Hidden *Plasmodium falciparum* parasites in human infections: different genotype distribution in the peripheral circulation and in the placenta

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Abstract

Sequestration of the mature *Plasmodium falciparum* forms complicates detection, quantification and molecular analysis of human infections. Whether the circulating parasites represent all or only a subset of co-infecting genotypes is unclear. We have investigated this issue and compared placenta and peripheral blood *msp1* and *msp2* genotypes in 58 women delivering with an ICT-positive placenta in Guediawaye, Senegal. Most placenta (91%) and blood samples (98%) were multiply infected. Multiplicity of infection was positively correlated in both tissues. However, the placental and circulating genotype profiles differed markedly. Only 10% of matched peripheral blood/placenta samples had identical genotypes, whereas 74% had only partially concordant genotypes, with some alleles detected in both tissues, together with additional allele(s) detected in one tissue only. Eight women (14%) had totally discordant placental and peripheral blood genotypes. Thus, in the vast majority of cases, some sequestered genotypes remain hidden, undetected in the peripheral circulation, indicating that analysis of peripheral parasites generates a partial picture of a *P. falciparum* infection.

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1. Introduction

The mature forms of the human malaria parasite *Plasmodium falciparum* do not circulate in peripheral blood. They are sequestered in microvessels, as a result of cytoadhesion to endothelial cells and/or to uninfected red blood cells or platelets (reviewed in Wahlgren et al. (1999)). Evidence is accumulating that some ring stages also cytoadhere (Silamut et al., 1999; Pouvelle et al., 2000). Sequestration impairs quantification of the actual parasite load, currently assessed by examining the circulating cells using peripheral blood smears, and complicates investigation of the molecular characteristics of the infecting parasites. Studies of association of disease severity with specific parasite characteristics, such as cytoadherence specificity (e.g. Carlson et al., 1990; Newbold et al., 1997; Roberts et al., 2000; Pain et al., 2001), multiplication rate (Chotivanich et al., 2000), and/or presence of specific genotypes (e.g. Robert et al., 1996; Kun et al., 1998; Arsey et al., 2001) have all been carried out using peripheral blood parasites, overlooking the sequestered population. The same shortcut has been done in the analysis of immune responses to the “infecting” parasites (e.g. Bull et al., 1998; Cavanagh et al., 1998; Cavanagh et al., 1998; Jouin et al., 2001) and of drug resistance profiles (Fidock et al., 2000; Djimdé et al., 2001; Hadzìtra et al., 2001). Likewise, the molecular epidemiology studies conducted so far on multiplicity of infection (e.g. Noëmi et al., 1995; Beck et al., 1997; Robert et al., 1996; Konaté et al., 1999; Arnott, 1996), infection dynamics (Daubersies et al., 1996; Farnert et al., 1997; Bruce et al., 2000), or population structure (Tibayrenc et al., 1999; Kyes et al., 1997; Babiker et al., 1997; Paul et al., 1998; Conway et al., 1999; Anderson et al., 2000), were based on analysis of the sole
peripheral blood stages. Yet, the basic assumption underlying these studies, namely that peripheral parasites constitute a representative sample of the overall population infecting the individual, remains unproven. This question is particularly critical, since a large number of infected individuals carry multiple *P. falciparum* genotypes in many endemic areas.

Daily samplings in holoendemic areas have shown that in some cases, parasite genotypes undetected in the periphery on one day could be detected on the next day (Duahiers, et al., 1996; Färnert et al., 1997). This reflects alternating periods of cytoadhesion and circulation of relatively synchronous parasites with a 24 h shifted cycle. How frequent such a situation occurs, and whether or not it is restricted to regions of intense transmission where the rate of inoculation of novel parasite genotypes is high, is unknown. The synchronicity of the infection(s) in humans is variable, so that the proportion of mature (sequestering) and young (circulating) parasites may differ from one infectious episode to the next and/or from one individual to the other. Thus, to date, the issue of how accurately the circulating parasites represent the infecting pool is unclear. In the work reported here, we have used placental sequestration as a model to explore the within-host distribution of multiple parasite genotypes.

The molecular basis of placental sequestration has been recently clarified (for a review see Benezet et al. (2001)). It is due to adhesion of infected erythrocytes to placenta-specific receptors, which is governed by expression of specific *P. falciparum* erythrocyte membrane protein 1 (EPIEMP1) encoded by the *var* repertoire. The distinct phenotypic characteristics of the placental and peripheral parasites are thus due to epigenetic regulation of *var* gene expression. This allows to explore the intra-host genotype distribution in a multiply infected host. If all co-infecting genotypes are detected by both tissues, the peripheral parasites would be “representative” of the overall infecting population, which they would not, if only a subset is detected in either tissue.

We have compared the genotype profiles of parasites collected from the placenta and from the peripheral blood at delivery in *P. falciparum*-infected women. Placenta and blood samples were collected from women delivering with an ICT-positive placenta in the maternity hospital of Guediawaye, a suburb of Dakar (Senegal), a hypoendemic area. Parasites were genotyped using a polymerase chain reaction (PCR)-based approach. To ear-mark the individual genotypes, we have analysed two unlinked single copy loci presenting a large allelic polymorphism. The markers chosen were the polymorphic block 2 of the chromosome 9-encoded *msp1* gene and the central domain of the chromosome 2-encoded *msp2* gene (Schützermacher et al., 2001).

Both markers have been extensively used in molecular epidemiology studies of field isolates (Contamin et al., 1995; Nsouka et al., 1995; Robert et al., 1996; Duahiers et al., 1996; Färnert et al., 1997; Beck et al., 1997; Kun et al., 1998; Arnot, 1998; Paul et al., 1998; Zwytsenga et al., 1998; Konaté et al., 1999; Sallenave-Sales et al., 2000). In particular, they have been used previously in an analysis of parasite diversity in the same area, which outlined the presence of a large number of alleles (Robert et al., 1996).

We have determined the *msp1* block 2 and *msp2* genotypes in matched placenta and peripheral blood samples, and compared multiplicity of infection and allele distribution of individual genotypes in both tissues.

2. Materials and methods

2.1. Study site, study population, collection of placental tissue and of fingerprick blood samples

In Guediawaye, a suburb of Dakar with about 1 million inhabitants, malaria transmission is seasonal, the rainy season usually lasting 4–5 months. The local entomological inoculation rate in the area was estimated as about 1 infective bite per person per year. *P. falciparum* prevalence in the area is low (Trape et al., 1992).

The study presented here was conducted over 3 weeks of a transmission season (20 October 1999 to 8 November 1999) to minimise local transmission heterogeneity. In order to recruit women with a *P. falciparum* placental infection, we screened placental smears collected immediately after delivery using the ICT Malaria *P. f. test* (ICT Diagnostics, Brookvale, Australia). Sixty of 442 placentas screened were ICT-positive. Women with an ICT-positive placenta were enrolled for the study. A fingerprick blood sample was obtained before anti-malarial treatment was given. Blood and placenta samples were stored at −20 °C. Informed consent was obtained from all participants.

Of the 60 placenta-positive women initially recruited, two were subsequently excluded, since one of the tissues was no longer available for parasite genotyping. In the group of 58 women studied here, there were 25 paucigravidae (18 first and 7 second pregnancies) and 29 multigravidae (≥3 pregnancies). In four cases, the number of previous pregnancies was unknown. Age at delivery ranged from 15 to 39 years (mean 24 years). Anti-malarial prophylaxis during pregnancy was taken, as recommended by the national health authorities (namely 300 mg chloroquine twice a week), but mostly on an irregular basis, by 43 women. Twenty three women reported a clinical malaria episode in the last trimester of pregnancy.

Parasite density in peripheral blood was determined by standardised readings of thick blood films (Trape et al., 1994). The parasite load of placental smears was determined by systematic microscopic examination of 200 oil-immersion fields.

2.2. Extraction of DNA and PCR genotyping

Approximately 100 mg of placenta were homogenised in a tissue grinder in 700 μl lysis buffer (0.1 M Tris-HCl pH 8.0, 0.1 M NaCl, 50 mM EDTA, 1% SDS), and incubated...
overnight with 40 μl of 50 mg/ml proteinase K. After one phenol extraction and one phenol/chloroform/isoamyl alcohol (25:24:1) extraction, DNA was precipitated with ethanol and resuspended in 100 μl of water. Peripheral blood DNA was extracted as described (Zwetyenga et al., 1998). PCR genotyping was performed by nested PCR, as described previously (Schleiermacher et al., 2001). PCR fragments were assigned to specific allelic families according to secondary PCR results. Individual alleles were identified by size polymorphism. The discrimination level was considered to be 10 bp. Distinct alleles were consequently assigned to 10 bp bins.

2.3. Distribution of allelic families, multiplicity of infection and statistical analysis

Multiplicity of infection in each tissue was estimated as the highest number of alleles detected for the msp1 and/or the msp2 locus. Statistical analysis was performed using EPI-INFO 6.0 (Center of Disease Control and Prevention, Atlanta, USA). P-values less than 0.05 were considered statistically significant. Geometric means of parasitaemias were calculated after log transformation of data, and were compared by analysis of variance, if variances were homogenous, or by a non-parametric test (Kruskall–Wallis) if not. Multiplicity of infection and allelic family frequency in blood and placenta were compared by using the Mann–Whitney test. Analysis by Kruskall–Wallis test of the distribution of individual msp1 and msp2 alleles in the placenta and peripheral blood was restricted to alleles detected in more than five women.

3. Results

3.1. Prevalence and parasite density

All placenta and peripheral blood samples were PCR-positive, indicating presence of parasites in both tissues. However, 24% of placenta infections, and 13% peripheral blood infections were not detectable by microscopy, and resuspended in 100 μl of water. Peripheral blood DNA was extracted as described (Zwetyenga et al., 1998). PCR genotyping was performed by nested PCR, as described previously (Schleiermacher et al., 2001). PCR fragments were assigned to specific allelic families according to secondary PCR results. Individual alleles were identified by size polymorphism. The discrimination level was considered to be 10 bp. Distinct alleles were consequently assigned to 10 bp bins.

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3. Results

3.1. Prevalence and parasite density

All placenta and peripheral blood samples were PCR-positive, indicating presence of parasites in both tissues. However, 24% of placenta infections, and 13% peripheral blood infections were not detectable by microscopy, probably because of too low a parasite density.

Schizonts were detected in 37 placentas (Table 1). The geometric mean density in the placental smears was 755 schizonts per 200 fields (95% CI 44–274, range 0–3380). Sixteen women with either trophozoites only, or no malarial parasites detected by microscopy examination of the placenta had a positive thick blood film. The geometric mean of the peripheral density was 142 trophozoites per μl blood.

Placental and peripheral blood parasite density did not differ in parous and multi-gravidae (P = 0.64 by Mann–Whitney test). Women who reported having had no clinical malaria in the last trimester of pregnancy tended to have higher parasite densities than those who reported a clinical episode (P = 0.09 by Mann–Whitney test). Parasite density was similar in women who declared having taken anti-malarials during pregnancy, and in those who had not (P = 0.29 by Mann–Whitney test). Whether this reflects irregular, and hence incomplete, treatment or inefficiency of chloroquine due to parasite resistance in the area is unknown.

3.2. Multiplicity of infection of matched placental/peripheral blood samples

The placental and peripheral blood msp1 block 2 and msp2 central domain genotypes were determined by nested PCR. All three allelic families K1, MAD20 and RO33 described for msp1 block 2, and both allelic families 3D7 and FC27 described for the msp2 central domain, were detected. A large diversity was observed, with 19 MAD20 alleles, 15 K1 alleles, a single RO33 allele, 25 3D7 alleles and 17 FC27 alleles.

For all criteria investigated, namely multiplicity of infection, family distribution and individual allele distribution, genotyping data from women with a schizont-positive placenta, i.e. with a bona fide sequestering population, were no different from women with no placental schizonts detected. Fifty seven peripheral blood (98%) and 54 placenta samples (93%) contained multiple msp1 and/or msp2 alleles. Up to six distinct allelic types were detected in peripheral blood. Most peripheral blood and placenta samples contained two or three alleles at each locus. The mean number of alleles per sample (“multiplicity of infection”) was similar in blood and placenta, when the msp1 and msp2 results are considered separately (not shown), and when msp1 and msp2 results are combined (Fig. 1). Overall, combined multiplicity

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Parasite prevalence as estimated by microscopic examination of the matched placenta and peripheral blood samples collected at delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placental smear</td>
<td>Thick blood film</td>
</tr>
<tr>
<td>No cases</td>
<td>No positive cases</td>
</tr>
<tr>
<td>Positive</td>
<td>Presence of schizonts</td>
</tr>
<tr>
<td></td>
<td>Trophozoites only</td>
</tr>
<tr>
<td>Negative</td>
<td>14</td>
</tr>
</tbody>
</table>

*In one woman, only gametocytes were detected.*
Fig. 1. Number of alleles per tissue as estimated by combined msp1 and msp2 genotyping in women with a *P. falciparum* infection delivering in Guediawaye, Senegal.

was 2.8 for peripheral blood and 2.6 for placenta (not statistically different, $P = 0.36$). Multiplicity of infection was positively correlated in both tissues (parametric test, correlation coefficient 0.43, $P = 0.01$).

Multiplicity of infection was not age-dependent and was similar in paucigravidae and in multi-gravidae. It was not different in women having or not having taken chemoprophylaxis and in women reporting or not reporting a recent clinical malaria episode.

3.3. Genotype comparison of matched placenta/peripheral blood samples

Comparison of alleles in the matched peripheral blood and placenta samples showed that the populations were often different. There were three situations, as illustrated in Fig. 2.

There were only six blood/placenta pairs with concordant combined msp1 and msp2 profiles. All six were multiple infections (2 msp1 alleles and 1 msp2 allele, or 3 msp1 alleles and 1 or 2 msp2 alleles). These probably represent identical parasite populations in both tissues. However, since the technique does not genotype each parasite clone individually, there is a slight chance that the two tissues in fact had different clones, with different combinations of msp1 and msp2 alleles.

The most frequent situation was a partial sharing of alleles, with some alleles detected in both peripheral blood and placenta, and other allele(s) detected in one tissue only. There were 43 women, who had 1–3 identical msp1 allele(s) in both tissues, as well as other msp1 alleles detected in one tissue only. There were 19, 18 and 6 women with one, two, or three identical msp1 alleles in both tissues, respectively, as well as other distinct msp1 alleles in one tissue only. For 25 women, 1–2 shared msp2 allele(s) were detected in both tissues, together with other distinct msp2 alleles in one tissue only. There were 23 cases where a single msp2 allele was shared, and 2 cases where 2 msp2 alleles were shared.

There were 19 women with $\geq 1$ identical msp1 allele(s) and $\geq 1$ identical msp2 alleles in both tissues, associated with otherwise distinct msp1 and msp2 genotypes in one or both tissues. Five women with concordant msp1 genotypes had partially discordant msp2 genotypes, and conversely, 19 women with concordant msp2 genotypes in both tissues had partially discordant msp1 genotypes. We failed to establish the msp2 genotype for one blood/placenta pair. Thus, a total of 43 women had partially concordant 2 locus genotypes. This partial overlap indicated limited contamination of placenta homing forms by circulating parasites.

The third situation, observed in eight women, was discordant peripheral and placental msp1 and msp2 genotypes (Table 2), suggesting completely separate populations of clones in the two tissues. There were two cases of completely discordant and partially concordant msp1 and msp2 genotypes, respectively, four cases where the opposite was observed (partially concurrent msp1 and completely discordant msp2) and two cases of discordance at both loci. Interestingly, six of eight discordant cases where from women who had no msp2 PCR.

<table>
<thead>
<tr>
<th>Combined msp1 block 2 and msp2 genotypes</th>
<th>All alleles shared</th>
<th>Partial allele sharing$^a$</th>
<th>No allele shared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire study population $(N = 57)$</td>
<td>6</td>
<td>43</td>
<td>8</td>
</tr>
<tr>
<td>Paucigravidae $(N = 25)$</td>
<td>3</td>
<td>19</td>
<td>3</td>
</tr>
<tr>
<td>Multi-gravidae $(N = 28)$</td>
<td>3</td>
<td>21</td>
<td>4</td>
</tr>
<tr>
<td>Unknown parity $(N = 4)$</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Schizont-positive placenta $(N = 37)$</td>
<td>3</td>
<td>32</td>
<td>2</td>
</tr>
<tr>
<td>Schizont-negative placenta$^a$ $(N = 20)$</td>
<td>3</td>
<td>11</td>
<td>6</td>
</tr>
</tbody>
</table>

$^a$ There was one blood/placenta pair with a negative msp2 PCR.
Fig. 2. Concordance of msp1 block 2, msp2, and combined genotypes in matched placental and blood samples from 58 women delivering in Guediawaye, Senegal. The proportion of women with identical, completely discordant and partially concordant genotypes is illustrated. The group of women with partially concordant genotypes was sub-divided into two sub-groups with additional allele(s) detected either in the placenta or in peripheral blood.

with no evidenced placental schizonts. This again suggests limited contamination of the placental sample with circulating blood parasites.

These results indicate that in 52 of 58 women, there was partial or complete discrepancy in the genotype profiles of the parasites in the placenta and those in the peripheral blood. This was observed in paucigravidae and multi-gravidae (Table 2). There was no influence of the gravidity status on the extent of allelic concordance in both tissues. A similar level of discrepancy was observed whether or not schizonts were evidenced in the placenta.

3.4. Allele distribution

There were 21 cases where individual msp1 alleles were detected only in the placenta (11 paucigravidae, 8 multi-gravidae, and 2 of unknown parity), and 25 cases where some msp1 alleles were detected only in peripheral blood (10 paucigravidae, 11 multi-gravidae, and 4 of unknown parity). Likewise, there were 12 cases of some msp2 alleles being detected only in the placenta (5 paucigravidae and 7 multi-gravidae) and 13 cases where some msp2 alleles were detected only in peripheral blood (7 paucigravidae, 5
When taking into consideration both tissues, the actual number of genotypes present at the time of infection from a single tissue (as above) underestimated two tissues indicated that calculation of the multiplicity of infection was not associated with a particular subset of strains, but rather no statistically significant difference in tissue distribution of those alleles detected in more than five women. There was the analysis of tissue distribution of individual alleles to was similar in both tissues (data not shown). We restricted whether there was some tissue-specific distribution bias of specific alleles.

The frequency of *msp1* block 2 and *msp2* allelic families was similar in both tissues (data not shown). We restricted the analysis of tissue distribution of individual alleles to those alleles detected in more than five women. There was no statistically significant difference in tissue distribution of any individual allele, suggesting that placental sequestration is not associated with a particular subset of strains, but rather with the parasite stage or the phenotypic placental tropism or both.

### 3.5. A more accurate estimation of multiplicity of infection using placenta and peripheral blood genotypes

The observed discrepancy in allelic composition in the two tissues indicated that calculation of the multiplicity of infection from a single tissue (as above) underestimated the actual number of genotypes present at the time of tissue sampling. When taking into consideration both tissues, the mean multiplicity of infection was 3.5 *msp1* genotypes, 2.1 *msp2* genotypes and 3.6 combined genotypes/woman (Table 3). This deduced “readjusted” multiplicity was similar in women with and without evidenced placental schizonts, and was not influenced by age or parity (Mann–Whitney test). This a minimal estimate, as additional possible sequestration sites exist, in which additional genotypes undetected in the periphery might sequester.

### Table 3

<table>
<thead>
<tr>
<th>Mean multiplicity of infection estimated using genotyping of</th>
<th>msp1 block 2</th>
<th>msp2</th>
<th>Two loci combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire study population</td>
<td>3.5</td>
<td>2.1</td>
<td>3.6</td>
</tr>
<tr>
<td>Paucigravidae</td>
<td>3.5</td>
<td>2.2</td>
<td>3.6</td>
</tr>
<tr>
<td>Multi-gravidae</td>
<td>3.5</td>
<td>2.1</td>
<td>3.6</td>
</tr>
<tr>
<td>&lt;22 years</td>
<td>3.3</td>
<td>2.3</td>
<td>3.4</td>
</tr>
<tr>
<td>≥22 years</td>
<td>3.6</td>
<td>2.0</td>
<td>3.6</td>
</tr>
<tr>
<td>With placental schizonts</td>
<td>3.4</td>
<td>2.1</td>
<td>3.7</td>
</tr>
<tr>
<td>Without placental schizonts</td>
<td>3.7</td>
<td>2.1</td>
<td>3.6</td>
</tr>
</tbody>
</table>

### 4. Discussion

The large allelic polymorphism, the high proportion of multiply infected women, and the high multiplicity of infection, confirm previous findings in the Dakar surroundings (Robert et al., 1996; our unpublished results). These characteristics are surprising for a hypoendemic region, where the overall diversity, the proportion of multiple infections and the multiplicity of infection are usually low (Haddad et al., 1999; Sallenave-Sales et al., 2000; Ariey et al., 1999, 2001). They are not attributable to pregnancy per se, because the proportion of multiple infections and the multiplicity of infection observed here were higher than observed previously using the same technique in pregnant women living in a mesoendemic Senegalese village (Schleiermacher et al., 2001). The entomological records available indicate that multiple, successive inoculations in the Guediawaye area are unlikely. Moreover, the low prevalence of infection and the absence of influence of age and parity on all parameters studied here, are consistent with a low endemicity. The molecular characteristics of infection observed here might reflect peculiarities of parasite infection in an urban setting, where the actual site of inoculation is difficult to trace back and may differ from the site of residence. The most plausible explanation is that the multiple infections detected in these women have been acquired during some travel to an area with more intense transmission. Alternatively, they might reflect long term carriage of infections, which would not be suppressed by a partially efficient and irregular chemoprophylaxis. The available data indicate that the parasite populations in African hypoendemic settings are more diverse (Babiker et al., 1997; Cavanagh et al., 1998) than in South American hypoendemic settings (Haddad et al., 1999; Sallenave-Sales et al., 2000; Ariey et al., 1999, 2001; Urdaneta et al., 2001). This might result in more complex infections and different population dynamics in both situations. This deserves further investigation.

Forty one of 44 women (93%) with a microscopically positive placenta also had a positive thick blood film. This contrasts with common occurrence of placental infection in the absence of visually detectable peripheral parasitaemia in high transmission areas (Watkinson and Rushton, 1983; Matteelli et al., 1997; Leke et al., 1999). This is probably to be attributed to the limited acquired immunity of adults living in this area (Trave et al., 1992). A limited previous exposure to placental infection may also explain why parasite density in peripheral blood or placenta was not influenced by parity, again contrasting with observations from areas with higher endemicity (Beeson et al., 2001).

The PCR genotyping technique used here tends to miss alleles present at a low concentration and favours detection of the dominant allelic population (Contamin et al., 1995). Thus, we compared the dominant population sequestered within the placenta with the dominant circulating population. In only 6 of 58 women were identical parasite genotypes detected in both tissues, whereas in 52 of 58 women (89.6%) was there partial or total discrepancy in the tissue-specific genotype profiles. The conservative conclusion is that the dominant population sequestered within the placenta and the dominant circulating population only partially overlap in the vast majority of cases.

Detection of the same genotypes in both tissues probably reflects an asynchronous infection, with circulating and sequestered forms with similar genotypes at the time of sampling. The possibility exists of massive contamination of the placental sample with circulating blood. We think
this unlikely in view of the limited to null overlap observed for the other 52 matched pairs studied. Conversely, complete discordance of circulating and sequestered forms (genotypes) at a given time point is the predicted figure for highly synchronous infections with two distinct populations shifted by 1 day as described by Färnert et al. (1997).

In the great majority of cases, the genotypes of placenta and peripheral blood parasites were partially concordant, with similar genotypes detected in both tissues in association with extra genotypes detected in only one of the tissues. Several possible explanations can be proposed to meet these observations. They probably reflect a median position between the two previous situations, namely a mixture of poorly synchronous infections by some genotypes and synchronous infections by others. An alternative explanation would be that the genotypes detected in the placenta but not in the periphery derive from a cryptic population, sequestered throughout the asexual blood cycle. Pouvelle et al. (2000) have recently reported in vitro evidence of ring stage cytoadhesion onto the syncitiotrophoblasts in placental cryosections. The molecular interactions involved in ring stage cytoadhesion remain to be clarified. Interestingly, the placenta-cytoadhering ring stages constitute a particular sub-population of parasites, namely parasites committed to cytoadhere at a later stage onto chondroitin sulfate A (CSA), one of the major placental receptors of trophozoites and schizonts (for a review see Bessou et al. (2001)). Therefore, the possibility exists of a cryptic placenta-cytoadhering ring and late stage population. We are currently trying to clarify this issue by in situ PCR of the placental smears using the methodology developed by Ranford-Cartwright and Walliker (1999).

One of the unknowns in these scenarios is the extent to which infections are synchronous in asymptomatic carriers (most women recruited here) on the one hand, and in pregnant women on the other hand. We need to know more about the dynamics of infection in humans exposed to Plasmodium falciparum in endemic areas. Whatever the actual mechanism(s) responsible for the observed picture, our results demonstrate that a fraction of the sequestered parasites is a hidden population, which would remain unnoticed had we not investigated a sequestration site, in this case the placenta. Presence of such a hidden population is a frequent occurrence in severe malaria. Developmental stages have been reported in severe malaria (Silamut et al., 1999). This implies that restricting the analysis of a P. falciparum infection to peripheral blood as usually done for reasons of practicality, is bound to yield a partial picture. Not only are specific phenotypes (e.g. propensity to bind to specific receptors) lost for analysis, but also some genotypes, precluding a proper evaluation of the genetic make up of the infecting population for other parasite factors likely contributing to pathology, such as drug susceptibility, multiplication rate, capacity to induce pro-inflammatory cytokines, etc. This also challenges previous studies, including ours, on the factors influencing multiplicity of infection in endemic areas and analysis of strain-specific immune responses.

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References


