

Review article

The epidemiology and consequences of maternal malaria: a review of immunological basis

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Abstract

Millions of women who become pregnant in malaria-endemic areas are at increased risk of contracting malaria infection that jeopardises the outcome of pregnancy. The complication of this infection for mother and baby are considerable. In absence of any other reason, it was thought that the increased risk of infection during pregnancy was related to suppression of pre-existing malaria immunity. Although this concept is plausible, the significantly higher risk of maternal malaria and consequences in primigravidae compared with multigravidae suggests that there are more to mere immunosuppression in pregnancy. The mechanisms underlying some of the striking epidemiological and clinical features of malaria in pregnancy could be related to differences in the strains of parasite populations infecting pregnant women occasioned by the cyto-adherent properties of human placenta, presence or absence of anti-adhesion antibodies acquired from previous pregnancies or the elevated production of some pro-inflammatory cytokines in response to parasitisation of human placenta. Malaria infection of placenta causes a shift from Th2 to Th1 cytokine profile that may be detrimental to pregnancy. The increased susceptibility in the first pregnancy can be explained by the absence of anti-adhesion antibody in the primigravida that is being exposed for the first time to a different strain of malaria parasite sub-population that adhere exclusively to chondroitin sulphate A and hyaluronic acid (HA) in the placenta. In reviewing the epidemiology and consequences of maternal malaria, we have highlighted possible immunological and molecular basis that could account for the higher impact of malaria in pregnancy especially among primigravidae. These factors could be the basis for future research and vaccine formulation.

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1. Introduction

Malaria, the most important parasitic disease of man, is endemic in 103 countries where more than

2000 million people live exposed to the infection (Menendez, 1995). An estimated 300–500 million cases of malaria each year result in about 1–2 million deaths, mainly in children less than 5 years of age living in sub-Saharan Africa (Snow et al., 1999, 2001). Besides children, some 24 million women that become pregnant each year in malaria-endemic regions are at increased risk of being

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infected with *Plasmodium falciparum* malaria and its associated complications (McGregor et al., 1983; Steketee et al., 1996b). In some sub-Saharan African countries endemic for malaria, almost half of all primigravidae will be parasitaemic at their first antenatal visit (Menendez, 1995). The complications of these infections, for the mother and baby are considerable and constitute one of Africa's major public health problems. The reasons for the increased susceptibility to malaria in pregnancy and its clinical manifestations are not as straightforward as has been reported previously. The previously held opinion that pregnancy depresses pre-existing immunity (Blacklock and Gordon, 1925; Ibanesebhor and Okolo, 1992) does not explain the differential impact of maternal malaria infection on primigravidae. The available data suggest that several mechanisms could be involved (Scherf et al., 2001; Beeson and Brown, 2002).

An extensive review of available data on immunological basis of the epidemiology and consequences of maternal malaria was carried out with the view of suggesting some therapeutic and control measures in this high-risk group.

2. Clinical description

2.1. Epidemiology of malaria in pregnancy

There are some distinctive features of the epidemiology of maternal malaria that are important in deciding appropriate control strategies. First of all, in all malaria-endemic areas, the frequency and severity of the infection are greater in pregnant women than in the same women before pregnancy and in their non-pregnant counterparts (McGregor et al., 1952; Gilles et al., 1984). In their analysis of a 14-year follow up study of 15–45-years-old women in the Gambia, McGregor et al. (1952) found a higher prevalence of parasitaemia among gravid females compared with non-gravid women in the same area. Both the frequency and density of malaria infections were higher in these pregnant women than in either the same individuals before pregnancy or in control (McGregor et al., 1952; Gilles et al., 1984).

The second feature is that susceptibility to infection and severity of clinical manifestation of malaria are determined by the level of pre-pregnancy immunity that largely depends on the intensity and stability of malaria transmission (Menendez, 1995; Singh et al., 2001; Luxemburger et al., 1997); thus, in areas where malaria transmission is low and/or unstable, the degree of acquired immunity of the women prior to pregnancy is likely to be low, rendering both the mother and her foetus susceptible to the most severe consequences of malaria infection. In such settings, pregnant women of any parity are comparably susceptible to malaria (Luxemburger et al., 1997) and pregnancy outcome associated with *P. falciparum* infection in such areas are enormous (Wickramasuriya, 1937; Luxemburger et al., 2001). In contrast, in areas of high malaria endemicity, women have acquired a significant protective immunity and the effects of malaria on mother and foetus are less severe. These two important epidemiological features give rise to two contrasting consequences of malaria in different zones (Menendez et al., 1995; Steketee et al., 1996a,b).

Parity is another important factor that determines susceptibility and severity of malaria infection in pregnancy. The susceptibility to infection among non-immune women is known to be similar in all parities (Wickramasuriya, 1937; Nosten, 1991; Luxemburger et al., 1997). However, in areas where transmission is high and the level of acquired immunity against malaria is expected to be significant, primigravidae are much more affected (Okoko et al., 2002). An intermediate situation may be seen in meso-endemic area where secundigravidae have been shown to suffer effects of infection similar to those of primigravidae (Nosten, 1991).

Furthermore, it has been reported that the increased risk of malaria varies during the course of the pregnancy. The prevalence of infection and the parasite density are highest in the first half of pregnancy and decrease progressively until delivery, while postpartum parasitaemia are usually similar to pre-gravid levels (McGregor and Smith, 1952; Menendez, 1995).

2.2. Consequences of malaria in pregnancy

Malaria in pregnancy jeopardises the outcome of pregnancy, affecting both the mother and foetus (McGregor et al., 1983; Ibanesebhor and Okolo, 1992; Okoko et al., 2002). Such consequences vary from place to place depending on the intensity of infection.

2.2.1. Maternal consequences

Consequences for the mother range from significant anaemia in pregnancy (Steketee et al., 2001) to severe complications, such as cerebral malaria, pulmonary oedema or renal failure (Wickramasuriya, 1937; Watkinson and Rushton, 1983; Nosten, 1991). These consequences are seen in areas of unstable transmission (Endeshaw, 1991), mes-oendemic (Nosten, 1991) and areas of high transmission (Steketee et al., 2001; Guyatt and Snow, 2001). In malaria endemic area of Mozambique for example, 15.5% of maternal deaths could be directly attributed to malaria especially among adolescent primigravidae (Granja et al., 2001); showing that poor pregnancy outcome expected in hypo-endemic regions are being observed in hyper-endemic regions as well.

2.2.2. Foetal and postnatal consequences

There is broad evidence for the adverse effects of parasitisation of placenta. These consequences on the foetus also differ from place to place depending on the endemicity of malaria. In the region of Saigon where malaria transmission is unstable, Hung (1951) documented malaria in pregnancy to be responsible for 14% of foetal death five decades ago. Other studies in hypoendemic regions have associated malaria in pregnancy with poor foetal outcome such as abortion, stillbirths and intrauterine growth retardation (Nosten, 1991; Endeshaw, 1991; Kasumba et al., 2000). Similarly, a review of studies from regions where malaria is highly endemic showed that malaria population attributable risk (PAR) account for the increased stillbirth rate (Okoko et al., 2002), intra-uterine growth retardation (IUGR) and prematurity (Ibanesebhor and Okolo, 1992; Steketee et al., 1996b, 2001; Verhoeff et al., 2001; Shulman et al., 2001). Both the prevalence of the risk factors in the

population and the magnitude of the associated risks for foetal anaemia, preterm-low birth weight (LBW), IUGR-LBW and infant mortality were substantial (Steketee et al., 2001). In both hyper- and hypo-endemic regions, timing and severity of infection determines whether an outcome is prematurity, IUGR or both (Menendez et al., 2000; Sullivan, 1999). Infection in the first or second trimester commonly results in intrauterine retardation whereas severe late infection is more likely to cause premature delivery than IUGR (Sullivan et al., 1999).

There is a dearth of information on the effects of malaria during pregnancy on subsequent mortality and morbidity in infancy. The complexity of the multiple associations of the different effects of malaria in pregnancy and other socio-economic problems in most developing countries even make this assessment more difficult. Maternal anaemia, for instance, is a risk factor for perinatal mortality (Zucker et al., 1994; Khan, 2001) and may also influence the infant's mortality and morbidity by reducing the baby's birth weight (Menendez, 1995). An association between maternal anaemia and the probability of dying at 1 year of age independent of LBW and prematurity has been observed by Dolan et al. (1994). This may be explained by an increased risk of developing anaemia in infancy, as several reports have found a direct correlation between maternal and cord blood haemoglobin levels in malaria endemic areas (Oppenheimer et al., 1986; Fleming, 1989; Brabin, 1992; Menendez, 1995).

The best-studied perinatal outcome of malaria infection in pregnancy is birth weight. It is adversely affected by both peripheral and placental parasitaemia (Ibanesebhor and Okolo, 1992; Rogerson and Beeson, 1999; Okoko et al., 2002). The increased risk of LBW could reasonably account for the poor neonatal and infant outcome associated with malaria infection (Luxemburger et al., 2001). It is estimated that, about 5.7% of infant deaths in malarious areas could be indirectly linked to malaria infection in pregnancy (Guyatt and Snow, 2001). Besides, higher susceptibility to malaria infection among infants delivered from parasitised placentae compared with control has

been observed in some malaria endemic regions (Le Hesran et al., 1997).

3. What happens to maternal immunity during pregnancy

The mechanisms underlying some of the striking epidemiological and clinical features of malaria in pregnancy are subjects of importance for research and proper control strategies. Several immunological hypotheses have been proposed to explain the increased risk of malaria in pregnant women. During pregnancy, a physiological immunosuppression mediated by pregnancy-associated hormones and proteins occurs. This regulation is seen by some as a necessary adjustment to maintain the antigenically different conceptus (Bray and Anderson, 1979; Marzi et al., 1996; Raghupathy, 1997). The state of immune depression affects both specific mechanisms (to maintain pregnancy) and non-specific, which lead to an increased risk of several infections, including malaria. However, some observations from areas of stable malaria run counter to the widespread view of loss of previously acquired immunity to malaria during pregnancy (McGregor and Smith, 1952; McGregor, 1969; Menendez, 1995; Rieke et al., 2000). Moreover, the generalised immuno-depression does not explain the observed influence of parity or gestational age on the frequency and/or severity of malaria infection (Okoko et al., 2002; Steketee et al., 2001).

Recent studies from malaria endemic regions show that the cytoadherent properties of the malaria parasite infecting human placenta and the host responses (humoral and cellular) explain to some extent, the striking epidemiological features seen in maternal malaria infection (Beeson and Brown, 2002; Rieke et al., 2000; Staalsoe et al., 2001).

3.1. Pregnancy and humoral immune response

The serum total IgG titres at different stages of pregnancy hardly reflect degree of protection in pregnancy. For instance, maternal malaria infection rate is lowest in the third trimester (Ibane-

sebhör and Okolo, 1992) when mean gamma-immunoglobulin concentrations (IgG) falls to its lowest level (Logie et al., 1973). This IgG level seems to be a relative rather than an absolute decrease as suggested mechanisms include hemodilution from fluid retention that occurs in the second half of pregnancy and by the transplacental transfer of IgG from the mother to the foetus (Logie et al., 1973; Okoko et al., 2001). It does not appear to result from impaired immunoglobulin (Ig) production as humoral responses to vaccination appear intact in pregnancy (Logie et al., 1973). Earlier, Billewicz et al. (1965) in the Gambia failed to establish any convincing evidence that humoral component of malaria immunity was depressed in association with pregnancy. This observation was consistent with Logie et al. (1973) study where adequate precipitins activity towards malaria antigens in 98% of maternal samples and 97% of samples from their newborns was reported, suggesting that in pregnancy, women remain immunologically reactive to the stimulus of plasmodia parasitaemia. Rather, recent evidences showed that there is an association between production of antibodies recognising antigens on the placental infected erythrocytes and protection against maternal malaria (Rieke et al., 2000; Staalsoe et al., 2001).

3.1.1. Cyto-adherent property of the malaria parasite infecting human placenta

After several years of exposure to malaria, women have acquired immunity to *P. falciparum* equivalent to their male counterparts. Gravid women, however, are uniquely susceptible to malaria infection that diminishes as their gravid status increases (Okoko et al., 2002). In the absence of evidence for other mechanisms, previous studies attributed this susceptibility to immuno-suppression of pregnancy (Bray and Anderson, 1979; Ibanesebhör and Okolo, 1992). But this theory cannot entirely explain the epidemiology of this infection. Rather, recent observations (Beeson et al., 2001; O'Neil-Dunne et al., 2001) seem to favour McGregor (1984) earlier hypothesis that pregnancy establishes within an otherwise effectively immune host an extremely vascular organ that shields parasite from destruc-

tion by extra-uterine immune effector mechanisms. This shelter is temporary because the presence of the parasite in the placenta induces an immune response, though least effective in first pregnancy, which strengthens and increases in effectiveness with subsequent exposures.

The placenta is the preferential site for the sequestration of infected red blood cells (IRBC) and can experience high parasitisation while the peripheral circulation is free of parasites (Ibane-sebhor and Okolo, 1992). During the past several years, a number of studies have demonstrated that specific proteins expressed on the endothelial surfaces of the vascular system support the binding of IRBCs. Some of the identified receptors include intracellular cell adhesion molecule-1 (ICAM-1), CD36, vascular cell adhesion molecule (VCAM-1), E-selectin, P-selectin, chondroitin sulphate A (CSA) and thrombospondin (Fried and Duffy, 1996; Chaiyaroj et al., 1996; Gowder and Ockenhouse, 1999; Newbold et al., 1997). The adherence of IRBCs in specific organs and consequent sequestration is believed to be responsible for the development of malaria pathology (Gowder and Ockenhouse, 1999). The affinity of binding to a particular endothelial receptor may be related to the pattern of disease. Some receptors such as CD36 and Thrombospondins are used by all parasite isolates but others may select some sub-populations of parasites (Newbold et al., 1997). In 1995, two studies independently showed that selected populations of IRBC could recognise and bind CSA chain in the placenta but not others (Rogerson et al., 1995; Robert et al., 1995). Soon after this discovery, CSA (Fried and Duffy, 1996) and hyaluronic acid (HA) (Beeson et al., 2000) were shown to be receptors for IRBCs adherence in human placenta. While the role of HA remains unclear, CSA remains the only uncontroversial receptor for the malaria-IRBCs in human placenta (Beeson et al., 1999; Fried et al., 2000; Alkhalil et al., 2000). IRBC sequestration results from adhesive interactions between parasite-derived proteins expressed on the surface of endothelial cells and placental cells (Pouvelle et al., 2000; Beeson and Brown, 2002). Studies have shown that there are structural requirements, including percentage of sulphated and non-sulphated disaccharides in

CSA, for either inhibition or adherence of IRBC in the placenta to take place (Alkhalil et al., 2000; Chai et al., 2002).

In placental malaria (PM) syndrome, the principal parasite ligand and antigen on the RBC surface, *P. falciparum* erythrocyte membrane protein 1 (PfEMP-1) encoded by a multigene family termed Var, is clonally variant, enabling evasion of specific immune responses (Scherf et al., 2001; Beeson and Brown, 2002). It has been shown that the PfEMP-1 domains (duffy binding-like [DBL-gamma]), expressed by the placental *P. falciparum* have affinity to CSA (Khatab et al., 2001). All parasite populations infecting placenta express only one variant of PfEMP-1, each of which contains a DBL-gamma domain with CSA binding capacity. Furthermore, Khatab et al. (2001) have shown that there is evidence for antigenic conservation among the DBL-gamma sequences expressed by different parasites. These findings reflect the process of parasite adhesion and pathogenesis of PM infection.

These adhesion studies suggest that maternal malaria arises when placenta selects a parasite population that binds to CSA. Infection with this parasite population does not occur in non-pregnant host and differs from other field isolates with altered antigenic determinants to evade immune surveillance (Scherf et al., 2001). Despite previous immunity, a woman becomes highly susceptible to infection during initial pregnancy when these selected CSA-binding parasites are presented to the placental substrate. With successive pregnancies, a woman develops increasing immunity to this parasites sub-population and reduces the frequency and severity of maternal malaria (Fried and Duffy, 1998).

3.1.2. *Gravidity-dependent susceptibility*

3.1.2.1. Earlier hypotheses. Following some observations, three hypotheses were postulated that attempted to explain the mechanism underlying the increased susceptibility of pregnant women especially primigravidae to malaria infection. First, McGregor (1983); McGregor (1984) hypothesised that the placenta and utero-placental circulations are immunologically “malaria-naive”

and, therefore, the development of a specific adaptive immune response in the utero-placental circulation was required. Repeated exposure to the parasite as gestation advances and subsequent pregnancies will make these local responses more effective. Another hypothesis suggests that the general immune depression associated with pregnancy as a result of hormonal changes is most marked in primigravid women (Stimpson, 1980). But this does not explain why the parasite multiplies and grows easily in the placenta more than any other organ.

Finally, the susceptibility was said to be due to the role of the local immunosuppressive factors synthesised in the placenta relative to peripheral blood causing a permissive effect on the parasite, (Rasheed et al., 1993) which leads to the increased number of parasites in the placenta. Since oestrogen level decreases with advancing pregnancy due to decreased synthesis, its suppressive effect on humoral and cell-mediated immunity is, therefore, expected to decrease (Rasheed et al., 1993). Thus, the documented reduction in cortisol and oestrogen levels with higher parity would also explain the lower susceptibility to malaria found in multigravidae compared with primigravidae.

3.1.2.2. Recent findings: anti-adhesion antibody. Part of the host's specific immune response to adhered *P. falciparum* IRBC is the production of an antibody, in this case anti-adhesion antibodies, which limit the adhesion and accumulation of IRBC in the placenta (Duffy and Fried, 1999; Ricke et al., 2000). The fact that the frequency and devastating effects of maternal malaria occurs mainly during the first couple of pregnancies in endemic region (Okoko et al., 2002) indicates that protective immunity to maternal malaria is a function of parity (Ricke et al., 2000). It is increasingly apparent that this acquired protective immunity relies on antibodies specifically recognising variant parasite antigens (Ags) expressed on IRBC (Fried and Duffy, 1998; Duffy and Fried, 1999; Staalsoe et al., 2001). Thus, it is only the parasite that expresses variant Ags, which the host does not possess adequate level of antibody that causes diseases (Staalsoe et al., 2001). These findings explain the epidemiology of maternal

malaria better than possible quantitative or qualitative changes in the cellular immune system during pregnancy. For instance, plasma from Kenyan multigravid, but not male or primigravid, women from endemic areas can inhibit adhesion of placental parasites to CSA (Fried et al., 1998). Similar results from West Africa showed that plasma antibodies from Ghanaian women in their third trimester of pregnancy could inhibit and even reverse binding of CSA-selected parasites to CSA. Furthermore, the level of antibody correlated strongly with this inhibitory capacity (Ricke et al., 2000). But adequate level of antibody depends on endemicity of infection (Duffy and Fried, 1999; Staalsoe et al., 2001), parity (Fried et al., 1998; O'Neil-Dunne et al., 2001) and Gestational age (Ricke et al., 2000; Staalsoe et al., 2001). Thus, the early onset of efficient antibody response in multigravidae and the delayed production of antibodies in primigravidae appear to account for the gravidity-dependent differential susceptibilities of pregnant women to PM (O'Neil-Dunne et al., 2001).

These evidences suggest that the production of anti-adhesion antibodies against strain-independent CSA-binding parasites is an acquired humoral response, which may be protective in different geographical location and is associated with greatly reduced prevalence and density of infection. And more importantly, the variant surface antigens that mediates the adhesion to CSA, could be a target for vaccination against maternal malaria infection.

3.2. Cellular immunity

3.2.1. Depressed cellular immunity and cytokine profile

In pregnancy, there is a transient depression of cell-mediated immunity (May and Sexton, 1980). Although the mechanism behind the depressed cellular immunity is not well understood, the pregnancy hormones are implicated as partly responsible. Indeed, cortisol, level of oestrogen and a-glyco-protein have been demonstrated in-vitro to depress cellular responses (Brabin, 1997; Beer and Billingham, 1988; Stimpson, 1980).

However, this transient depression of immunity is seen as an event of immunologic tolerance that

allows a woman to accept implantation of the foetal allograft in her uterus (Wegmann et al., 1993). The lack of strong maternal cell-mediated anti-foetal immunity and a dominant hormonal immune response has prompted workers to suggest that the cellular immune system in normal pregnancy is biased towards type II helper cell (Th2)-like response (Wegmann et al., 1993; Clerici and Shearer, 1994). Th1 type immune responses characterised by the production of interferon gamma (IFN- γ), IL-12 and tumour necrosis factor beta (TNF- β) is seen as anti-foetal immunity in rodents, while Th2 mediated responses associated with the production of IL-4, IL-5, and IL-13, sustain pregnancy (Marzi et al., 1996). This shift in the balance of cytokine profiles away from Th1-type reactivities to Th2 type reactivities during pregnancy occurs in both human and rodent placentas (Wegmann et al., 1993; Raghupathy, 1997; Marzi et al., 1996). Although this immunological change in pregnancy is useful to the foetus, the consequences of this weakened cellular immunity (shift away from complement-fixing IgG2a type antibodies) is susceptibility to a number of infectious diseases caused by intracellular pathogens including viral, malaria and other parasitic infections in pregnancy (Clerici and Shearer, 1994; Krishnan et al., 1996) with potential of interactions between these infections.

3.2.2. Interaction between HIV and *P. falciparum* in pregnancy

There is a potential for interaction between malaria and human immunodeficiency virus (HIV) infection. Malaria could enhance the progression of HIV infection to AIDS (Bloland et al., 1995); conversely HIV infection might reduce immunity to malaria resulting in more frequent and severe infections (Moore et al., 2000). These two infections have serious consequences in pregnant women, their foetuses, and infants in sub-Saharan Africa. A study in Malawi showed that PM rates were higher in HIV positive compared with HIV negative women (Steketee et al., 1996c). This association was surprisingly strongest in multigravidae. Compared with infants born to HIV(–) women, newborns born to HIV(+) women had higher rates of cord blood parasitaemia in the Malawian study (Steketee et al., 1996c).

A previous study in the same country strongly suggested that exposure to both PM and maternal HIV infections increases post-neonatal mortality beyond the independent risk associated with exposure to either maternal HIV or PM infection (Bloland et al., 1995).

One of the major reasons advanced for the increased susceptibility of HIV-positive pregnant women to malaria is the HIV-mediated cytokine dysregulation and impairment of the protective IFN-gamma response (Moore et al., 2000). Moore et al. (1999) had observed previously that elevated production of IFN-gamma, as part of a carefully regulated cytokine network, is important in the control of PM infection. Loss of the IFN-gamma response observed in HIV-positive pregnant women (Moore et al., 2000), especially after malarial antigen stimulation, may impair their ability to control PM infection. Furthermore, substantial impairment of IL-12, not IL-18 or IFN-gamma inducible protein (IP)-10, production by intervillous mononuclear cells was observed in HIV/PM co-infection (Chaisavaneeyakorn et al., 2002). The implication of this impairment is increased susceptibility to PM and its consequences as IL-12 is also implicated as a potentially critical regulator of malaria antigen-specific IFN-gamma (IFN- γ) responses in HIV-infected and HIV/PM coinfecting women (Chaisavaneeyakorn et al., 2002).

4. Pathology and consequences of placental malaria: immunological basis

4.1. Pathological features

Parasites infecting the placenta are commonly observed in intervillous erythrocytes with or without pigment deposition in the intervillous spaces or intravillous regions. The chorionic villous syncytiotrophoblast and stroma are also commonly observed to contain malaria pigment (Bulmer et al., 1993; Galbraith et al., 1980; Leopardi et al., 1996).

Changes that have been described in parasitised microvilli include proliferation of cytotrophoblast, focal syncytiotrophoblastic necrosis, loss of syncy-

tial microvilli and irregular thickening of trophoblastic membrane (Bulmer et al., 1993; Leopardi et al., 1996). The unusual prominence of the villous cytotrophoblast cells in some heavily parasitised placentae and a variable degree of thickening of the trophoblastic basement membrane in an irregular focal or diffuse manner has been described (Galbraith et al., 1980). Other abnormalities noted in the intervillous spaces were mononuclear cells containing pigment, intra-syncytial pigment accumulation and necrosis, loss of microvilli, increased amounts of Clq, C4, C3, C9, fibrinoid fibrinogen and plasmin. These pathological changes of the parasitised placenta may reduce the area of syncytium exposed to maternal blood and thus impair materno-foetal exchanges. Likewise, the considerable abnormality in the intervillous spaces may jeopardise the nutritional function of the placenta, hence the increased frequency of IUGR seen in pregnancy complicated with PM.

The cause of the placental pathology is not clear but it is speculated that direct effects of malaria infection and pigment deposition with its resultant immunological reaction (increased deposition of complement and immunoglobulin) could be responsible for the cytotrophoblastic prominence and fibrinoid necrosis (Bulmer et al., 1993). Suggesting that the parasite causes foetal pathology through other mediators.

Studies have shown that maternal malaria elicits Th1 cytokines, increasing the concentration of IFN- γ , IL-2 and TNF- α in the placenta. In Kenya, Fried et al. (1998) demonstrated that placentae of pregnant women, particularly primigravidae, from a malaria holo-endemic area had higher concentrations of Th1 cytokines and TNF- α , but lower IL-10, than those of gravid women from non-endemic region who were not exposed to malaria infection. These pro-inflammatory cytokines could be embryotoxic or damages the placental trophoblast in both rodents and humans (Krishnan et al., 1996; Raghopathy, 1997).

4.2. Consequences of maternal malaria: immunological basis

The implication of the production of pro-inflammatory cytokines in human placenta in

relation to malarial infection has been the subject of recent studies. The deleterious effects of their increased production on placental trophoblast and consequently on the foetus have been demonstrated in rodents and speculated in human pregnancy (Krishnan et al., 1996; Raghopathy, 1997).

4.2.1. Abortion

In hypo-endemic area, maternal malaria has been shown to be associated with spontaneous abortion (Nair and Nair, 1993). But the direct association between malaria and wastage of human foetus has not been properly studied in malaria-endemic region. However, a higher concentration of IFN- γ , IL-2 and TNF- α , known to cause foetal loss in rodents (Chaouat et al., 1990), have been observed among gravid women with parasitised placenta in Kenya (Fried et al., 1998); and their increase (Th1 cytokine) has also been correlated with recurrent spontaneous abortions in human (Hill et al., 1995). Th1 cytokines, particularly TNF- α and TNF- γ have been shown to be embryotoxic (Hill, 1995), cause necrosis of the implanted embryo and increases the risk of uterine contraction resulting in foetal expulsion (Kwak et al., 1995). Furthermore, IFN- γ could act indirectly via induction or activation of natural killer (NK) cells. In humans, the levels of NK cells in peripheral blood of non-pregnant women who had had repeated spontaneous abortion (RSA) have been found to be significantly raised as compared with normal women (Kwak et al., 1995), suggesting that NK cells could be involved in the premature expulsion of foetuses. However, more human studies that will correlate maternal malaria, cytokine production and abortion are needed.

4.2.2. Stillbirth

Hypotheses advanced for abortion hold for stillbirth. Some of these have been outlined in the Table 1. Furthermore, an inhibitory effect of Th1 cells may be important in both human and animal pregnancy. IFN- γ has been shown to inhibit IL-10 production and the secretion of granulocyte-macrophage colony-stimulating factor (GM-CSF) from uterine epithelium; these

Table 1

Cytokine production and pregnancy outcome: hypotheses of the underlying mechanisms

Pregnancy outcome	Cytokine production	Underlying mechanism	References
<i>Low birth weight</i>			
(1) IUGR	IFN- γ , TNF- α (maternal malaria)	Inhibition of GM-CSF \rightarrow \downarrow growth	Rasheed et al., 1993
(2) Prematurity		Thrombosis of foetal blood supply; uterine contraction. \downarrow Placental nutrition. Maternal anaemia	Richards, 1997
Stillbirth	\uparrow IL2, TNF- α (Rodent leishmaniasis)	IFN- γ \rightarrow \uparrow NK cells. Placental damage. Foetal resorption. Inhibition of GM-CSF	Robertson et al., 1994
Abortion	\uparrow IL2, TNF- α , and IFN- γ (mice and human); \uparrow NK cells	Embryotoxicity, uterine contraction	Chaouat et al., 1990; Hill et al., 1995; Kwak et al., 1995
Maternal anaemia	\uparrow TNF- α (maternal malaria)	? Inhibition of erythropoiesis	Frede et al., 1994; Udupa and Sharma, 1996
Normal pregnancy	Th2 cytokines especially IL10	\downarrow T cell rejection of foetus. Inhibition of INF- γ	Raghupathy, 1997; Wegmann et al., 1993

Cytokine production and possible associated pregnancy outcome. Factors like intracellular infections e.g. PM and/or maternal HIV infection may favour Th1 response in the human placenta. GM-CSF, granulocyte-macrophage colony stimulating factor; IFN- γ , interferon gamma; TNF- α , tumor necrosis factor; NK cell, natural killer cells.

cytokines are known to promote the growth and differentiation of trophoblast. Their inhibition, therefore, may be deleterious to the trophoblast and the foetus (Robertson et al., 1994).

4.2.3. Low birth weight (LBW), intra-uterine growth retardation (IUGR) and prematurity

Malaria-associated LBW has different causes in different epidemiologic settings. In malaria holoendemic areas, an infection early in pregnancy is associated with IUGR whereas it is associated with preterm delivery in late pregnancy (Steketee et al., 1996b). The table below illustrates some of the suggested mechanisms. However, many of these observations and hypotheses are subjects for further research.

Significant elevations of Th1 cytokines in human placenta have been positively correlated with IUGR and prematurity (Rasheed et al., 1993; Richards, 1997). For instance, IFN- γ and TNF- α were significantly higher in primigravidae who delivered LBW babies than in those delivering normal birth weight (NBW) babies in malaria endemic area of Kenya (Fried et al., 1998). This finding was in agreement with the study from Malawi where Moormann et al. (1999) correlated an induction of Th1 cytokines among malaria-infected primigravidae with IUGR.

However, in a separate study from Kenya, Moore et al. (1999) observed that the production of IFN- γ was highest in cells from uninfected placentae of multigravidae; furthermore, there was an impairment of an in-vitro production of these cytokines from intervillous mononuclear cells from PM positive mothers (Moore et al., 1999; Chaisavaneeyakorn et al., 2002), suggesting that the elevation of IFN- γ observed in endemic regions was rather a critical factor (protective) in the immunologic response against malaria infection.

4.2.4. Maternal anaemia

Maternal anaemia is a major consequence of maternal malaria in malaria endemic region (Brabin et al., 1990). Fried et al. (1998) demonstrated a strong relationship in Kenyan primigravidae between severe maternal anaemia and placental TNF- α . Although the mechanism(s) underlying this correlation is not very clear, TNF- α has been known to inhibit erythropoiesis in mice by suppressing erythropoietin-induced erythroid cell formation (Udupa and Sharma, 1996). Given the role of erythropoietin, the suppression of its production from the human trophoblast may lead to maternal anaemia (Conrad et al., 1996; Frede et al., 1994). Indeed, similar elevation of TNF- α in children

with high-density parasitaemia, as compared with control was also observed in malaria endemic areas of Kenya (Othoro et al., 1999).

5. Conclusion and challenges ahead

Although other factors may be important in the epidemiology and consequences of maternal malaria, accumulated data suggest strongly that immunological process play a substantial role. A previously immuned woman becomes more susceptible to malaria infection during pregnancy as Th2-biased changes in the placenta and presence of CSA receptors favour the adhesion and sequestration of IRBC in the placenta of unprotected primigravid women. Anti-adhesion antibodies against CSA-binding parasites are associated with protection from PM, but these antibodies develop over successive pregnancies, accounting for the susceptibility of primigravid women to infection.

There are also speculations that the pro-inflammatory process in the placenta triggered by *P. falciparum* alters the local cellular immune response and cytokine profile and consequently may become deleterious to the survival of the foetus.

What does the future hold for research in this area? Much of these findings should be confirmed in multi-centre studies that may focus on methods of monitoring changes in gravid women with the view to predict outcome and strategise control of maternal infection. Such strategies may include: (1) prediction of pregnancy outcome based on detection of the level of cytokine in the circulation or in the amniotic fluid and possible means of intervention; (2) examination of the association between anti-adhesion antibody and foetal outcome, and ultimately, development of vaccine against the parasitic sub-population infecting human placenta. Until when these new tools are developed, priority areas of malaria control in pregnancy should still involve multidimensional approach involving use of drugs, insecticide impregnated bed nets, intermittent and early treatments of malaria infection in pregnancy, including iron supplementation.

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