Variant-Specific Immunity to *Plasmodium berghei* in Pregnant Mice

Rosette Megnekou,1,2,3 Lars Hviid,1,2* and Trine Staalsoe 1,2

Centre for Medical Parasitology, Department of International Health, Immunology and Microbiology, University of Copenhagen,1 and Centre for Medical Parasitology, Department of Clinical Microbiology and Department of Infectious Diseases, Copenhagen University Hospital (Rigshospitalet),2 Copenhagen, Denmark, and Biotechnology Centre and Faculty of Science, University of Yaounde I, Yaounde, Cameroon3

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We have investigated the immunological basis of pregnancy-related *Plasmodium berghei* recrudescence in immune mice with substantial preexisting immunity. Specifically, we examined the relevance of this experimental model to the study of pregnancy-associated malaria (PAM) caused by *P. falciparum* in women with substantial preexisting protective immunity. We used mice with immunity induced prior to pregnancy and employed flow cytometry to assess their levels of immunoglobulin G (IgG) recognizing antigens on the surfaces of infected erythrocytes (IEs) in plasma. After immunization, the mice did not possess IgG specific for antigens on IEs obtained during pregnancy-related recrudescence but they acquired recrudescence-specific IgG over the course of several pregnancies and recrudescences. In contrast, levels of antibodies recognizing IEs from nonpregnant mice did not increase with increasing parity. Furthermore, maternal hemoglobin levels increased and pregnancy-related parasitemia decreased with increasing parity. Finally, parasiticemic mice produced smaller litters and pups with lower weights than nonparasitemic mice. Taken together, these observations suggest that levels of antibodies specific for recrudescence-type IEs are related to the protection of pregnant mice from maternal anemia, low birth weight, and decreased litter size. We conclude that the model replicates many of the key parasitological and immunological features of PAM, although the *P. berghei* genome does not encode proteins homologous to the *P. falciparum* erythrocyte membrane protein 1 adhesins, which are of key importance in *P. falciparum* malaria. The study of *P. berghei* malaria in pregnant, immune mice can be used to gain significant new insights regarding malaria pathogenesis and immunity in general and regarding PAM in particular.

Pregnancy-associated malaria (PAM) is a major cause of mother-offspring morbidity and mortality in areas with stable transmission of *Plasmodium falciparum* parasites, despite protective immunity to *P. falciparum* malaria acquired by the mother prior to the first pregnancy (7, 29). Susceptibility to PAM declines with increasing parity due to the acquisition of protective immunoglobulin G (IgG) recognizing antigens on the surfaces of infected erythrocytes (IEs) in plasma. After immunization, the mice did not possess IgG specific for antigens on IEs obtained during pregnancy-related recrudescence but they acquired recrudescence-specific IgG over the course of several pregnancies and recrudescences. In contrast, levels of antibodies recognizing IEs from nonpregnant mice did not increase with increasing parity. Furthermore, maternal hemoglobin levels increased and pregnancy-related parasitemia decreased with increasing parity. Finally, parasiticemic mice produced smaller litters and pups with lower weights than nonparasitemic mice. Taken together, these observations suggest that levels of antibodies specific for recrudescence-type IEs are related to the protection of pregnant mice from maternal anemia, low birth weight, and decreased litter size. We conclude that the model replicates many of the key parasitological and immunological features of PAM, although the *P. berghei* genome does not encode proteins homologous to the *P. falciparum* erythrocyte membrane protein 1 adhesins, which are of key importance in *P. falciparum* malaria. The study of *P. berghei* malaria in pregnant, immune mice can be used to gain significant new insights regarding malaria pathogenesis and immunity in general and regarding PAM in particular.

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In a series of papers published in the '80s, Van Zon and coworkers developed a mouse model to study the impact of pregnancy on immunity to *P. berghei* infection. Importantly, they used the model to demonstrate pregnancy-related recrudescences accompanied by severe clinical symptoms in mice with preexisting acquired protective immunity (38). Furthermore, they found that susceptibility to recrudescence appeared to decrease with increasing parity (39, 40). In these aspects, their model resembles PAM caused by *P. falciparum* in areas where malaria is endemic, where women generally develop substantial clinical immunity to malaria before reproductive age. In the present study, we reevaluated the model developed by Van Zon et al. in view of the recent evidence pointing to the clinical importance of VSA-specific antibody responses in PAM. We show that the apparent breakdown of preexisting protective immunity to *P. berghei* K173 infection during pregnancy is in fact the consequence of the emergence of parasites expressing pregnancy-specific VSA to which the animals do not possess antibodies if they have never been pregnant before. Furthermore, antibodies to these pregnancy-specific VSA are

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* Corresponding author. Mailing address: Department of International Health, Immunology and Microbiology, University of Copenhagen, CSS Building 22, Øster Farimagsgade 5, 1014 Copenhagen K, Denmark. Phone: 45 3545 6099. Fax: 45 3532 7851. E-mail: lhcmp @rh.dk.

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acquired in a parity-dependent manner and appear to be related to protection from pregnancy-related recrudescence, maternal anemia, low birth weight, and reduced litter size.

MATERIALS AND METHODS

Mice. We used BALB/c mice purchased from Taconic, Lille Skensved, Denmark (http://www.taconic.com). The animals were maintained on a 12-h/12-h dark/light cycle with food and water ad libitum at the Department of Experimental Medicine, University of Copenhagen, Copenhagen, Denmark, in accordance with institutional, Danish, and European guidelines for animal experimentation and welfare. All mice used were specific-pathogen free. The Danish Animal Experiments Inspectorate (Dyreforstigilfen) approved all experiments reported in this article (permission code no. 2006/561-1093), as required under Danish law.

Parasites and infections. We used P. berghei strain K173 parasites (12) for all the experiments reported herein. The parasites were originally obtained as a kind gift from Wijnand Eling. The parasites were maintained by weekly passage in the blood of nonimmunized mice. Infections were initiated by the intraperitoneal injection of 10^5 IEs in 200 μl of normal saline, and parasitemias were monitored from the day of infection by microscopic examination of thin Christmas-stained smears of blood obtained from tail nicks. This blood was also used to assess hemoglobin levels by using a HemoCue instrument (http://www.hemocue.com). Mice with fulminant parasitemia or severe clinical symptoms were killed.

Immunization. We used a modification of the immunization protocol described by Eling and Jerusalem (10). In brief, 6- to 8-week-old mice were infected as described above. The infection was suppressed by adding 15 mg/liter sulfadiazine (http://www.sigmaaldrich.com) to the drinking water on day 4 (D4) to D11 and D18 to D25. On D32, the mice were challenged using the same inoculum and route used for immunization. Mice showing very low-level or microscopically undetectable parasitemia after 1 week were considered immune.

Mating and pregnancy monitoring. The weights and peripheral blood parasitemia levels of females to be mated were recorded. On the following day (D0), they were put together with males (two to three females and one male per cage) for 4 days. The animals were not disturbed during this period to minimize stress-induced early pregnancy failure. The females were weighed when the males were removed on D4 and then left undisturbed until D10. We took an increase in body weight (from D4 to D10) as evidence of pregnancy. Subsequent abrupt weight loss was taken as an indicator of pregnancy interruption. The parasitemia levels and body weights of the animals were monitored daily from D10. Although parasitic recrudescence often occurred spontaneously in pregnant mice (see Results), we generally reinjected mice on D11 to D12 (with 0.2 × 10^7 to 1 × 10^8 IEs from pregnant mice) to increase the frequency of recrudescence in immune mice. To determine litter sizes and pup weights, female mice were kept in separate cages from D19. The cages were examined every morning, and all newborn pups were counted and weighed. The mating protocol was repeated until mice of parities 1 through 4 had been obtained.

Plasma for analysis of VSA-specific IgG. Blood samples were obtained at various time points from different groups of mice, identified as described below. We used samples obtained from immunized females (IF) before the first pregnancy (IF0 group), around D16 of the first pregnancy (IP1 group), or shortly after the first delivery (IPP1 group). We also used similar samples obtained from IF during or shortly after the second pregnancy (IP2 or IPP2 group) or the third pregnancy (IP3 or IPP3 group) or during the fourth pregnancy (IP4 group). It should be noted that mating did not always result in pregnancy, and therefore, there was no clear-cut relationship between age and the time of exposure to parasites for the different IF and IPP samples. Control samples were collected from immunized, nonpregnant females with parities of 1 to 3 (IF>0), from immunized males (IM), and from age-matched, nonimmunized animals that had never been mated or infected (nonimmunized, noninfected [NI] mice). Finally, we used blood samples collected from primigravid, nonimmunized, and never-infected mice around D16 of gestation (NP1 group).

Measurement of VSA-specific antibodies. Levels of plasma IgG antibodies reacting with antigens on the surfaces of P. berghei IEs were measured by flow cytometry, using a modification of the protocol we developed previously for P. falciparum IEs (33). In brief, IEs from the peripheral blood of either primigravid mice (at D14 to D16 of pregnancy) or nonpregnant mice with parasitemia levels of >5% were collected in heparinized Eppendorf tubes. After centrifugation and the removal of plasma, 50 μl of packed IEs was resuspended in 10 ml of Krebs-Henseleit medium (Sigma-Aldrich) supplemented with 1% bovine serum albumin and the IEs were matured overnight (under an atmosphere of 3% O_2, 6% CO_2, and 91% N_2 at 37°C). The next day, the cultures were enriched with hemozoin-containing late developmental stages by exposure to a strong magnetic field. The DNA of purified late-stage IEs (3 × 10^3/ml) was labeled with dihydroethidium bromide (Hydroethidine; 1 μg/ml). Labeled cells were incubated at 37°C for 20 min in 96-well microtiter plates (100 μl/well) with murine plasma samples (5 μl/well), washed, and stained with secondary fluorescein isothiocyanate conjugated horse anti-mouse IgG(H+L) affinity-purified antibody (FL-2000 [1:100]; http://www.vectorlabs.com) at 4°C for 30 min. The samples were analyzed by flow cytometry, and levels of IgG reacting with antigens on the IE surface were quantified as the mean fluorescence isothiocyanate fluorescence index (MFI) of dihydroethidium bromide-positive cells by using WinMDI software (http://facs.scripps.edu/software.html). Although levels of VSA-specific antibodies measured by flow cytometry depend on a number of variables that make interspecies comparisons difficult, the levels we observed in the present study were comparable to levels observed with placental P. falciparum isolates.

Histology. Mice were anesthetized by intraperitoneal injection (10 μg/l of body weight) of a 1:1 mixture of Hypnorm (http://www.janssenpharmaceutica.be) and Dormicur (http://www.roche.com), each reconstituted 1:1 in sterile water. After the collection of a blood sample from the retro-orbital plexus, the animals were killed by cervical dislocation. Tissues from the placenta, kidneys, livers, spleens, lungs, and brains were collected and fixed by immersion in Zamboni’s fixative solution for 24 h at room temperature. The organs were then transferred into 70% ethanol, dehydrated, embedded in paraffin, and cut in a microtome. For the illustrations, we used 4-μm sections, whereas 2-μm sections were used for the evaluation of parasitemia in solid tissues. All sections were stained by hematoxylin and eosin and examined by light microscopy. Hemozoin pigment crystals were examined under polarized light to increase their visibility.

Statistical analysis. We used the SigmaStat (http://www.systat.com) and CIA (2) software packages for the statistical analyses. Results are reported as means or medians with corresponding 95% confidence intervals. Student’s t-test and the Mann-Whitney rank-sum test (T) were used to evaluate intergroup differences. The Spearman rank-order coefficient (r_s) was used to evaluate parameter association, while multiple linear regression analysis was used to identify significant predictors of hemoglobin levels and birth weights. Differences with P values of <0.05 were considered statistically significant.

RESULTS

Infection and subcurative treatment result in immunity to virulent P. berghei infection. The infection of nonimmune BALB/c mice with the K173 strain of P. berghei is uniformly lethal (12). However, the mice can be rendered immune to P. berghei by repeated infection and subcurative treatment (10, 11). Using a modification of this protocol, we obtained immunized animals that were highly resistant to challenge. Thus, all of 40 animals infected with 10^6 IEs and given two rounds of suppressive sulfadiazine treatment had parasitemias of <0.6% 1 week following challenge on D32 (Fig. 1). In contrast, three of three nonimmunized mice infected on the same day developed parasitemias of >15% over this period (Fig. 1). Hemoglobin levels in immunized animals about 14 days after challenge were slightly lower than those in NI control animals [median difference (95% confidence interval), 1.1 g/dl (0.5 to 1.9 g/dl); P(T) = 0.001], probably because of the episodes of patent parasitemia during the immunization period. These results show that immunization by infection and subcurative treatment induces marked but nonsterile immunity that provides a high degree of resistance to challenge infection.

Pregnancy causes recrudescence of P. berghei parasitemia in immunized mice. It has long been known that a proportion of mice immune to P. berghei infection develop recrudescence parasitemia during pregnancy (38, 39). This finding was confirmed in the present study, in which we observed recrudescence in 24 of 24 primigravid mice immunized prior to mating (median maximum parasitemia, 2.8%; range, 0.02 to 35.8%), including 7 with fulminant parasitemia (Fig. 2). Although episodes of patent parasitemia in all of 12 immune nonpregnant control
females during the same period of time were also observed, the parasitemia levels were much lower (median maximum parasitemia, 0.15%; range, 0.008 to 6.8%) and the difference in the highest observed parasitemia levels between pregnant and nonpregnant animals was significant [median difference, 2.5% (95% confidence interval, 0.73 to 12%); \( P < 0.01 \)]. These results show that pregnancy can cause recrudescence of parasitemia previously controlled at very low levels by acquired immunity.

previously that recrudescence rates are lower during the second pregnancy than during the first pregnancy (38) and that this appears to be due to some kind of pregnancy-dependent immune response (40). Our preliminary data supported these findings and also showed that pregnancy-associated recrudescences during second and third pregnancies were more common if mice were reinfected around D11 of their second or third pregnancies with IEs obtained from primigravid mice with pregnancy-associated recrudescence (data not shown). Taking this approach, we found that hemoglobin levels around D16 of pregnancy correlated with parity (Fig. 3A, groups IP1, IP2, and IP3) \(P(r = 0.40; P < 0.001)\), as did the proportion of animals with anemia (hemoglobin levels of \(12 \text{ g/dl}\)) (Fig. 4A, groups IP1, IP2, and IP3) \(P(\chi^2 = 14.5; P < 0.001)\). Corresponding correlations were observed with respect to levels of parasitemia (Fig. 3B, groups IP1, IP2, and IP3) \(P(r = -0.40; P < 0.001)\) and the proportion of mice with patent parasitemia (Fig. 4B) \(P(\chi^2 = 9.3; P = 0.009)\). Both parasitemia and parity were significant predictors \((P < 0.001\) for each\) of hemoglobin levels in a multiple linear regression model. Taken together, these results show that \(P. \text{berghei}\) parasitemia adversely affects maternal hemoglobin levels and that acquired immunity reduces recrudescent parasitemias and thereby protects the pregnant mice from anemia.

### Immunized mice acquire high levels of IgG with specificity for antigens on the surfaces of IEs

The immunization protocol used here has been shown previously to result in the acquisition of antibodies with specificity for antigens on the surfaces of \(P. \text{berghei}\) IEs (32) (Fig. 5). In agreement with these data, we found that levels of surface-reactive IgG with specificity for VSA expressed on the surfaces of \(P. \text{berghei}\) IEs from nonpregnant mice were significantly different \([\text{median difference, 17.6 MFI units (95\% confidence interval, 15.3 to 19.9 MFI units); } P(T < 0.001)\] in NI animals and immunized (IF and IM) mice of comparable ages (Fig. 5A). Also as expected, levels of IgG in the immunized animals did not depend on sex \([\text{median difference between IF and IM mice, 0.7 MFI units (95\% confidence interval, -2.5 to 3.3 MFI units); } P(T = 0.64)\] or parity \([\text{median difference between IF and IF>0 mice, 2.1 MFI units (95\% confidence interval, -0.7 to 5.3 MFI units); } P(T = 0.15)\] (Fig. 5A). These results show that immunization results in the acquisition of IgG with specificity for antigens on the surfaces
of IEs obtained from nonpregnant mice and that this acquisition is independent of sex and parity.

**Pregnancy-related recrudescence is caused by parasites expressing distinct variant antigens on the surfaces of IEs.** Based on the above-described findings, we proceeded to address the hypothesis that the susceptibility to pregnancy-related recrudescence in mice immune to *P. berghei* infection is due to the expression of pregnancy-specific VSA by the recrudescing parasites, VSA to which the mice do not have antibodies, despite high levels of antibodies to VSA expressed on the surfaces of *P. berghei* IEs from nonpregnant animals. We found that levels of IgG specific for VSA expressed by pregnancy-associated recrudescence-type IEs in nonimmunized (NI) and immunized (IF and IM) mice were significantly different [median difference, 25.2 MFI units (95% confidence interval, 17.6 to 31.2 MFI units); *P* (*T*) < 0.001] (Fig. 5B), similar to the levels of IgG specific for VSA expressed by IEs from nonpregnant mice (Fig. 5A). However, among the immunized animals (IF and IM), the levels of IgG antibody to VSA expressed on the surfaces of IEs obtained from pregnant mice varied with both sex and parity. Thus, antibody levels in IM and IF were different [median difference, 7.4 MFI units (95% confidence interval, −0.2 to 13.2 MFI units); *P* (*T*) = 0.06] (Fig. 5B) due to the difference between males (IM) and previously pregnant females [IF > 0; median difference, 9.7 MFI units (95% confidence interval, 4.1 to 14.8 MFI units); *P* (*T*) = 0.002] (Fig. 5B). Levels in the males (IM) were similar to levels in never-pregnant females [IF0; median difference, 0.6 MFI units (95% confidence interval, −9.1 to 9.1 MFI units); *P* (*T*) = 0.84] (Fig. 5B). Taken together, these results show that the antigens on *P. berghei* IEs from nonpregnant and pregnant mice are partially different. The simplest explanation for this finding is that there are antigens on the recrudescence-type IEs that are not found on other *P. berghei* IEs, in addition to antigens that are similar or identical to antigens on IEs from nonpregnant animals.

**Levels of antibodies with specificity for the VSA expressed by parasites causing pregnancy-related recrudescences increase with increasing parity.** To further substantiate the hypothesis that the susceptibility to pregnancy-related recrudescence in mice immune to *P. berghei* infection is due to the expression of pregnancy-specific VSA to which the host does not have specific antibodies, we examined the relationship between IE-specific IgG levels and parity. While there was no apparent association between parity and levels of IgG with specificity for VSA on the surfaces of IEs from nonpregnant animals [Pr (z = 0.2) = 0.23] (Fig. 6A), there was a clear correlation between parity and levels of IgG with specificity for recrudescence-type IEs [Pr (z = 0.5) = 0.002] (Fig. 6B). These results show that IgG with specificity for recrudescence-type IEs is acquired in a selective and parity-dependent manner, reinforcing the hypothesis of a causal relationship between resistance to pregnancy-related recrudescence and IgG with specificity for antigens on the surfaces of erythrocytes infected with recrudescence-type *P. berghei* parasites.

**Erythrocytes infected by mature *P. berghei* parasites accumulate in the placenta.** Erythrocytes infected by parasites of all maturation stages can be seen in the peripheral blood of *P. berghei*-infected mice. Nevertheless, erythrocytes infected by mature *P. berghei* parasites can be sequestered in various tissues (1, 24). Of particular importance here is the recently observed chondroitin sulfate A (CSA)-dependent binding of *P. berghei* IEs in the placenta (24), which resembles that observed in PAM (13). We found that the placental interstitial spaces in mice with pregnancy-associated recrudescence showed numerous erythrocytes infected with mature, pigment-containing parasites (median, 19.0% [95% confidence interval, 15.1 to 27.3%] of all erythrocytes) but were almost devoid (<1%) of early-developmental-stage parasites (ring forms) (Fig. 7A). In contrast, ring-stage parasitemia (median, 15.0% [95% confidence interval, 8.6 to 18.2%]) dominated over mature-stage parasitemia (median, 7.2% [95% confidence interval, 4.0 to 9.8%]) in the peripheral blood [median difference, 6.8% (95% confidence interval, 3.0 to 11.0%); *P* (*T*) < 0.001] (Fig. 7B). These results support earlier evidence that placental accumulation of *P. berghei* IEs is a feature of pregnancy-related recrudescence (24). We also looked for evidence of the sequestration of IEs in brain, kidney, liver, lung, and spleen tissues. Brains were essentially free of both IEs and hemozoin (the presence of hemozoin is evidence of the phagocytosis of IEs) (data not shown). Hemozoin dominated in spleens, livers, and lungs, consistent with the expected phagocytosis of IEs in these or-
gans (Fig. 7C and data not shown). Low levels of IEs were seen in the kidneys (Fig. 7D). Taken together, these results provide additional support for the hypothesis of preferential sequestration of recrudescence-type IEs in the placenta.

Pregnancy-associated recrudescence is associated with small litters and low pup weights. Pregnancy-related parasite recrudescence in nonimmune mice has been reported previously to be associated with intrauterine growth retardation and reduced pup weight (24). We found offspring from immunized mice to be smaller \((n = 44);\) mean birth weight, \(1.33 \text{ g} \) [95% confidence interval, \(1.27\) to \(1.38 \text{ g}\)] than offspring from NI mice \((n = 9);\) mean birth weight, \(1.40 \text{ g} \) [95% confidence interval, \(1.24\) to \(1.56 \text{ g}\)] (Fig. 8). Furthermore, the average litter size among the control mice (median litter size, \(9.0 \text{ pups}\) [95% confidence interval, \(6\) to \(9 \text{ pups}\)]) was bigger than that among immunized mice (median litter size, \(5.5 \text{ pups}\) [95% confidence interval, \(5.0\) to \(7.0 \text{ pups}\)]). The median difference in litter size was \(3 \) [95% confidence interval, \(1\) to \(4\); \(P(T) = 0.004\)] (Fig. 8), and litter size was the strongest predictor of birth weight in a linear regression model \((P = 0.013)\). Among immunized mice, those with above-average parasitemia levels between D10 and D18 of pregnancy produced smaller pups (mean birth weight, \(1.26 \text{ g} \) [95% confidence interval, \(1.17\) to \(1.39 \text{ g}\)]) than those with below-average peak parasitemia levels (mean birth weight, \(1.39 \text{ g} \) [95% confidence interval, \(1.32\) to \(1.47 \text{ g}\)]). The mean difference was thus \(0.12 \text{ g} \) [95% confidence interval, \(0.04\) to \(0.23 \text{ g}; P(T) = 0.008\)]. In contrast, the average birth weight of pups from immunized mice with only low-grade parasitemia was not significantly different from that of the pups from non-immunized mice \((P = 0.01 \text{ g} \) [95% confidence interval, \(-0.14\) to \(0.15 \text{ g}; P(T) = 0.95\)])

We could not demonstrate that immunized primigravidae produced smaller litters or pups than mice of higher parity, as might have been expected. However, litter size and pup birth weights can be determined only after a successful pregnancy, which renders these markers unreliable for primigravid mice, in which pregnancies are often unsuccessful (resulting in maternal death, fetal resorption, or miscarriage) if high-level parasitemia develops. Our results show that the level of parasitemia in pregnancy-associated \(P. \)berghei recrudescence adversely affects litter size and pup weights.

**DISCUSSION**

In 1915, Clark noted that it “has long been known that it is possible to find an abundance of [malaria] parasites in the placenta” but that it was generally regarded as “a curious feature sometimes encountered” (5). Later, when it was discovered that pregnancy modulates the immune system in order to protect the developing fetus from maternal immune attack (15), many malaria researchers started to see PAM as the inevitable consequence of pregnancy-associated immunosuppression (20). It was known early that primigravidae are particularly susceptible to placental infection (3), but this finding, which is at variance with the immunosuppression hypothesis, was largely ignored—with some notable exceptions (18). Eventually, a coherent understanding of the pathogenesis and immunology of PAM emerged when it was found that \(P. \)falciparum IEs being sequestered in the placenta have unique adhesive properties (13) and that susceptibility to PAM is related to levels of antibodies recognizing particular parasite-encoded interclonally variant proteins (the so-called VSAPAM) on the IE surface (14, 34). These insights have spurred the current intensive and worldwide effort to develop vaccines against PAM. However, progress is being hampered by the lack...
of animal models that exhibit the characteristic features of PAM. Although the chimpanzee parasite *P. reichenowi* possesses a gene homologous to the *P. falciparum* var2csa gene implicated in the pathogenesis of PAM (37), higher primates are very impractical experimental model systems for malaria in general, let alone for PAM. The situation is not much better with respect to lower primates, and essentially nothing is known about the relationship between immunity and susceptibility to infection in pregnant monkeys (8). Rodents are the most accessible and therefore best-studied experimental malaria model system, and a number of studies of malaria in pregnant animals are available, including a recent study advocating murine *P. berghei* infection as a useful model of PAM (24). However, the report by Neres et al. (24) was based exclusively on data from nonimmune animals, and available studies of immunity and susceptibility to infection in pregnant mice either predate the discovery of or ignore the apparent importance of VSA-specific immunity in PAM. In the present study, we show that most, if not all, previously described features of *P. berghei* infection during pregnancy are consistent with the current, VSA-based understanding of PAM pathogenesis and immunity (29). Thus, in the *P. berghei*-infected mouse model, pregnancy-associated recrudescences are associated with placental and CSA-dependent sequestration of IE(s) (9, 24, 36), decrease in frequency and severity with increasing parity (38, 39), have adverse consequences for the pregnant mouse and their offspring (24), and occur despite immunity acquired before the first pregnancy (38, 39). Furthermore, we show for the first time that pregnancy-associated recrudescence in *P. berghei*-infected mice leads to the acquisition of antibodies that are specific for variant antigens expressed only during recrudescence. The acquisition of these antibodies appears to be associated with clinical protection from the consequences of pregnancy-associated recrudescence, such as maternal anemia and low birth weight. However, direct evidence of a causal relationship must await the identification of the antigen(s) involved and its use in vaccination studies.

In common with most other malaria parasites, *P. berghei* does not possess genes homologous to the var genes encoding the PfEMP1 proteins thought to be the major *P. falciparum* IE adhesion ligands. Nevertheless, IE sequestration, including placental sequestration during pregnancy, is clearly not restricted to *P. falciparum* infection (25–27, 36). The capacity for glycosaminoglycan-dependent IE sequestration in the placenta may thus have evolved independently several times. Alternatively, it may be a truly ancient feature, raising the possibility that orthologs of the parasite genes involved in placental IE sequestration in mice exist in *P. falciparum*. In either case, the identification of the pregnancy recrudescence-related *P. berghei* genes and the characterization of their products are of considerable interest and a current priority in our laboratories.

In conclusion, we have demonstrated many similarities between PAM in *P. falciparum*-exposed women and pregnancy-related *P. berghei* recrudescence in immune mice. This finding opens new opportunities for research on the pathogenesis and immunity of PAM, which remains a major source of poor mother-infant health in large parts of the world.

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