Antibodies That Inhibit *Plasmodium falciparum* Adhesion to Chondroitin Sulfate A Are Associated with Increased Birth Weight and the Gestational Age of Newborns

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Children born to mothers with pregnancy malaria commonly suffer low birth weight (LBW), and *Plasmodium falciparum*-induced LBW kills 62,000 to 363,000 neonates in sub-Saharan Africa each year (4, 5). Women develop increasing resistance to pregnancy malaria over successive pregnancies (12). This pattern of parity-specific resistance has been related to the acquisition of antibodies that inhibit adhesion of *P. falciparum*-infected erythrocytes (IEs) to the placental receptor, chondroitin sulfate A (CSA) (7).

These antiadhesion antibodies are related to a reduced risk of placental parasitemia (7). The antigens targeted by the antiadhesion antibodies have conserved features (7, 13), suggesting that a pregnancy malaria vaccine comprising a limited number of antigens may be globally effective.

No previous studies have related a specific antimalarial immune response to an improvement in pregnancy outcomes. We collected plasma samples from parturients in an area of high malaria transmission in western Kenya and assayed them for their ability to inhibit placental parasite adhesion to CSA. We then examined relationships between plasma antiadhesion activity and newborn birth weight, gestational age, or maternal hemoglobin levels.

All parturients at the New Nyanza General Provincial Hospital in Kisumu, Kenya, 18 years of age 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. This study was approved by the ethical committees of the Walter Reed Army Institute of Research and Kenya Medical Research Institute. Infants were weighed immediately after delivery, and gestational age was estimated according to the modified Dubowitz examination. Placental blood samples were obtained by compressing fresh tissue in a tissue grinder. Hemoglobin levels were measured by Coulter model T-890. Placental parasite densities (percent IEs) were determined by microscopic examination of Giemsa-stained blood smears.

Representative plasma samples were randomly selected from among the samples donated by women of different parities and from infected and uninfected women. Plasma samples were tested for their ability to inhibit adhesion of a median of two placental parasite isolates (mean, 2.8; range, 1 to 9 isolates). Plasma samples collected early in the series and those with common ABO type (plasma samples were assayed against ABO-matched IEs) were assayed more frequently than plasma collected later in the series or those with uncommon ABO type. Individual plasma samples gave highly similar results against different parasite isolates (7).

The antiadhesion antibody assay was described previously (7). Thirty-microliter volumes of CSA (Sigma, St. Louis, Mo.) at 10 μg/ml in phosphate-buffered saline (PBS) were adsorbed onto petri dishes (8). Wells were blocked with bovine serum albumin (Sigma) at 2 mg/ml in PBS. The parasites used in the binding assay were freshly collected isolates from infected placentas in Kisumu, Kenya. Placental IE suspensions (5 to 20% parasitemia, 5% hematocrit) in RPMI medium (GIBCO, Grand Island, N.Y.) were preincubated for 1 h at 37°C with placental plasma (diluted 1:5) and then allowed to bind to the immobilized CSA for 30 min. After three gentle washes with PBS, bound cells were fixed in 0.5% glutaraldehyde (Sigma) in PBS and stained with 1% modified Giemsa (Sigma) in tap water.

Adhesion was quantified as the number of parasites bound per 20 high-power fields. Antiadhesion activity was measured as the percentage of binding of the control, where control binding is binding that occurred in pooled AB plasma from the United States. Plasma that reduced parasite binding to a level below 35% of control binding was considered to have antiadhesion activity. The level of 35% is one standard deviation (14%) above the mean level of binding (21%) in the presence of plasma from multigravid women (n = 53) in western Kenya.
and has been used previously to define protective levels of antiadhesion activity (7).

Differences between groups were analyzed by nonparametric methods (Mann-Whitney or Kruskal-Wallis tests). Correlations were examined by Spearman rank test. Analysis of variance (ANOVA) was used to study the effects of antibody levels and other variables on pregnancy outcomes. The significance limit was chosen at a P of 0.05, and tied P values are given. Statistical analyses were performed with Statview version 5.0.1 (SAS Institute, Cary, N.C.) on a Macintosh computer.

Among 1,485 mothers of singleton, live, vaginally delivered infants who participated, maternal age, maternal hemoglobin, infant birth weight, and gestational age were fully documented in 1,329 mothers, and these constitute the whole cohort for our analysis. Demographic characteristics were similar among the whole cohort compared to the subset of women whose plasma was selected for assays.

Within gravid groups, women without antiadhesion activity did not differ significantly in age from women with antiadhesion activity (P > 0.2 for all comparisons, Mann-Whitney test). Only 1 of 47 plasma samples from primigravid women reduced binding below 35% of the control level, compared to 47 of 68 samples from secundigravid women and 48 of 55 samples from multigravid women (Fig. 1). Increasing levels of antiadhesion activity correlated significantly with decreasing placental parasite density in both primigravid (ρ = 0.317; P = 0.03) and secundigravid (ρ = 0.469; P = 0.0001) women. Among multigravid women, no relationship was observed between levels of antiadhesion activity and parasite density in the placenta—likely because antiadhesion activity was fairly uniform among multigravid women. Parasite density of infected placentas was significantly lower in multigravid women than in primigravid women (mean percentages of IEs, 0.5% versus 6.1%, respectively; P = 0.03).

Among secundigravid women, antiadhesion activity in the plasma correlated with increasing birth weight in the newborn (Fig. 2; p = −0.239, P = 0.050), and women with antiadhesion antibodies had significantly heavier babies than did women without these antibodies (P = 0.019, Mann-Whitney test). By ANOVA, antiadhesion antibody levels in secundigravidas retained a significant influence on birth weight (P = 0.05) when placental parasitemia was included as a covariate. Women without antiadhesion activity delivered babies that were, on average, 398 g smaller than infants of women with antiadhesion activity in their plasma: birth weight (mean ± standard error) 2.907 ± 0.145 versus 3.305 ± 0.059 kg.

Secundigravid women with antiadhesion activity (Fig. 3A) delivered infants with significantly higher gestational ages (P = 0.002, Mann-Whitney test) compared to women without activity (Fig. 3B). By ANOVA, antiadhesion activity in secundigravid plasma retained a significant influence on gestational age (P = 0.04) when placental parasite density was included as a covariate. Secundigravid women without antiadhesion activity delivered babies that were, on average, 2 weeks more premature than did women with antibodies: gestational age (mean ± standard error), 35.14 ± 0.65 versus 37.09 ± 0.31 weeks.

Antiadhesion activity and maternal hemoglobin levels were not related within the gravid groups. In the subset of women studied for plasma antiadhesion activity, differences in hemoglobin level in women with or without placental malaria did not achieve significance (P = 0.29).

Our principal finding is that antiadhesion antibodies in maternal plasma are associated with significantly increased birth weights and gestational ages in neonates from western Kenya, where malaria transmission is intense. LBW is the strongest risk factor for mortality during infancy (10). Reducing the incidence of LBW deliveries should reduce infant mortality in sub-Saharan Africa, which has ranged between 133 and 176 deaths per 1,000 live births during recent studies in areas of high transmission (3, 11, 14).

Secundigravid women with antiadhesion antibodies delivered infants who were on average 398 g heavier and 2 weeks older than infants born to women without antibodies. In a recent study from an area of Kenya where transmission is
seasonal, neonates born to chronically infected mothers were 395 to 636 g larger when antibodies (VSA\textsubscript{PAM} immunoglobulin G [IgG]) specific for variant surface antigens of CSA-binding parasites were present (T. Staalsoe et al., personal communication). The findings from these studies support efforts to develop a vaccine against pregnancy malaria and suggest that birth weight may be used as an end point for defining the efficacy of such a vaccine during clinical trials.

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