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The Sick Placenta—The Role of Malaria

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The human placenta is an ideal site for the accumulation of *Plasmodium falciparum* malaria parasites, and as a consequence serious health problems arise for the mother and her baby. The pathogenesis of placental malaria is only partially understood, but it is clear that it leads to a distinct epidemiological pattern of malaria during pregnancy. The objectives of this review are: (1) To review recent data on the epidemiology of malaria in pregnancy, with emphasis on placental malaria; (2) to describe the pathological changes and immunological factors related to placental malaria; and (3) to discuss briefly the functional consequences of this infection for the mother and her baby. The review attempts to bring together local events at the maternal-fetal interface which encompass immunological and pathological processes which relate to the epidemiological pattern of malaria in pregnancy in areas of both high and low malaria transmission. An integrated understanding of the epidemiological, immunological and pathological processes must be achieved in order to understand how to control malaria in pregnancy. The yearly exposure of at least 50 million pregnancies to malaria infection makes it the commonest and most recurrent parasitic infection directly affecting the placenta. These statistics and our limited understanding of its pathogenesis suggest the research priorities on this subject.
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INTRODUCTION

At least 50 million pregnancies are exposed every year to malaria infection [1]. These infections may result from single or mixed infections with any of the four species of *Plasmodium* which cause human malaria. These are *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. The importance of this infection is related not only to its frequency, as it is the commonest parasitic infection of pregnant women in the world, but also to its consequences. Firstly, pregnancy malaria relates to malaria attributable maternal anaemia, which may be severe and increase maternal morbidity and the risk of mortality [2]. Secondly the consequences of pregnancy malaria and placental malaria for the fetus and infant are enormous. *P. falciparum* infection is the most important non-genetic factor contributing to low birthweight in first pregnancies in Africa and is associated with increased neonatal mortality [3] and infant anaemia [4]. There is an increased prevalence and parasite density of *P. falciparum* infection in primigravidae across locations with widely different levels of malaria transmission. Placental as well as peripheral parasitaemia occurs more frequently in first pregnancies indicating that the malaria immunity acquired with increasing parity reduces placental as well as peripheral parasitaemia [5]. The availability of novel placental receptors may select for malaria parasites that are uncommon in non-pregnant hosts and this could increase the density and duration of placental malaria [6]. These consequences indicate that placental malaria presents a unique set of problems.

This review focuses on the epidemiology, immunology, pathology, and functional consequences of placental malaria in the human.

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EPIDEMIOLOGY AND PLACENTAL INFECTION

Epidemiology of placental malaria

Various methods have been employed to identify placental malaria infections possibly contributing to prevalence differences observed between studies. In some studies placental malaria was defined only by the presence of parasites between the villi while in others the definition includes the presence of

pigment. The appearance of malaria parasites early in pregnancy and subsequently pigment has been used to characterize the chronology of infection in the placenta [7]. Intervillous spaces, where parasites sequester later in pregnancy, form as lacunae in the trophoblast between days 10 and 21 of gestation, but it is unclear whether maternal blood enters these spaces before 12 weeks of gestation [8] and this may explain why parasites do not sequester in the placenta before the fourth month of gestation [9].

Parasite data on placental malaria are available only at delivery when peripheral and placental parasite prevalence estimates show reasonable correlation [10]. However some studies have shown high density placental parasites with scanty peripheral parasitaemia [9,11,12], whereas other studies have shown peripheral parasitaemias in the absence of placental infection [13–15]. Ismail et al. [16] showed that as many as 68.8 per cent of women with negative peripheral blood parasitaemia had some evidence of malarial infection (either active or past) in placental samples. In this study, 46.3 per cent of actively infected placentae did not show parasites in the peripheral blood examination, whereas only 5.2 per cent of cases with no parasites in the placental histological study had a positive peripheral blood. In contrast, Walter et al. [13] reported that only 9.5 per cent women had negative peripheral blood and positive placental parasitaemias, while 61 per cent of cases were positive in peripheral blood and negative in the placenta. On the Thai–Burmese border, a very low malaria transmission area, where all women with positive peripheral parasitaemia at weekly antenatal screening were treated, positive placental parasitaemia only occurred in women with positive peripheral parasitaemia at the time of delivery, and then only in women with recent infection: median (range) of 1 (0–3) day from the end of pregnancy [15]. Variations in epidemiological characteristics of malarial infection between different areas as well as differences in methodology, particularly the use of polarized light, may partially explain these various findings. A regression plot of placental against peripheral parasitaemia for 20 published studies estimated an average frequency of placental parasitaemia of 9.2 per cent in the absence of peripheral parasitaemia [10]. Cord blood parasitaemias have not infrequently been reported in blood smears examined by light microscopy and are more closely associated with placental parasitaemia than with maternal parasitaemia [13,15,16]. In a holoendemic area of Kenya, microscopy indicated no *Plasmodium* species in cord blood, in contrast the polymerase chain reaction (PCR) method in cord blood showed a prevalence of 32 per cent for *P. falciparum*, 23 per cent for *P. malariae*, and 21 per cent for *P. ovale* [17]. These findings, and comparable data from Malawi, indicate that cord blood *Plasmodium* infections are common in areas of high malaria transmission and may be acquired before delivery [18].

There are similarities and differences between *P. falciparum* and *P. vivax* infection of the placenta. *P. falciparum* can achieve high parasite densities (greater than 90 per cent of red cells in the placenta infected) and invades erythrocytes of all ages. *P. vivax* exclusively invades young red cells (reticulocytes) and consequently achieves relatively low parasite densities. The mature stages of *P. falciparum* sequester in deep organ vasculature and do not appear in peripheral blood, except in severe clinical infections [19]. All asexual stages of *P. vivax* appear in the peripheral circulation and this species has only recently been reported to occur in the placenta although less frequently and at less density than *P. falciparum* [15].

Placental parasitaemia occurs more frequently in first pregnancies. Peripheral parasite prevalence is also higher in the first half of pregnancy and decreases with advancing gestational age [20]. Interestingly, placental involvement in malarial infections is closely related to parity and both frequency and parasite density decrease as parity increases in higher transmission areas [21–23]. This effect is considered to be largely parity specific, but there is also an age-dependent component [5,24,25]. An important inter-related factor is co-infection with HIV, which increases the prevalence of peripheral and placental parasitaemia [26–28]. Although HIV-infected women demonstrate parity-specific immunity, they have higher parasite prevalence than HIV-uninfected women as multigravidae [28].

As mentioned previously, placental parasites may occur in the absence of peripheral parasites, and during seasonal periods of low malaria transmission placental malaria infection may persist. Table 1 shows a summary of data on the seasonal prevalence of placental parasitaemia for women of all parities for areas of high (rural) and low (urban) transmission in Africa. Five of these eight studies showed no significant difference in parasite prevalence (*P. falciparum*) between wet and dry seasons suggesting that in urban areas of lower transmission, as well as in areas with higher transmission, placental infection persists despite the expected reduced incidence of infection during the dry season. Hidden placental *P. falciparum* parasites, undetected by microscopy, have been demonstrated by PCR in a study from a low transmission area of Senegal which showed different parasite genotype profiles in the peripheral circulation and in the placenta [29]. In the vast majority of cases, some sequestered genotypes remained hidden, undetected in the peripheral circulation, indicating that analysis of peripheral parasites generates only a partial picture of *P. falciparum* infection. Likewise, analysis of placental parasites only gives a partial picture of *P. falciparum* infection during pregnancy. Similar findings are reported from Kenya [17]

Placental malaria and low birthweight

Both *P. falciparum* and *P. vivax* malaria are associated with increased low birthweight (<2500 gms) risk [30,31]. The increased low birthweight prevalence in primigravidae attributable to malaria is substantial, ranging from below 10 per cent in low endemic areas to over 50 per cent in high transmission areas. Figure 1 shows the association of low birthweight in primigravidae (13 studies) and multigravidae (10 studies) with placental parasite prevalence. The correlation of low birthweight and placental parasite prevalence is significant for

Table 1. Placental parasitaemia prevalence (*P. falciparum*) in wet and dry seasons (all parities)

Location	Years	Prevalence (%)		Relative risk 95% CI	References
		Wet	Dry		
Urban					
Gambia (Banjul)	1966–1972	12.8 (1637)	11.1 (1290)	1.16, 0.95–1.41	[21]
Nigeria (Ibadan)	1970–1973	10.2 (560)	8.6 (525	1.19, 0.82–1.78	[147]
Senegal (Thies)	1975–1976	7.2 (193)	5.2 (250)	0.39, 0.67–2.90	[133]
Rural					
Gambia (3 coastal villages: Sukuta, Bakau, Gunjur)	1966–1972	27.6 (1926)	26.4 (1574)	1.05, 0.94–1.17	[21,43]
Gambia (5 inland locations)	1992–1993	26.4 (121)	9.3 (97)	2.88, 1.44–5.74	d'Alessandro (unpublished data)
Malawi (Mangochi)	1987–1989	27.5 (559)	16.2 (1184)*	1.70, 1.41–2.05	[144]
Malawi (Shire Valley)	1992–1994	19.3 (680)	16.6 (799)	1.16, 0.93–1.44	Verhoeff (unpublished data)
Tanzania (Ifakara)	1994–1995	38.4 (614)	31.4 (561)	1.16, 1.04–1.29	Menendez (unpublished data)

Parentheses: sample size.

* Includes post-rainy and dry seasons; prevalence in post-rainy season 21.1% (160/760).

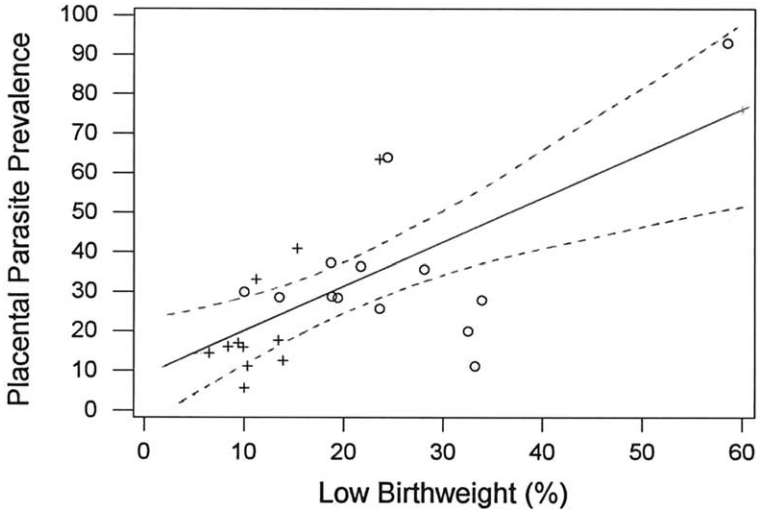

Figure 1. Regression plot of placental parasite prevalence (per cent) and low birthweight incidence in primigravidae (0) and multigravidae (+) for 23 cross-sectional studies with available data. $y=8.089+1.113X$. $R^2=42.6$ per cent. Correlation coefficient (r)=0.653. Stippled line: 95 per cent confidence interval.

primigravidae (correlation coefficient 0.57, $P=0.04$) and multigravidae (correlation coefficient 0.84, $P<0.01$), although low birthweight and placental parasite prevalence are much lower in multigravidae. Figure 2 likewise shows the correlation of mean birthweight with placental parasite prevalence. Fewer studies were available for comparison and although some correlation is apparent, this fails to reach statistical significance (primigravidae, $r=-0.64$, $P=0.08$; multigravidae, $r=-0.73$, $P=0.06$). The outliers in these graphs may reflect study heterogeneity, relate to prior use of malaria chemoprophylaxis during pregnancy, bacterial co-infection, or unknown causes. The graphical summary strongly supports the conclusion that placental malaria is associated with reduced birthweight. The regression slope indicates that for every 10 per cent increase in placental malaria prevalence there is a 9.0 per cent increase in low birthweight risk. The incidence of low birthweight has also been related to placental parasite density, in the Gambia, with the main effect observed at parasite densities >10 000 parasites/μl [43]. In Thailand mean (SD) birthweight was considerably lower in placenta positive compared with negative women known to have had *P. falciparum* in pregnancy [2425 (625) ($n=8$) vs 2878 (479) ($n=123$); $P=0.081$] (McGready et al., unpublished data).

The reduced birthweight is thought to be effected through placental insufficiency which leads to intra-uterine growth retardation and premature delivery, although the exact mechanisms remain unclear. The peak prevalence of peripheral parasitaemia occurs at the end of the first trimester (between 13–20 weeks' gestation) in some studies, gradually falling as gestation advances [20]. This coincides with the second wave of trophoblast invasion of the maternal vasculature between 16 and 20 weeks' gestation and it has been hypothesized that placental malaria infection will interfere with this invasion and the transformation of the maternal vasculature leading to impaired placental development and function [30,32]. Utero-placental haemodynamics are altered in the presence of

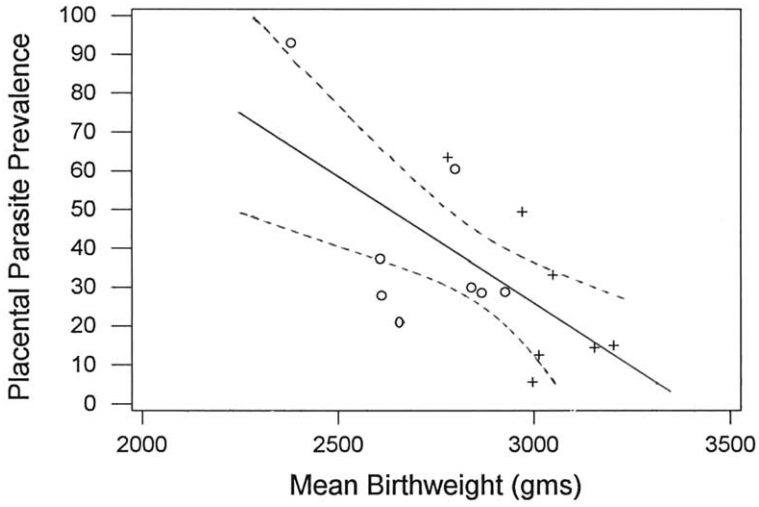

Figure 2. Regression plot of placental parasite prevalence (per cent) and mean birthweight in primigravidae (0) and multigravidae (+) for 15 studies with available data. $y=231.9-0.069X$. $R^2=43.3$ per cent. Correlation coefficient (r)=0.658. Stippled line: 95 per cent confidence interval.

maternal falciparum malaria and this may account for some of the excess of low birthweight babies [32]. Also, fever (which is common in the tropics) in the week before birth due to malaria or other causes may induce premature birth leading to increased neonatal mortality [33].

Several reports have focused on the impact of the histological and immuno-histochemical abnormalities on birthweight. Mononuclear intervillous inflammatory infiltration has consistently been associated with increased risk of low birth weight [15,34–37]. The reduction in birthweight is especially severe in cases of massive chronic intervillositis [36]. Monocyte infiltration has been associated with maternal anaemia and low birthweight [38] although gestational age was not controlled for. Malarial pigment deposition has also been associated with birthweight reduction in most studies, although this association rarely persists after multivariate analysis [35]. Several malaria associated placental lesions, particularly erythrocyte parasitization and fibrin deposition have been associated with increased risk of premature delivery [35]. The presence of pigment in maternal erythrocytes in the intervillous space was an independent predictor of decreased birthweight in Karen women, whose malaria was treated. All women with pigment in maternal erythrocytes had symptomatic malaria in the last few days before delivery [15].

Table 2 summarizes birthweight outcomes in the Gambia, Malawi, Tanzania, Kenya and Thailand in relation to placental histological classification of malaria according to Ismail et al. [16]. These studies are all from highly endemic areas except Thailand where transmission is low. The worst birthweight outcomes were associated with chronic infection. There is a progressively increasing low birthweight risk from the group with no infection to active, then past infection, with the highest risk (30–66.7 per cent) for chronic infections. There are broadly comparable low birthweight risks for the different histological groups in mothers from Malawi, Tanzania and Kenya, all areas of high malaria transmission. The high rate of low birthweight in Thailand mothers, even though they were treated for malaria, may reflect their lack of premunition. Chronic infection substantially increases low birthweight risk, which approximately doubles compared to acute infection. The highest mean birthweights were observed in placentae with no evidence of malaria infection, past or present, in all three areas. For the Kenya study there was a significant interaction between chronic or past malaria infection and severe maternal anaemia [5].

The rural Malawi birthweight data in Table 2 were further stratified according to fetal growth retardation (less than 10th percentile [39]), or pre-term birth (less than 37 weeks' gestation). The results indicated that the risk of preterm birth was commonest for acute and chronic infection, compared to past or no infection. Fetal growth retardation was commoner than pre-term delivery for all histological types of placental malaria, except that of acute infection. A separate study also from rural Malawi showed that late malaria parasitaemia was associated with pre-term delivery, and chronic malaria with growth retardation [40]. Disproportionate growth [defined as a Rohrers index less than 10th (percentile)] was most common with chronic infection (Table 3).

Malaria and fetal-placental weight ratios

In humans a linear relationship between fetal weight and placental weight has been documented for both early and late gestational ages [41]. Mean placental weight and fetal : placental (F/P) weight ratios continue to increase through 42 weeks' gestation in appropriate for gestational age infants. In small for gestational age infants in non-malarious areas, higher placental weights and lower mean birthweights were reported to be associated with increased neonatal morbidity [42]. McGregor et al. [43] reported lower birthweights in Gambian infants in the presence of placental malaria defined by positive blood

Table 2. Birthweight and placental histological classification

Location	No infection	Acute	Past	Chronic	All
Gambia*					
Low birthweight, %	22.2 (18)	25.0 (8)	31.6 (19)	40.0 (35)	32.5 (80)
Mean birthweight, g	2863 (±490)	2539 (±326)	2601 (±464)	2576 (±544)	2643 (±502)
Malawi Rural†					
Low birthweight, %	13.0 (23)	50.0 (2)	17.1 (292)	29.9 (117)	20.5 (434)
Mean birthweight, g	2930 (±342)	2660 (±339)	2894 (±439)	2756 (±530)	2857 (±462)
Malawi Urban‡					
Low birthweight, %	9.6 (10)	10.5 (4)	11.3 (16)	–	–
Mean birthweight, g	3101 (455)	2923 (423)	2978 (425)	–	–
Tanzania§					
Low birthweight, %	14.9 (289)	8.9 (112)	13.7 (475)	29.6 (301)	17.6 (1177)
Mean birthweight, g	–	–	–	–	2819 (±429)
Kenya¶					
Low birthweight, %	13.2 (423)	11.5 (61)	22.3 (215)	29.3 (116)	17.9 (815)
Mean birthweight, g	–	–	–	–	2900 (±510)
Thai–Burmese border‖					
Low birthweight, %	10.0 (1)	–	10.0 (1)	66.7 (4)	21.4 (6)
Mean birthweight, g	3085 (±325) (10)	2700 (±n.a) (2)	2860 (±370) (10)	1992 (±754) (6)	2742 (±600) (28)

Histological classification follows Ismail et al. [16]. Brackets: sample size; square brackets: standard deviation.

* d'Alessandro—unpublished data.

† Verhoeff—unpublished data, only singleton, live births included.

‡ [62].

§ Menendez—personal communication and [35].

¶ [5].

‖ Only includes women with parasites and pigment detected histopathologically in women with a known positive peripheral *P. falciparum* infection detected and treated antenatally i.e. the classification of 'no infection' in the placenta at delivery does not mean the woman was not infected during pregnancy.

Table 3. Fetal growth retardation, pre-term delivery and placental histological classification in Malawian and Thai* pregnancies

Pregnancy outcome (%)	No infection	Acute	Past	Chronic	All
IUGR					
Malawi	22.7 (22)	50.0 (2)	29.3 (290)	30.4 (115)	29.4 (429)
Thailand	6.3 (16)	–	11.1 (18)	33.3 (9)	13.0 (46)
Pre-term					
Malawi	9.1 (22)	50.0 (2)	9.7 (290)	20.9 (115)	12.8 (429)
Thailand	–		–	18.2 (11)	4.2 (48)
Low birthweight					
Malawi	13.0 (23)	50.0 (2)	17.1 (292)	29.9 (117)	20.5 (434)
Thailand	6.3 (16)	–	11.1 (18)	45.5 (11)	16.7 (48)
Disproportionate					
Malawi	0.0 (22)	0.0 (2)	5.2 (292)	9.6 (115)	6.1 (429)

Brackets: sample size. IUGR: intra-uterine growth retardation less than <10th percentile [39]. Pre-term: less than 2500 g. Disproportionate: less than 10% Rohrers index [135].

* Thai data includes women known to have had only *P. falciparum* infection detected and treated antenatally, including all women in the 'No infection' group.

slides (especially in first-born children), but not depressed placental weight. F/P ratios recalculated from their data are summarized in Table 4 and consistently show lower F/P ratios with placental malaria. As placental weight was not affected, the effect of malaria on the infant weight was considered due to placental insufficiency, leading to growth retardation, or an increased incidence of pre-term deliveries, or a combination of both. However, data on gestational age were not presented in this report. The data shown in Table 4 from a low transmission area in Thailand also shows significantly reduced F/P ratios in primigravidae, but with a much reduced effect in multigravidae.

Table 4. Fetal : placental weight ratios in relation to placental malaria parasitaemia in the Gambia* and Thailand

Area	Fetal : placental weight ratio	
	Malaria positive	Malaria negative
Urban Gambia		
Primigravidae	5.55 (100)	5.87 (448)
Multigravidae	5.88 (43)	5.98 (602)
Rural Gambia		
Primigravidae	5.49 (183)	5.70 (157)
Multigravidae	5.98 (246)	6.01 (816)
Thailand†		
Primigravidae	4.3 (4)	5.8 (20)
Multigravidae	7.3 (2)	6.0 (52)

Parentheses: sample size for placental values.

* Estimated from mean birthweight and placental weights reported by McGregor et al. [21].

† P=0.047 (primigravidae); P=0.229 (multigravidae). Thailand [unpublished data]

An analysis of F/P ratios by gestational age in relation to placental malaria has been possible from data available from a large cohort study of mothers and babies delivering in a rural area of southern Malawi where malaria transmission is perennial [44]. Gestational age was assessed using a modified Ballard Scale [45]. The quantitative data is summarized in Table 5. In primigravidae from 36 weeks' gestation mean placental weight was increased and mean birthweight reduced. This results in an overall reduction in the F/P ratio between 36–40 weeks' gestation. Conversely in multigravidae only small differences were found in mean placental weight or birthweight between infected and non-infected mothers and no consistent difference in the F/P ratios was apparent. These patterns are illustrated in Figure 3 for primigravidae and multigravidae. These results suggest that in African primigravidae the low F/P ratio was due not only to a reduced birthweight, but also to increased placental weight. This supports the placental microscopic structural features model proposed in the term variety of fetal growth retardation [46]. Severe anaemia associated with malaria, which is commoner in primigravidae in highly malarious areas, may contribute to this process [47].

THE PATHOLOGY OF PLACENTAL INFECTION

Placental findings associated with malarial parasites or products

During *P. falciparum* infections, the placenta can harbour a striking density of parasites, macrophages and pigment. Bignami [48] and Serini [49] first described these features while studying congenital malaria. Blacklock and Gordon [12] subsequently reported the placental involvement in malarial infection describing the presence of parasites and malarial pigment in thick smears from placental blood.

Both parasites and haemozoin (or malarial pigment) can be easily detected in the histological examination of the placenta [156]. When present, parasites are always detected in the intervillous space, mostly within maternal erythrocytes but occasionally can also be present in the cytoplasm of macrophages and free in the intervillous space. The parasitized erythrocytes do not form rosettes, which is a well-known sequestration mechanism in cerebral vessels [25,50], but lie free in the maternal blood of the placenta [13,16]. Sequestration of infected erythrocytes due to cytoadherence of several strains of *P. falciparum* to chondroitin sulphate A, hyaluronic acid and other molecules expressed by the trophoblast is considered a key point in the pathogenesis of placental malaria [6]. However, parasitized erythrocytes in the histological studies are not usually attached to the trophoblast [13,16] (Figure 4). On the other hand, Beeson et al. [19] have recently demonstrated a selective accumulation of mature asexual-stage infected erythrocytes in the intervillous space with scant young forms (rings). This correlates with the appearance of the antigen PfEMP1, which is known to be involved in the parasite adhesion phenomenon. Other experience shows that most parasites in placental blood show pigment accumulation [16,36], known to appear in mature rings and schizonts [51], which is in keeping with placental adhesion of mature parasites. Species identification cannot be confidently performed in histologic studies [13,16]. However, most reports on placenta and malaria include only infections by *P. falciparum*, and very little is known on placental findings associated with infections by other species. McGready et al. [15] examined the placenta of women known to have had positive peripheral parasitaemia of *P. falciparum, P. vivax* or both infections detected and treated antenatally. *P. vivax* was only associated with increased haemozoin deposition but this was still significantly lower than for *P. falciparum*. Detection of *P. vivax* and *P. falciparum* in the same women in the antenatal period did not present with placental changes different from those of *P. falciparum* alone.

Haemozoin is also detected in the maternal part of the placenta [16], either free in the intervillous space or in perivillous fibrin. In both locations the haemozoin can be found free or in the cytoplasm of maternal macrophages. Haemozoin is a product of the digestion of haemoglobin by the parasites present in all species of human malaria [52,53]. The digestion of haemoglobin, used by intraerythrocytic malaria parasites as a major source of nutrients, releases haem, which is converted by the parasite into the insoluble microcrystalline material haemozoin to avoid the toxicity associated with soluble haem [52].

Several techniques have been described to improve the detection of malarial haemozoin. Galbraith et al. [54] reported that the use of fluorescent light enhanced haemozoin detection in histological sections. However, the relative complexity and expensiveness of this technique has precluded widespread use. Polarized light has been reported as a simple, fast, sensitive, and specific alternative method for localizing intracellular pigmented malarial parasites both in histological slides [7,16] and in wet preparations of blood (Lawrence et al., 1994), due

Table 5. Placental malaria, birthweight and fetal-placental weight ratios in Malawi

Gestation weeks	Birthweight*				Placental weight*†				F : P ratio*			
	Malaria −		Malaria+		Malaria −		Malaria+		Malaria −		Malaria+	
	PG	MG	PG	MG	PG	MG	PG	MG	PG	MG	PG	MG
35	2332 (13)	2608 (11)	2383 (3)	2383 (6)	490 (12)	457 (11)	490 (3)	398 (6)	5.0	6.1	4.9	6.3
36	2619 (29)	2810 (85)	2420 (16)	2782 (17)	482 (29)	520 (84)	483 (16)	532 (17)	5.6	5.6	5.2	5.4
37	2656 (35)	2959 (114)	2569 (16)	2995 (15)	488 (35)	527 (114)	504 (15)	503 (15)	5.6	5.8	5.2	6.1
38	2856 (37)	3023 (180)	2842 (13)	3044 (25)	534 (36)	535 (176)	563 (13)	523 (24)	5.8	5.9	5.1	6.0
39	2888 (45)	3101 (198)	2983 (27)	3045 (26)	523 (45)	547 (195)	602 (24)	552 (26)	5.8	5.9	5.1	5.6
40	2925 (50)	3147 (236)	3048 (19)	3019 (32)	547 (50)	557 (234)	585 (19)	515 (31)	5.7	5.9	5.3	6.2

PG: primigravidae; MG: multigravidae. Parentheses: sample size.

* Mean values.

† Placenta weighed, wiped clean, with membranes and cord.

to the marked birefringence of haemozoin (Figure 4). We have recently compared the sensitivity and specificity of polarized light and non-polarized light to detect malarial pigment in routinely processed placental histological slides (Romagosa et al., manuscript in preparation). The use of polarized light significantly increased the sensitivity of detection of pigment and parasites (100 per cent and 98.1 per cent respectively), compared with non-polarized light (40.5 per cent and 50.3 per cent respectively). The sensitivity of non-polarized light is especially poor in cases with scant parasites (12.7 per cent for <1 per cent vs 97.8 per cent for >5 per cent parasitized erythrocytes, $P<0.001$), or minimal pigment deposition (42.4 per cent vs 84.5 per cent when severe, $P<0.001$). However, careful attention to the fixation process is essential due to the similar physical properties of formalin and haemozoin. The use of neutral buffered formalin reduces the formation of formalin pigment, even after long fixation periods, and should be stressed when planning histological studies of placental malaria. Formation of formalin pigment can be avoided by using streck tissue fixative (STF) (manufactured by Streck Laboratories, Omaha, NE, USA).

Haemozoin can be quantified by a sensitive and specific assay [55]. In an area of high transmission, recent falciparum malaria infections (many of which were untreated) resulted in significantly higher placental haemozoin concentrations [53]. On the Thai–Burmese border, the same laboratory found placental haemozoin concentration was strongly positively correlated with *P. falciparum* malaria infection in pregnancy but not with *P. vivax* infection, being a primigravidae, and with malaria infection (positive peripheral parasitaemia) in the last trimester of pregnancy [15].

Most evidence indicates that malarial products do not accumulate in fetal structures. Although malarial parasites are detected in a small proportion of cases in the cord blood by standard light microscopic examination of Giemsa stained blood smears, malarial parasites have never been described in fetal erythrocytes or in fetal structures in histological studies [13,16,56]. This suggests that malarial parasites cannot cross the placental barrier until delivery, when multiple vessel ruptures and mixing of maternal and fetal blood takes place. A few reports have described the presence of malarial pigment in villous stroma but not in fetal vessels [15,54,57], but this finding has not been confirmed in most studies. McGready et al. observed one case with pigment in fetal monocytes in fetal vessels in a primigravid woman with hyperparasitaemia (>4 per cent RBC prarasitized) who started premature labour spontaneously but delivered by Caesarean section for fetal distress. As suggested previously the mode of delivery may affect the placental histopathological findings of some features including sequestration [58]. Only trophoblastic cells covering the fetal villi and acting as a barrier between maternal and fetal blood have consistently been shown to contain haemozoin [13,15,54,59,155].

Classification of malarial infection

Bulmer et al. [7] introduced the first classification of placental malarial infection. This classification was later slightly modified by Ismail et al. [16]. The rationale for histological classifications is based on the different significance of haemozoin and parasites and on the assumption of the progression of the infection that is often left untreated. Thus, the presence of parasites indicates active infection whereas haemozoin deposition, either free or in macrophages, indicates chronic haemolysis. During the early stages of infection, only parasites can be detected. After the initial haemolytic episodes, malarial pigment is detected in intervillous monocytes. Later, haemozoin is covered by fibrin and is detected in the perivillous fibrin, either free or within the cytoplasm of entrapped macrophages, or in the decidua. Obviously, parasites can coincide with haemozoin in macrophages and in fibrin. After clearance of the infection, pigment persists only within the fibrin for a period of time, but it eventually disappears. Thus, active infections, defined by the presence of parasitized red blood cells in the intervillous space of the placenta, includes two categories, acute infections (only parasites and minimal haemozoin deposition in the macrophages, but not fibrin), and chronic infections (parasites and haemozoin deposition). The category 'past infection', includes cases with haemozoin, usually mixed with fibrin, but no parasites.

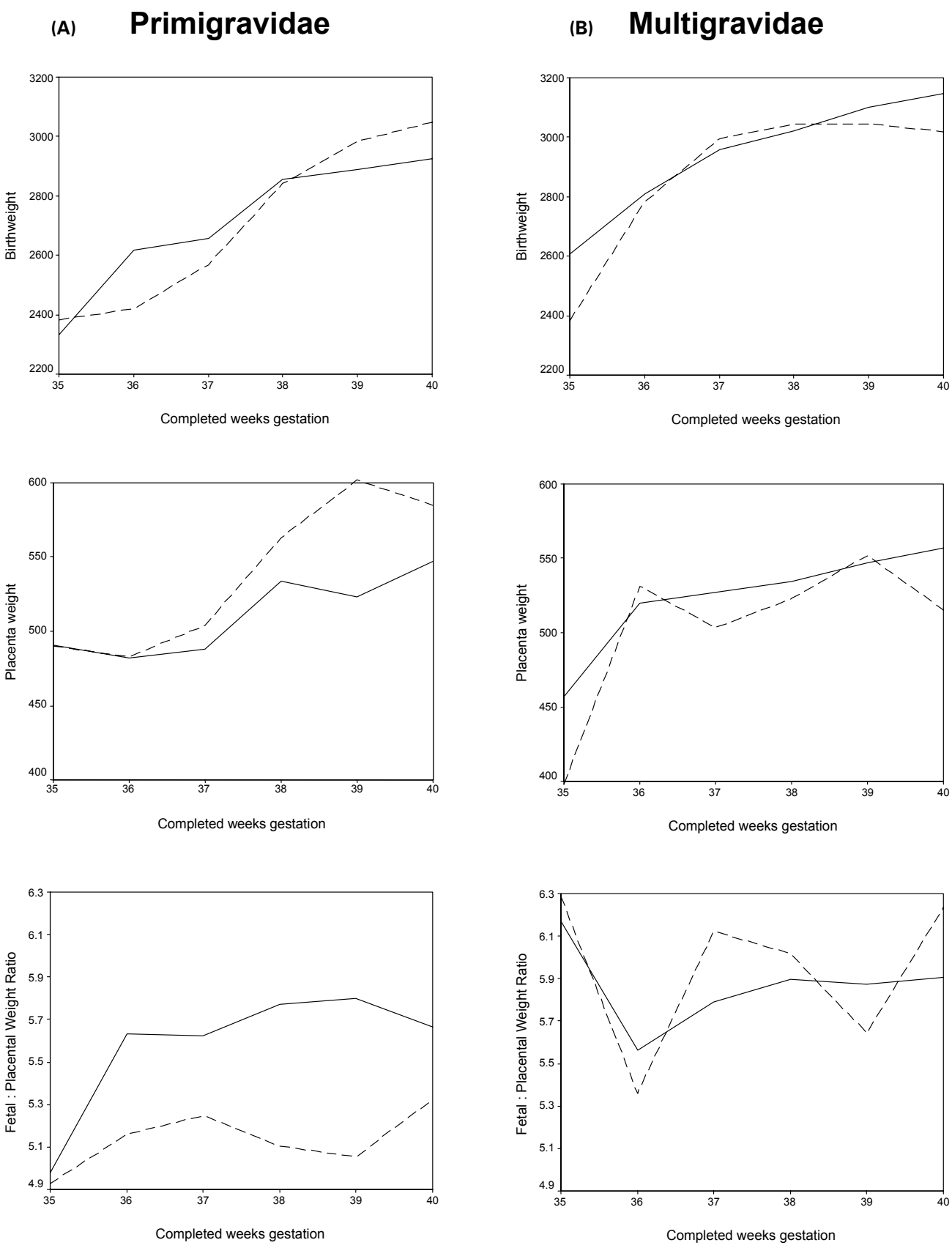

Figure 3. Gestational changes in placental weight, birthweight and fetal : placental ratios in primigravidae or multigravidae with or without malaria parasitaemia at delivery. Continuous line: no peripheral parasitaemia. Stippled line: peripheral falciparum parasitaemia. Left side graphs: primigravidae. Right side graphs: multigravidae.

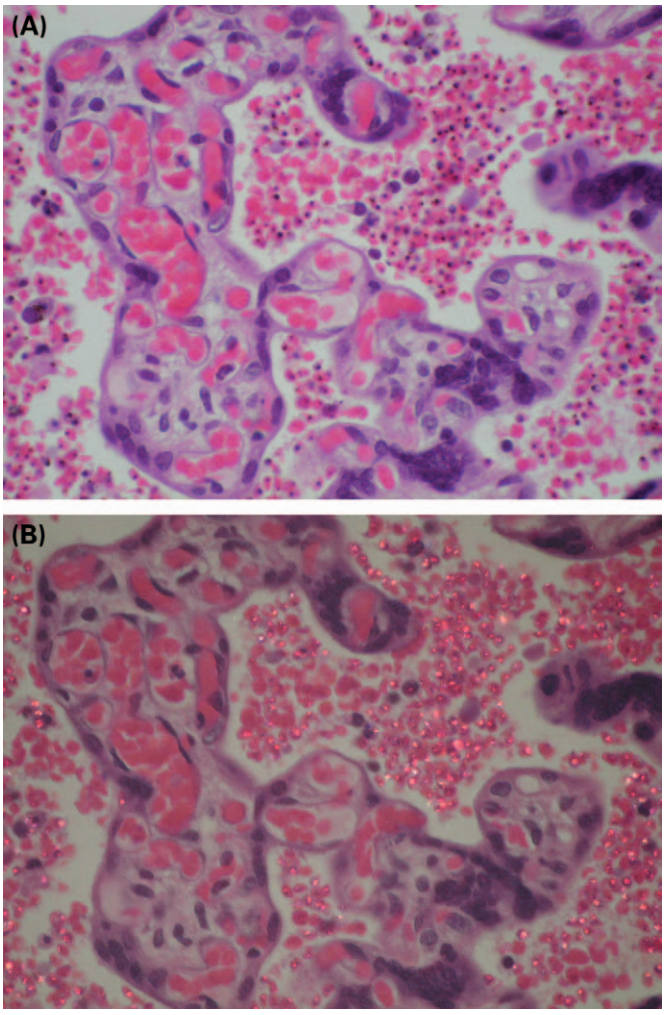

Figure 4. Massive malarial infection involving the placenta. Many maternal erythrocytes in the intervillous space are parasitized. (B) The same field with polarized light. Note that no parasites are detected in the villi. (Haematoxylin and eosin, 200 × .)

Rogerson et al. [37] have reported a new modification which subdivides the chronic active infections depending on the presence or absence of haemozoin in the cytoplasm of monocytes, because of the strong association between haemozoin in macrophages and low birth weight. Any classification of placental malaria should account for treatment, prophylaxis or intermittent preventive therapy (IPT), especially when given close to delivery. More than one-quarter of pregnant women who had *P. falciparum* parasitaemia (defined by positive peripheral blood smear), were placental malaria negative using the Bulmer classification [15]. On the Thai–Burmese border weekly malaria screening of pregnant women was a much more sensitive indicator of malaria in pregnancy than placental histopathology. The absence of parasites and pigment on placental histopathology (the Bulmer definition of negative [7]) does not eliminate the possibility of malaria infection during pregnancy [15].

Histology versus placental impression smears for detection of placental infection

Histology has been shown to be a very sensitive method to detect placental malaria but other methods such as thick or thin smears of placental blood and impression smears of placenta have also been used [12,21]. These methods allow the detection of parasites and pigment and have the advantage of being easier, faster and cheaper than histology. They also permit the recognition of the different *Plasmodium* species, which is not possible in histology slides [13,16,54,60]. These techniques also avoid the problem of fixation and formation of formalin pigment that, as discussed earlier, can easily be confused with malarial pigment [16]. On the other hand, histology permits a good classification of the infection detecting also past infections [7,16,22] and gives more information on placental lesions. Another advantage of histological processing with tissue fixation and paraffin embedding, is that tissue samples can be kept for a long time, allowing the possibility of performing ancillary techniques such as immunohistochemistry or molecular studies [16,59,61]. An analysis of data from Malawi has shown 85.5 per cent specificity and 40.7 per cent sensitivity of placental blood smears for detecting histological evidence of active or chronic placental malaria (Verhoeff, personal communication). Comparable results have been shown in a further study from Malawi [62]. Histological assessment has also been reported to be more sensitive than placental smears or peripheral films in a study from Kenya [5]. These findings are based on light microscopy and PCR detection of parasites in the placenta would show more sensitive detection [18].

Other placental lesions associated with placental malaria

Several histological changes have been described in malarial placentae involving the villi and the villous surfaces and have been considered as secondary to the local parasitaemia [13]. Excess of perivillous fibrinoid deposits, excessive syncytial knotting, and trophoblastic basement membrane thickening are the lesions most frequently associated with malarial infection [7,13,16,54,59]. These changes have been associated with syncytiotrophoblastic damage and cytotrophoblastic proliferation in ultrastructural studies [13,59,60], and some of them are controversial [16]. It has been hypothesized that these placental lesions, especially the thickening of trophoblastic basement membrane may alter the materno-fetal exchange and contribute to the deleterious effect of malaria-associated placental lesions on fetal growth. These changes are usually related to chronic infection and severe parasitaemia and are present in only a small proportion of cases, and may be secondary to a deficient utero-placental blood flow [63,64], and probably play a small role in the pathogenesis of fetal alterations. Past infections show only minor histological abnormalities indicating that most resolved malarial infections leave few or no residual changes in the placenta.

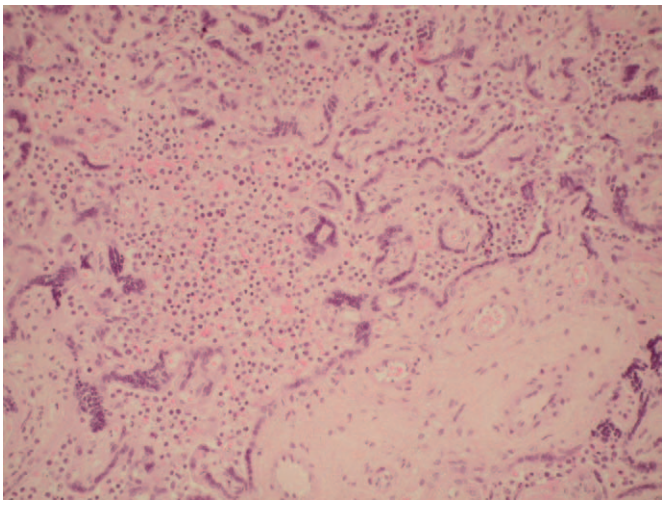

Figure 5. Massive chronic intervillositis. Massive infiltration of the intervillous space with complete sparing of the villi. (Haematoxylin and eosin, 200 × .)

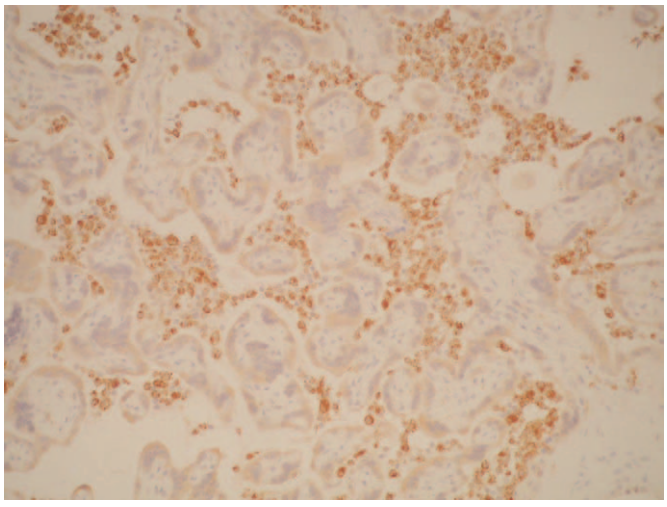

Figure 6. Massive chronic intervillositis. Most inflammatory cells show strong positivity for the monocyte/macrophage marker CD68. (CD68, EnVision, haematoxylin counterstaining, 100 × .)

The most significant association with active malarial infection is intervillous infiltration by inflammatory cells, mainly mononuclear cells, with no increase in villous inflammatory cells [16,34,36,65]. In contrast, past infections are not associated with an increased inflammatory reaction. This inflammatory infiltrate can be prominent, with a pattern called 'massive chronic intervillositis' (Figure 5) that can cause confusion with the dissemination of a malignant maternal neoplasm to the placenta, or autoimmune lesions [36]. Massive chronic intervillositis might be considered as a proxy indicator of malaria control in pregnancy, as its presence has been shown in studies where ineffective antimalarial control measures were in place (i.e. a high level of chloroquine drug resistance). The mononuclear infiltration in the intervillous spaces is always associated with placental parasitaemia and is considered to play an important role in local *Plasmodium* clearance [13,66]. In the series by McGready et al. [15] where all episodes of malaria were promptly treated, increased polymorphonuclear cells were only significant when malaria infection occurred in the last 7 days of pregnancy; the rate of massive chronic intervillositis was also very low 1.7 per cent (3/175). However, inflammatory infiltration may impair the materno-fetal exchange of nutrients either due to a decrease of the maternal blood output or to the release of cytokines [151,154]. Intervillous infiltration is mild or absent in acute infections, suggesting that local parasitaemia precedes placental inflammation, and is later suppressed by the onset of the inflammatory infiltration.

Information on placental abnormalities at earlier stages of gestation, and the possible relationship between malaria and abortion is very scant and mostly limited to studies in animal models [58,67]. A comparison of placental histopathological changes in women with a single treated *P. falciparum* episode in pregnancy, detected by weekly antenatal screening on the Thai–Burmese border, showed only an increase in the presence of parasites and pigment with increasing trimesters and no significant increase in other indicators of placental damage: inflammatory cell infiltrate, syncytial knotting, fibrin deposition, fibrinoid necrosis and cytotrophoblastic prominence [15].

Immunohistochemical studies

Only a few immunohistochemical studies in malarial infected placentae have been conducted. Initial studies were focused on immunoglobulins and complement. Thus, C3 and C9 deposits in trophoblastic basement membrane were detected [59,60].

Recent studies focused on the typing of inflammatory cells [16,36,61,65]. A marked increase in the number of CD68+ monocytes and macrophages and cytotoxic CD8 cells in the intervillous space of placentae with active malaria infection, with increases associated with the severity of the infection (Figure 6). This increase in cytotoxic T cells, which is associated with marked reduction in birth weight, is in keeping with the immunological evidence of type 1 helper T-cell (TH1) cytokine responses that have been observed in placental malaria [151,154]. These are described in more detail in the next section. Other inflammatory cells such as granulocytes and B-cells constitute a significant proportion of inflammatory cells in the intervillous space but do not seem to effect fetal weight. Finally, a selective absence of CD56 natural killer cells has also been described [65] and the possible role of this finding in the pathogenesis of placental hiding and accumulation of *P. falciparum* parasites needs confirmation.

IMMUNOLOGY OF PLACENTAL MALARIA

The precise mechanisms controlling malaria infection are incompletely understood. Both specific and non-specific mechanisms are important, with the monocyte/macrophage series as the major effector cells in the direct attack on

parasitized erythrocytes. Adults and older children uncommonly develop severe malaria in areas of high transmission. This partial and developing immunity is associated with variant-specific agglutinating antibodies against different parasite isolates, which reflects previous exposure [68]. This naturally acquired immunity reduces the frequency and density of parasitaemia. During pregnancy the immune system alters to accept the fetal allograft while maintaining host defences against foreign antigens. A degree of immunomodulation occurs, which increases susceptibility of pregnant women to certain infections. There are several well-described examples of these infections, including *P. falciparum* malaria.

Immune pathways in placental malaria

Increased susceptibility of pregnant women to malaria has been attributed to either localized uterine cell-mediated immune (CMI) suppression, or to a systemic reduction in CMI. Figure 7 outlines several of the mechanisms discussed in this section. The human placenta is a major anti-inflammatory organ favouring the type 2 helper T-cell (TH2) pathway, through interleukin 10 (IL-10) [69], tissue growth factor-β (TGF-β) [70], and progesterone production [71]. The placental anti-inflammatory cytokines are likely to suppress CMI. Expression of type 1 helper T-cell (TH1) pathway cytokines is associated with spontaneous abortion while TH2 pathway cytokines are mandatory for pregnancy maintenance [72]. Gamma-interferon (INF-γ) and tumour necrosis factor α (TNF-α) have direct cytotoxic effects towards both intracellular organisms and the villous trophoblast. Malaria stimulates the production of pro-inflammatory mediators, shifting the cytokine balance away from the TH2 response [66]. Both the type of inflammatory activity and the chronicity of the inflammatory response, rather than parasite density or peak time of the inflammatory activity, have been associated with poor pregnancy outcome.

Placental parasite adhesion

McGregor [43] viewed the placenta as an immunologically naïve organ during the first pregnancy lacking the local immune responses previously developed in other organs during malaria infection. The improved control of falciparum malaria shown by multigravidae could result from uterine sensitization to malaria antigens recognized during the first pregnancy. The model proposed by Fried and Duffy [6] has provided a molecular explanation for placental parasite sequestration and parity-specific susceptibility with the placenta representing a vascular bed displaying receptors not commonly accessible for parasite adhesion elsewhere. Primigravidae who lack immunity to parasite sub-populations expressing epitopes which could adhere to these receptors would be highly susceptible to parasitaemia and with malaria exposure during pregnancy would develop increasing immunity. This hypothesis was tested in Kenya and among several molecules examined only chondroitin sulfate A (CSA), a glycosaminoglycon, supported placental parasite binding. Adhesion to CSA in vitro is a common feature of placental parasite isolates in Kenya [6], Malawi [73], and Cameroon [74]. Placental parasites bind to CSA but not to other ligands such as CD36 [6]. CSA proteoglycans can mediate the sequestration of infected erythrocytes in the placental intervillous spaces during the entire second and third trimesters and possibly the later part of the first trimester [75]. These CSA proteoglycans efficiently bind to infected erythrocytes due to the presence of 4-sulfated disaccharide clusters [76]. The identification and characterization of the CSA proteoglycans involved in placental infected erythrocyte adherence provides a valuable tool for understanding the molecular interactions as a basis for therapeutic strategies for maternal malaria.

Naturally occurring anti-adhesion antibodies against CSA-binding parasites are globally (Kenya, Malawi and Thailand) cross-reactive suggesting that epitopes targeted by these antibodies are conserved [66]. Mature infected erythrocytes (IE) adhere to CSA receptors via a parasite ligand, which is a duffy binding-like (DBL)-gamma 3 domain and is part of the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family [77]. The adhesion of infected erythrocytes to syncytiotrophoblast correlates with the appearance of PfEMP1 at the erythrocyte surface, after merozoite invasion during the early ring stage [78]. During blood stage development of these infected erythrocytes, trophozoites switch to an exclusviely CSA adhesion phenotype. Therefore adhesion may occur throughout the blood stage cycle. Placental adhesion of infected erythrocytes also involves a rhoptery-derived ring surface protein (RSP-2) which is discharged on the erythrocyte membrane during contact with merozoites [79].

PfEMP1 is an antigenically diverse malaria protein of 200–350 kDa [80]. Multiple forms of the PfEMP1 gene are apparent in malaria parasite chromosomes and some are in clustered arrangements. The var gene, which encodes PfEMP1 switches to alter the antigenic and adhesive phenotype properties of the parasite [82]. Each var gene contains copies of a motif that has been previously shown to bind diverse host receptors. These findings are consistent with the involvement of var genes in antigenic variation and binding to endothelium. The multigene var family codes for approximately 50 variant adhesive proteins expressed in a mutually exclusive manner at the erythrocyte surface. Selective upregulation of a single distinctly structured var gene (var 2 CSA) in CSA-adhering *P. falciparum* parasites involved in pregnancy-associated malaria is described [83]. Vaccines based on DBL gamma CSA domains may only need to target a limited number of variants more commonly expressed by placental isolates [84]. Beeson and colleagues in Malawi [85] have also demonstrated in vitro binding of placental parasites to hyaluronic acid (HA), a glycosaminoglycan which lacks sulfation.

In Tanzania the expression of the receptor ICAM-1 on the surface of monocytes and macrophages within the placental vascular bed significantly correlated with the degree of

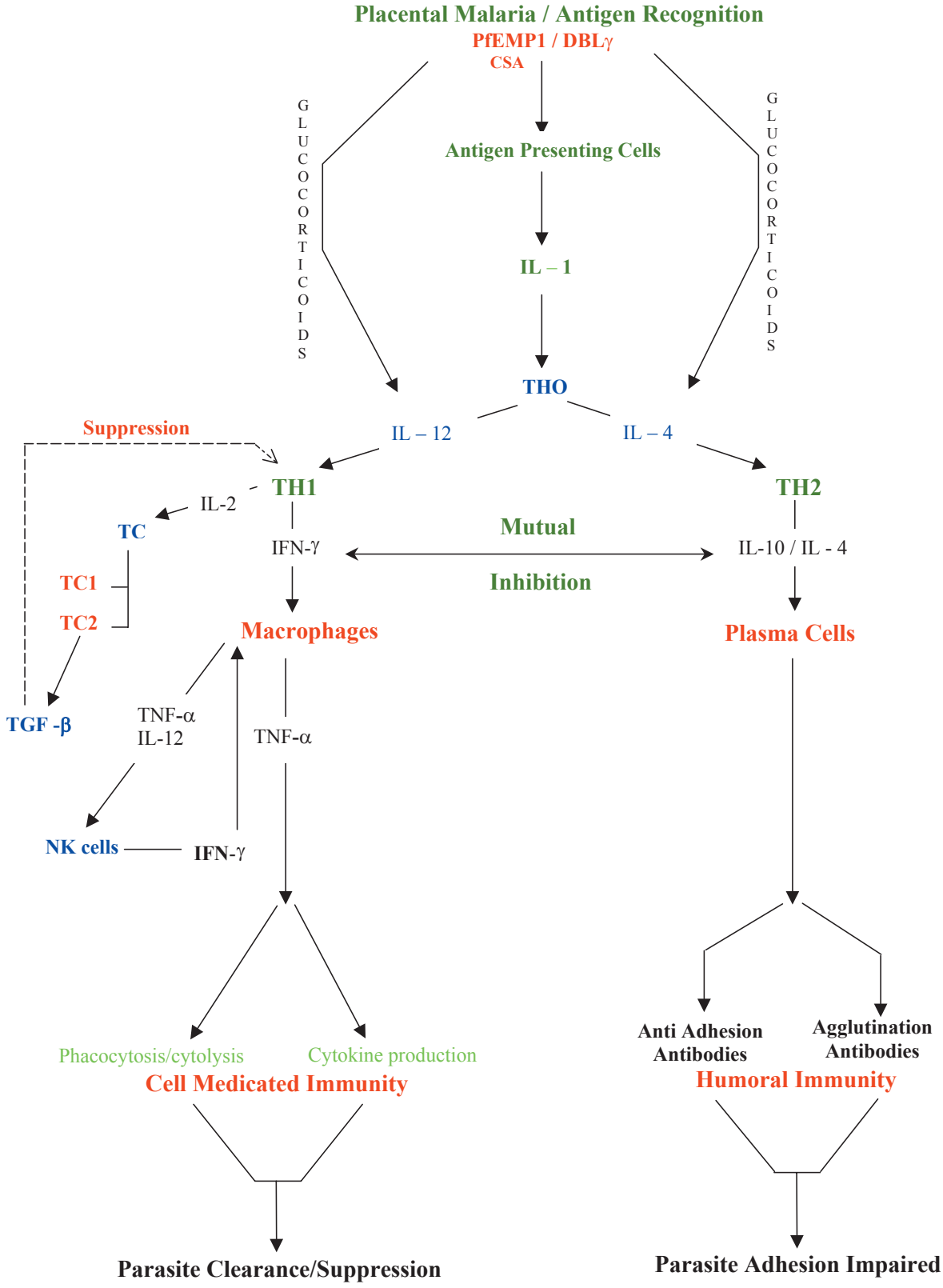

Figure 7. Placenta-related mechanisms of malaria parasite clearance, suppression and adhesion. PfEMP, *P. falciparum* erythrocyte membrane protein; DBLγ, Duffy binding like domain-γ; CSA, Chondroitin sulphate A; NK, Natural killer cells; IFNγ, Interferon γ; TGF-β, Transforming growth factor β; TC, T cell cytotoxic; THO, T helper (precursor).

intervillous leukocyte infiltration [86]. It was suggested that this might contribute indirectly to the sequestration of infected erythrocytes within the intervillous spaces. Pro-inflammatory cytokines increase the expression of HA on microvascular endothelial cells [87], but do not alter expression of CSA in the placenta [50]. CSA and HA are widely distributed in peripheral vascular beds and it is unclear whether malaria parasites use these molecules as receptors for adhesion outside the placenta in non-placental tissue during pregnancy [88].

Placental parasites are also unable to form rosettes, which is another differentiating feature from parasite isolates infecting non-pregnant hosts [89]. Little is known about the relative contribution of different receptors in rosetting and their prevalence in nature [90]. Parasitized RBCs form rosettes more readily with RBCs belonging to blood group A or B, than with those belonging to group O [91]. Blood group A has been reported as a risk factor for severe malaria [152]. This may be due to the fact that group A blood antigens have an important role as co-receptors in *P. falciparum* rosetting [90]. Host blood group in combination with a parasitic preference for the same blood group may provide a favourable biological environment for *P. falciparum* infection. No information has been identified examining blood group types and their relation to placental malaria.

Sequestration in the placenta, via cytoadherence, is related to parasite pathogenesis and antigenic phenotype. Pathological events may occur through several possible mechanisms: obstruction of blood flow; systemic or local production of pro-inflammatory cytokines; blockage of signal transduction [92].

Placental parasites express unique antigens (CSA and HA binding epitopes) different from which are usually seen in other parasite isolates. A specific form of immunity is required to confer protection against these parasite subpopulations via anti-adhesion antibodies and/or variant-specific agglutinating antibodies [157].

Anti-adhesion antibodies

There is growing evidence that anti-adhesion antibodies provide immune protection against placental malaria [66,93,150]. Anti-adhesion antibodies against CSA-binding parasites are associated with reduced prevalence and density of placental malaria. Malaria susceptibility in primigravidae has been related to the lack of these antibodies [66]. In Cameroon this antibody response towards pregnancy-associated parasites was related to parity, explaining the difference in susceptibility between primigravidae and multigravidae [94]. The difference in the levels of anti-adhesion antibodies rather than the degree of parity-dependent humoral immunity has been related to protection from placental malaria [93,95]. In Ghana antibody recognition of placental parasites at term correlated with donor parity, although adhesion inhibition was related to the antibody level regardless of parity. Plasma samples from primigravidae at term, with high plasma levels of anti-adhesion antibodies, were as efficient as those from multigravidae in inhibiting CSA-specific adhesion [93]. O'Neil-Dunne and colleagues [91] reported that pregnant women with placental malaria in Cameroon, regardless of parity, lacked anti-adhesion antibodies during early gestation. Eighty-eight per cent of their sample acquired antibodies during the second trimester. Multigravidae produced anti-adhesion antibodies earlier at 12 weeks' gestation, whereas primigravidae started at 20 weeks' gestation. At term most pregnant women, regardless of parity, had adequate anti-adhesion antibody and these protective antibodies after pregnancy can block binding to CSA of parasites from different parts of the world. The delayed response in primigravidae reduces protection against placental malaria during the second trimester. Agglutination antibodies may be found more commonly in multigravidae with placental malaria, but have not been shown to be associated with protection from infection. Immunization of monkeys with recombinant duffy binding-like-gamma 3 has been shown to induce pan-reaction and adhesion-blocking antibodies against placental CSA-binding *P. falciparum* parasites suggesting that the development of a vaccine to prevent placental adhesion is feasible [96]. Strategies exploring these conserved epitopes as vaccine candidates against placental malaria should be investigated.

Cell mediated immunity

Without anti-adhesion antibodies an extensive CMI response occurs leading to chronic inflammatory placental malaria. Pro-inflammatory cytokines, inducing a TH1 response, dominate in primigravidae in the absence of an adequate anti-inflammatory TH2 cytokine response. Disequilibria between the TH1/TH2 responses results in adverse outcomes in placental malaria in the non-immune pregnant host. The development of acquired specific immunity over one or two successive pregnancies limits both parasite sequestration and inflammatory infiltrates. This will decrease both the intensity and duration of pro-inflammatory cytokines in the placenta.

With placental malaria transcription of certain cytokines is up-regulated (IL-1, INF-γ), leading to increased levels of placental cytokines (INF-γ TNF-α, IL-2) (Figure 7). Their levels, as well as soluble cytokine receptors, generally exceed normal peripheral circulatory levels indicating a local inflammatory process [66,97]. Dense accumulation of intervillous inflammatory cells takes place in active placental malaria [65]. Macrophages are the predominant cells, considered to be the principal arm of protective immunity to placental malaria. In murine pregnancy placental macrophages have been shown to be as effective as peritoneal exudate cells in phagocytosing parasite derived material in vitro [98]. Pro-inflammatory cytokine secretion (TNF and IL-8) was shown by immunohistochemistry to be localized in haemozoin-laden macrophages actively participating in phagocytosis [99]. Macrophages present in infected placentae express tissue factor, the initiator of the clotting system, and the subsequent perivillous clot

formation leads to a narrowing and plugging of the intervillous space and disturbance of the blood supply [100]. Macrophage tissue factor expression in malarial placentae could be associated with retarded placental growth and low birthweight. The quantitative response of these inflammatory cells may not be the only determinant factor for outcome in placental malaria. The rate of appearance of these cells is important as the reduced proliferative capacity of the placental mononuclear cell in comparison to peripheral mononuclear cells may contribute to heavy placental parasite colonization [101,102].

Ordi and colleagues [65] in Tanzania reported that placental malaria does not appear to be associated with CMI suppression, as there was a selective absence of NK cells in placental malaria, which may contribute to the failure of parasite clearance. The interaction of NK cells and other lymphocytes may determine pregnancy outcome via the regulation of the NK cell/MQ inflammatory engine. While there is good evidence for the protective role of humoral immunity in placental malaria, it is not yet clear whether inappropriate and/or insufficient CMI is linked to pregnancy outcome.

Cytokines and placental malaria

Placental malaria has been associated with elevated levels of placental TNF-α [38,66,99,102,103] and high levels of this cytokine were related to poor pregnancy outcome [37,66,99]. The concentration of placental TNF-α has been correlated with placental parasite densities and intervillous monocyte infiltrates [37,38]. Peripheral blood levels of TNF-α were weakly associated with placental malaria and/or pregnancy outcome [37]. Elevated levels of placental INF-γ during first pregnancies may be a contributory factor to poor outcome in pregnancy. Multigravidae showed a high placental INF-γ response early in the acute phase of placental malaria while primigravidae showed a high level even in the absence of placental parasitaemia. Parity differences in susceptibility to placental malaria may be associated with differential ability to mount local cytokine responses in the placenta [102,104]. The pregnant women whose intervillous inflammatory cells produce a high level of INF-γ should more effectively control infection than women whose INF-γ response is less efficient. Primigravidae placentae induce weak primary INF-γ responses and further exposure to placental malaria amplifies the INF-γ response as multigravidae more effectively clear parasites. In primigravidae placentae, with cord blood parasitaemia from Malawi, neither INF-γ nor IL-12 was detected using measurements of cytokine mRNA [99]. Rogerson and colleagues [38] have also reported, in a study from Malawi, a slight but significantly elevated placental plasma INF-γ response with placental parasites but concluded that elevated INF-γ levels were not associated with poor pregnancy outcomes. Maternal HIV infection may partly explain these differences between studies [148].

Impaired production of INF-γ in HIV sero-positive pregnant women is associated with increased susceptibility to placental malaria [105,148]. The triad of pregnancy, HIV sero-positivity and placental malaria seems to affect the IL-12 response, leading to decreased parasite clearance [106]. Multigravidae with placental malaria can produce high levels of TGF-β [66] and this 'mature' response, together with a specific humoral immunity, would reduce an extensive CMI response. TGF-β levels have been reported to be lower in primigravidae with, placental malaria compared to those without [99], which could lead to an uncontrolled TH1 response, especially in the absence of specific humoral immunity (Figure 7). Although both primigravidae and multigravidae with placental malaria produce high levels of IL-2, only multigravidae have been reported to produce high levels of TGF-β [66]. Parity seems to affect placental cytokine synthesis and inflammatory pathway maturation. The IL-2 response to different malaria antigens gradually increased with increasing parity suggesting the construction of a T cell memory response during successive pregnancies [104]. IL-10 acts as a regulatory cytokine and its production may serve to regulate INF-γ and TNF-α, as its synthesis in placental malaria paralleled that of INF-γ and to lesser extent TNF-α [102]. In placenta from holoendemic areas, in contrast to low endemic areas, the cytokine balance was shifted toward a TH1 response reducing the level of IL-10 via the mutual inhibitory effect [66]. Pregnancy may influence malaria parasite dynamics in ways that alter immune responses, rather than influence immune responses in ways that alter parasite dynamics. Placental parasite proteins or peptides could be selected in the placenta, or be specifically induced by pregnancy hormones [107]. The trigger for the pro-inflammatory responses in placental malaria may be parasite cytoadhesion to CSA, HA and other placental receptors. Conversely pro-inflammatory cytokines may induce these placental vascular receptors, which promote parasite cytoadhesion.

FUNCTIONAL CONSEQUENCES OF PLACENTAL MALARIA

Infant morbidity and survival

Fetal growth restriction and pre-term delivery are recognized major consequences of placental malaria. These have important implications as low birthweight babies have increased morbidity and mortality. Maternal placental infection with *P. falciparum* has been positively associated with malaria morbidity during the child's first 2 years of life ([108]; Menendez et al., in preparation), infant anaemia ([4]; Menendez et al., in preparation); fetal anaemia [109,110,149]; cord malaria parasitaemia [17], prenatal immune priming to malaria antigens [111], and perinatal and neonatal mortality [3,28,112,113]. Placental infection with *P. falciparum* appears to have a much more significant role in infant survival in Africa than has been previously assumed [114].

These risks occur even though symptomatic congenital malaria is an unusual event for babies born to semi-immune

Table 6. Effects of placental malaria and/or maternal hypergammaglobulinaemia on transfer of specific antibodies

Year	Country	Tetanus toxoid	HSV	Strep O	Men Gp A	Men Gp C	*S.pn*	Measles	*H.inf*	Reference
1994	Papua New Guinea	↓	–	–	–	–	–	–	–	[131]
1996	Brazil	↓	↓	↓	–	–	↓	–	–	[153]
1996	Gambia	–	–	–	↓	→	–	–	–	[137]
1996	Gambia	–	–	–	–	–	↓	–	–	[138]
1998	Malawi	→	–	–	–	–	↓	↓	–	[122]
2001	Gambia	→	–	–	–	–	–	↓	–	[139]
2001	Gambia	→	↓	–	–	–	→	–	→	[140]

Strep O, Streptolysin O; *S.pn*, *S. pneumoniae*; *H.inf*, *H. influenzae*; MenGp A/C, Meningococcal group A or C. ↓ Reduced transfer; → Transfer not affected.

mothers who are living under holoendemic conditions [81]. Accumulation of infected red cells at the interface between the maternal and fetal circulation does however result in a small number of cases as symptomatic congenital malaria. Importantly, placental malaria and anaemia are associated with the production of *P. falciparum*-specific immunoglobulins by the fetus leading to early immune priming and acquisition of immunity by infants [115,116]. This may partly explain why neonates and infants are relatively protected from clinical malaria if born to semi-immune mothers [117,118]. Yet in low transmission areas placental malaria can lead to congenital infection and infant death [119]. Placental parasitaemia can be used as an alert signal to identify which infants need active screening and treatment of peripheral parasitaemia, to prevent death from malaria [15].

Placental transfer of maternal antibody

Placental transfer of maternal malaria antibody relates to infant immune status and the protection of young infants from severe malaria morbidity and mortality [109,118,120]. The data on the effect of placental malaria on transfer of maternal antibody to a variety of antigens is of particular interest because of effects on infant immunity to infectious illness. This is an active process and believed to be mediated by specific receptors at the materno-fetal interface, or syncytiotrophoblast [121]. There are a number of candidate glycoproteins but recently, attention has focused on the human analogue of a rat pup enterocyte receptor, FcRn. In the proposed model, immunoglobulin is taken across the apical surface of the syncytiotrophoblast, and bound to FcRn within endosomes. The receptor protects antibody from degradation within the cell, before orchestrating its release into the chorionic stroma [121].

In a study in [122] in Malawi, reduced placental transfer of antibodies was associated with placental malaria and maternal hypergammaglobulinaemia. Similar effects on placental antibody transfer have been reported from Papua New Guinea and The Gambia (Table 6). The pathophysiology is uncertain but may reflect gross, inflammatory disruption of the syncytiotrophoblast. Alternatively, saturation of receptors with non–specific immunoglobulin secondary to malarial hypergammaglobulinaemia may be responsible. Interestingly, recent evidence suggests that maternal non-immune immunoglobulin may play a role in parasite sequestration at the syncytiotrophoblast [123].

There are implications for the vaccination of infants born to malaria-infected mothers. Such children may become susceptible to measles earlier than those born to non-infected mothers, requiring measles vaccination at a younger age [124]. Similar concerns have been raised about the effect of placental malaria on the success of antenatal tetanus vaccination as a strategy to control neonatal tetanus [125].

Placental nutrient transfer

Abnormalities of placental blood flow related to placental malaria could influence several indices of placental function including proliferative, metabolic, transfer, and secretory capacities [126,127]. In addition, *P. falciparum* infection could disturb the folate-B_{12}-metabolic pathway and the evidence for this mechanism as a contributor to intrauterine growth retardation (IUGR) has been reviewed [110]. This review also highlighted the likely consequences disturbance of this pathway would have on the availability of the essential amino acid methionine and also of vitamin B_6, not only to the developing fetus but also to the enlarging placenta.

Sibley et al. [126] also stated that so-called placental insufficiency, arising from pathological changes of the trophoblast may be an important cause of IUGR. These workers emphasized that not only were abnormalities of uterine or umbilical blood flow involved but specific placental transport mechanisms were likely to be disturbed, as diffusional transfer of solutes cannot explain the findings in fetal IUGR. There is now good evidence for active aminoacid transport mechanisms and that these can be deranged in placental insufficiency. A more recent report by the same group [128] has enlarged on these mechanisms with particular reference to the Na+/K+ exchanger group of transport proteins. They showed that there were a wide variety of placental transporters deranged by IUGR, which as mentioned before is a common finding in *P. falciparum* malaria, especially in primigravidae.

It is likely that larger molecules such as IgG and transferrin-bound iron are transferred across the trophoblast by receptor-mediated endocytosis/exocytosis mechanisms. There is evidence that IgG transfer across the placenta is reduced in IUGR [127,129]. Christensen [130] has reported that the placenta participates not only by specific transporter mechanisms in the amino acid needs of the fetus, but also by metabolism, which allows for control of the rate of supply of various amino acids. The importance of a plentiful but controlled supply of amino acids via the placenta to the fetus is well illustrated by the findings of Jackson [134], in relation to just one amino acid, glycine.

Dietary trace metal requirements for the fetus are obviously of great importance [141] although this is an area which has been little studied. Very little is known about their transplacental transfer. Transport mechanisms have been described for vitamins [132,143]. The placenta also has a hepatobiliary-like excretory function, necessary because this activity is immature in the fetus [136].

The metabolic activities of the placenta have been little studied, and it is inevitable that pathological changes related to malaria in the placenta are likely to have serious metabolic consequences for both the placenta and the developing fetus.

CONCLUDING COMMENTS

There is much we do not know about pathogenesis of placental malaria. McGregor [43] proposed that the accumulation of parasites in the placenta resulted from the placenta actually shielding the parasites from destruction. More recent studies have indicated that a placental selection process is operating leading to the accumulation of *P. falciparum* parasites that adhere to the surface of the syncytiotrophoblast and the immunity which develops to this adhesion, partly explains the epidemiology of falciparum malaria in pregnancy. However, other interactions may explain parasite accumulation in the placenta not least with other malaria species. The role of maternal hormones has been little studied [142] although raised cortisol levels are positively associated with peripheral parasitaemia during pregnancy [145], and low oestradiol levels have been associated with past malaria infection and fetal growth retardation [146].

In spite of the increase in the amount of information in recent years on placental malaria, it is still unclear exactly how malaria in pregnancy causes low birthweight. It is hoped that by the application of knowledge derived from a number of fields, that the pathogenesis of this infection will be better understood, as herein lies the means for better control of this serious public health problem. A placenta with parasites is sick and the adverse effects on the woman and infant have been clearly summarized. Every effort must be made to prevent placental infection and failing that, to rid any infected placenta of parasites. The only method we have to determine infection antenatally is peripheral blood smear or rapid diagnostic kits, but these are rarely used in Africa. Adequate malaria treatment or intermittent preventive treatment with effective antimalarials must be accessible and available for all pregnant women.

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