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Malaria Elicits Type 1 Cytokines in the Human Placenta: IFN-γ and TNF-α Associated with Pregnancy Outcomes[1]

Michal Fried,[2]* Richard O. Muga,[†] Ambrose O. Misore,[‡] and Patrick E. Duffy[2]*

Pregnant women, especially primigravidas, are highly susceptible to malaria infection, resulting in maternal anemia and low birth weight infants. Because circulating parasitemia is rare in the newborn, the cause of poor fetal outcomes has been unclear. We measured cytokine concentrations in placentas collected from women delivering in urban hospitals in malaria-holoendemic or nonendemic areas of Kenya. Normal placentas displayed a bias toward type 2 cytokines; type 1 cytokines IFN-γ and IL-2 were absent in placentas not exposed to malaria but present in a large proportion of placentas from a holoendemic area. TNF-α and TGF-β concentrations were significantly higher, and IL-10 concentrations significantly lower, in placentas from the holoendemic area. Among primigravidas, placental TNF-α concentrations were significantly higher in the presence of severe maternal anemia, and both IFN-γ and TNF-α were significantly elevated when a low birth weight, rather than normal weight, infant was delivered. We conclude that maternal malaria decreases IL-10 concentrations and elicits IFN-γ, IL-2, and TNF-α in the placenta, shifting the balance toward type 1 cytokines. This is the first demonstration that these placental cytokine changes are associated with poor pregnancy outcomes in humans. ***The Journal of Immunology,*** **1998, 160: 2523–2530.**

Plasmodium falciparum, the most lethal of the malaria species infecting humans, incapacitates and kills millions each year. Because infection occurs in areas where health needs, including surveillance, are unmet, and because the disease has protean presentations, the precise toll inflicted on mankind is difficult to quantify. Among the many syndromes elicited by the protozoan, maternal malaria may cause the greatest number of unrecognized deaths, in the forms of fetal loss and mortality early in life to low birth weight (LBW)[3] babies.

Pregnant women are at increased risk of malaria infection because the parasite is able to adhere to the trophoblastic villous epithelium (1) and sequester in the placenta (2, 3). Sequestration in the placenta relies on parasite adhesion to chondroitin sulfate A (CSA) (1), a molecule that may not be accessible to the parasite in other tissue beds (4-6), and parasites that bind CSA do not commonly infect a host who is not pregnant (1, 7). Therefore, primigravidas have had little or no immunologic experience with the CSA-binding parasite and are most susceptible to infection; protection develops over successive pregnancies (2, 3). While *P. falciparum* commonly causes anemia in the mother (8, 9), the frequency of other complications depends on a woman's pre-exisiting immunity to malaria. Women in high transmission (and therefore high immunity) areas are typically asymptomatic (2); among women with incomplete immunity, severe syndromes, such as cerebral malaria and pulmonary edema, frequently occur (10–12).

Maternal malaria is associated with poor fetal outcomes. Stillbirths, abortions, and LBW result from infection in areas of low transmission (2, 13), and in holoendemic (high transmission) areas, malaria contributes substantially to the high incidence of LBW babies born to primigravidas (2, 3, 14). LBW infants are at higher risk to die early in life (15); because the immediate cause of these deaths is often not malaria, the overall impact of maternal malaria is underestimated. Although the relationship between maternal malaria and poor fetal outcomes is recognized, the basis for the association is unclear. *P. falciparum* can be found in the cord blood of up to 16% of infants born to infected mothers (16–18) but rarely establishes a circulating congenital infection (16, 19), suggesting that the parasite elicits fetal pathology through other mediators.

Pregnancy is an event of immunologic tolerance, whereby a woman accepts the implantation of the fetal allograft in her uterus (20). Murine studies suggest a bias toward type 2 responses and away from type 1 responses during successful pregnancy (20–22); studies in humans have not been adequate to resolve this issue (21, 22). Type 2 cytokines feature prominently in the mouse placenta (20, 23, 24), and type 2 cytokines (25, 26) and TGF-β (26) appear in the human placenta, possibly to promote implantation (26) and inhibit inflammatory responses (20, 26). Infections thought to require a type 1 response for protection, such as tuberculosis, listeriosis, toxoplasmosis, malaria, and leishmaniasis, are more severe during human pregnancy (27); similarly, pregnancy compromises protective type 1 responses known to confer resistance to leishmaniasis in mice (28).

In rodent models of pregnancy, interventions that increase concentrations of type 1 cytokines and TNF-α induce stillbirths, abortions, and maternal anemia (29, 30). In humans, TNF-α is frequently increased during severe malaria syndromes other than maternal malaria, and elevated concentrations of this inflammatory mediator are associated with mortality (31). No data exist regarding the cytokine changes that result from placental malaria in humans or the effect that these may have on fetus and mother.

In this study, we examined cytokine concentrations in placentas collected from Kenyan parturients presenting for delivery at urban

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[3] Abbreviations used in this paper: CSA, chondroitin sulfate A; LBW, low birth weight; NBW, normal birth weight; LPW, low placental weight; NPW, normal placental weight; hgb, hemoglobin.

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hospitals. We show that type 1 cytokines are absent and type 2 cytokines are present in normal placentas at parturition. Exposure to malaria elicits TNF-α, IFN-γ, and IL-2 in the placenta, and these cytokine changes are associated with poor pregnancy outcomes among primigravidas.

Materials and Methods

Subjects

Nine hundred thirty volunteers were recruited at New Nyanza Provincial General Hospital, Kisumu, Kenya, between April 1995 and August 1996, and forty-nine volunteers at Kenyatta National Hospital, Nairobi, Kenya, during November 1996. All parturients 18 years and older were asked to participate in the study and gave signed informed consent after receiving a study explanation form and oral explanation from a nurse in their native language. Women involved in other research studies and women delivering in Nairobi who had traveled to endemic areas during pregnancy were excluded. This study was approved by the ethical committees of Walter Reed Army Institute of Research, Kenya Medical Research Institute and Kenyatta National Hospital, Nairobi.

Clinical data

Infants and placentas were weighed immediately after delivery. A medical history of the pregnancy was obtained from parturients using a standard questionnaire. Peripheral blood samples were collected by venipuncture from women after delivery. Cord blood samples were obtained by manual compression of the umbilical cord. After removal of the umbilical cord and fetal membranes, placental blood samples were obtained by compressing fresh tissue in a tissue grinder. Hemoglobin (hgb) concentrations were determined on a Coulter cell counter, model T-890 (Coulter Corp., Hialeah, FL); samples were categorized as severely anemic for hgb <7 g/100 ml, mildly anemic for hgb 7 to 10 g/100 ml, and normal for hgb >10 g/100 ml. Peripheral and placental parasitemias at the time of delivery were determined by microscopic examination of Giemsa-stained blood smears. Placental blood smears were examined for the presence of either pigment within macrophages (as a sign of parasite death) or parasites, or both, to define the stage of infection: no infection (no parasites, no pigment); early infection (parasites, no pigment); late infection (parasites and pigment); resolved infection (pigment, no parasites).

Cytokine assays

Serum cytokine concentrations were measured by the sandwich ELISA method (32). The following Abs were used: IFN-γ, mAb 1-D1K and mAb 7-b6-1-biotin (MABTECH, Nacka, Sweden); TNF-α, mAb, and polyclonal Ab (Genzyme, Cambridge, MA); IL-2, IL-4, and IL-6, DuoSet kits (Genzyme); IL-10, mAb JES3–9D7, and mAb JES-12G8-biotin (PharMingen, San Diego, CA); TGF-β1, ELISA system (Promega, Madison, WI). Detection limits for cytokines were: IFN-γ, TNF-α, and IL-10, 10 pg/ml; IL-2, IL-4, and IL-6, 50 pg/ml; TGF-β1, 30 pg/ml.

Assays were performed on all samples collected in Nairobi ($n = 49$), and on a representative number of randomly chosen samples collected in Kisumu.

Statistical analyses

Differences between groups were analyzed by nonparametric methods (Mann-Whitney or Kruskal-Wallis). Proportions were compared by contingency table analysis. Correlations were examined by Spearman rank test. Tied p values are given. The significance limit was chosen at $p = 0.05$.

Results

Placental cytokine concentrations differ between a nonmalarious area and a malaria-holoendemic area

Type 2 cytokines predominate in a normal mouse placenta (20, 23, 24); data concerning the cytokine bias in human placentas are incomplete (21, 22). To define the effect of malaria, we compared placental concentrations of TNF-α, IFN-γ, IL-2, IL-4, IL-6, IL-10, and TGF-β1 among exposed women with concentrations among unexposed women. Only aparasitemic women were included in this analysis. "Exposed" women were recruited in Kisumu, Kenya, a malaria-holoendemic area where the incidence of infection among male residents can exceed 90% over 4 mo (P. E. Duffy, unpublished data). "Unexposed" women were recruited in Nairobi, where transmission is sporadic.

Table I. *Proportions of women with detectable cytokine concentrations in placental sera at delivery*

Cytokine	Exposed (%)	Unexposed (%)	p
TGF-β1	84.3	77.5	0.25
IL-4	16.7	14.3	0.67
IL-6	42	44.8	0.72
IL-10	29.4	53	0.001
IL-2	38.3	0	<0.001
IFN-γ	42	0	<0.0001
TNF-α	37	22.4	0.046

The proportions of samples with detectable TGF-β1, IL-4, and IL-6 were similar in the two groups (Table I). While placental concentrations of IL-4 and IL-6 were also similar among the groups (Fig. 1, *B* and *C*), the placental concentration of TGF-β1 was significantly lower in unexposed women (Fig. 1*A*). IL-10 was detected more frequently (Table I) and was measured at a significantly higher concentration (Fig. 1*D*) in placentas from unexposed women.

Strikingly, IL-2 and IFN-γ were not detected in any samples from unexposed women but were detected in 38.3 and 42%, respectively, of samples from exposed, uninfected women (Table I; Fig. 2, *A* and *B*), both highly significant differences. TNF-α was also detected more frequently (Table I) and at a significantly higher concentration (Fig. 2*C*) in placental samples obtained from these exposed women.

Placental cytokine concentrations differ based on gravidity and infection

Systemic malaria infection elicits an array of immune responses (33). Exposure to malaria during pregnancy elicits type 1 cytokines in the placenta, altering the cytokine balance observed in unexposed women (Figs. 1 and 2), and we questioned whether active infection predictably altered the cytokine profile. We stratified women in a holoendemic area (Kisumu, Kenya) by the presence or absence of parasites in their placentas at the time of delivery. Type 2 cytokines (IL-4, IL-6, and IL-10) in the placenta did not significantly differ on the basis of infection or parity (Fig. 3). Among multigravidas, but not other women, placental TGF-β1 concentrations were significantly higher during infection (Fig. 3*A*).

Our interest in placental cytokines arose, in part, from earlier reports that pregnancy may impair cellular immune responses against the parasite (34); others have cited this impairment as the basis for the susceptibility of pregnant women, particularly primigravidas, to malaria (13). TNF-α, IFN-γ, and IL-2 are soluble mediators of the cellular response, and we analyzed placental concentrations of these cytokines with regard to infection and gravidity. TNF-α concentrations did not differ between gravid groups but did vary within groups on the basis of infection. Among primigravid and multigravid women, TNF-α concentrations were significantly higher when the placenta was parasitized (Fig. 4*A*); the elevation of TNF-α was not significant among secundigravid women. Like TNF-α, IL-2 concentrations did not differ among gravid groups. IL-2 concentrations were higher during infection in all groups, although these elevations (compared with uninfected samples) were not significant (Fig. 4*B*).

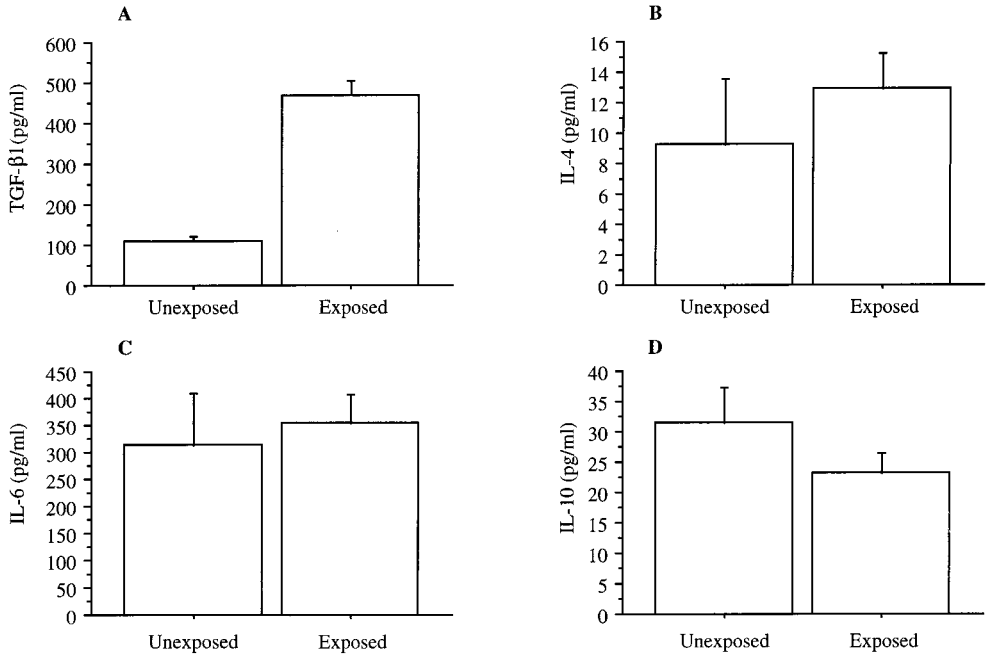

FIGURE 1. Comparison of placental type 2 cytokine concentrations between unexposed and exposed, uninfected women. Mean concentrations and SEs for cytokines are shown. Numbers that follow in parentheses indicate number of samples from unexposed women and number of samples from exposed, uninfected women. *A*, TGF-β1 ($n = 49, 242$), $p < 0.0001$; *B*, IL-4 ($n = 49, 287$), $p = 0.7$; *C*, IL-6 ($n = 49, 142$), $p = 0.8$; *D*, IL-10 ($n = 49, 248$), $p = 0.0018$.

Overall, placental concentrations of IFN-γ were significantly higher in primigravidas than other groups ($p = 0.01$, Kruskal-Wallis). Within gravid groups, multigravid women had significantly elevated concentrations of IFN-γ in association with infection while primigravid women had decreased concentrations (Fig. 4*C*). We defined the stage of infection at the time of delivery (no infection, early infection, late infection, or resolved infection) and compared IFN-γ concentrations between different stages (Fig. 4*D*). In multigravidas, IFN-γ concentrations were significantly elevated during early infections compared with other stages of infection; among primigravidas, the elevation of IFN-γ did not differ significantly between stages. The significant elevation of IFN-γ in uninfected placentas of primigravidas ($p = 0.02$, compared with similar placentas from other gravid groups) may indicate a sustained pattern of cytokine secretion after infection or may indicate the cumulative effect of more frequent (2, 3) or more chronic infections.

IFN-γ and TNF-α are associated with poor pregnancy outcomes among primigravidas

Placental concentrations of IFN-γ and TNF-α were significantly higher in primigravidas delivering LBW babies than in those delivering normal birth weight (NBW) babies (Fig. 5, *A* and *B*). The elevation of IFN-γ among primigravidas in the absence of infection (Fig. 4*D*) was specifically associated with LBW babies: among uninfected primigravidas, IFN-γ concentrations were significantly higher with the birth of a LBW rather than a NBW infant ($p = 0.0075$, Mann-Whitney), a difference not seen among infected primigravidas or other groups. Because small placentas are associated with LBW babies and often reflect a general growth disturbance, we also examined the relationship between cytokine concentrations and placental weight. Among primigravidas, but not other women, deliveries characterized by low placental weight (LPW), compared with normal placental weight (NPW), had significantly higher concentrations of IFN-γ and TNF-α (Fig. 5, *C* and *D*).

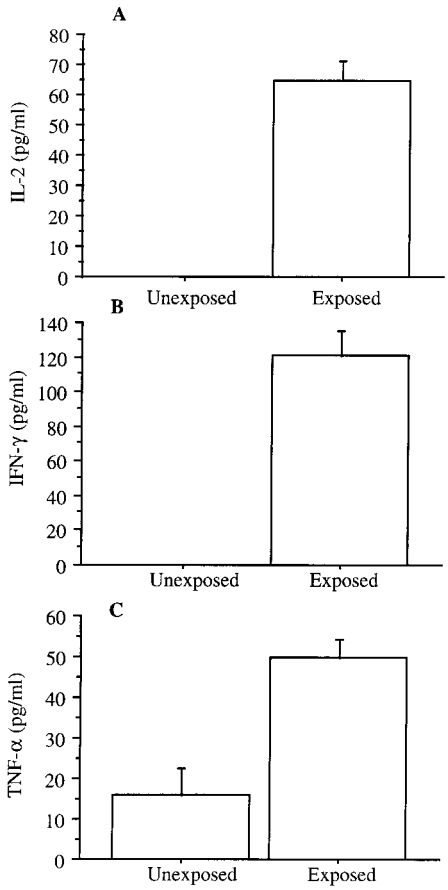

FIGURE 2. Comparison of placental type 1 cytokine concentrations between unexposed and exposed, uninfected women. Mean concentrations and SEs are shown. Numbers that follow in parentheses indicate number of samples from unexposed women and number of samples from exposed, uninfected women. *A*, IL-2 ($n = 49, 308$), $p < 0.0001$; *B*, IFN-γ ($n = 49, 294$), $p < 0.0001$; *C*, TNF-α ($n = 49, 322$), $p = 0.028$.

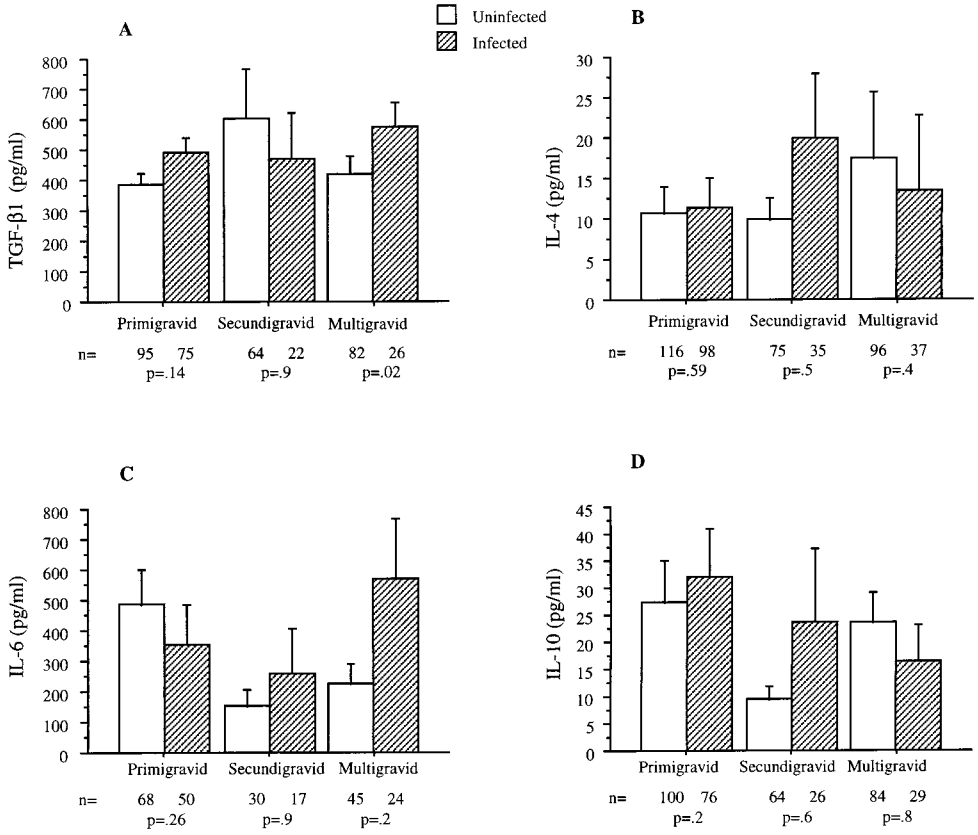

FIGURE 3. Comparison of placental type 2 cytokine concentrations between uninfected and infected placentas of exposed women. Mean concentrations and SEs are shown. *A*, TGF-β1; *B*, IL-4; *C*, IL-6; *D*, IL-10.

In holoendemic areas, women with maternal malaria suffer anemia as the most common complication, and anemia in the mother has been associated with LBW deliveries (35). Among primigravidas, but not other groups, significantly higher placental TNF-α concentrations (Fig. 6) and significantly smaller infants ($p = 0.01$, Mann-Whitney, severe anemia vs no anemia) occurred in association with maternal anemia. Because TNF-α and anemia might both contribute to LBW, we stratified the data from primigravidas: anemia was associated with significantly smaller babies when TNF-α was detected in the placenta ($p < 0.05$, Kruskal-Wallis), but not if TNF-α was not detected; conversely, detection of TNF-α in the placenta was associated with significantly smaller babies in anemic ($p < 0.05$, Mann-Whitney) but not in normal women. In sum, TNF-α concentrations are related to anemia in primigravid women, and both appear to contribute to the development of LBW infants.

Placental blood samples were collected by compressing tissue in a grinder, and they therefore contained a fraction of fetal blood. We examined the contribution of cord cytokine concentrations to our analyses (data not shown). The correlation between cytokine concentrations in the cord and those in the placenta was poor to moderate (TNF-α, $\rho = 0.096$; IFN-α, $\rho = 0.49$; IL-4, $\rho = 0.18$), suggesting a marginal effect of fetal blood on overall cytokine measurements. Further, cord cytokine concentrations had no significant association with pregnancy outcomes. Therefore, the presence of fetal blood would tend to reduce the significance of associations between placental cytokine concentrations and outcomes, and our analyses may understate the relationship between placental cytokine concentrations in maternal blood and pregnancy outcomes.

Peripheral cytokine concentrations do not predict pregnancy outcomes

Peripheral and placental concentrations of cytokines correlated to varying degrees (TNF-α, $\rho = 0.081$, $n = 285$; IFN-γ, $\rho = 0.5$, $n = 173$; IL-4, $\rho = 0.3$, $n = 143$: Spearman rank correlation). Frequently, TNF-α was detected in the placenta but not in the peripheral circulation; less commonly, TNF-α was detected in the periphery but not in the placenta (Fig. 7*A*). IFN-γ and IL-4 followed patterns similar to that of TNF-α (data not shown). Peripheral TNF-α concentrations were significantly higher among multigravid women than among other women ($p = 0.03$, Kruskal Wallis) but did not significantly differ on the basis of infection (Fig. 7*B*). Peripheral concentrations of IFN-γ (data not shown) or TNF-α (Fig. 7*C*) were not elevated in association with LBW infants. The relationship between peripheral TNF-α and anemia was more complicated (Fig. 7*D*): primigravidas with mild anemia had significantly decreased concentrations; secundigravidas with mild anemia had significantly increased concentrations; concentrations did not differ between groups with severe anemia and normal hgb counts. Thus, despite the strong relationship between placental cytokines and pregnancy outcomes, peripheral cytokine concentrations were not associated with either LBW or severe anemia among primigravidas.

Discussion

Maternal malaria occurs with great frequency in endemic areas, and it threatens the life of both mother and child. In areas of high transmission, maternal malaria most commonly manifests as anemia in the mother and LBW in the infant, with subsequent risk for

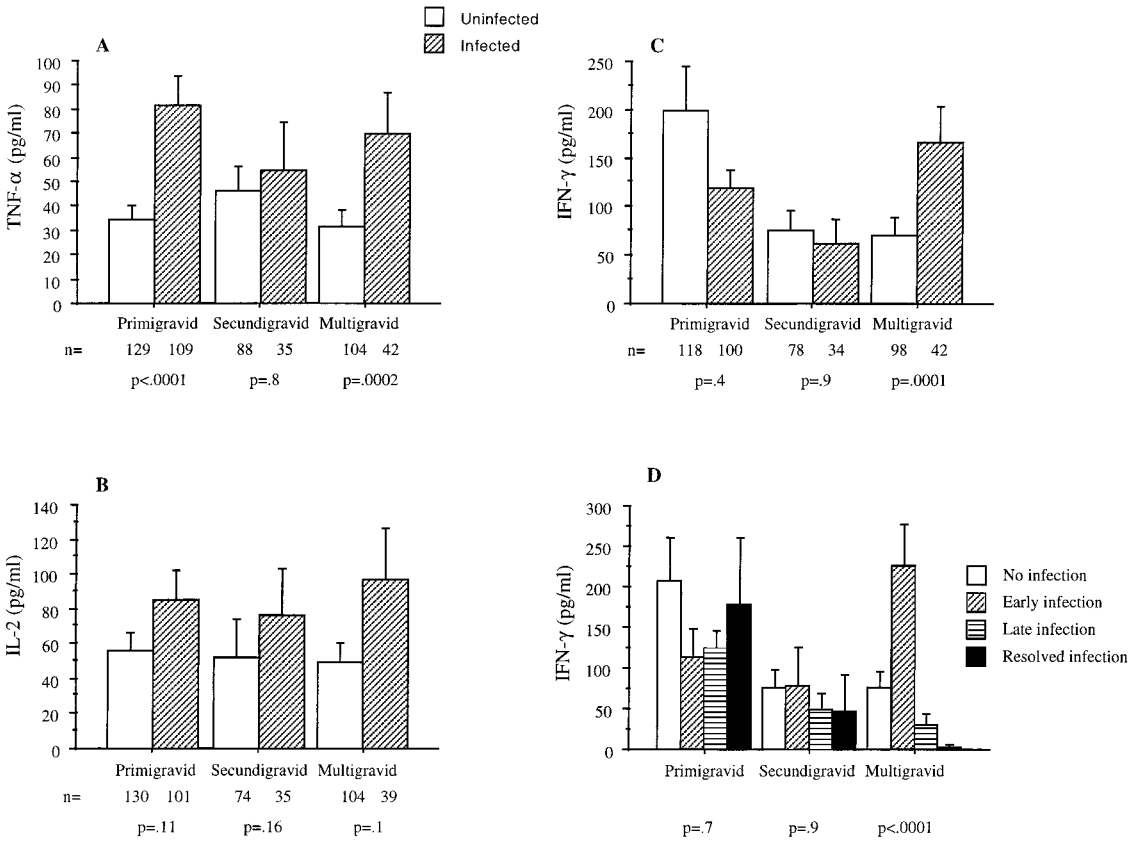

FIGURE 4. Comparison of placental type 1 cytokine concentrations between uninfected and infected women exposed to malaria. *A*, TNF-α; *B*, IL-2; *C*, IFN-γ; *D*, placental IFN-γ concentrations segregated by four stages of infection. Numbers that follow indicate the number of samples, respectively, with no infection, early infection, late infection, or resolved infection in each gravid group: primigravidas (n = 96, 30, 70, 19); secundigravidas (n = 71, 17, 16, 6); multigravidas (n = 90, 29, 13, 4).

morbidity and mortality. The genesis of either complication has been unclear; *P. falciparum* rarely establishes circulating congenital parasitemia, suggesting that the parasite disrupts fetal development through other mediators.

In this study, exposure to malaria altered the balance of placental cytokines. In placentas from Nairobi, type 2 cytokines (IL-4, IL-6, and IL-10) predominated, and type 1 cytokines (IFN-γ and IL-2) were absent. These data support the idea that successful pregnancy in humans is accompanied by a bias away from type 1 cytokines and toward type 2 cytokines (27). Samples from malaria-exposed women were markedly different: pro-inflammatory cytokines like TNF-α, IFN-γ, and IL-2 appeared in abundance; IL-10 concentrations were decreased (possibly down-regulated by type 1 cytokines); and TGF-β1 concentrations were increased. In studies of mice, IL-10 and the inflammatory cytokines play counter-regulatory roles in the placenta (23, 36), a finding consistent with our observations. Conversely, placental concentrations of the anti-inflammatory cytokine TGF-β1 were higher among women exposed to malaria in this study. The uterine epithelium and placental trophoblasts are known to be sources of TGF-β during pregnancy (26). Increases in TGF-β may have occurred in response to the appearance of inflammatory cytokines, perhaps to ameliorate immune-mediated damage to the fetoplacental unit.

TNF-α is believed to have a role in parturition (37), which may explain our detection of this cytokine in some placentas donated by unexposed women. However, exposure to malaria was associated with significant elevations of placental TNF-α (Fig. 2*C*). Further, type 1 cytokines (IFN-γ and IL-2) appeared only in women exposed to malaria. TNF-α and IL-2 concentrations were always higher during infection (Fig. 4, *A* and *B*), but the pattern of IFN-γ secretion was not similarly uniform (Fig. 4, *C* and *D*): compared with other groups, primigravidas had elevated concentrations of IFN-γ at all stages of infection; among multigravidas, IFN-γ was elevated during early infection, but not at other stages of infection.

Primigravid women are at particular risk to deliver LBW babies, and maternal anemia, most commonly seen in primigravidas, increases that risk (35). In Kisumu, primigravid women delivered significantly smaller babies than other women did ($p < 0.05$, data not shown), and maternal age did not account for these differences. Elevations of TNF-α and IFN-γ were both significantly associated with LBW and LPW in primigravid women. The association of elevated IFN-γ and LBW was strongest in aparasitemic primigravidas, suggesting that the sustained elevation of IFN-γ after resolution of infection may be particularly harmful. Rodent models of pregnancy provide findings consistent with our own: administration of IFN-γ and TNF-α (29, 30, 38), or increases in these cytokines that occur during infection of C57BL/6 mice with *Leishmania major* (39), are associated with impaired development and fetal loss. In particular, the sustained administration of low doses of IFN-γ to pregnant rodents, which may approximate the pattern of IFN-γ secretion in malaria-exposed primigravidas (Fig. 4*D*), increases fetal abortion and decreases fetal weight (30). Malaria, like other infections that activate macrophages, potentiates the abortifacient effects of TNF-α in mice (40).

For the mother, anemia is the commonest consequence of maternal malaria. Anemia frequently results from malaria infection in other populations and is an important cause of death among small children in holoendemic areas (41, 42). Among

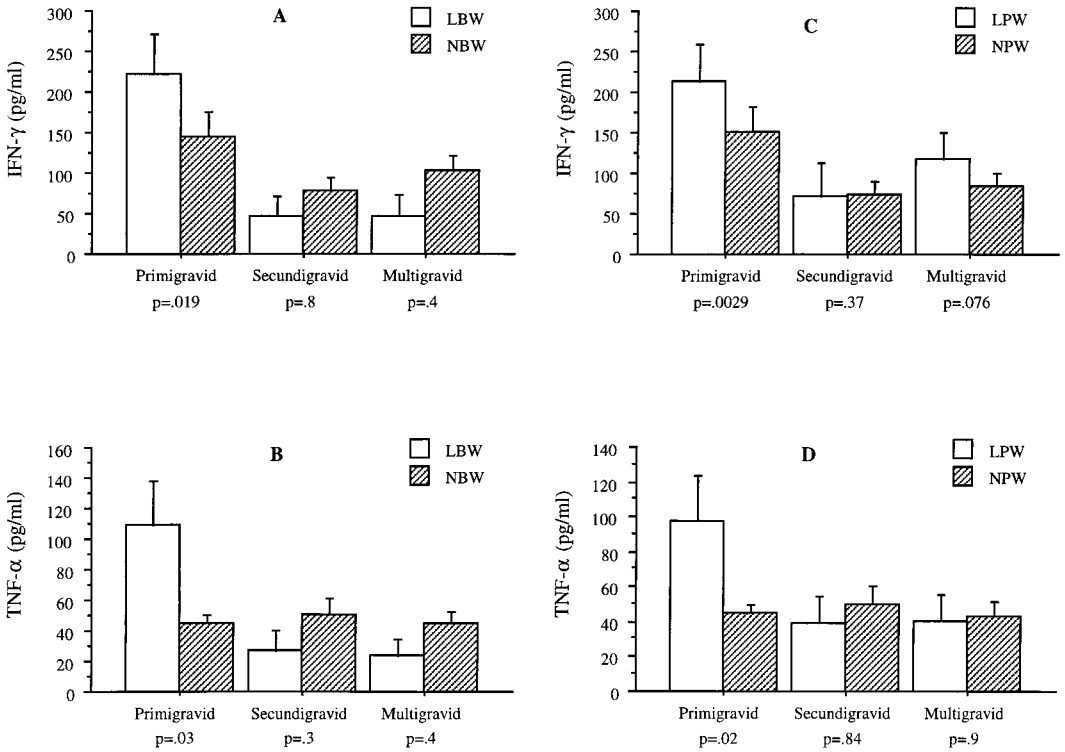

FIGURE 5. Comparison of placental cytokine concentrations between groups stratified by pregnancy outcomes. *A*, *B*, Comparisons between groups stratified by infant birth weight. Numbers that follow in parentheses indicate number of samples from LBW deliveries and number of samples from NBW deliveries. *A*, IFN-γ: primigravidas (n = 33, 177); secundigravidas (n = 10, 98); multigravidas (n = 15, 124). *B*, TNF-α: primigravidas (n = 34, 191); secundigravidas (n = 11, 108); multigravidas (n = 14, 133). *C*, *D*, Comparisons between groups stratified by placental weight. LPW defined as <320 g (320 g = 1 SD below mean placental weight among deliveries of primigravidas in Kisumu). Numbers that follow in parentheses indicate number of samples from LPW deliveries and number of samples from NPW deliveries. *C*, IFN-γ: primigravidas (n = 34, 174); secundigravidas (n = 12, 195); multigravidas (n = 20, 113). *D*, TNF-α: primigravidas (n = 39, 184); secundigravidas (n = 13, 104); multigravidas (n = 21, 119). In regression analysis, the age of the mother had a negligible effect as an explicative variable to predict birth weight or placental weight (coefficient = −0.002, p = 0.68 and coefficient = −0.0002, p = 0.82, respectively).

subjects in this study, placental TNF-α concentrations were significantly higher among primigravidas with severe anemia compared with other primigravidas (Fig. 6). Anemia places the mother at risk for mortality (9) and is an independent risk factor for poor fetal outcomes (9, 35, 43).

The shift to a type 1 immune response and elevation of TNF-α were associated with adverse pregnancy outcomes among our primigravid subjects. Elevated serum TNF-α has been associated with severe nonmaternal malaria (31), as have specific alleles of the TNF-α promoter region (44). While these earlier findings suggested that TNF-α up-regulation of downstream immune mediators, like nitric oxide, might contribute to syndromes such as cerebral malaria (45), subsequent work has not consistently supported this hypothesis (46). In rodent models of malaria, TNF-α can be associated with survival or death, depending on which tissues express the cytokine, and the point in the course of infection when concentrations peak (47).

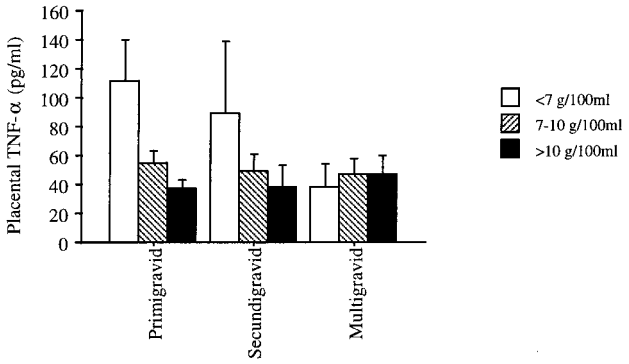

FIGURE 6. Comparison of placental TNF-α concentrations between groups stratified by peripheral hgb concentration at delivery. Numbers that follow in parentheses indicate number of samples from women with severe anemia, number from women with mild anemia, and number from women with normal hgb. Primigravidas (n = 33, 110, 72), p = 0.03; secundigravidas (n = 14, 51, 42), p = 0.6; multigravidas (n = 19, 65, 49), p = 0.8.

Human studies generally measure peripheral concentrations of soluble mediators, which may not correlate with concentrations in inflamed tissues. Here, peripheral concentrations of TNF-α, IFN-γ, and IL-4 correlated with placental concentrations to varying degrees, from minimal to moderate. The correlations between peripheral and placental concentrations suggest that immune responses occurring in the placenta can influence the systemic profile of cytokines, but this influence can vary according to the cytokine of interest. We were unable to identify significant associations between peripheral cytokine concentrations and clinical outcomes. Perhaps because cytokine effects are local, our measurements within the placenta were best able to describe the relationship between the immune response and fetal outcomes. The strong relationship in primigravidas between severe maternal anemia and placental TNF-α, but not peripheral TNF-α, was unexpected. TNF-α is known to inhibit erythropoiesis in mice, in part by suppressing erythropoietin-induced erythroid cell formation (48), and the human trophoblast was recently identified as an extrarenal site

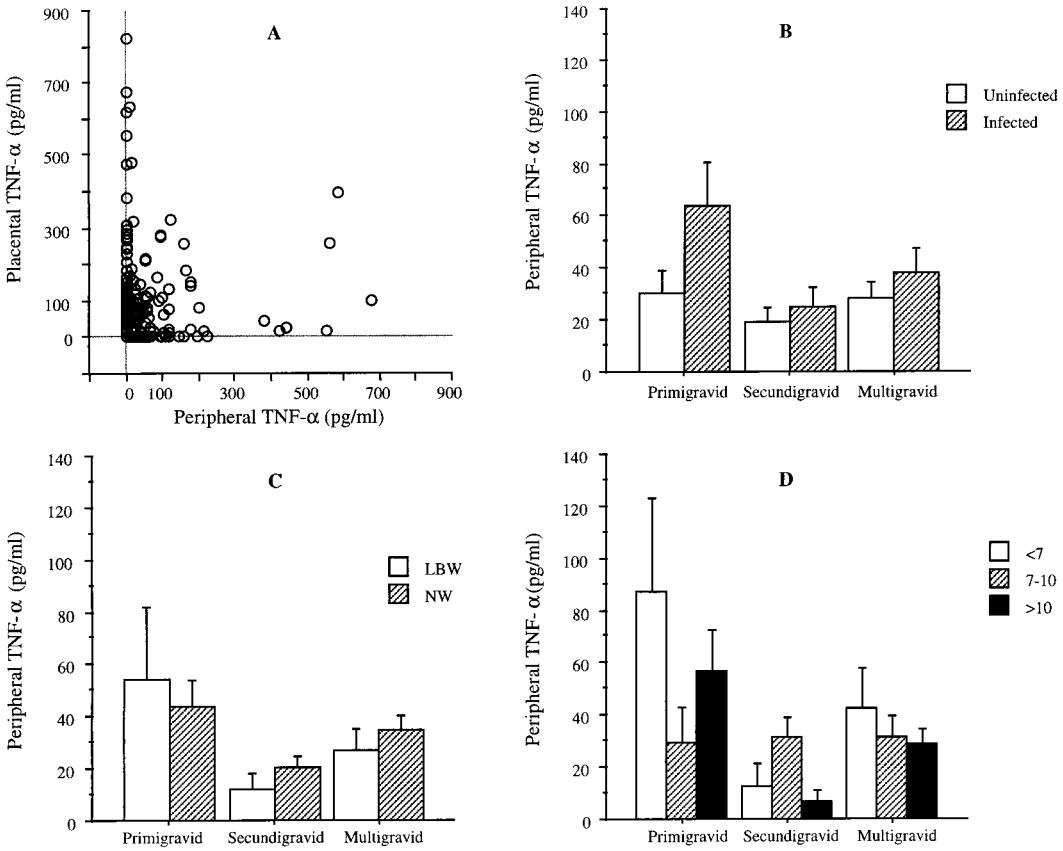

FIGURE 7. Peripheral TNF-α and pregnancy outcome. *A*, Scattergram of placental and peripheral concentrations of TNF-α. $\rho = 0.081$, $n = 285$. *B*, Concentrations of peripheral TNF-α stratified by infection status and gravidity. Numbers that follow in parentheses indicate number of samples from uninfected placentas and number of samples from infected placentas: primigravid ($n = 63, 74$), $p = 0.38$; secundigravid ($n = 57, 25$), $p = 0.217$; multigravid ($n = 47, 25$), $p = 0.36$. *C*, Comparisons of peripheral TNF-α between groups stratified by infant birth weight. Numbers that follow in parentheses indicate number of samples from LBW deliveries and number of samples from NBW deliveries: primigravid ($n = 22, 111$), $p = 0.57$; secundigravid ($n = 8, 70$), $p = 0.94$; multigravid ($n = 9, 59$), $p = 0.85$. *D*, Comparison of peripheral TNF-α concentrations between groups stratified by peripheral hgb concentration and gravidity. Numbers that follow in parentheses indicate number of samples from women with severe anemia, mild anemia, and normal hgb: primigravid ($n = 21, 61, 50$), $p = 0.02$; secundigravid ($n = 10, 42, 23$), $p = 0.03$; multigravid ($n = 13, 34, 23$), $p = 0.61$.

for the expression of erythropoietin (49). Although the role of placental erythropoietin in erythropoiesis remains undefined, its production may be suppressed by placental TNF-α (50), with maternal anemia as a potential consequence. Alternatively, maternal anemia and placental TNF-α may both be related to another variable, such as the frequency or chronicity of infection, but are not causally related to each other.

Parasites infecting the placenta have a unique adhesive phenotype (1). Women may have limited immunologic experience with this subpopulation until their first pregnancy, making primigravidas relatively naive, and therefore more susceptible to malaria. In a holoendemic area, malaria exposure altered the placental cytokine profile, shifting the balance in favor of a type 1 environment. Although *P. falciparum* may initiate the events leading to disease and death in the mother and her newborn, other mediators, such as placental cytokines, may play a critical role in pathophysiology.

In this study, all parities produced TNF-α and type 1 cytokines in response to malaria. In primigravidas, elevations of placental IFN-γ at all stages of infection, in concert with raised concentrations of TNF-α and possibly IL-2, are associated with poor outcomes for both mother and child. The distinct pattern of cytokines observed in primigravidas may be the result of more frequent parasitemia or could represent cellular responses occurring in the absence of established acquired immunity. In either case, a vaccine against the parasite subpopulation infecting the human placenta could offer nulligravid women the immunologic protection enjoyed by multigravid women, averting the cytokine responses associated with poor outcomes during first pregnancy.

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