Infection and Immunity

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***Infect. Immun.* 2005, 73(12):7988. DOI: 10.1128/IAI.73.12.7988-7995.2005.**

INFECTION AND IMMUNITY, Dec. 2005, p. 7988–7995 Vol. 73, No. 12
0019-9567/05/$08.00+0 doi:10.1128/IAI.73.12.7988–7995.2005

Neonatal and Maternal Immunological Responses to Conserved Epitopes within the DBL-γ3 Chondroitin Sulfate A-Binding Domain of *Plasmodium falciparum* Erythrocyte Membrane Protein 1

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Received 1 June 2005/Returned for modification 9 July 2005/Accepted 7 August 2005

***Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) mediates the adherence of *P. falciparum*-infected erythrocytes to placental syncytiotrophoblasts via interactions with chondroitin sulfate A (CSA), a characteristic of pregnancy-associated malaria. Pregnancy-associated malaria predicts increased susceptibility of newborns to malaria, and it is postulated that transplacental passage of parasite antigen induces immune regulatory activity in the neonate. We wished to examine the immune responsiveness to a CSA-binding domain of PfEMP1, the DBL-γ3 domain, in cord and maternal venous blood obtained from pregnancies with various histories of *P. falciparum* infection. We assessed in vitro T-cell cytokine and plasma immunoglobulin G (IgG) and IgM responses to four peptides corresponding to highly conserved regions of a DBL-γ3 domain common to central African parasite isolates. The presence of placental *P. falciparum* infection at delivery was associated with elevated frequencies of DBL-γ3 peptide-specific $CD3^+$ interleukin-10-positive T cells in cord blood, while treatment and clearance of infection prior to delivery was associated with elevated frequencies of $CD3^+$ gamma interferon-positive T cells. DBL-γ3 peptide-specific IgM antibodies were detected in 12 of 60 (20%) cord plasma samples from those born to mothers with *P. falciparum* infection during pregnancy. Consistent with polyclonal anti-PfEMP1 antibody responses that are associated with protection against pregnancy-associated malaria, the presence of maternal IgG antibodies with specificity for one of the DBL-γ3 peptides showed a parity-dependent profile. These data demonstrate that peptides corresponding to conserved regions of the DBL-γ3 domain of PfEMP1 are immunogenic in *P. falciparum*-infected mothers and their offspring.**

In malaria-endemic regions, pregnancy is associated with increased risk of *Plasmodium falciparum* infection that has deleterious consequences for both maternal and neonatal health (32). Susceptibility to pregnancy-associated malaria is related to the abundant expression of chondroitin sulfate A (CSA) on placental syncytiotrophoblasts. Chondroitin sulfate A is a proteoglycan that acts as a receptor for *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) (24) expressed on the surface of infected erythrocytes (26). Infected erythrocytes accumulate in the intervillous spaces of the placenta (8), and naturally acquired antibodies that interfere with CSA-mediated adherence of infected erythrocytes are associated with protection against pregnancy-associated malaria (9) and increase with parity (25).

Variants of PfEMP1 are encoded by individual members of the *var* multigene family and most comprise at least one cysteine-rich interdomain region with a variable number of Duffy binding-like (DBL) domains (29). The repertoire of PfEMP1 variants expressed on infected erythrocytes found in association with pregnancy-associated malaria is narrower than that expressed on infected erythrocytes of non-pregnancy-associated malaria parasites, perhaps due to constraints imposed by receptor specificity, which may help to explain the relatively rapid acquisition of immunity to pregnancy-associated malaria (12). The DBL-γ3 domain of PfEMP1 expressed by placental parasite isolates binds to CSA (4, 10), and antibodies directed against recombinant DBL-γ3 block infected erythrocyte adhesion to CSA (5). Monoclonal antibodies raised against DBL-γ3 bind to the surface of CSA-adhering parasites obtained from different geographic areas (19), which is itself probably a reflection of the relatively conserved nature of the DBL-γ3 domain that supports the feasibility of a vaccine against pregnancy-associated malaria.

The knowledge of B- and T-cell activity directed to specific epitopes of PfEMP1 in naturally exposed humans is very limited (1), and no studies have reported PfEMP1-specific immune responses in the cord blood from neonates born to mothers with malaria. Epidemiological studies suggest that pregnancy-associated malaria increases the likelihood of early infection in the newborn (6, 18), possibly as a result of antigen exposure inducing immunosuppressive pathways during fetal development (2, 3). In this study, we wished to determine whether DBL-γ3 domain-specific antibody and T-cell responses are present in cord blood and maternal venous blood.

We tested a panel of peptides corresponding to conserved regions of the DBL-γ3 domain present in closely related PfEMP1 variants expressed by placental parasites isolated

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from Cameroon and Gabon (15, 16). For comparative purposes, we also used recombinant glutamate-rich protein, a *P. falciparum* antigen shown to be present in cord blood (14). DBL-γ3 domain sequence-specific peptide selection was based both on amino acid conservation and HLA-DR allele-binding agretope prediction (23). Our results show that maternal *P. falciparum* infection during pregnancy is associated with increased frequencies of DBL-γ3 peptide-specific T cells and IgM in cord blood.

MATERIALS AND METHODS

Study population. The study was carried out at the Albert Schweitzer Hospital in Lambaréné, Gabon, a site with perennial transmission of *P. falciparum* (33). Informed consent for participation was obtained from mothers prior to inclusion in the study. From May to December 2003, 85 maternal venous and umbilical cord blood samples were collected into heparinized Vacutainer tubes (BD Biosciences, Heidelberg, Germany). The presence of *P. falciparum* parasites in the maternal peripheral, placental, and cord blood at the time of delivery was determined through microscopic examination of Giemsa-stained thick and thin smears. The medical records of uninfected mothers were examined to verify those who had been appropriately diagnosed and treated for *P. falciparum* malaria episodes during their pregnancy. The majority of those with such a history received chemotherapy with quinine, a drug with 100% efficacy for the treatment of uncomplicated *P. falciparum* malaria in the study area (22), at least 2 weeks prior to delivery.

Based on these criteria the following distinct groups were defined: (i) negative: no evidence of *P. falciparum* parasites or active infection in any compartment at delivery and no record of malaria during pregnancy; (ii) placenta positive: *P. falciparum* asexual stage parasites present in placental blood; and (iii) treated: no evidence of *P. falciparum* parasites or active infection in any compartment at delivery but recorded history of a diagnosed and treated malaria episode during pregnancy. A significantly higher proportion of primiparous mothers had placental *P. falciparum* infection compared with those who were multiparous ($P < 0.001$). Ethical clearance for the study was given by the ethics committee of the International Foundation of the Albert Schweitzer Hospital in Lambaréné.

Isolation of mononuclear cells and cell culture. Cord blood mononuclear cells were isolated by standard density-gradient centrifugation on Ficoll-Paque (Amersham, Freiburg, Germany). Freshly isolated cells were resuspended at 5×10^6 cells/ml in complete medium comprising RPMI 1640 medium (Sigma, Diesenhofen, Germany) supplemented with 10% (wt/vol) human serum (AB) (Sigma), 1 mM L-alanylglutamine (Life Technologies, Grand Island, NY), 100 U/ml penicillin, 100 μg/ml streptomycin (Life Technologies), and 1 mM sodium pyruvate (Life Technologies). To facilitate costimulation 0.5 μg/ml of both anti-human CD28 and CD49d antibodies were added (BD Biosciences, San Jose, CA) (11). A total of 0.6 ml of cell suspension was placed in a 48-well flat-bottomed tissue culture plate (Falcon). Peptides (10 μg/ml), purified protein derivative of *Mycobacterium tuberculosis* (10 μg/ml) (StatensSerumInstitut, Copenhagen, Denmark), or phytohemagglutinin (5 μg/ml) (Sigma, Diesenhofen, Germany) was added to the cells followed by incubation at 37°C in a humidified 5% CO_2 atmosphere. For intracellular cytokine analysis, 10 μg/ml brefeldin A (Sigma) was added after 18 h of culture and the cells were incubated for a further 4 hours prior to fixation and antibody staining.

Measurement of intracellular cytokine production by flow cytometry analysis. For the analysis of intracellular cytokines and surface markers cells were washed twice in cold phosphate-buffered saline (PBS) immediately after culture, fixed with 2% paraformaldehyde/PBS, and stored at 4°C. For staining with fluorescently labeled antibodies cells were washed in PBS and incubated on ice for 15 min with PBS-10% fetal bovine serum containing 2 μl of Fc receptor blocking reagent (Miltenyi Biotec, Bergisch Gladbach, Germany), followed by surface-staining antibody specific for human CD3 (SK7) (conjugated to peridinin chlorophyll protein) or isotype control antibody mouse IgG1-peridinin chlorophyll protein (MOPC-31C). For intracellular staining of gamma interferon (IFN-γ), interleukin-10 (IL-10), and IL-13, Cytofix/Cytoperm Plus kits (BD Biosciences, Heidelberg, Germany) were used according to the manufacturer's protocol using antibodies IFN-γ-fluorescein isothiocyanate (B27), IL-10-allophycocyanin (JES3-19F1), IL-13-phycoerythrin (JES10-5A2) or isotype control antibodies mouse IgG1-fluorescein isothiocyanate (MOPC-21), rat IgG2a-allophycocyanin (R35-95), and rat IgG1-phycoerythrin (A11-01). Flow cytometry was performed on a FACScan flow cytometer with CellQuest (version 3.3) data analysis software (BD Biosciences). A minimum of 100,000 lymphocytes were analyzed. The responses measured from seven malaria-unexposed Europeans were used to determine the cutoff value for a positive response in the study subjects (mean of net response + 3 standard deviations).

Detection of cord blood and maternal venous blood IgM and IgG antibodies specific for DBL-γ3 peptides, glutamate-rich protein, and purified protein derivative by ELISA. The antibody responses to the four DBL-γ3 peptides, glutamate-rich protein (gift from Michael Theisen) (34), and purified protein derivative of *Mycobacterium tuberculosis* (StatensSerumInstitut, Copenhagen, Denmark) were determined by a standard enzyme-linked immunosorbent assay (ELISA). Briefly, the wells of flat-bottomed microtiter plates (Immulon2; Dynatech) were coated either with 100 μl (10 μg/ml) of individual DBL-γ3 peptides, 100 μl (100 ng/ml) of glutamate-rich protein, or 100 μl (100 ng/ml) of purified protein derivative, all diluted in PBS (0.01 M, pH 7.2). After overnight incubation at 4°C, the plates were washed twice with PBS containing 0.05% Tween 20 (PBS-T) and blocked with PBS-T containing 2% bovine serum albumin (PBS-TB). Plasma samples diluted at 1:200 in PBS-TB were added in duplicate wells and allowed to react for 2 hours at room temperature. Unbound antibodies were removed by washing the plates four times with PBS-T. Peroxidase-conjugated goat anti-human IgG or IgM antibody (Biosource, Solingen, Germany) at a dilution of 1:50,000 in PBS-TB was added. After 1 hour, plates were washed four times with PBS-T, and 100 μl/well of TMB (tetramethylbenzidine) substrate (Biosource) was added. The reaction was stopped after 10 min by the addition of 100 μl of 1.8 N H_2SO_4 and the absorbance was read at 450 nm.

In order to calculate the relative antibody concentrations of the test plasma samples, standard curves using serial dilutions of pooled plasma samples from five Gabonese adults who tested positive for DBL-γ3-specific antibodies were generated. In order to determine the cutoff value for a positive result, we calculated the mean optical density plus three standard deviations of plasma samples from negative controls (10 Europeans with no known exposure to *P. falciparum*).

Peptides. Synthetic peptides corresponded to a DBL-γ3 sequence from a placentally derived *P. falciparum* isolate obtained in Cameroon in 1996 (isolate 732, GenBank accession number AF334807) (16) that showed >98% amino acid sequence identity to two isolates obtained in Lambaréné, Gabon in 2000 (15). The SYFPEITHI algorithm (23) was used to predict motifs most likely to bind to HLA-DR molecules present in the Gabonese population (21), and belonging to the main HLA-DR super type (30). Figure 1 shows the amino acid sequences with the algorithm scores of the four peptides used in this study. The DBL-γ3 peptides were synthesized at >98% purity by ThermoHybaid (Ulm, Germany), and resuspended in sterile PBS.

Statistical analyses. All figures were drawn using StatView. The significance of differences in continuous variables between groups was assessed using the nonparametric Kruskal-Wallis test and the Mann-Whitney *U* test (two groups), for associations between two continuous variables the nonparametric Spearman rank correlation test, and for differences in proportions the Fisher exact test.

RESULTS

In vitro T-cell cytokine responses to DBL-γ3 peptides in cord and maternal blood. Flow cytometric analysis was used to measure intracellular IFN-γ, IL-13, and IL-10 in $CD3^+$ cells after overnight stimulation with the four DBL-γ3 peptides of cord blood mononuclear cells, maternal peripheral blood mononuclear cells, and malaria-unexposed European peripheral blood mononuclear cells. The proportion of cord blood mononuclear cell samples that produced cytokine in response to peptide stimulation ranged from 0.09 to 0.23, with DBL120 inducing the greatest response (Fig. 2A), while the proportion of maternal peripheral blood mononuclear cell samples with positive responses ranged from 0.19 to 0.29 (Fig. 2A). Cord blood mononuclear cells samples from the treated group of neonates contained significantly higher percentages of DBL-γ3 peptide-specific $CD3^+IFN\text{-}\gamma^+$ cells compared to the negative group, while cord blood mononuclear cells samples from the placenta-positive group contained significantly higher percentages of DBL-γ3 peptide-specific $CD3^+IL\text{-}10^+$ cells (Fig. 2B).

Cord blood mononuclear cells samples from both the placenta-positive and treated groups of neonates contained ele-

A

DBL-γ3 peptide	Amino Acid Sequence
DBL25	EAFTKTAAAETFLAW
DBL78	DICLGTDDISVKQGDV
DBL120	PQTWWEKNAKDIWEG
DBL132	WEGMLCALTNGLTDA

B

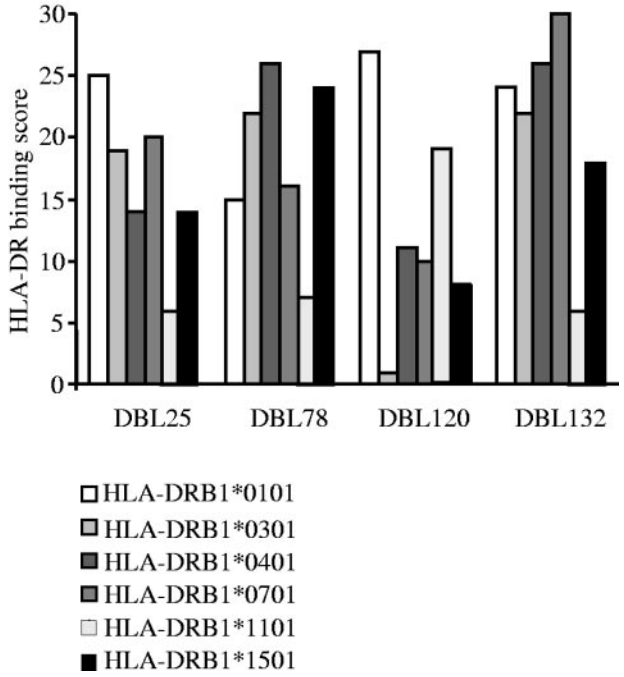

FIG. 1. A) Amino acid sequences of peptides corresponding to conserved regions of DBL-γ3, and B) their SYFPEITHI score for HLA-DR phenotypes.

vated numbers of $CD3^+IL\text{-}13^+$ cells compared to negative neonates, though the differences were not statistically significant. The percentages of DBL-γ3 peptide-specific $CD3^+$ cells displaying either IFN-γ, IL-13, or IL-10 activity were statistically similar among cocultures from the three groups of mothers, though there were trends toward higher percentages of $CD3^+IL\text{-}13^+$ cells in the negative group, and of $CD3^+IFN\text{-}\gamma^+$ and $CD3^+IL\text{-}10^+$ cells in the placenta-positive group (Fig. 2B). Strong associations between maternal DBL-γ3 peptide-specific cytokine activity and parity were not observed (data not shown). Comparison of the cytokine activity of cord and maternal $CD3^+$ cells following nonspecific stimulation with phytohemagglutinin showed no significant differences between the groups segregated according to maternal *P. falciparum* infection status (Fig. 2C).

IgM and IgG antibodies with specificity for DBL-γ3 peptides are present in cord and maternal plasma. Plasma samples collected from cord blood and maternal venous blood were analyzed for IgM and IgG antibodies with specificity for the four DBL-γ3 peptides, and for comparative purposes recombinant glutamate-rich protein and purified protein derivative protein preparations. Maternally derived IgM antibodies do not cross the placental barrier and *P. falciparum*-specific IgM detected in cord plasma is therefore assumed to reflect stimulation of fetal B cells by transplacentally transferred antigen. With the exception of DBL78, the highest proportions of cord blood samples that contained IgM antibodies specific for the DBL-γ3 peptides or glutamate-rich protein were found in the placenta-positive group (Fig. 3A).

The proportions of positive responses for each peptide among all neonates were DBL25 (8%), DBL78 (8%), DBL120 (10%) and DBL132 (4%). One-third of cord plasma samples with IgM specific for DBL120 contained IgM antibodies specific for DBL132. In the treated group of neonates, the proportions of plasma samples with IgM responses to DBL25, DBL78, DBL120, and glutamate-rich protein were more than 1.5-fold higher than found in plasma of the negative group (Fig. 3A). Overall, IgM with specificity for any DBL-γ3 peptide was detected in 7 of 32 (22%) cord plasma samples from neonates born to mothers with placental *P. falciparum* infection at delivery, and 5 of 28 (18%) cord samples from neonates whose mothers were treated for *P. falciparum* infection during pregnancy, compared with only 1 of 25 (4%) cord samples from neonates whose mothers did not have *P. falciparum* infection during pregnancy (Fig. 3B).

The mean + 3 standard deviations of IgM antibody levels detected in plasma samples of malaria-naïve (unexposed) Europeans was used to establish the cutoff value for a positive response (illustrated for DBL120-specific IgM in Fig. 3C). The proportion of mothers' plasma samples from all groups with IgM specific for at least one peptide was 77 of 85 (90%). No correlations were observed between cord and maternal IgM antibody levels for any of the DBL-γ3 peptides, or for glutamate-rich protein or purified protein derivative (data not shown). There was a strong positive correlation ($\rho = 0.72$, $P < 0.001$) between cord and maternal venous blood DBL-γ3 peptide-specific IgG antibody levels that was not affected by maternal infection history at the time of delivery (Fig. 3D).

Compared with plasma from negative mothers, plasma from placenta-positive mothers contained significantly higher levels of IgG antibodies with specificity for DBL120 ($P = 0.009$) and of IgM antibodies with specificity for DBL25, DBL78, and glutamate-rich protein ($P = 0.030$, 0.002, and 0.007, respectively) (Fig. 4A and B). Treatment for malaria during pregnancy was not associated with significantly different DBL-γ3 peptide-specific IgG and IgM antibody levels compared to the levels observed in negative mothers (Fig. 4A and B).

We found a positive association between parity and levels of IgG antibodies in the plasma of negative mothers with specificity for either DBL78 or DBL120, and levels of IgM antibodies specific for DBL120 ($\rho = 0.47$ and $P = 0.050$; $\rho = 0.51$ and $P = 0.043$; and $\rho = 0.47$, and $P = 0.038$, respectively) (Fig. 5A and B). No such relationships were found between parity and the levels of either glutamate-rich protein- or purified protein derivative-specific IgG or IgM antibodies (Fig. 5).

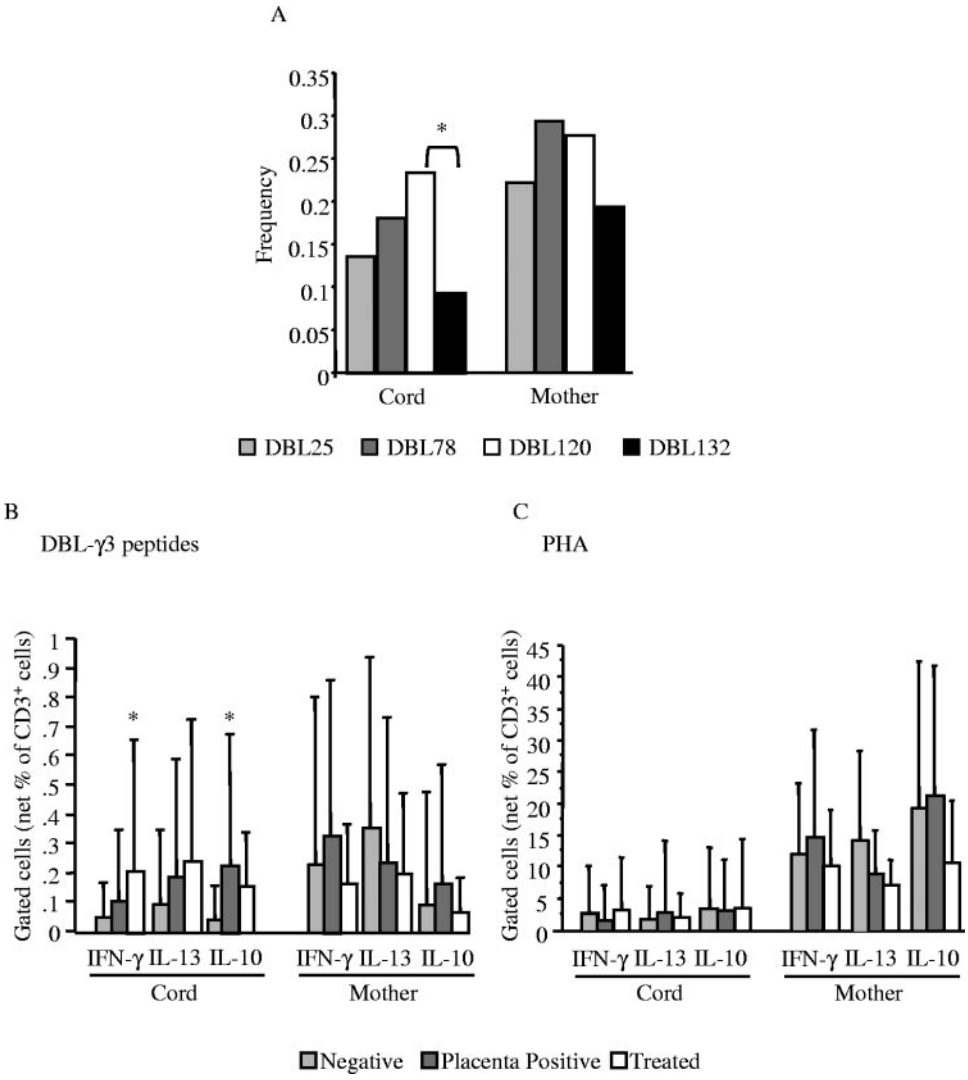

FIG. 2. Intracellular cytokine activity of cord and maternal T cells following stimulation of cord blood mononuclear cells and peripheral blood mononuclear cells with DBL-γ3 peptides. A) Percentage of samples containing $CD3^+$ cells with a cytokine response to DBL-γ3 peptides in cord and maternal blood. The mean net percent gated $CD3^+$ cells in cord and maternal blood that produce IFN- γ, IL-13, or IL-10 in response to B) DBL-γ3 peptides or C) phytohemagglutinin (PHA) in groups segregated by maternal *P. falciparum* infection history during pregnancy. Bar graphs illustrate mean percentages with standard deviations. Sample sizes: European control, 7; Cords: negative, 19; placenta positive, 14; treated, 17; Mothers: negative, 8; placenta positive, 7; treated, 7. *, $P < 0.05$ by either Fisher exact test or the nonparametric Mann-Whitney test.

DISCUSSION

This study had as its foundation the evidence-based premise that conserved regions of a domain of a *P. falciparum* protein antigen implicated in parasite persistence during pregnancy would be immunogenic, and furthermore, that the presence of antigen in the placental compartment could lead to transplacental transfer and in utero sensitization of the fetal immune system. Thus, we selected four peptides from conserved regions of the PfEMP1 CSA-binding domain, DBL-γ3, which displays a very high degree of spatial and temporal conservation within Central African *P. falciparum* placental isolates (15, 16). This level of conservation in PfEMP1 suggests that these regions may be fundamental to the parasite's survival in the placental compartment, perhaps via participation in CSA binding. B-cell epitopes that also bind CSA have been identified in other DBL-γ3 domain variants, and a CSA-binding PfEMP1 variant associated with pregnancy-associated malaria that contains no DBL-γ3 domain has been described (10, 27, 28). The latter findings clearly imply the existence of a degree of diversity in the receptor-ligand interactions that are thought to be integral to the development and persistence of pregnancy-associated malaria.

The algorithm-based method we used to identify potential agretopes, and by extension T-cell epitopes, within the DBL-γ3 domain revealed peptides that are recognized by both B and T cells from the mother and fetus. We found that antibody responses with specificity for at least one of the peptides (DBL120) were significantly enhanced in the peripheral blood of mothers with pregnancy-associated malaria, and that they showed the pattern of parity-dependence commonly associated

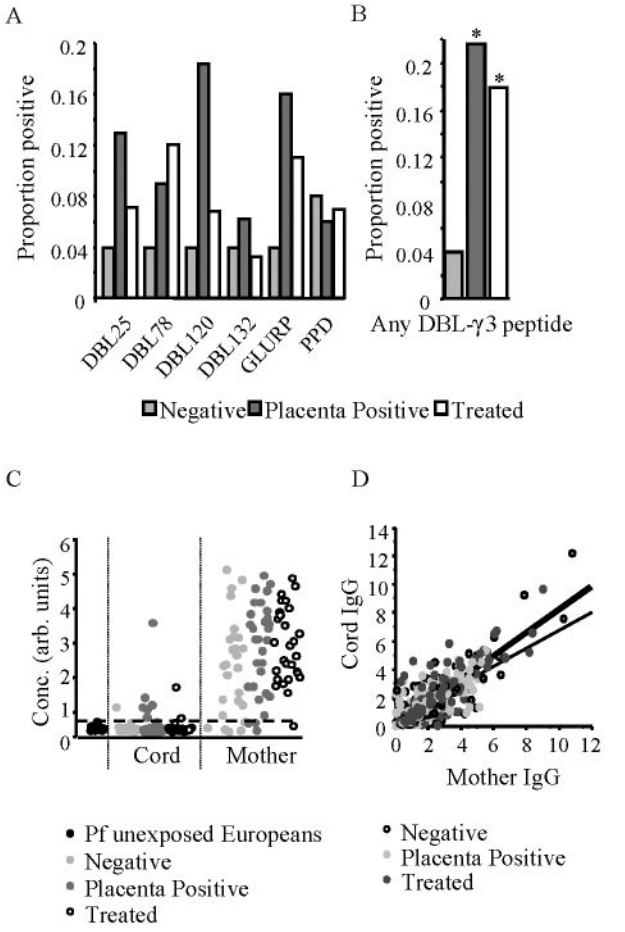

FIG. 3. IgM and IgG antibodies with specificity for DBL-γ3 peptides are present in cord blood. A) The proportions of cord blood samples containing IgM antibodies with specificity for DBL-γ3 peptides, glutamate-rich protein (GLURP), or purified protein derivative (PPD) in groups segregated by maternal *P. falciparum* infection history during pregnancy. B) The proportions of cord plasma samples containing IgM with specificity for at least one DBL-γ3 peptide. C) Dot plot of DBL120 IgM antibody concentrations in plasma from malaria-unexposed Europeans (positivity threshold: mean + 3 standard deviations, shown as a dotted line), Gabonese mothers' peripheral blood and cord blood segregated by maternal *P. falciparum* infection history during pregnancy. D) Correlation between cord and maternal venous blood IgG antibody levels for all DBL-γ3 peptides. Sample sizes: negative, 25; placenta positive, 32; treated, 28; European controls, 12. *, $P < 0.05$ compared to negative group by Fisher exact test.

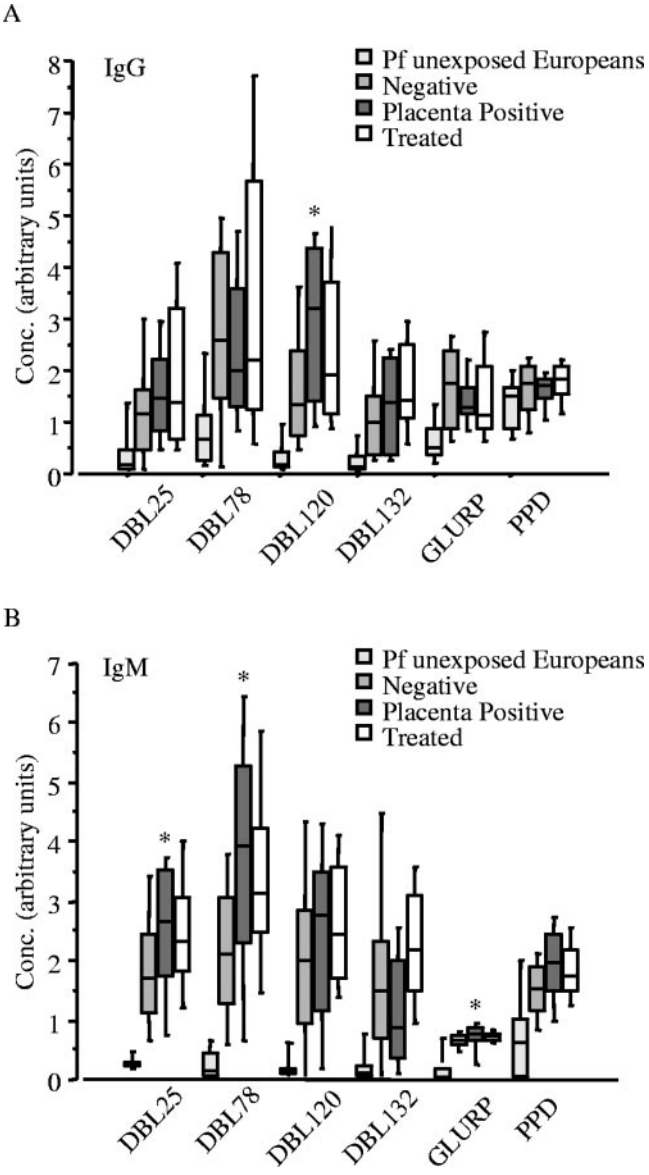

FIG. 4. A) IgG and B) IgM antibodies with specificity for the DBL-γ3 peptides, glutamate-rich protein (GLURP), and purified protein derivative (PPD) in peripheral blood plasma from mothers at the time of delivery. Samples are segregated by *P. falciparum* infection history during pregnancy. Box-whisker plots show medians with 25th and 75th and whiskers for 10th and 90th percentiles. Sample sizes: negative, 25; placenta positive, 32; treated, 28; European controls, 12. *, $P < 0.05$ compared to negative group, Mann-Whitney test.

with protection against pregnancy-associated malaria (25, 31). In the same context it is noteworthy that similar although mostly statistically nonsignificant patterns were observed for the antibody responses to the DBL78 peptide, which was predicted to bind most strongly to HLA-DRB1*1501, which is a particularly common allele among the Gabonese.

We interpret the presence of DBL-γ3 peptide- as well as glutamate-rich protein-specific IgM in cord blood plasma samples primarily of those born to mothers with past or present *P. falciparum* infection as confirmation of our premise concerning in utero sensitization. The proportions of cord samples we found with IgM specific for DBL-γ3 peptides (4 to 10%) are comparable to the proportion of Kenyan cord samples containing IgM antibodies specific for recombinant *P. falciparum* MSP-1_{19} (6%) (17), and to the proportion of Cameroonian cord samples with IgM specific for crude parasite lysate preparations (14%) (36). These findings, coupled with our observation of DBL-γ3 peptide-specific cytokine activity, identify PfEMP1, or at least components thereof, as a parasite antigen that is transferred transplacentally and that sensitizes fetal T and B cells.

There is mounting evidence that prolonged placental *P. falciparum* infection, as reflected by high titers of anti-pregnancy-associated malaria antibodies in cord blood, leads to greater

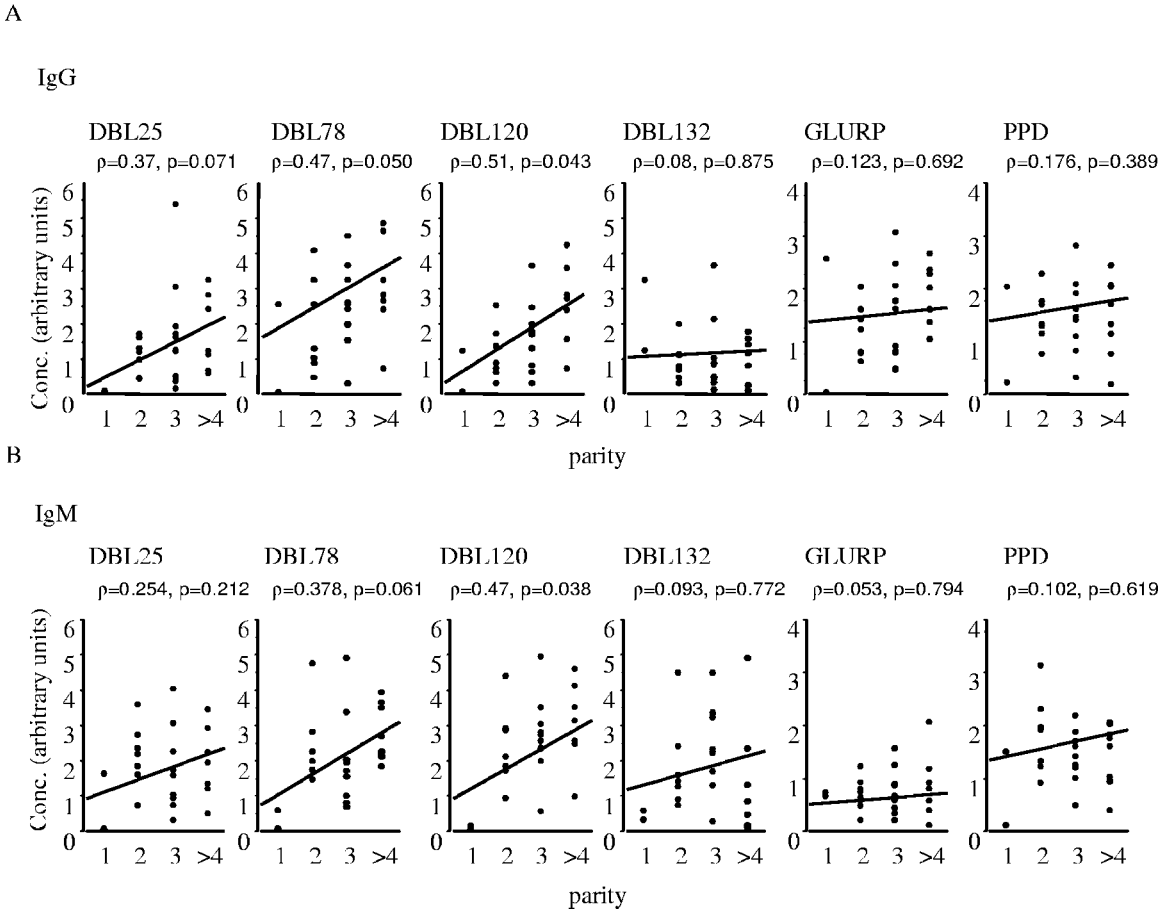

FIG. 5. Relationship between parity and maternal A) IgG and B) IgM antibody levels with specificity for DBL-γ peptides, glutamate-rich protein (GLURP), or purified protein derivative (PPD). *, $P < 0.05$. Spearman rank correlation.

susceptibility to malaria during infancy (6, 18). Untreated placental *P. falciparum* infection appears to further reduce already poor nonspecific neonatal T-cell responsiveness (13), while our own work has shown that the presence of placental *P. falciparum* infection at delivery is associated with reduced major histocompatibility complex class I and II expression on monocytes, and IL-10-mediated suppression of *P. falciparum* antigen-specific Th1-type responses (2, 3). In the study presented here, cord blood samples of those born to mothers with placental *P. falciparum* infection at delivery generally had the highest anti-DBL-γ3 IgG antibody titers and the highest DBL-γ3 peptide-specific IL-10 responses, findings that are consistent with those of the studies cited above.

Although no statistically significant associations in cytokine activity and *P. falciparum* infection during pregnancy were found in mothers, peripheral blood mononuclear cells from negative mothers contained elevated frequencies of DBL-γ3 peptide-specific $CD3^+IL\text{-}13^+$ cells. These results indicate that increased frequencies of Th2-type cells (35), as well as Th1-type 1 cells (20), are likely required for protection against *P. falciparum* malaria in semi-immune adults. We did not see significant enhancement of DBL-γ3 peptide-specific IgG or IgM in plasma from negative mothers, although a trend toward enhanced DBL78-specific IgG antibodies was observed. An evaluation of IgG subtypes may reveal a different profile indicative of protective antibodies in this group.

Although short peptides do not have conformations representative of the native protein displayed on the surface of the infected red blood cell, the controls that we used in this study were intended to confirm sensitivity and to differentiate nonspecific responses. For example, the absence of strong antibody responses to the DBL-γ3 peptides in nonexposed Europeans validates an association of these response to our cohort, while the parity dependency of maternal plasma antibody levels specific for peptides DBL78 and DBL120, but not glutamate-rich protein, supports the idea that these two DBL-γ3 peptides are specifically associated with pregnancy-associated malaria. Additional studies are needed to determine the degree of specificity of the antibodies interacting with these DBL-γ3 peptides, for example whether these antibodies are present in malaria exposed adults who have not been pregnant, whether the addition of recombinant DBL-γ3 to the plasma prior to detection of antibodies by ELISA reduces peptide-specific responses, and whether the addition of the DBL-γ3 peptides would block antibody binding to infected erythrocytes.

In summary, this study is the first to analyze maternal and neonatal immune responses directed to peptides corresponding to conserved regions of the DBL-γ3 domain of PfEMP1.

Two of the four peptides were particularly immunogenic, associated with both B- and T-cell responses in pregnant women and their offspring. On the maternal side, higher levels of antibodies recognizing one of these peptides were associated with pregnancy-associated malaria and parity. On the fetal side, the presence of both cytokine- and IgM peptide-specific responses demonstrates that components of the DBL-γ3 domain of PfEMP1 cross the placental barrier and sensitize the fetal immune system.

ACKNOWLEDGMENTS

We are especially grateful to the mothers for their participation in this study, and to the staff of the Maternity Unit of the Albert Schweitzer Hospital in Lambaréné for their unreserved cooperation and assistance. We extend special thanks to Saadou Issifou for his continuous help and support, Michael Theisen and Francine Ntoumi for providing the recombinant glutamate-rich protein preparation, and Ayman Khattab for technical assistance regarding the DBL-γ3 amino acid sequences.

Financial support was provided by the German Government (DAAD; DFG-BMZ Lu812/1-3) and the fortüne program of the Medical Faculty of the University of Tübingen.

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Editor: W. A. Petri, Jr.