Although this relation was inconsistently observed [15,32] and remains uncertain, no similar relationship was reported for IgG2. In addition, the functional role of IgG isotypes, as well as the effector mechanisms of protective immunity against malaria parasites, may differ between young infants and immune adults.

The relationship of fetal immunity to maternal infection and to infant survival is difficult to assess, but is of primary importance given the variety of malaria control strategies targeted at both pregnant women and young children, including vaccination [33]. Additional studies on malaria throughout the pregnancy period, as well as during the first years of life, are required for a better assessment of the relevance of malaria-specific immune response at birth.

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REFERENCES

1 Garnham PCC. Malarial immunity in Africans: effects in infancy and early childhood. Ann Trop Med Parasitol 1949; **43**:47–61.

2 Bruce-Chwatt LJ. Malaria in African infants and children in Southern Nigeria. Ann Trop Med Parasitol 1952; **46**:173–200.

3 Kitua AY, Smith T, Alonso PL, Masanja H, Urassa H, Menendez C, Kimario J, Tanner M. *Plasmodium falciparum* malaria in the first year of life in an area of intense and perennial transmission. Trop Med Int Hlth 1996; **1**:475–84.

4 Steketee RW, Wirima JJ, Slutsker L, Heymann DL, Breman JG. The problem of malaria and malaria control in pregnancy in sub-Saharan Africa. Am J Trop Med Hyg 1996; **55** (Suppl. 1):2–7.

5 Brabin B. An analysis of malaria parasite rates in infants: 40 years after MacDonald. Trop Dis Bull 1990; **87**:R1–R21.

6 Carnevale P, Frezil JL, Besseno MF, Le Pont F, Lancien J. Etude de l'agressivité de *An. gambiae* en fonction de l'ge et du sexe des sujets humains. Bull Wlrd Hlth Org 1978; **56**:147–54.

7 Chizzolini C, Trottein F, Bernard FX, Kaufmann M-H. Isotypic analysis, antigen specificity, and inhibitory function of maternally transmitted *Plasmodium falciparum*-specific antibodies in Gabonese newborns. Am J Trop Med Hyg 1991; **45**:57–64.

8 Desowitz RS, Elm J, Alpers MP. *Plasmodium falciparum*-specific immunoglobulin G (IgG), IgM, and IgE antibodies in paired maternal–cord sera from East Sepik Province, Papua New Guinea. Infect Immun 1993; **61**:988–93.

9 Eaton BM, Contractor SF. *In vitro* assessment of trophoblast receptors and placental transport mechanisms. In: Redman CWG, Sargent IL, Starket PM, eds. The human placenta. Oxford: Blackwell Scientific Publications, 1993:**471**–503.

10 Perlmann H, Berzins K, Wahlgren M, Carlsson J, Björkmann A, Patarroyo ME, Perlmann P. Antibodies in malarial sera to parasite antigens in the membrane of erythrocytes infected with early asexual stages of *Plasmodium falciparum*. J Exp Med 1984; **159**:1686–704.

11 Trager W, Jensen J. Human malaria parasite in continuous culture. Science 1976; **193**:673–5.

12 Wahlgren M, Berzins K, Perlmann P, Björkmann A. Characterization of the humoral immune response in *Plasmodium falciparum* malaria. I. Estimation of antibodies to *P. falciparum* or human erythrocytes by means of microELISA. Clin Exp Immunol 1983; **53**:127–34.

13 Wahlgren M, Berzins K, Perlmann P, Wahlin B, Persson M. Characterization of the humoral immune response in *Plasmodium falciparum* malaria. II. IgG-subclass levels of anti-*P. falciparum* antibodies in different sera. Clin Exp Immunol 1983; **54**:135–42.

14 Wahlgren M, Perlmann H, Berzins K, Björkman A, Larsson A, Ljungström I, Patarroyo ME, Perlmann P. Characterization of the humoral immune response in *Plasmodium falciparum* malaria. III. Factors influencing the coexpression of antibody isotypes (IgM and IgG-1–4). Clin Exp Immunol 1986; **63**:343–53.

15 Dubois B, Deloron P, Astagneau P, Chougnet C, Lepers JP. Isotypic analysis of *P. falciparum*-specific antibodies and their relation to protection: a prospective study in Madagascar. Infect Immun 1993; **61**:4498–500.

16 Quakyi I. The development and validation of an enzyme linked immunosorbent assay for malaria. Tropenmed Parasitol 1980; **31**: 1–8.

17 Rasheed FN, Bulmer JN, De Francisco A, Jawla MFB, Jakobsen PH, Jepson A, Greenwood BM. Relationships between maternal malaria and malarial immune responses in mothers and neonates. Parasite Immunol 1995; **17**:1–10.

18 Desowitz RS. Plasmodium-specific immunoglobulin E in sera from an area of holoendemic malaria. Trans Roy Soc Trop Med Hyg 1989; **83**:478–9.

19 Loke YW. Transmission of parasites across the placenta. Adv Parasitol 1982; **21**:155–228.

20 Carlier Y, Truyens C. Influence of maternal infection on offspring resistance towards parasites. Parasitol Today 1995; **11**:94–99.

21 Achidi EA, Perlmann H, Salimonu LS, Asuzu MC, Perlmann P, Berzins K. Antibodies to Pf155/RESA and circumsporozoite protein of *Plasmodium falciparum* in paired maternal–cord sera from Nigeria. Parasite Immunol 1995; **17**:535–40.

22 Hagan P, Blumenthal UJ, Dunn D, Simpson AJG, Wilkins A. Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium*. Nature 1991; **349**:243–5.

23 Pritchard DI, Quinnell RJ, Walsh EA. Immunity in humans to *Necator americanus*: IgE, parasite weight and fecundity. Parasite Immunol 1995; **17**:71–75.

24 Perlmann H, Helmby H, Hagstedt M, Carlson J, Larsson PH, Troye-Blomberg M, Perlmann P. IgE elevation and IgE anti-malarial antibodies in *Plasmodium falciparum* malaria: association of high IgE levels with cerebral malaria. Clin Exp Immunol 1994; **97**:284–92.

25 Fievet N, Ringwald P, Bickii J, Dubois B, Maubert B, Le Hesran JY, Cot M, Deloron P. Malaria cellular immune responses in neonates from Cameroon. Parasite Immunol 1996; **18**:483–90.

26 Michaux JL, Heremans JF, Hitzig WH. Immunoglobulin levels in cord-blood serum of Negroes and Caucasians. Trop Geogr Med 1966; **18**:10–14.

27 Logie DE, McGregor IA, Rowe DS, Billewicz WZ. Plasma immunoglobulin concentrations in mothers and newborn children with special reference to placental malaria. Studies in the Gambia, Nigeria, and Switzerland. Bull Wld Hlth Org 1973; **49**:547–54.

28 Aribot G, Rogier C, Sarthou JL, Trape JF, Balde AT, Druilhe P, Roussilhon C. Pattern of immunoglobulin isotype response to *Plasmodium falciparum* blood-stage antigens in individuals living in a holoendemic area of Senegal (Dielmo, West Africa). Am J Trop Med Hyg 1996; **54**:449–57.

29 Bouharoun-Tayoun H, Druilhe P. *Plasmodium falciparum* malaria: evidence for an isotype imbalance which may be responsible for delayed acquisition of protective immunity. Infect Immun 1992; **60**:1473–81.

30 Luty AJF, Mayombo J, Lekoulou F, Mshana R. Immunologic response to soluble antigens of *Plasmodium falciparum* in Gabonese children exposed to continuous intense infection. Am J Trop Med Hyg 1994; **51**:720–9.

31 Taylor RR, Smith DB, Robinson VJ, McBride JS, Riley EM. Human antibody response to *Plasmodium falciparum* merozoite surface protein 2 is serogroup specific and predominantly of the immunoglobulin G3 subclass. Infect Immun 1995; **63**:4382–8.

32 Ferreira MU, Kimura EAS, De Souza JM, Katzin AM. The isotype composition and avidity of naturally acquired anti-*Plasmodium falciparum* antibodies: differential patterns in clinically immune Africans and Amazonian patients. Am J Trop Med Hyg 1996; **55**:315–23.

33 Menendez C. Malaria during pregnancy: a priority area of malaria research and control. Parasitol Today 1995; **11**:178–83.