Reduced Cord Blood Immune Effector-Cell Responsiveness Mediated by CD4+ Cells Induced in Utero as a Consequence of Placental Plasmodium falciparum Infection

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To determine mechanisms of neonatal parasite antigen (Ag)–specific immune suppression associated with placental Plasmodium falciparum infection, we isolated cord blood mononuclear cells (CBMCs) from Gabonese neonates born to mothers with differing histories of P. falciparum infection and performed ex vivo and in vitro studies to evaluate immune regulatory activity. We found increased ex vivo percentages of CD4+CD25hi and CD4+CD25+CTLA-4+ cells and increased interleukin (IL)–10 responses to parasite Ag in vitro in CBMCs from neonates born to mothers with placental P. falciparum infection at delivery. Depleting CBMCs of CD4+CD25+ cells before cell culture led to the abrogation of parasite Ag–specific IL-10 responses, to enhanced interferon-γ responses, and to enhanced expression of CD25 on CD8+ T cells and of major histocompatibility complex class I and II on monocytes. These data demonstrate that parasite Ag–specific CD4+ regulatory cells are generated in utero as a consequence of placental P. falciparum infection.

The impact of placental Plasmodium falciparum infection on the immune competence of the neonate remains poorly understood. Congenital malaria infections are rare, but children born to mothers with placental P. falciparum infection at delivery are more susceptible than other children to infection and/or malaria [1, 2]. These observations are strongly suggestive of some form of immune modulation occurring in utero—a process that the results of other studies have indicated arises as a consequence of the transplacental transfer of soluble P. falciparum antigen (Ag) [3–6]. We previously demonstrated that, in neonates born to mothers with placental P. falciparum infection, P. falciparum Ag–specific immune responses from cord blood mononuclear cells (CBMCs), including the production of interferon (IFN)–γ by T cells, are markedly enhanced in the presence of neutralizing anti–interleukin (IL)–10 antibody [7]. We hypothesize that exposure of the fetal immune system to P. falciparum Ag induces the generation of both effector and regulatory/suppressor T cells.

It has been proposed that Ag-induced regulatory T cells (a subset of IL-10–dependent, IL-4–independent, Ag-specific regulatory T cells), which are otherwise known as Tr1 cells, are generated during priming in the presence of IL-10 and/or immature dendritic cells (DCs) [8–11], 2 conditions that prevail during fetal immune development. Neonatal DCs represent immature forms of Ag-presenting cells, because they express low levels of costimulatory molecules and Th1-inducing cytokines when they encounter Ags [12, 13]. IL-10 levels are enhanced in the fetal circulation [14], presumably to con-
trol the pathological effects of inflammation. The cogeneration of effector T cells and regulatory T cells has been documented in various human infections, particularly chronic infection [15–18]. We asked whether placental *P. falciparum* infection, which exposes the fetus to potentially chronic and high levels of soluble parasite Ag during gestation, induces the generation of activated regulatory T cell populations. To this end, we examined CBMCs from neonates born to mothers with differing histories of malaria living in a region of Gabon where malaria is endemic. Experiments conducted both ex vivo and in vitro demonstrated that placental *P. falciparum* infection is associated with enhanced regulatory CD4+ cell activity that suppresses both effector T cell responses and major histocompatibility complex (MHC) class I and II expression on monocytes.

**SUBJECTS, MATERIALS, AND METHODS**

**Study population.** The study was performed at the Albert Schweitzer Hospital in Lambaréné, Gabon (a site with perennial transmission of *P. falciparum*; entomological inoculation rate, 50) [19], from May to December 2003, and informed consent for participation was obtained before subjects were included in the study. Venous umbilical cord blood from 54 neonates was obtained immediately after birth. The collection of cord blood involved direct aspiration via puncture of the ethanol-sterilized umbilical vein at a site distal to the placenta, to reduce to a minimum the possibility of cross-contamination by maternal lymphocytes. The presence of *P. falciparum* in cord blood, placental blood, and maternal venous blood was determined by microscopic examination of Giemsa-stained thick and thin smears. The mothers’ medical records were examined to identify those who had received a diagnosis of and been treated for malaria during pregnancy. Most women who had malaria during pregnancy had been treated with quinine, which has been reported to retain 100% efficacy for the treatment of uncomplicated *P. falciparum* malaria arising during pregnancy in the study area [20]. For comparative analyses, the following distinct groups were thus defined on the basis of the presence or absence of placental *P. falciparum* infection by blood smear and of assessment of documented medical history: (1) negative, no evidence of *P. falciparum* parasites in the placental and maternal venous blood at delivery and no record of maternal malaria during pregnancy; (2) placenta positive, *P. falciparum* asexual-stage parasites present in the placental blood; and (3) treated, no evidence of *P. falciparum* parasites in the placental and maternal venous blood at delivery but a recorded history of a diagnosed and treated maternal episode of malaria during pregnancy, at least 2 weeks before delivery. None of the cord blood samples in the study had detectable levels of parasitemia. Table 1 provides a summary of the study population. Ethical clearance for the study was given by the ethics committee of the International Foundation of the Albert Schweitzer Hospital in Lambaréné.

**Blood-stage parasite Ag (pRBC lysate) preparation.** pRBC lysate was prepared from schizont-infected erythrocytes using a local *P. falciparum* isolate cultured and synchronized by temperature cycling through 37°C, 40°C, and 17°C. Schizont-infected erythrocytes or uninfected erythrocytes (RBC lysate) were Percoll purified, resuspended in RPMI 1640, and frozen and thawed 1 time before addition to the culture.

**Isolation of CBMCs and cell culture.** CBMCs were isolated by standard density-gradient centrifugation at 800 g on Ficoll-Paque (Amersham). For ex vivo surface-marker analysis, cells were immediately resuspended in 2% paraformaldehyde/PBS and stored at 4°C. For in vitro studies, cells were immediately resuspended at 5 × 10^6 cells/mL in complete medium, and 0.5 μg/mL of both anti–human-CD28 and -CD49d antibodies (BD Biosciences) was added for the acquisition of optimal T cell responses [21]. Complete medium was composed of RPMI 1640 medium (Sigma) supplemented with 10% (wt/vol) human serum (AB; Sigma), 1 mM L-α-aminoglutamine (Life Technologies), 100 U/mL penicillin, 100 μg/mL streptomycin (Life Technologies), and 1 mM sodium pyruvate (Life Technologies). Antibodies specific for human CD3 (HIT3a, 10 μg/mL; BD Biosciences), uninfected red blood cells (uRBC lysate), or pRBC lysate (1 CBMC:10 RBCs) were added to the cells, followed by incubation at 37°C in a humidified 5% CO₂ atmosphere. For analysis of cytokines in culture supernatants, cells were incubated for 48 h, and harvested supernatants were stored at −80°C. For intracellular cytokine analysis, 10 μg/mL brefeldin A (Sigma) was added after 18 h of culture, and the cells were incubated for an additional 4 h before fixation. Cells were washed in PBS, fixed with 2% formaldehyde for 20 min, resuspended in Hanks’ balanced salt solution/0.1% azide/0.1% bovine serum albumin (BSA), and stored at 4°C before staining for flow-cytometric analysis.

For CD4+CD25+ depletion studies, 35 × 10^6 CBMCs were resuspended in MACS buffer (PBS, 2 mM/L EDTA, and 0.5% 

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Negative (n = 20)</th>
<th>Placenta positive (n = 20)</th>
<th>Treated (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of mother, years</td>
<td>26.6 ± 7.1</td>
<td>21.8 ± 4.9b</td>
<td>23.5 ± 6.4</td>
</tr>
<tr>
<td>P. falciparum infections</td>
<td>4.2 ± 3.6</td>
<td>3.1 ± 2.7</td>
<td>2.4 ± 2.1</td>
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<tr>
<td>Hemoglobin level, %</td>
<td>14.3 ± 1.7</td>
<td>13.6 ± 3.2</td>
<td>14.8 ± 1.9</td>
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*NOTE.* Values are geometric mean ± SD, unless noted otherwise. In the treated group, the median (range) no. of days from treatment to delivery was 92.5 days (14-203 days).

b *P* < 0.05, compared with the negative group.
human serum albumin or BSA). CD4+ cells were collected using anti-CD4 microbeads and a Mini-MACS magnetic separation column (Miltenyi Biotec), in accordance with the manufacturer’s instructions, followed by enzymatic cleavage and removal of the anti-CD4 microbeads. The washed CD4+ cell population was then fractionated into CD25+ and CD25− subsets using anti-CD25 microbeads (Miltenyi Biotec). The washed CD4+CD25− fraction was added back to the CD4− CBMC preparation. Of the 20 paired samples analyzed, flow-cytometric analysis of CBMCs and CD4+CD25+–depleted CBMC samples showed that the median percentage of CD4+ cells expressing CD25 before depletion was 9.3% (interquartile range [IQR], 5.3%); after depletion, it was 1.2% (IQR, 0.82%)—an overall reduction of 87.1% from the CD4+ cell population. The median percentage of CD25+CD4+ cells in CBMC cultures was 29.0% (IQR, 7.8%), and that in CD25−CD4+–depleted CBMC cultures was 30.1% (IQR, 6.9%)—a difference that was not statistically significant ($P = .5$, Mann-Whitney U test).

Measurement of cytokine production by ELISA and of intracellular cytokine production and surface markers by flow-cytometric analysis. ELISA was used in accordance with the manufacturer’s recommendations (Biosource) for the detection of IL-2, IL-10, and IFN-γ in 48-h culture supernatants. For intracellular cytokine and cell surface marker analyses, cells were incubated on ice for 15 min with PBS/10% fetal calf serum (FCS) that contained 2 μL of FcR Blocking Reagent (Miltenyi Biotec), followed by staining with the following panel of fluorescently labeled antibodies (all supplied by BD Biosciences): CD3 (UCHT1), CD4 (SK3), CD8 (RPA-T8), CD25 (M-A251), CTLA-4 (BN13), CD14 (M5E2), HLA-A,B,C (G46-2.6), and HLA-DR (L243); or isotype control antibodies mouse IgG1 conjugated to fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin-chlorophyll-protein (PerCP), or allophycocyanin (MOPC-21) and mouse IgG2a conjugated to FITC, PE, or PerCP (G155-178). For the intracellular staining of IFN-γ and IL-10, the Cytofix/Cytoperm Plus kit (BD Biosciences) was used in accordance with the manufacturer’s protocol with the antibodies IFN-γ–FITC (25723.11) or IL-10–PE (JES3-9D7) or the isotype control antibodies mouse IgG1-FITC (MOPC-21) and mouse IgG2a–PE (G155-178). Flow-cytometric analysis was performed on a FACSscan flow cytometer with CellQuest data analysis software (version 3.3; BD Biosciences). A mean of ~150,000 viable cells was gated.

Statistical analyses. The significance of differences between continuous variables was assessed using the Kruskal-Wallis test. If the Kruskal-Wallis test gave $P < .05$, the Mann-Whitney U (2 group) test was performed with the level of statistical significance in all cases set at $P < .05$.

RESULTS

Placental P. falciparum infection and increased frequencies of CD4+ cells expressing CD25 and CTLA-4 in cord blood. We wished to determine whether placental P. falciparum infection induces T cell activation in utero. The presence of CD25 and CTLA-4 on T cells is generally associated with the extent of activation of both effector and regulatory T cells [10, 22–24]. We compared the percentages of CD4+ cells expressing CD25, CD25+, and CTLA-4 in freshly isolated CBMCs from the 3 groups of neonates described in Subjects, Materials, and Methods: negative (n = 20), placenta positive (n = 20), and treated (n = 14). Representative flow-cytometric analysis data (expressed as dot plots in figure 1A) show that CBMCs from a placenta-positive neonate contained discrete populations of CD4+ cells expressing CD25 (10.43%) and CD25+ (1.90%) and coexpressing CD25 and CTLA-4 (0.71%), whereas CBMCs from a negative neonate contained relatively reduced percentages of CD4+ cells expressing CD25 (6.10%) and no CD4+ cells coex-
pressing detectable levels of CD25 and CTLA-4 or CD25hi. A comparison of the samples, segregated according to maternal history of *P. falciparum* infection, showed that CBMCs from the placenta-positive neonates contained significantly greater median percentages of CD4+ cells expressing either CD25 (figure 1B) or CD25hi (figure 1C) or coexpressing CD25 and CTLA-4 (figure 1D) than did CBMCs from negative neonates. The percentages of CD4+ cells expressing CD25 or CD25hi in CBMCs from treated neonates were higher than those of negative neonates, although these differences were not statistically significant (figure 1B and 1C).

**Production of IL-10 by cord blood CD4+ cell subsets in response to *P. falciparum* Ag.** In an earlier study, we found that neutralizing anti–IL-10 antibody increased *P. falciparum* Ag–specific IFN-γ responses from cord blood T cells [7]. In the present study, we detected elevated concentrations of IL-10 in the culture supernatants of *P. falciparum* Ag–stimulated CBMCs from placenta-positive neonates, compared with those from negative and treated neonates (figure 2A). Nonspecific stimulation induced significantly higher IL-10 production by CBMCs from both placenta-positive and treated neonates, compared with those from negative neonates (figure 2B). To clarify the source of IL-10 in CBMC samples, we performed flow-cytometric analysis with gating on CD4+ cells and segregated the source of IL-10 within CBMCs from placenta-positive neonates as the population with the highest median percentage of *P. falciparum* Ag–specific IL-10+CD4+CD25+ cells in CBMCs (figure 2C). Nonspecific stimulation of CBMCs with anti-CD3 plus anti-CD28 antibody revealed a similar profile (figure 2D). Figure 2E shows a representative flow-cytometric analysis dot plot of IL-10 production from uRBC lysate–stimulated, pRBC lysate–stimulated, and anti-CD3 plus anti-CD28 antibody–stimulated CD4+ cells that were gated on CD25+ (upper panels) and CD25– (lower panels) populations. CTLA-4 was expressed both on CD4+CD25+ and CD4+CD25– cells, as well as on IL-10+ and IL-10– CD4+ cells (figure 2F). After stimulation with pRBC lysate, a majority of IL-10+CD4+CD25+ cells expressed CTLA-4 (median, 78%; IQR, 14.1%).

**Depletion of CD4+CD25+ cells from freshly isolated CBMCs in vitro reduction in IL-10 production and increase in IFN-γ production.** Given that CBMCs from placenta-positive neonates contained increased percentages of CD4+CD25+ cells, a proportion of which produced IL-10 in response to *P. falciparum* Ag, we explored the effect of their depletion on *P. falciparum* Ag–specific IL-10 and IFN-γ production. The depletion of CD4+CD25+ cells before CBMC culture significantly reduced the concentration of IL-10 in culture supernatants that was detectable after stimulation with *P. falciparum* Ag (figure 3A) and also reduced the median percentage of *P. falciparum* Ag–specific IL-10+CD4+CD25+ cells in CBMCs from placenta-positive neonates (figure 3B). We speculate, therefore, that the *P. falciparum* Ag–specific IL-10+CD4+CD25+ cells that we detected either expressed CD25 before stimulation with *P. falciparum* Ag or that the differentiation of CD4+CD25+ cells into IL-10+CD4+CD25+ cells after incubation with *P. falciparum* Ag required the presence of CD4+CD25+ cells. The depletion of CD4+CD25+ cells before culture significantly increased the median concentrations of *P. falciparum* Ag–specific IFN-γ in culture supernatants from both placenta-positive and treated neonates (figure 3C). Accordingly, CD4+CD25+ cell depletion also increased the percentages of IFN-γ+CD8+ and IFN-γ+CD8+ T cells (figure 3D), although these differences were not of statistical significance. These data clearly imply the existence, within CBMCs from neonates presumed to have been sensitized as a result of maternal *P. falciparum* infection, of a population of in utero–induced *P. falciparum* Ag–specific IL-10+CD4+CD25+ cells that modulate *P. falciparum* Ag–specific T cell IFN-γ activity.

**Suppression of CD25 expression on CD8+ T cells by CD4+CD25+ cells.** Because CD4+CD25+ cells were found to suppress the *P. falciparum* Ag–specific IFN-γ responses of CD8+ T cells, we postulated that the activation marker CD25 on CD8+ T cells might also show signs of modulated surface expression. The removal of CD4+CD25+ cells before CBMC coculture increased the median percentage of *P. falciparum* Ag–specific CD25hiCD8+ cells in CBMCs from both placenta-positive and treated neonates (figure 4A). Figure 4B is an example of a flow-cytometric analysis dot plot showing CD25 expression on CD8+ T cells after overnight coculture of CBMCs with uRBC lysate or pRBC lysate in the presence or absence of CD4+CD25+ cells. Given that CD25 expression is regulated by IL-2, we measured the concentration of anti-CD3 plus anti-CD28 antibody–driven IL-2 in CBMC culture supernatants and found that those from placenta-positive neonates contained significantly less IL-2 than that found in supernatants from treated neonates (figure 4C). Little *P. falciparum* Ag–specific IL-2 was detected in the culture supernatants from any of the samples (data not shown).

**CD4+CD25+ cell suppression of MHC class I and II expression on monocytes during stimulation with *P. falciparum* Ag.** Both IL-10 and CD4+CD25+ regulatory cells have been shown to suppress the expression of costimulatory molecules on DCs and monocytes, which impairs their function and their ability to activate T cells [25–27]. Similar to observations that we have previously reported [7], MHC class I and II expression on monocytes was reduced in response to stimulation with pRBC lysate relative to that in uRBC lysate–stimulated cultures (100 × pRBC mean fluorescence intensity [MFI] – uRBC MFI/uRBC MFI) in CBMCs from placenta-positive neonates (figure 5A). The depletion of CD4+CD25+ cells before CBMC coculture with pRBC lysate led to significantly increased levels of MHC class I and II on monocytes from placenta-positive neonates, although not on monocytes from negative and treated neonates.
Figure 2. Association of placental *Plasmodium falciparum* infection with an increased frequency of interleukin (IL–10) $^{+}$CD4$^{+}$CD25$^{+}$ cells. *P. falciparum* antigen (Ag)–specific (A and C) or anti-CD3 plus anti-CD28 antibody–driven (B and D) IL–10 from cord blood mononuclear cell culture supernatants (A and B) and the percentages of IL–10 $^{+}$CD4$^{+}$CD25$^{+}$/CTLA-4$^{+}$ cells of CD4$^{+}$ cells (C and D) among cord samples were segregated according to maternal *P. falciparum* infection history. E, Representative fluorescence-activated cell sorting dot plots of IL–10 production by CD4$^{+}$CD25$^{+}$/CTLA-4$^{+}$ cells from a placenta-positive neonate. Sample sizes: for IL–10 levels in supernatants, $n = 20, 20$, and 14 for negative, placenta-positive, and treated neonates, respectively; for analysis of intracellular IL–10, $n = 12, 14$, and 12 for negative, placenta-positive, and treated neonates, respectively. Box plots illustrate medians with 25th and 75th percentiles and whiskers for 10th and 90th percentiles. Net responses are values obtained for blood-stage parasite Ag (pRBC) lysate–stimulated cells minus values obtained for uninfected red blood cell (uRBC) lysate– or medium-stimulated cells. * $P < .05$, Mann-Whitney *U* test.
Figure 3. Depletion of CD4^+CD25^+ cells from cord blood mononuclear cells (CBMCs) before in vitro culture and alterations in *Plasmodium falciparum* antigen (Ag)–specific interleukin (IL)–10 and interferon (IFN)–γ production. *P. falciparum* Ag–specific IL-10 and IFN-γ responses from CBMCs cultured with or without CD4^+CD25^+ cells: A, concentration of *P. falciparum* Ag–specific IL-10 detected in culture supernatants; B, percentages of *P. falciparum* Ag–specific IL-10^+^CD4^+^CD25^−^ and IL-10^+^CD4^+^CD25^+^CD4^−^ cells; C, concentration of *P. falciparum* Ag–specific IFN-γ detected in culture supernatants; and D, percentages of *P. falciparum* Ag–specific IFN-γ^+^CD4^+^ and IFN-γ^+^CD8^+^ cells of respective T cell populations. For CD4^+^CD25^+^ depletion experiments, n = 6, 8, and 6 negative, placenta-positive, and treated neonates, respectively. *P < .05, Mann-Whitney U test.

(figure 5A). Figure 5B is an example of a flow-cytometric analysis histogram showing MHC class I expression on monocytes from a placenta-positive neonate after coculture with uRBC lysate or pRBC lysate in the presence or absence of CD4^+^CD25^+^ cells. These data show that *P. falciparum* Ag–specific CD4^+^CD25^+^ cells can modulate in vitro MHC expression on CBMCs that potentially contributes to the modulated *P. falciparum* Ag–specific proinflammatory T cell activity that we detected.

**DISCUSSION**

We present evidence that a subset of CD4^+^ cells suppress *P. falciparum* Ag–specific Th1 and CD8^+^ T cell IFN-γ responses and MHC class I and II expression on CBMCs from neonates who were exposed to *P. falciparum* Ag as a result of placental *P. falciparum* infection. Immune suppression was associated with IL-10 production and was dependent on the presence of CD4^+^CD25^+^ cells during in vitro culture. The role and effectiveness of CD8^+^ T cells on asexual blood-stage parasites is likely to be limited, compared with that of CD4^+^ cells, but it has been speculated that they may contribute to parasite clearance through IFN-γ production [28]. We found that CD8^+^ T cell responses—both IFN-γ production and CD25 surface expression—were enhanced in the absence of CD4^+^CD25^+^ cells during culture with Ag. Other researchers have shown that Ag-induced CD4^+^CD25^+^ regulatory cells suppress CD8^+^ T cell responses by inhibiting the up-regulation of CD25 and IL-2 production [29]. There was significantly less anti-CD3 plus anti-CD28–driven IL-2 detected in CBMC culture superna-
Figure 4. CD4+CD25+ cells and inhibition of CD25 expression on CD8+ T cells. A, Plasmodium falciparum antigen (Ag)–specific CD8+CD25+ cell percentages of CD8+ T cells from cord blood mononuclear cells (CBMCs) cultured in the presence or absence of CD4+CD25+ cells. B, Representative fluorescence-activated cell sorting dot plots showing the percentages of CD8+ T cells expressing CD25 and CTLA-4 after CBMC coculture with uninfected red blood cell (uRBC) lysate or blood-stage parasite Ag (pRBC) lysate and in the presence or absence of CD4+CD25+ cells. Dot plots show cells that were previously gated on CD8+ T cells. C, Net concentrations of interleukin (IL)–2 measured in supernatants from cultures of CBMCs stimulated for 48 h with anti-CD3 plus anti-CD28 antibody. *P < .05, Mann-Whitney U test.

Under normal culture conditions, P. falciparum Ag–specific IFN-γ responses were highest in CBMCs from treated neonates. Although IFN-γ responses were highest in treated neonates, we also found evidence of CD4+CD25+ cell–mediated suppression of these responses. The results from treated neonates were less homogeneous than those observed in placenta-positive neonates. We postulate that the duration and intensity of placental infection in treated neonates—variables that are impossible to measure before delivery—are likely to have been associated with effector and regulatory T cell activity. Ag-induced regulatory T cells require the presence of chronic low levels of Ag and have a relatively limited effect in the absence of persistent Ag [30]. The homogeneous and relatively reduced profile of cytokine responses observed in CBMCs from negative neonates is a good indication that parasitemia below the limit of detection of blood-smear analysis is too low to induce immune sensitization in the fetus. It should be noted that CBMCs from treated neonates, like those from placenta-positive neonates, contained elevated percentages of CD4+ cells expressing CD25hi that may also have imposed regulatory activity in an IL-10–independent manner [31], although perhaps not as strongly as activated IL-10+ regulatory cells isolated from placenta-positive neonates.

To date, the identification of both natural and Ag-induced CD4+ regulatory cells by surface phenotyping has relied, in part, on the presence of CD25 and CTLA-4, because regulatory cells are enriched in this population. Thymic-derived CD4+CD25+ regulatory cell activity is associated with expression of the transcription factor Scurfen, which is encoded by the forkhead/winged helix (foxp3) gene [32, 33]. We measured foxp3 gene expression using real-time polymerase chain reaction with mRNA
purified from freshly isolated CBMCs from 15 neonates and found similar levels of foxp3 mRNA among the 3 groups (relative to the ribosomal 18s RNA gene) (data not shown). Furthermore, foxp3 expression was up-regulated in some CBMCs in response to culture with pRBC lysate, irrespective of maternal P. falciparum infection history. Other researchers have also reported a disassociation between foxp3 expression and Ag-induced regulatory T cell activity [22, 34]. The current capacity to differentiate thymic-derived from Ag-induced regulatory T cells in humans is limited. It has been proposed that the expression of CD25hi by CD4+ cells distinguishes thymic-derived regulatory T cells [35]; however, we observed a higher percentage of circulating CD4+CD25hi cells in cord blood samples from neonates born to P. falciparum–infected mothers, compared with those born to uninfected mothers. Cancer patients with advanced disease have a significantly higher percentage of CD4+CD25hi cells in their lymph nodes than did patients with early or no disease [36]. These observations suggest that CD4+CD25hi cells expand in the periphery during the course of infection or disease progression. The separation of CD25lo- and CD25hi-expressing CD4+ cells using flow-cytometric analysis rather than by magnetic bead–based methods would provide more-detailed information on the precise cell subset that is responsible for the Ag-driven suppressive effects that we observed. Although our data showed that the presence of IL-10 and CD4+CD25+ cells induced a degree of suppression on T cell IFN-γ responses and MHC expression on monocytes in response to P. falciparum Ag, we did not exclude the contribution of other regulatory cells—such as thymic-derived CD4+CD25+ regulatory cells [37], CD8+ T cells [38], or cytokines such as transforming growth factor–β [39]—in controlling inflammatory responses to P. falciparum Ag.

In conclusion, we provide evidence that cord blood from neonates whose mothers were infected with P. falciparum during pregnancy contains an expanded population of Ag-specific IL-10+CD4+CD25+CTLA-4+ regulatory cells that suppress in vitro P. falciparum Ag-specific Th1-type and CD8+ T cell IFN-γ responses and that down-regulate MHC expression on monocytes. Thus, although potentially protective P. falciparum–specific immune responses are initially induced in the fetus, these responses can be restricted by regulatory T cell activity. If so, controlling the induction of regulatory T cell responses may increase beneficial effector T cell responses against P. falciparum infection that would reduce parasitemia and associated illness in children at risk of infection early in life.

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