Clin Exp Immunol 1997; **107**:462–467

Immune response to *Plasmodium falciparum* antigens in Cameroonian primigravidae: evolution after delivery and during second pregnancy

N. FIEVET*†, M. COT†, P. RINGWALD†, J. BICKII†, B. DUBOIS*, J. Y. LE HESRAN†, F. MIGOT* & P. DELORON* *Institut National de la Santé et de la Recherche Médicale (INSERM) Unité 13 and Institut de Médecine et d'Épidémiologie Africaines (IMEA), CHU Bichat, Paris, France, and †Institut Français de Recherche Scientifique pour le Développement en Coopération (ORSTOM) and Laboratoire de Recherche sur le Paludisme, Organization de Coordination pour la Lutte contre les Endémies en Afrique Centrale (OCEAC), Yaoundé, Cameroon

(Accepted for publication 22 November 1996)

SUMMARY

Mechanisms responsible for the increase in malaria susceptibility during pregnancy, and in particular during the first pregnancy, have not been elucidated. T and B cell responses to leucoagglutinin, bacille Calmette–Guérin (BCG) and to six *Plasmodium falciparum* antigens were longitudinally investigated in 33 pregnant women during their first pregnancy, after delivery, and during second pregnancy. Parasitological data obtained from the same women during and after the first pregnancy demonstrated the higher risk of *P. falciparum* infection during this pregnancy. Plasma levels of antibodies to Pf155/RESA were lower during pregnancy than after delivery. Conversely, antibodies to *P. falciparum* asexual blood stages were higher during pregnancy than after delivery, suggesting that during pregnancy the regulation of antibody production may be variously impaired depending upon the antigens. The most striking finding of the present study is the impairment of the IL-2 cellular response during the first pregnancy. Conversely, proliferative responses, as well as IL-4 and interferon-gamma (IFN-γ) responses, were either unaffected or moderately enhanced. No difference in humoral and cellular responses was observed between first and second pregnancy. The impairment of the IL-2 responses involved the response to malaria peptides and proteins, as well as the response to non-malarial antigens and to the mitogen leucoagglutinin. Thus, the alteration of malaria immunity might rather fall into the general frame of the depression of cellular immunity during pregnancy than involve a specific malaria phenomenon.

Keywords malaria *Plasmodium falciparum* pregnancy immunity

INTRODUCTION

Malaria during pregnancy is a priority within malaria research and control. The increased risk of malaria in pregnant women is associated with fetal abortions, stillbirths and placenta infection, the latter being responsible for low birth weight of the offspring. Several epidemiological studies demonstrated these effects are most frequent and marked in primigravidae [1,2]. Hitherto, the mechanisms responsible for this increase in malaria susceptibility during pregnancy, and in particular during the first pregnancy, have not been elucidated. Indeed, pregnancy has been shown to be associated with a general immunosuppression [3], being more marked in primigravidae [4]. Pregnancy is characterized by a transient depression of cell-mediated immunity of the mother that allows fetal allograft retention, but also interferes with resistance of the mother to various infectious diseases. Recently, experimental evidence indicated that the immune system of the pregnant woman is biased towards antibody production and away from cell-mediated immunity [5–7]. It has been suggested that Th2-type cytokines may inhibit Th1 responses, protecting the fetus from rejection [7,8], but also increasing the incidence of several infectious diseases that are usually under the control of $CD4^+$ T lymphocytes [3].

In a preliminary study, we showed that cells from primigravidae exhibited a reduced *in vitro* proliferative response to *Plasmodium falciparum* crude schizont extract and *Mycobacterium bovis* antigens, compared with cells from non-pregnant women. Moreover, the IL-2 *in vitro* production in response to Pf155/RESA, a major *P. falciparum* antigen [9], was reduced. IL-4 and interferon-gamma (IFN-γ) *in vitro* production did not appear to be affected in primigravidae [10,11].

M. C. present address: ORSTOM, BP434, Antananarivo, Madagascar.

F. M. present address: Centre International de Recherche Médicale, BP 769, Franceville, Gabon.

Correspondence: Philippe Deloron, INSERM U13/IMEA, Hôpital Bichat-Claude Bernard, 46 Rue Henri Huchard, 75018 Paris, France.

© 1997 Blackwell Science

To further dissect the immunological components of this system and to explore the mechanisms responsible for the increased susceptibility of primigravidae to *P. falciparum*, we designed a longitudinal study that allowed us to assess T and B cell response parameters in the same individuals. We enrolled a cohort of primigravidae from a semi-rural area of Cameroon at 6 months of pregnancy and followed them until next pregnancy to investigate the evolution of their T and B cell responses to *P. falciparum* antigens.

SUBJECTS AND METHODS

Subjects

The study took place in Ebolowa, a town of 35 000 inhabitants located 160 km south of Yaounde, Cameroon, in the rain forest area where *P. falciparum* malaria is hyperendemic with perennial transmission. Each individual receives a mean 62 *P. falciparum*-infected mosquito bites per year. The survey was conducted on 33 primigravidae who were enrolled with their informed consent at the first visit in the antenatal clinic and were followed during and after pregnancy. Assessment of humoral and cellular immune responses was performed three times: at 6 (± 2) months of first pregnancy, at 6 (± 2) months after delivery, and at 6 (± 2) months of the second pregnancy. For this purpose, 10 ml heparinized venous blood samples were drawn at each instance.

Haematological measurements

At each blood sampling, whole blood cell count and haemoglobin measurements were done. Malaria parasites were searched for on Giemsa-stained thick blood smears against 1000 leucocytes.

Antigens

A crude preparation of asexual falciparum components was obtained by sonication of an *in vitro* culture of the Palo Alto strain of *P. falciparum* (35% parasite density, 55% late stages). The resulting preparation, referred to as schizont antigen, was used at a final concentration of 5 μg/ml.

Pf155/RESA has long been considered a major candidate for a vaccine against blood stages of *P. falciparum* [12]. Numerous studies led to extensive knowledge of the protein, including the identification of epitopes [12,13], and characterization of antibodies [14,15]. One major T and B cell epitope is located in the 3′ repeat region with the repeat sequence EENVEHDA [12]. Other T and/or B cell epitopes have been identified, among which the best immunogenic are SLRWIFKHVAKTHLK, IVGYIMHGISTINTEMK [13], and NADMNEITERYFKLAENYY [16].

The response to Pf155/RESA was tested using synthetic peptides. Six synthetic peptides, P1 to P6, reproducing parts of the Pf155/RESA molecule, were used for the measurement of humoral and cellular responses. These peptides were obtained from the Emory University Protein Structure Facility (Atlanta, GA) and Bachem Co. (Bubendorf, Switzerland). Their structure and localization are shown in Table 1. Peptides P1 and P2 reproduced an overlapping T and B cell epitope, and thus were used either to measure cellular (P1) or humoral (P2) response. The other four peptides reproduced both a T and B cell epitope, and were used in both measurements. Antibody titres to peptides P2, P3, P4, P5 and P6 were measured by FAST-ELISA. Peptides P1, P3, P4, P5 and P6 were used to assess the lymphocyte response at a final concentration of 1 μM.

Table 1. List of synthetic peptides

No.	Residues*	Sequence†	Epitope
P1	181–195	LGRSGGDIIKKMQTL	T
P2	192–206	MQTLWDEIMDINKRK	B
P3	533–551	NADMNEITERYFKLAENYY	T + B
P4	700–714	SLRWIFKHVAKTHLK	T + B
P5	862–878	IVGYIMHGISTINTEMK	T + B
P6	944–959	$(EENVEHDA)_4$	T + B

* Position according to [17].

† The sequences are given in one-letter code.

Cellular responses to non-malarial proteins were also assessed. A fraction (referred to as BCG 45/47) highly enriched in a proline-rich 45/47-kD antigen complex from *M. bovis* eliciting DTH reactions in immunized guinea pigs [18] was used at a final concentration of 10 μg/ml. Bacille Calmette–Guérin (BCG) was used as a recall antigen as the BCG vaccine is included in the Cameroon Extended Programme of Immunization (EPI), and as epidemiological studies have shown that more than 90% of adults present with a positive tuberculin reaction (unpublished data). Leucoagglutinin (Sigma, St Louis, MO) was used at a final concentration of 10 μg/ml.

Antibody measurement

Plasma anti-*P. falciparum* antibodies were measured using a conventional immunofluorescent assay (IFA) (threshold of positivity, a reciprocal titre of 64). Anti-Pf155/RESA antibodies were measured by erythrocyte membrane immunofluorescent (EMIF) assay using a glutaraldehyde-fixed and air-dried monolayer of ring-infected erythrocytes (threshold of positivity, a reciprocal titre of 4) [9]. Anti-peptide antibodies were tested by FAST-ELISA using peptides conjugated to bovine serum albumin (BSA), as described [19].

Lymphocyte proliferative assay

Within 16 h after bleeding, peripheral blood mononuclear cells (PBMC) were isolated on Ficoll–Paque (Pharmacia, Uppsala, Sweden), and cell viability was confirmed by trypan blue staining. Purified PBMC were suspended at 10^6 cells/ml in buffered RPMI 1640 containing 10% human serum, and 100-μl aliquots were plated in triplicate in round-bottomed 96-well plates. Mitogen, antigens or RPMI alone were added in 100-μl amounts at the indicated concentrations. Plates were incubated at 37°C in a humidified chamber with 5% CO_2. After 6 days, 110 μl culture supernatants were removed and 50 μl fresh medium containing 0·5 μCi methyl-^{3}H-thymidine (specific activity 2 Ci/mmole; Amersham, Les Ulis, France) were added to each well. After an additional 16 h, cells were collected on glassfibre filter paper and radioactivity was counted. Stimulation indices (SI) were calculated by dividing the geometric mean ct/min of antigen-stimulated cultures by the geometric mean ct/min of unstimulated cultures. Threshold of positivity for all antigens was a SI $\geqslant$ 2·5 [20].

In vitro *production of IFN-γ, IL-2 and IL-4*

The 6-day culture supernatants from each triplicate were pooled and stored at −80°C. IFN-γ was assayed by a commercial two-site ELISA according to the manufacturer's instructions (Mabtech, Roslagvägen, Sweden). Absorbance was read at 405 nm. IFN-γ concentrations were determined by reference to human IFN-γ standard (Gg 23-901-530, 4000 U/ampoule; National Institute of Allergy and Infectious Diseases, National Institute of Health,

Table 2. Haematological and parasitological data on 33 women during first pregnancy, 6 months after delivery, and during second pregnancy

	During first pregnancy	After delivery	During second pregnancy
n	33	33	12
Haemoglobin (g/*l*)*	10·3 (9·6–11·1)	12·0 (11·2–12·9)	11·3 (10·6–12·0)
Plasmodium falciparum prevalence	48·5%	15·1%	0%
IFA†	1845 (963–3531)	522 (318–859)	456 (205–1011)
(%+)	(100%)	(100%)	(100%)
EMIF†	10·1 (6–17)	25·1 (15–42)	12 (7–20)
(%+)	(45·5%)	(92·8%)	(91·6%)

* Mean ± s.e.m.
† Geometric mean positive antibody titre (95% confidence interval).

Bethesda, MD). The threshold of sensitivity was 2·5 U/ml. For statistical purposes, values under this threshold were assigned a concentration of 1·25 U/ml. Mitogen- or antigen-induced IFN-γ production was derived from the difference between the IFN-γ content in stimulated cultures and the spontaneous IFN-γ content in unstimulated cultures.

Similar cultures were incubated for 72 h, and triplicate supernatants were pooled and stored at −80°C for assaying IL-2 and IL-4 contents. Cells from an IL-2-dependent mouse cytotoxic T cell line (CTLL2) were used as responder cells. Briefly, 10^4 CTLL2 cells/well were plated in 96-well round-bottomed plates in 100 μl volume and 100 μl of undiluted supernatants were added. After 24 h, cells were pulsed for 12 h with 0·5 μCi ^{3}H-thymidine/well, harvested and processed as previously. Data were expressed as SI as for lymphoproliferation. The threshold of positivity for all antigens was set to an SI $\geqslant$ 1·77 according to the mean + 2 s.d. of IL-2 production by cells from 14 non-immune European donors [21].

IL-4 was assayed by a two-site immunometric assay as previously described [10]. A mouse MoAb (IL4-38) was used as capture antibody while a second MoAb (IL4-3; Fab + -acetylcholinesterase conjugate) was used as tracer antibody. Bound enzyme was detected with Ellman's reagent and absorbance read at 412 nm. IL-4 concentrations were determined by reference to a standard (recombinant IL-4 produced in chinese hamster ovary (CHO) cells, a gift from Dr J. Banchereau, Schering-Plough, Dardilly, France). The threshold of sensitivity was 12 pg/ml. As for IFN-γ, values under this threshold were assigned a concentration of half this value (6 pg/ml). Mitogen- and antigen-induced IL-4 production was calculated as for IFN-γ (IL-4 content in stimulated cultures minus IL-4 content in unstimulated cultures).

Statistical analysis

Differences in prevalence rates between groups were tested by the χ^2 test. Differences between matched pairs of variables (during and after first pregnancy, and during first and second pregnancies) were tested by paired *t*-test or by the Wilcoxon signed rank test. Relations at the individual level were tested by the correlation test. All tests were two-tailed; *P* values $\leqslant$ 0·05 were considered significant. Statview 4.5 (Abacus Concept, Berkeley, CA) was used for all calculations.

RESULTS

All 33 women were followed during their first pregnancy and were studied again 6 (± 2) months after delivery. In addition, 12 of these women were investigated again at 6 (± 2) months of their second pregnancy. Ten other women were followed for 2 years after delivery, but did not become pregnant again during this time. The other women were lost to follow up, most having moved away from the study area. All women were permanently living in the Ebolowa area, thus it is likely that all have been exposed to similar levels of similar *P. falciparum* strains for a long time. No woman was febrile at the time of bleeding, although several presented with *P. falciparum* parasitaemia.

Comparison of women during and after first pregnancy

Haematological and parasitological data are given in Table 2. Haemoglobin levels were lower during the first pregnancy ($P = 0{\cdot}01$). Asexual *P. falciparum* parasitaemia was more frequent in primigravidae than in mothers after delivery (48·5% *versus* 15·1%, $P = 0{\cdot}004$). Parasite density were also higher during pregnancy ($P = 0{\cdot}02$).

All serum samples were positive for *P. falciparum* antibodies (by IFA), whereas the prevalence rates of anti-Pf155/RESA antibodies, as detected by EMIF, were lower during first pregnancy than after delivery ($P < 0{\cdot}0001$) (see Table 2). Mean titres of antibodies to *P. falciparum* and P4 were higher during the first pregnancy (both $P < 0{\cdot}05$), whereas those to Pf155/RESA, P2, P3, P5 and P6 were lower during pregnancy (the difference being significant for Pf155/RESA, P3 and P5; all $P < 0{\cdot}05$) (Table 3).

In vitro proliferative responses were measured after 7 days of *in vitro* culture. Cytokine production was assayed in 3-day (IL-2 and IL-4) or 6-day (IFN-γ) supernatants. Results of these assays

Table 3. Humoral responses to *Plasmodium falciparum* antigens in 33 women during first pregnancy and 6 months after delivery

	During pregnancy	After delivery	Paired *t*-test*
P2	0·355 ± 0·027	0·426 ± 0·035	0·002
P3	0·103 ± 0·007	0·113 ± 0·071	0·4
P4	0·206 ± 0·018	0·179 ± 0·014	0·01
P5	0·077 ± 0·004	0·119 ± 0·007	< 0·0001
P6	0·185 ± 0·024	0·211 ± 0·034	0·5

* *P* value of the paired *t*-test.

Data are expressed as arithmetic mean antibody titres (± s.e.m.). Peptides are designated according to Table 1.

Table 4. Proliferative and IL-2 responses to leucoagglutinin, bacille Calmette–Guérin (BCG), and *Plasmodium falciparum* antigens in 33 women during first pregnancy and 6 months after delivery

	LPA				IL-2			
	During pregnancy		After delivery		During pregnancy		After delivery	
Leucoagglutinin	100%	41·6 (24·4–71·2)	97%	39·2 (26·8–57·5)	73%	33·3 (13·2–83·9)	79%*	124·7 (66·4–234·1)**
BCG	70%	12·1 (7·5–19·6)	36%*	13·8 (7·8–24·4)**	61%	3·9 (2·9–5·3)	54%	6·5 (3·7–11·4)
Schizont	18%	8·4 (3·9–18·0)	33%	12·0 (6·5–22·0)	24%	2·9 (1·8–4·7)	39%*	3·1 (2·3–4·3)
P1	6%	4·0 (1·9–8·4)	15%	4·2 (3·2–5·7)	13%	2·0 (1·8–2·3)	30%*	2·4 (2·0–2·7)**
P3	14%	4·8 (2·7–8·4)	6%	3·3 (3·3–3·4)**	14%	2·7 (1·6–4·5)	25%	3·0 (2·2–4·0)
P4	10%	3·9 (2·6–5·9)	12%	3·9 (3·1–4·9)	10%	2·0 (1·9–2·1)	36%*	6·6 (4·4–10·0)**
P5	7%	2·7 (2·5–2·9)	15%	6·3 (2·5–15·9)	24%	2·1 (1·8–2·4)	27%	2·7 (2·1–3·4)
P6	9%	3·1 (2·7–3·5)	12%	8·3 (3·0–22·9)	19%	2·2 (1·9–2·6)	12%	7·4 (2·9–19·0)

* $P < 0{\cdot}05$ (χ^2 test); ** $P < 0{\cdot}05$ (Wilcoxon signed rank test).

Peptides are designated according to Table 1. Data are expressed as percentage of responders and geometric means (95% confidence intervals) of stimulation indices (stimulation index = (ct/min of stimulated cultures)/(ct/min of unstimulated cultures)) of responders.

are shown in Fig. 1 and Table 4. Most of the differences between the cellular responses of women during and after their first pregnancy concerned the IL-2 response, which was highly reduced during the first pregnancy. Indeed, the prevalence rates of the IL-2 response to all antigens tested were lower during pregnancy than after delivery, the difference being significant for leucoagglutinin, schizont, and two peptides from Pf155/RESA (P1 and P4) (all $P < 0{\cdot}05$). Similarly, the mean production of IL-2 in response to leucoagglutinin, P1 and P4 were lower during pregnancy than after delivery (all $P < 0{\cdot}05$). Conversely, the proliferative responses, as well as IL-4 and IFN-γ responses, were either unchanged or marginally enhanced (proliferative response to BCG, IL-4 response to leucoagglutinin and IFN-γ response to BCG and P6) during pregnancy compared with after delivery.

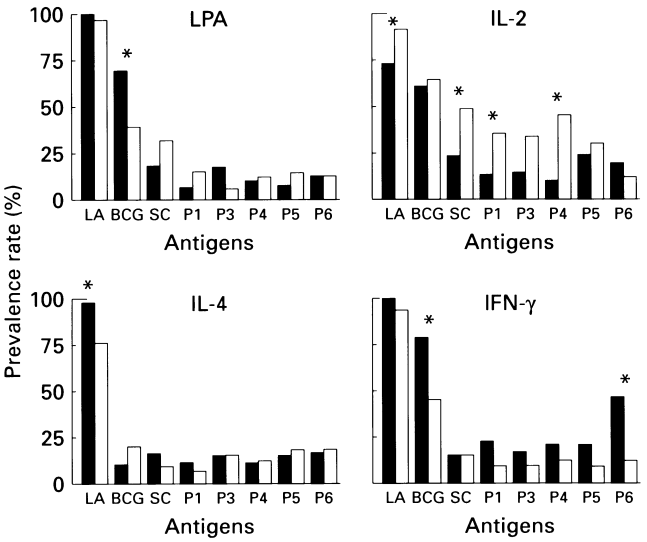

Fig. 1. Percentage of T cell responders to leucoagglutinin, bacille Calmette–Guérin (BCG) 45/47, crude *Plasmodium falciparum* schizont extract, and to derived peptides from Pf155/RESA in 33 Cameroonian women during first pregnancy (■) and 6 months after delivery (□). Peptides are designated according to Table 1. Lymphocyte proliferation and IL-2 production were measured by thymidine incorporation. IFN-γ and IL-4 release were measured by ELISA as the difference between stimulated and unstimulated culture supernatants. For threshold of positive results, see text. *$P < 0{\cdot}05$, χ^2 test.

Induction of proliferative and cytokine responses to schizont extract or peptides were not related in individual donors. Similarly, there was no relation in individual donors between antibody levels and their T cell responses (proliferation and/or cytokine release).

Comparison of women during first and second pregnancies

Haemoglobin levels were similar between the two pregnancies (Table 2). Asexual *P. falciparum* parasitaemia was more frequent in primigravidae than in secondigravidae ($P = 0{\cdot}03$). Mean titre and prevalence rate of antibodies to *P. falciparum* were similar during both pregnancies, whereas the prevalence rate of Pf155/RESA antibodies was higher during the second pregnancy ($P = 0{\cdot}01$). Mean antibody titres to Pf155/RESA, P2, P3, P4 and P6 were similar in both pregnancies. Mean antibody titre to P5 was higher in the second pregnancy ($P = 0{\cdot}006$). IL-2 response to BCG45/47 was lower during the first pregnancy than during the second; this decrease concerned both the prevalence rate ($P = 0{\cdot}04$) and the mean IL-2 index ($P = 0{\cdot}01$). The proliferative, IL-4, IFN-γ responses to all antigens, as well as the IL-2 responses to other antigens, were similar during both pregnancies.

DISCUSSION

We designed a longitudinal survey of the immunological responses to malarial antigens in order to characterize the potential changes that occur during pregnancy. A cohort of primigravidae from a semi-rural area of Cameroon was enrolled and followed after delivery until next pregnancy. This design allowed use of each woman as her own control, to assess better the nature of the immunological parameters engaged in the increased susceptibility to malaria during pregnancy. We assumed that the immunological status of these women evolved from disruption (during pregnancy) to normality (6 months after delivery).

The parasitological data obtained from the same women during and after their first pregnancy clearly demonstrate the higher risk of *P. falciparum* infection during this pregnancy. In addition, the *P. falciparum* infection rate during the first pregnancy was significantly higher than during the second pregnancy. These results are in agreement with previous data indicating the increase of

clinical impact of falciparum malaria during pregnancy [1,2,22], as well as the increased susceptibility of primigravidae compared with multigravidae [1,2,22].

Previous studies made to assay malaria antibody levels in relation to pregnancy have generally failed to yield consistent results. However, most studies reported that antibody levels to falciparum blood stages were either unchanged [2,10] or reduced in pregnant women compared with non-pregnant women [23,24]. Similarly, previous studies that assessed antibody titres to Pf155/RESA demonstrated that these titres were either similar in pregnant and non-pregnant women [10] or were significantly lower in pregnant than in non-pregnant women [24]. In the present study, the humoral response to *P. falciparum* during and after first pregnancy evolved according to two different profiles. Plasma antibody levels to Pf155/RESA, and to the peptides P2, P3, P5 and P6 were lower during pregnancy than after delivery. Conversely, antibodies to *P. falciparum* asexual blood stages and to P4 were higher during pregnancy than after delivery. The higher titres of antibodies to *P. falciparum* asexual blood stages and to the P4 peptide during pregnancy were unexpected and remain unexplained, as such an increase in antibody titres during pregnancy has never been reported. In pregnancy, it is considered that the progressive haemodilution and the transfer of IgG from the maternal to the fetal circulation is responsible for a progressive fall in IgGs. On the other hand, the deviation of immunity towards a Th2-mediated immune response that occurs during pregnancy [3,7] should be responsible for an increase of antibody synthesis, that might counterbalance the fall. In addition, it has been proposed that malaria infection in pregnancy provides an opportunity to develop antibodies to antigens unique to the placental form of the parasite [25]. Indeed, a study in Kenyan pregnant women demonstrated that the anti-*P. falciparum* antibody titre increased with parity, even after adjustment on age [26]. Overall, during pregnancy the regulation of antibody production and the triggering of antibody-secreting cells may be variously impaired depending on the specificity of the antigen. During the second pregnancy, antibody titres to Pf155/RESA and P5 were higher than during the first pregnancy. This difference between successive pregnancies seems related rather to a pregnancy-related phenomenon than to age variation, as the increase in anti-Pf155/RESA antibodies was not paralleled by an increase of other anti-*P. falciparum* antibodies.

The most striking finding of the present study was the impairment of the IL-2 cellular response during the first pregnancy. Conversely, proliferative responses, as well as the IL-4 and IFN-γ responses, were either unaffected or moderately enhanced, suggesting that the corresponding T cell subsets were not altered during pregnancy, and that the immunosuppressive phenomenon that occurs during pregnancy may variously impair the different T cell subsets. No difference in cellular responses was observed between first and second pregnancy. This lack of difference may be related to the fact that all investigated parameters were similarly affected during first and second pregnancy, but this also may be the consequence of the low power of our analysis, given the limited number of women that we have been able to study during both first and second pregnancies.

Antigen-specific suppression of cellular responses has been observed in both acutely ill and asymptomatic malaria-infected individuals [27]. However, in the present study, the cellular and humoral responses of parasitaemic and non-parasitaemic women were similar (data not shown), demonstrating that the suppression of selected immune responses during pregnancy is not primarily due to the higher infection rate of pregnant women. Riley *et al.* [4] suggested that the pregnancy-induced immunosuppression may persist several months after delivery, as they did not observe differences in cellular responses to malaria antigens 14 weeks after delivery. Conversely, a longitudinal study conducted during and after pregnancy showed that the lymphocyte transformation results after stimulation with antigens, such as purified protein derivative (PPD) or *Candida albicans*, were depressed towards the end of pregnancy but were increased again 3–5 months after delivery [28]. In the present study, the immune responses had varied 6 months after delivery, although all women were nursing. Indeed, prompt restoration of resistance to *P. falciparum* infection following parturition has been observed, even in nursing women [29].

The impairment of the IL-2 responses involved the response to malaria peptides and proteins, as well as the response to non-malarial antigens and to the mitogen leucoagglutinin. Thus, the alteration of malaria immunity might rather fall into the general frame of the depression of cellular immunity during pregnancy than involve a malaria-specific phenomenon. This suppression of the IL-2 responses is in agreement with the hypothesis of a transient depression of Th1 immunity during pregnancy [3,4]. However, this suppression of the IL-2 response was not paralleled by a reduction of the IFN-γ response, suggesting that T cells from the Th1 subset might be differentially affected by the pregnancy-related immunosuppression. Similarly, we did not observe a Th2 enhancement of the peripheral cellular response (according to IL-4 *in vitro* production). However, in mice, this enhancement is higher at the maternal–fetal interface than at the peripheral level [5,6]. Unprimed T cells, when first stimulated, proliferate and produce large amounts of IL-2, but no other T cell cytokines. Conversely, T cells producing multiple cytokines are considered to be memory helper T cells. Thus, the response of naive T cells may be more suppressed during pregnancy than that of memory cells. Alternatively, given the role of IL-2 as a growth and differentiation factor for Th1, Th2, and $CD8^+$ cells, it is also possible that the potential for cellular response during pregnancy is limited by the lack of IL-2.

Overall, our results demonstrate the usefulness of longitudinal studies for a better assessment of immune response modifications. Most studies that previously evaluated immune modifications during pregnancy only involved the lymphoproliferation assay. Examining several parameters of T cell activation allows a better evaluation of the ability of lymphocytes to respond to malaria antigens. Further investigations of the alterations of malaria-specific immune mechanisms during pregnancy will be useful in understanding malaria immunity in general.

ACKNOWLEDGMENTS

We thank all the staff and patients of the Ebolowa Hospital for their cooperation. We would also like to thank Jacques Grassi (CEA Service de Pharmacologie et Immunologie, Saclay, France) for the gift of anti-IL-4 antibodies. This work was supported by the French Ministry of Research and Space (grant 92S0034), and by the French Ministry of Cooperation and Development.

REFERENCES

1 Brabin BJ. The risks and severity of malaria in pregnant women. Applied field research in malaria reports, TDR/FIELDMAL/1. Geneva: World Health Organization, 1991.

2 McGregor IA. Epidemiology, malaria and pregnancy. Am J Trop Med Hyg 1984; **33**:517–25.
3 Weinberg ED. Pregnancy-associated depression of cell-mediated immunity. Rev Infect Dis 1984; **6**:814–31.
4 Riley EM, Schneider G, Sambou I, Greenwood BM. Suppression of cell-mediated immune responses to malaria antigens in pregnant Gambian women. Am J Trop Med Hyg 1989; **40**:141–4.
5 Delassus S, Coutinho GC, Saucier C, Darche S, Kourilsky P. Differential cytokine expression in maternal blood and placenta during murine gestation. J Immunol 1994; **152**:2411–8.
6 Lin H, Mosmann TR, Guilbert L, Tuntipopipat S, Wegmann TG. Synthesis of T helper 2-type cytokines at the maternal–fetal interface. J Immunol 1993; **151**:4562–73.
7 Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal–fetal relationship: is successful pregnancy a Th2 phenomenon? Immunol Today 1993; **14**:353–6.
8 Hunt JS. Immunobiology of pregnancy. Curr Opinion Immunobiol 1992; **4**:591–6.
9 Perlmann H, Berzins K, Wahlgren M, Carlsson J, Björkman A, Patarroyo ME, Perlmann P. Antibodies in malarial sera to parasite antigens in the membrane of erythrocytes infected with early asexual stages of *P falciparum*. J Exp Med 1984; **159**:1686–704.
10 Fievet N, Cot M, Chougnet C *et al.* Malaria and pregnancy in Cameroonian primigravidae: humoral and cellular immune responses to *Plasmodium falciparum* blood stages antigens. Am J Trop Med Hyg 1995; **53**:612–7.
11 Fievet N, Maubert B, Cot M *et al.* Humoral and cellular immune responses to synthetic peptides from a *Plasmodium falciparum* blood stage antigen, Pf155/RESA, in Cameroon. Clin Immunol Immunopathol 1995; **76**:164–9.
12 Perlmann H, Perlmann P, Berzins K *et al.* Dissection of the human response to the malaria antigen Pf155/RESA into epitope specific components. Immunol Rev 1989; **112**:115–32.
13 Troye-Blomberg M, Riley EM, Perlmann H *et al.* T and B cell responses of *Plasmodium falciparum* malaria-immune individuals to synthetic peptides corresponding to sequences in different regions of the *P. falciparum* antigen Pf155/RESA. J Immunol 1989; **143**:3043–8.
14 Berzins K, Perlmann H, Wåhlin B *et al.* Rabbit and human antibodies to a repeated amino acid sequence of a *Plasmodium falciparum* antigen, Pf155, react with the native protein and inhibit merozoite invasion. Proc Natl Acad Sci USA 1986; **83**:1065–9.
15 Udomsangpetch R, Lundgren K, Berzins K *et al.* Human monoclonal antibodies to Pf155, a major antigen of malaria parasite *Plasmodium falciparum*. Science 1986; **231**:57–59.
16 Rzepczyk CM, Ramasamy R, Ho PC-L *et al.* Identification of T epitopes within a potential *Plasmodium falciparum* vaccine antigen. A study of human lymphocyte responses to repeat and nonrepeat regions of Pf155/RESA. J Immunol 1988; **141**:3197–202.
17 Favaloro JM, Coppel RL, Corcoran LM, Foote SJ, Brown GV, Anders RF, Kemp DJ. Structure of the RESA gene of *Plasmodium falciparum*. Nucl Acids Res 1986; **14**:8265–77.
18 Romain F, Augier J, Pescher P, Marchal G. Isolation of a proline-rich mycobacterial protein eliciting delayed-type hypersensitivity reactions only in guinea pigs immunized with living mycobacteria. Proc Natl Acad Sci USA 1993; **90**:5322–6.
19 Deloron P, Campbell GH, Brandling-Bennett AD *et al.* Antibodies to the *Plasmodium falciparum* ring-infected erythrocyte surface antigen and to the *P. falciparum* and *P. malariae* circum-sporozoite proteins: seasonal prevalence in Kenyan villages. Am J Trop Med Hyg 1989; **41**:395–9.
20 Chougnet C, Deloron P, Lepers JP, Rason MD, Savel J, Coulanges P. Longitudinal study of the cellular response to Pf155/RESA and circumsporozoite protein in Madagascar. Immunol Letters 1990; **25**:231–5.
21 Migot F, Chougnet C, Raharimalala L, Astagneau P, Lepers JP, Deloron P. Human immune responses to the *Plasmodium falciparum* ring-infected erythrocyte surface antigen (Pf155/RESA) after a decrease in malaria transmission in Madagascar. Am J Trop Med Hyg 1993; **48**:432–9.
22 Mangochi Malaria Research Project. Malaria prevention in pregnancy: the effects of treatment and chemoprophylaxis on placental malaria infection, low birthweight, and fetal, infant, and child survival. ACSI-CCCD (ed.). Atlanta: US-AID & US Dept Hlth Human Services, 1993.
23 Campbell CC, Martinez JM, Collins WE. Seroepidemiological studies of malaria in pregnant women and newborns from coastal El Salvador. Am J Trop Med Hyg 1980; **29**:151–7.
24 Mvondo JL, James MA, Sulzer AJ, Campbell CC. Malaria and pregnancy in Cameroonian women—naturally acquired antibody responses to asexual blood-stage antigens and the circumsporozoite protein of *Plasmodium falciparum*. Trans R Soc Trop Med Hyg 1992; **86**:486–90.
25 Williams AIO. Preparation and use of malarial placental antigen for immunodiffusion studies in Nigerian population. Trans Roy Soc Trop Med Hyg 1973; **67**:621–30.
26 Brabin L, Brabin BJ. Parasitic infections in women and their consequences. Adv Parasitol 1992; **31**:1–81.
27 Riley EM, Jepsen S, Andersson G, Otoo LN, Greenwood BM. Cell-mediated immune responses to *Plasmodium falciparum* antigens to adult Gambians. Clin Exp Immunol 1988; **71**:377–82.
28 Birkeland SA, Kristoffersen K. Lymphocyte transformation with mitogens and antigens during normal human pregnancy: a longitudinal study. Scand J Immunol 1980; **11**:321–5.
29 Watkinson M, Rushton DI. Plasmodial pigmentation of placenta and outcome of pregnancy in West African mothers. Br Med J 1983; **287**:251–4.