Infectious diseases are associated with substantial morbidity and mortality in very young infants in developing countries [1]. The main thrust toward preventing such diseases has been vaccination shortly before and/or after birth. However many vaccines administered at birth are either poorly immunogenic or contraindicated [2, 3]. Thus, the protection of young infants depends, to a large extent, on antibody acquired by materno-fetal transfer [3, 4]. The antibody transfer from mother to the fetus is an active process mediated by Fc\_Rgamma and hFcRn receptors on the placental syncytiotrophoblast [5]. Although the exact mechanisms of the selective and active process of transfer are not fully known, receptors for the Fc part of IgG (Fc\_Rgamma) may play a role [5]. Some studies have provided evidence for important associations among these receptors and transcytosis of IgG [5–8].

These Fc receptors could be altered by damage to placental architecture. A number of conditions, such as human immunodeficiency virus and parasitic infections, have been shown to be associated with pathologic changes in the placenta that could interfere with the materno-fetal transfer of specific antibodies [9–11]. Changes that have been described in parasitized microvilli include proliferation of cytotrophoblasts, focal syncytiotrophoblastic necrosis, loss of syncytial microvilli, and irregular thickening of trophoblastic membrane [12–15]. Earlier studies have described the unusual prominence of the villous cytotrophoblastic cells in some heavily parasitized placentae and a variable degree of thickening of the trophoblastic basement membrane in an irregular focal or diffuse manner [16]. In light of the above, the pathologic changes of the parasitized placenta may reduce the area of syncytium exposed to maternal blood and thus impair materno-fetal exchanges, including antibody transfer.

Present immunization schedules were designed on the basis of studies delineating disease epidemiology, immunogenicity of vaccines, and transplacentally acquired maternal IgG [17, 18]. Thus, we saw the urgent need to evaluate the combined influence of some factors in The Gambia, where postneonatal vaccination with *Haemophilus influenzae* type b (Hib) and pneumococcal conjugate vaccines is gradually being introduced.

We studied the influence of maternal hypergammaglobulinemia and placental malaria infection on the efficiency of placental transfer of specific IgG antibodies to a variety of viral and bacterial antigens and IgG isotypes in Gambian mothers and babies from a rural population, where pregnant women rarely use malaria chemoprophylaxis. These data allowed us to describe the impact of malaria on the transplacental transfer of antibodies.
Subjects, Materials, and Methods

Study Design

The study was carried out in the labor and postnatal wards of Bansang Hospital, Bansang, The Gambia, during the wet season (July through December 1997), the time of the year when malaria infection occurs most often. The hospital serves a rural population of >300,000 in a region of West Africa where malaria is hyperendemic.

Enrollment of Subjects

Mothers. Parturient (≥24 weeks of gestation) women who consented to be enrolled were consecutively recruited at delivery in the labor ward. Gravid women who had hypertension (essential or pregnancy induced), had twin deliveries, or had received a blood transfusion ≤24 h before delivery were excluded.

At enrollment, basic demographic data and antenatal care were documented on a prepared questionnaire for each patient. The information was obtained from the patient’s antenatal health card, if available; patients who did not have such a card were questioned directly. Information included maternal age, last menstrual period, parity, obstetric history, medication used during pregnancy, immunizations, and hemoglobin level. The use of antimalarial drugs for treatment or prophylaxis during pregnancy also was recorded. The antepartum weight for each mother was measured to the nearest 0.05 kg.

Babies. All babies delivered (alive or stillborn) after the 24th week of gestation were enrolled. On delivery, each baby was resuscitated and was assessed by using the Apgar scoring system, and a clinical examination was done by a pediatrician within the first 12 h of delivery. Each baby was weighed, and crown-heel length was measured on an infantometer. Three consecutive readings of each parameter were obtained, and mean values were recorded to the nearest 0.1 cm or 0.05 kg.

Babies’ gestational ages were assessed by using Dubowitz scores [19]. Live babies were classified as appropriate for gestational age, small for age, or large for age on the basis of their gestational age and birth weight. Mothers and babies were followed up daily in the labor or pediatric wards until discharge.

Collection of Specimens

Maternal blood (5 mL) was obtained from a peripheral vein within 12 h of delivery. Cord blood (5 mL) was collected from large veins on the fetal side of the placenta immediately after delivery. Serum samples separated from these samples were stored at −20°C until they were assayed for total and specific IgG and IgG subclasses.

Biopsy samples were obtained from 2 off-center positions of the placenta, as described elsewhere [13], and were stored in 20 mL of 10% formaldehyde in phosphate buffer until they were processed according to standard technique [16]. Thick and thin Giemsa-stained films also were prepared with blood from biopsy sites.

Laboratory Methods

Paraffin-embedded sections (5-µm thick) of the placental biopsy specimens were stained with hematoxylin-eosin or Giemsa or by the periodic acid–Schiff techniques. They were examined by light microscopy (×40) and under polarized light. Thick and thin blood smears prepared from the placental biopsy sites for malaria parasite detection were stained with Giemsa or Field stain and were examined at ×100 for the presence and species of parasites. A minimum of 200 fields was examined each time.

Placental malaria infection was defined by the presence of parasites and/or malaria pigments on thick and thin Giemsa-stained films and/or the presence of histologic features of malaria, as described elsewhere [13].

Assessment of IgG Antibody Levels

Total serum IgG and IgG subclasses were assayed by laser nephelometry, using an Array Protein System (Beckman). Specific antibodies against herpes simplex virus type 1 (HSV-1), respiratory syncytial virus (RSV), varicella-zoster virus (VZV), diphtheria toxoid (DT), and the capsular polysaccharides of Streptococcus pneumoniae and Hib in maternal and neonatal serum samples were tested by use of an in-house indirect ELISA, as described elsewhere [11].

The reference serum samples employed were of international units. The absorbance obtained for the test samples were transformed into antibody units (international units per milliliter), using standard curves [11]. Arbitrary units (AU) were used for some specific antibodies.

The tested serial dilutions of a reference-positive serum (with the antibody level expressed in IU or AU per milliliter) for the different antibody specificities were studied. The optical density (OD) values of the diluted serum samples were plotted against the log10 values of the units of antibody in each dilution to generate a standard curve. A line of best fit (asymmetric sigmoid) was drawn, and the OD values of the test serum sample were transformed into antibody units (IU or AU per milliliter) by interpolating the mean OD value of duplicate wells from the standard curve [10].

Statistical Analysis

The effect of a number of variables on placental antibody transfer was assessed by use of multiple regression analysis. For each specific antibody type, the logarithm of the antibody transfer ratio (neonatal:maternal antibody level) was regressed on the following predictors: placental malaria infection status, the logarithm of maternal total serum IgG titer, the logarithm of maternal and neonatal serum IgG subclass titer, parity, maternal age (classified as adolescent if <20 years old and as adult if ≥20 years old), maternal weight (classified as <45 kg or ≥45 kg), and parity (primigravida and multigravida). Parity, maternal age, weight, and gestational age were taken as both binary and continuous variables. Placental malaria, active or past, has no bearing on antibody transfer, so data for infected subjects were analyzed as a single group.

Variables were removed from the model if proven to be nonsignificant. The geometric mean maternal and neonatal antibody or IgG subclass levels and the geometric mean antibody or IgG sub-
class transfer ratios between the placental malaria–positive and malaria–negative groups were compared by use of Student’s t tests. Independent sample Student’s t tests also were used to compare the geometric mean maternal and neonatal IgG subclass levels and the geometric mean IgG subclass transfer ratios between hypergammaglobulinemic (≥15 g/L total IgG) [6] and nonhypergammaglobulinic mothers and their newborns. Antibody levels that were not detectable in the mother and/or neonate (n = 3) were removed from log regressions.

Results

Characteristics of the Study Population

Overall, 213 mother-baby pairs were recruited consecutively. The average age of study women at delivery was 23.7 years. The male-to-female ratio of the babies delivered was 1.3:1. Forty-four (20.7%) babies were delivered preterm (<37 weeks), and 51 (23.9%) were low-birth-weight (LBW) babies (<2.5 kg). Thirty-six (69.7%) of the LBW babies were preterm LBW. Characteristics of the studied population are shown in table 1.

As determined by placental histology, 109 (51.1%) of the study population had placental malaria infection; of these, 27 (24.7%) had active infection, whereas 49 (45%) and 33 (30.3%) had a chronic infection and chronic infections, respectively. Placental blood smears detected only 79 infected women (37.1%), and 95.6% of malaria parasites were Plasmodium falciparum.

Mothers <20 years old were more likely than older women to have infected placentas (P < .001), and a significantly higher proportion of the primigravidae than the multigravidae mothers were infected (65.3% vs. 44.7%; P = .01). Women who delivered a baby with a gestational age <37 weeks were more likely to have had infected placentas than those who delivered babies with a gestational age ≥37 weeks (P = .001; table 1).

Maternal Serum Immunoglobulin and Antibody Levels

Thirty-seven women (17.4%) in the study had serum total IgG levels ≥15 g/L (maternal hypergammaglobulinemia) [6]; 35 (94.6%) of these women were in the infected group.

The geometric mean serum total IgG level in mothers with placental malaria infection was significantly higher than the level in mothers without placental malaria infection (22.0 vs. 11.3 g/L; P < .01). Mothers with malaria-positive placentas had significantly higher levels of specific antibodies for HSV-1, RSV, and VZV compared with mothers without placental malaria infection. Both groups of mothers had similar levels of IgG3 and IgG4 subclasses (P = .43 and P = .31, respectively). Hypergammaglobulinic mothers had significantly higher IgG1 (20.15 vs. 7.17 g/L; P < .01) and IgG2 (11.32 vs. 6.59 g/L; P < .01) levels than did nonhypergammaglobulinic mothers. The levels of IgG3 and IgG4 were not significantly different between the 2 groups (P = .32 and P = .29, respectively).

There were no significant differences between the total IgG titers or specific IgG titers in mothers of preterm–appropriate birth weight (ABW) newborns and term-ABW newborns or mothers of term-LBW newborns and term-ABW newborns (data not shown).

Placental Antibody Transfer

Influence of placental malaria infection. Placental transfer of antibodies for HSV-1, RSV, and VZV was significantly lower in the placental malaria–positive mother–baby pairs than in the placental malaria–negative pairs (tables 2 and 3). The proportional reduction in antibody transfer (the difference between these 2 regression coefficients expressed as a percentage of the regression coefficient of the placental malaria–negative group) in association with placental malaria infection is shown in table 3. In contrast, placental malaria infection did not have a significant effect on the transfer of antibodies for DT, S. pneumoniae, or Hib (table 2).

Mothers with placental malaria infection transmitted significantly lower levels of IgG1, IgG2, and IgG4 to their newborns than did mothers without placental malaria infection (P < .01, P = .01, and P = .03, respectively; table 4). However, the placental transfer of IgG3 was similar in the infected and noninfected groups (P = .52; table 4).

Maternal hypergammaglobulinemia. Maternal hypergammaglobulinemia (≥15-g/L total IgG), which occurred in 17.4% of the mothers, was associated with a significant reduction in IgG transfer for HSV-1, RSV, VZV, and S. pneumoniae (table 3), without impairing antibody transfer for DT and Hib (table 3).

Table 1. Characteristics of the maternal study population in rural West Africa.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>Placental malaria infection (histology)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age</td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>&lt;20 years</td>
<td>117 (55)</td>
<td>74 (63.3)</td>
</tr>
<tr>
<td>≥20 years</td>
<td>96 (45)</td>
<td>35 (36.5)</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primigravida</td>
<td>66 (31)</td>
<td>43 (63.5)</td>
</tr>
<tr>
<td>Multigravida</td>
<td>147 (69)</td>
<td>66 (44.9)</td>
</tr>
<tr>
<td>Gestational age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥37 weeks</td>
<td>169 (79.2)</td>
<td>76 (44.8)</td>
</tr>
<tr>
<td>&lt;37 weeks</td>
<td>44 (20.7)</td>
<td>33 (75.6)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of subjects, unless otherwise indicated.
Levels of maternal IgG1, IgG2, IgG3, and IgG4 were not correlated with maternal IgG2, and IgG4 and antibody transfer for HSV-1, RSV, and VZV, but there were no correlations between maternal levels of IgG1, IgG3, and IgG4.02 levels, and transfer for HSV-1, RSV, and VZV (), maternal IgG3 and IgG4 was not significantly different between the 2 groups (P = .62 and P = .53, respectively; table 4).

Influence of Maternal IgG Subclasses on Antibody Transfer

There was a significant positive correlation between maternal levels of IgG4 (but not IgG1, IgG2, or IgG3) and transplacental antibody transfer of DT antibodies (P < .01), maternal IgG3 levels, and transfer for HSV-1, RSV, and VZV (P = .01–.04), but there were no correlations between maternal levels of IgG1, IgG2, and IgG4 and antibody transfer for HSV-1, RSV, and VZV. Levels of maternal IgG1, IgG2, IgG3, and IgG4 were not correlated with antibody transfer for S. pneumoniae and Hib.

Parity and maternal age, weight, and height did not influence placental antibody transfer for any of the antibody specificities and IgG subclasses studied. These variables were removed from multiple regression analyses.

Mean Neonatal Antibody Levels

Geometric mean total serum IgG levels were significantly lower in newborns of mothers with plasental malaria infection than in newborns of mothers without placental malaria (9.0 vs. 14.3 g/L; P < .01). Compared with neonates of mothers without plasental malaria, the neonates of mothers with placental malaria had significantly lower geometric mean levels of RSV-specific IgG (0.20 vs. 0.54 AU/mL; P < .01) and VZV-specific IgG (1.34 vs. 3.56 AU/mL; P = .01).

Compared with neonates of mothers without malaria-infected placentae, newborns of mothers with malaria-infected placentae had significantly lower levels of IgG1 (6.39 vs. 10.1 g/L; P = .02) and IgG2 (2.53 vs. 4.32 g/L; P = .01). The IgG3 and IgG4 levels were similar in newborns of mothers with and without placental malaria infection (P = .19 and P = .52, respectively). In a similar manner, compared with neonates born to nonhypergammaglobulinemic mothers, those born to hypergammaglobulinemic mothers had significantly reduced levels of IgG1 (6.53 vs. 10.1 g/L; P = .01) and IgG2 (1.32 vs. 4.32 g/L; P = .03), whereas levels of IgG3 (P = .39) and IgG4 (P = .23) were similar.

Discussion

This study shows that placental malaria infection and maternal hypergammaglobulinemia were 2 characteristics that differentiated Gambian mothers in this population, with incidences of 51.1% and 17.4%, respectively. Placental malaria infection was associated with significantly increased maternal concentrations of total serum IgG, IgG1, and IgG2 and significantly higher specific IgG titers to HSV-1, RSV, and VZV. The materno-fetal transfer of some specific antibodies (HSV-1, RSV, and VZV) and the transfer of IgG1 and IgG2 were significantly impaired when the placenta was parasitized. However, the transfer of antibodies to DT, S. pneumoniae, and Hib was not affected. After adjustments were made for different characteristics of the population (e.g., maternal hypergammaglobulinemia, gestational age and birth weight, and maternal age, weight, and parity), the effect of malaria on antibody transfer was retained.

Placental malaria infection has been shown to cause a number of pathologic changes in the placenta [13–16], and these changes probably affect the Fc receptors that ferry immunoglobulins across the placenta. In addition, repeated malaria infection that causes specific and/or nonspecific stimulation of B lymphocytes is probably the main reason for the increased antibody concentrations in this Gambian population. This view is supported by previous studies in West Africa that showed

Table 2. Antibody transfer in relation to placental malaria infection.

<table>
<thead>
<tr>
<th>Antibody specificity</th>
<th>Placental malaria (n = 77)</th>
<th>No placental malaria (n = 104)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herpesvirus type 1</td>
<td>0.86 (0.17)</td>
<td>1.40 (0.15)</td>
</tr>
<tr>
<td>Diptheria toxin</td>
<td>1.31 (0.14)</td>
<td>1.43 (0.22)</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>0.64 (0.23)</td>
<td>0.75 (0.12)</td>
</tr>
<tr>
<td>Haemophilus influenza type b</td>
<td>0.70 (0.22)</td>
<td>0.72 (0.26)</td>
</tr>
<tr>
<td>Respiratory syncytal virus</td>
<td>0.62 (0.13)</td>
<td>1.18 (0.17)</td>
</tr>
<tr>
<td>Varicella-zoster virus</td>
<td>0.83 (0.16)</td>
<td>1.36 (0.16)</td>
</tr>
</tbody>
</table>

NOTE. Data are the geometric mean (SD) of the neonatal maternal antibody levels.

* Controlled for the 22 preterm babies in the infected group.

b P < .01, Student’s t test.

c P = .01–.05, Student’s t test.

The transfer of IgG1 and IgG2 subclasses was significantly lower in hypergammaglobulinemic mothers and their neonates than was the transfer in nonhypergammaglobulinemic mothers and their neonates (P < .01), whereas the transfer of IgG3 and IgG4 was not significantly different between the 2 groups (P = .62 and P = .53, respectively; table 4).

Table 3. Proportional reduction in antibody transfer associated with placental malaria infection and maternal hypergammaglobulinemia.

<table>
<thead>
<tr>
<th>Antibody specificity</th>
<th>Placental malaria infection</th>
<th>Hypergammaglobulinemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herpes simplex virus type 1</td>
<td>69 (60–88)</td>
<td>89 (79–95)</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>—</td>
<td>88 (77–98)</td>
</tr>
<tr>
<td>Respiratory syncytal virus</td>
<td>58 (52–65)</td>
<td>90 (81–99)</td>
</tr>
<tr>
<td>Varicella-zoster virus</td>
<td>55 (48–62)</td>
<td>91 (88–99)</td>
</tr>
</tbody>
</table>

NOTE. Estimates were derived from regression analysis. For illustration, proportional reduction was taken as the difference in the regression coefficient groups, with malaria-negative placenta and malaria-positive placenta expressed as a percentage of the regression coefficient of the group with malaria-negative placenta. The SE, 95% confidence interval (CI), and the t value of the proportional reduction were calculated by using the following formula: SE(b1/b2) = [(b1/b2) - (SE[b1/b2] / (SE[b1/b2] + SE[b2/b2]) (obtained via logarithms), where the t value is the proportional reduction/SE, b1 is the regression coefficient of neonatal antibody level and maternal antibody level for the malaria-negative placenta group, and b2 is the regression coefficient of neonatal antibody level and maternal antibody level for the malaria-positive placenta group. For the antibody specificities not included in the table, no proportional reduction in antibody transfer associated with placental malaria infection or maternal hypergammaglobulinemia was observed. In all cases, P < .01.

CI = 1.96 × SE.
Table 4. IgG subclass transfer in relation to placental malaria infection and maternal hypergammaglobulinemia.

<table>
<thead>
<tr>
<th>IgG subclass</th>
<th>Positive* (n = 109)</th>
<th>Negative (n = 104)</th>
<th>&gt;15 g/L (hypergamma-globulinemia; n = 37)</th>
<th>&lt;15 g/L (nonhypergamma-globulinemia; n = 176)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1</td>
<td>0.73 (0.15)a</td>
<td>1.51 (0.26)</td>
<td>0.63 (0.21)c</td>
<td>1.51 (0.26)</td>
</tr>
<tr>
<td>IgG2</td>
<td>0.71 (0.16)b</td>
<td>1.02 (0.19)</td>
<td>0.53 (0.14)c</td>
<td>1.02 (0.19)</td>
</tr>
<tr>
<td>IgG3</td>
<td>1.13 (0.13)</td>
<td>1.29 (0.23)</td>
<td>1.24 (0.11)</td>
<td>1.29 (0.23)</td>
</tr>
<tr>
<td>IgG4</td>
<td>1.09 (0.09)d</td>
<td>1.33 (0.12)</td>
<td>1.26 (0.14)</td>
<td>1.33 (0.12)</td>
</tr>
</tbody>
</table>

NOTE. Data are the geometric mean (SD) of the neonatal:maternal antibody levels.

- * Controlled for the 22 preterm babies in the infected group.
- a, Student's t test; b, Student's t test; c, Student's t test; d, Student's t test.

...that maternal total IgG levels were significantly higher in association with placental malaria, and measles IgG concentrations were higher in the wet season, when malaria transmission is at its peak [20, 21].

Reduced transfer of some antibodies has been documented elsewhere [6, 16], but this differs from one population to another. Placental malaria was significantly associated with impairment of tetanus-toxoid antibody transfer in a Papua New Guinean population [22] and reduced transfer of S. pneumoniae and measles virus antibodies in Malawian mothers [10]. However, P. falciparum infection of the placenta did not influence antibody transfer for tetanus toxoid in the Malawian study and antibody transfer for S. pneumoniae in the present study.

Why placental malaria infection reduces antibody transfer is not yet clear how maternal hypergammaglobulinemia impairs placental antibody transfer. Perhaps, as Brambell [26] originally postulated, at high concentrations of IgG, large amounts of IgG may need to compete with a finite number of hFcRn or FcRp receptors that normally divert endocytosed IgG from catabolism within the placenta by transporting it to the fetal circulation [27]. The study in Malawi has shown that maternal hypergammaglobulinemia independently impaired placental transfer of some antibodies; however, since most of the hypergammaglobulinemic mothers (94.6%) in this study were from the infected group, these 2 variables (malaria infection and high IgG) could be seen to be associated in these individual mothers.

Neonates born to mothers in the infected group had significantly lower IgG1 and IgG2 levels than those born to mothers without placental malaria infection and hypergammaglobulinemia. Passive acquisition of adequate concentrations of IgG1 and IgG2 subclasses is important for the newborn for several reasons. IgG1 and IgG2 are important for complement activation and are involved in efficient opsonization of infectious agents [28]. The newborn synthesizes small amount of IgG1 and IgG2 subclasses during the first 6 months of life, and adult levels are attained in late childhood [29]. Therefore, failure of neonates to acquire sufficient levels of these antibodies and IgG subclasses transplacentally may result in increased susceptibility to some encapsulated bacterial and viral infections during infancy [28]; this is particularly important in infants who are not breast-fed.

Finally, maternal vaccination strategies to provide immunity (IgG) to neonates in malarious areas may be less successful because of the reduced transplacental antibody transfer. On the other hand, because of the lower level of maternally acquired antibodies in these newborns, the blocking effect of pre-existing maternal IgG on the neonate’s immune response to active immunization may be less. Alternative vaccination strategies, including earlier vaccination schedules and/or development of vac-
cines that are directed toward stimulation of IgA production, may provide better protection to these neonates. Studies investigating immunogenicity of vaccines in neonates and those born to mothers with placental malaria infection and hypergammaglobulinemia are needed. Furthermore, there is an urgent need to review the policy on use of antimalaria chemoprophylaxis in pregnancy in regions where the disease is endemic.

Acknowledgments

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References