Comparative Medicine
Copyright 2007
by the American Association for Laboratory Animal Science

Vol 57, No 4
August 2007
Pages 396–401

Anemia and Antibodies to the 19-kDa Fragment of MSP1 During *Plasmodium falciparum* Infection in *Aotus* Monkeys

Alfonso S Gozalo,[1,†,*] Carmen M Lucas,[1] Jing Qin,[2] B Ted Hall,[3] and Alan J Magill[1,‡]

To determine whether antibodies to the 19-kDa fragment of merozoite surface protein 1 ($MSP1_{19}$) help to control blood-stage *Plasmodium falciparum* infection, we performed a rechallenge experiment of previously infected *Aotus* monkeys. Monkeys previously exposed to the FVO strain of *P. falciparum* that did or did not develop high antibody titers to $MSP1_{19}$ and malaria-naïve monkeys were challenged with erythrocytes infected with the same strain. Prepatent periods were prolonged in previously infected monkeys compared with malaria-naïve monkeys. Previously infected monkeys with preexisting anti-$MSP1_{19}$ antibodies showed low peak parasitemias that cleared spontaneously. Previously infected monkeys that had no or low levels of pre-existing anti-$MSP1_{19}$ antibodies also showed low peak parasitemias, but because of low hematocrits, all of these animals required treatment with mefloquine. All previously malaria-naïve animals were treated because of high parasitemias. The results of this study suggest that antibody to the 19-kDa carboxy-terminal fragment of MSP1 plays a role in preventing the development of anemia, an important complication often associated with malaria.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin; $MSP1_{19}$, 19-kDa fragment of merozoite surface protein 1; OD, optical density

Merozoite surface protein 1 (MSP1), an approximately 200-kDa surface-associated antigen, is expressed during schizogony in all species of *Plasmodium* as a precursor protein that undergoes processing and cleavage during merozoite invasion. During invasion of erythrocytes, the major fragment from the carboxyl terminus (the 42-kDa $MSP1_{42}$) is processed to produce a 19-kDa fragment ($MSP1_{19}$) that remains bound to the merozoite.[10] When administered with complete Freund adjuvant, recombinant $MSP1_{19}$ and $MSP1_{42}$ offer some protection to *Aotus nancymae* against homologous challenge.[3,4,16,24,26] Because protection seemed independent of antibody response in some cases and because Freund adjuvant stimulates both strong cellular immune responses and antibodies, a passive transfer study was performed to further characterize the relative contribution of antibody alone to protection in the *Aotus* monkey model.[8] In the cited study, *A. nancymae* monkeys that received rabbit immunoglobulin (Ig) against $MSP1_{19}$ from either the homologous, challenge strain of *Plasmodium falciparum* (FVO) or a heterologous strain of *P. falciparum* (3D7) failed to develop antibodies (or developed only low titers) to $MSP1_{19}$ when challenged with the FVO strain of malaria. In contrast, *Aotus* monkeys that received rabbit immunoglobulin raised against an unrelated *P. falciparum* antigen (Pfs-25) did produce high titers of their own, 'native' antibodies to the $MSP1_{19}$ fragment after challenge with FVO strain parasites. To determine whether native antibodies to $MSP1_{19}$ help to control blood-stage infection, we performed homologous rechallenge of the monkeys from the previous trial.[8]

Received: 22 Nov 2006. Revision requested: 2 Jan 2007. Accepted: 14 Jan 2007.
[1]US Naval Medical Research Center Detachment, Lima, Peru; [2]Biostatistics Research Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD; [3]Department of Immunology, Walter Reed Army Institute of Research, Silver Spring, MD.
[†]Present address: Comparative Medicine Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health and SoBran, Bethesda, MD.
[‡]Present address: Malaria Vaccine Development Unit, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD.
[*]Corresponding author. Email: gozaloa@niaid.nih.gov

Materials and Methods

Parasites. Mefloquine-sensitive *Plasmodium falciparum* FVO strain asexual-stage parasites were used for challenge, because a standard inoculum induces highly reproducible patent infections in *Aotus* monkeys.[22]

Primates. Captive-born, genetically unrelated, young adult, *Aotus nancymae* monkeys were obtained from the Peruvian Primate Center in Iquitos, Peru, had been used in a previous passive-transfer study 1030 d before,[8] and were assigned to 1 of 3 groups according to their immunologic status. Group 1 included 3 monkeys that developed high antibody titers to $MSP1_{19}$ in a previous study. Group 2 consisted of 3 monkeys who never developed antibodies, or showed very low titers, to $MSP1_{19}$ after previous challenge with FVO strain parasites, and Group 3 consisted of 2 research-naïve, captive-born, young adult monkeys as controls. The study was approved by the US Naval Medical Research Center Detachment Animal Care and Use Committee. The experiment was conducted according to the principles set forth in the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals*.[21]

Primate handling and treatment. Ketamine chlorhydrate (10 mg/kg) was given intramuscularly in the thigh to immobilize the monkeys each time they were removed from their cages. Mon-

keys were challenged via the saphenous vein with 10,000 *P. falciparum* (strain FVO)-infected erythrocytes obtained from a donor monkey. Malaria-infected monkeys were treated with mefloquine (20 mg orally in a single dose) when parasitemia was greater than 300,000 parasites/µl blood or if the hematocrit decreased to 50% of preinfection values.

Baseline and postchallenge sampling. A blood sample (3 ml) was drawn from each monkey's femoral vein 1 mo prior to and at various times after challenge, and serum was collected and stored at –20 °C. Beginning on day 4 after rechallenge with *P. falciparum*, blood samples (0.05 ml) were obtained daily from a saphenous vein for preparation of Giemsa-stained thick and thin smears and for microhematocrit determinations. Monkeys were monitored daily for 30 d and then 3 times weekly for days 31 to 60.

Detection of parasitemia. Giemsa-stained thick smears were examined microscopically at 1000× magnification (200 fields) to detect parasites. Low parasite counts (below 1,000 parasitized erythrocytes/µl) were estimated by assuming that 200 fields of a thick blood smear contained approximately 0.2 µl of blood. Subsequently, when parasitemias increased, the percentage of infected erythrocytes on thin blood smears was calculated directly from the smear.

Serology. A standard enzyme-linked immunosorbent assay (ELISA) was modified for use with yeast-expressed recombinant $MSP1_{19}$ as capture antigen, as described elsewhere.[9] Triplicate assays were averaged for each serum sample. Corrected optical density (OD) readings were calculated by subtracting the mean OD reading of triplicate wells without antigen from the mean OD reading of triplicate wells with antigen.

Statistical analysis. Statistical analyses were carried out using S-Plus statistical software (Insightful Corporation, Seattle, WA). For each monkey, hematocrits and parasitemia were summarized over the observation period by using the area under the time-hematocrit and time-parasitemia curves. Groups 1 and 2 then were compared by using *t* tests.

Results

Prechallenge ELISA. Prior to the rechallenge (1030 d after the first challenge), a serum sample from each of the 8 monkeys was tested in ELISA for antibody activity to $MSP1_{19}$. Of the 3 monkeys in group 1, 2 (nos. 477 and 489) still had measurable IgG antibodies to $MSP1_{19}$ (Table 1). In the third monkey, IgG antibodies to $MSP1_{19}$ had waned such that they were undetectable at the time of challenge. Monkeys in groups 2 and 3 did not have any measurable titers of anti-$MSP1_{19}$ IgG.

Postchallenge ELISA. A serum sample was obtained from each monkey at day 30 after rechallenge and tested in ELISA for antibody activity to $MSP1_{19}$ (Table 1). The 3 monkeys in group 1 had high IgG titers to $MSP1_{19}$. Only 1 animal in group 2 (monkey no. 471) developed high titers against $MSP1_{19}$ comparable to those of group 1 animals. The other 2 monkeys in group 2 showed measurable titers to $MSP1_{19}$. The 2 control monkeys in Group 3, infected with *P. falciparum* for the first time, showed lower titers than the animals in the first 2 groups.

Prepatency. Prepatent periods were delayed in the test animals (groups 1 and 2) compared with controls (Group 3), except for 1 animal in group 1 (Table 2). The first parasitemias in group 1 animals were detected at 5, 10, and 22 d; in group 2 at 7, 7, and 10 d; and in group 3 (malaria naïve-control animals) at 5 and 5 d (Table 2).

Table 1. Antibody status to $MSP1_{19}$ before and after challenge with 10,000 *P. falciparum*-infected *Aotus* erythrocytes

		Enzyme-linked immunosorbent assay optical density value	
Monkey no.	Group	before challenge	after challenge
477	1	0.73	1.74
489	1	0.15	1.65
496	1	0.06	1.81
471	2	0.06	1.50
547	2	0.06	0.73
579	2	0.06	0.41
581	3	0.06	0.22
1045	3	0.06	0.13

Optical density values greater than 0.06 were considered positive.

Daily mean parasitemia. Monkeys in group 3 (controls) had the highest daily mean parasitemias (3963 and 4621 parasites/µl). In addition, 2 monkeys in group 2 had slightly higher daily mean parasitemias (498 and 3218 parasites/µl) than did animals in group 1 (1013, 363, and 391 parasites/µl, respectively). However, 1 monkey in group 2 (no. 579) had the lowest daily mean parasitemia (160 parasites/µl) among all monkeys in the study (Table 2).

Maximal parasitemia. Group 1 animals showed low peak parasitemias that spontaneously cleared between days 35 through 39. Group 2 animals showed low peak parasitemias, but all monkeys required treatment with mefloquine between days 18 through 22 because of low hematocrits (Table 2, Figures 1 and 2). Both monkeys in Group 3 were treated on day 11 because of high parasitemia (greater than 300,000 parasites/µl).

Days to peak parasitemia. Days to peak parasitemia varied among animals in the same group except for the malaria-naïve monkeys (group 3). Days to peak parasitemia for monkeys in group 1 was 18, 21, and 24 d; for group 2 monkeys was 15, 15, and 16 d; and for group 3 was 11 and 11 d (Table 2).

Day of treatment. Only the 2 malaria-naïve monkeys in group 3 required treatment because of parasitemias greater than 300,000 parasites/µl; both monkeys were treated on day 11. Group 2 monkeys were treated for depressed hematocrit between day 18 through 22 (Table 2).

Hematocrits. All animals in Group 2 experienced a decrease (exceeding 50% of preinfection value) in their hematocrits (51%, 53%, and 69% decrease) compared with monkeys in group 1 (13%, 28%, and 36% decrease; Figure 1; Table 3) and group 3 (7% and 4% decrease). The hematocrits in group 2 monkeys continued to decrease despite treatment with mefloquine (Figure 1).

Statistical analysis. Group 2 monkeys were treated on day 22, 22, and 18 because of anemia. However, the treatment would not have affected the hematocrit until after day 29. Therefore for each monkey, the observed hematocrits over days 4 to 29 were summarized by using area under the time-hematocrit curve. Groups 1 and 2 then were compared by using *t* tests on the calculated area-under-the-curve values. The area-under-the-curve values for hematocrits in group 1 were 1339, 1168, and 1020.5 (cumulative hematocrit times days), whereas those for group 2 were 933, 850, and 853.5 (cumulative hematocrit times days) ($P = 0.036$). Therefore the native antibody level has a significant effect on hematocrit. In a similar analysis of parasitemia, the area-under-

Table 2. Parasitemia and treatment data during days 4 through 29 of the follow-up period after challenge of *Aotus* monkeys with 10,000 *P. falciparum*-infected erythrocytes

			no. of parasites/μl				
Monkey no.	Group	Prepatency (no. of days)	Mean[a]	Maximum	Day of peak parasitemia	Treatment day[b]	Reason for treatment[c]
477	1	22	1013	12,840	24	nt	nt
489	1	5	363	2040	21	nt	nt
496	1	10	391	4560	18	nt	nt
471	2	10	498	3000	16	22	HCT
547	2	7	3218	31,200	15	22	HCT
579	2	7	160	6,800	15	18	HCT
581	3	5	3963	326,000	11	11	para
1045	3	5	4621	304,000	11	11	para

[a]Geometric mean.
[b]Day on which animal was treated with mefloquine (20-mg single dose); nt, not treated.
[c]Reason for treatment: nt, not treated; HCT, greater than 50% decrease in hematocrit; para, parasitemia in excess of 300,000 parasites/μl.

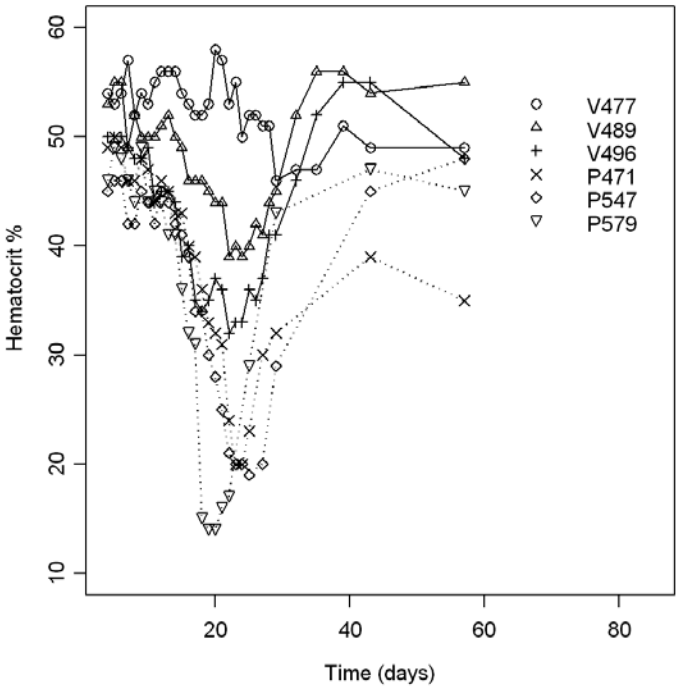

Figure 1. Hematocrit (%) in individual *Aotus* monkeys challenged intravenously with 10,000 *Plasmodium falciparum*-infected *Aotus* erythrocytes. Group I, *Aotus* monkeys with native antibodies against $MSP1_{19}$ (solid lines); group II, *Aotus* monkeys previously exposed to *P. falciparum* but with no or low native antibody titers against $MSP1_{19}$ (dashed lines).

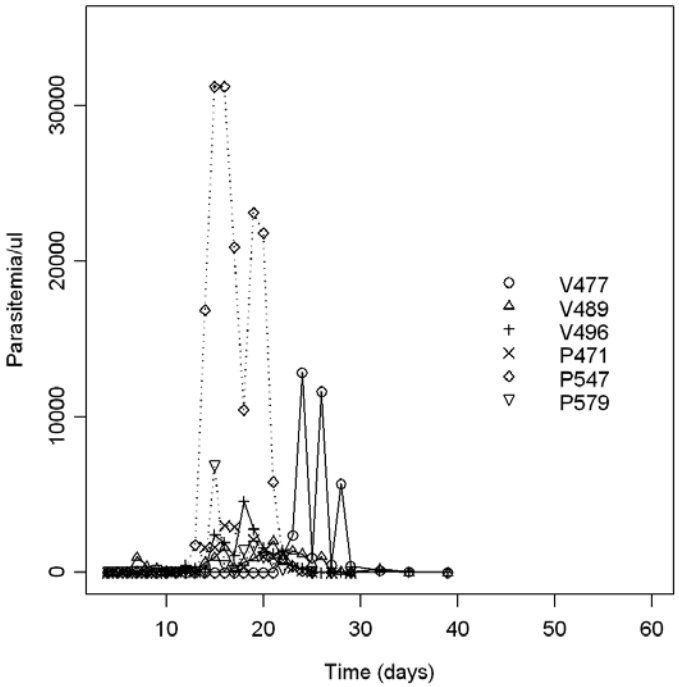

Figure 2. Parasitemia (no. of parasites/μl) curves in individual *Aotus* monkeys challenged intravenously with 10,000 *Plasmodium falciparum*-infected *Aotus* erythrocytes. Group I, *Aotus* monkeys with native antibodies against $MSP1_{19}$ (solid lines); group II, *Aotus* monkeys previously infected with *P. falciparum* but with no or low native antibody titers against $MSP1_{19}$ (dashed lines).

the-curve values were 35280, 15500, and 18640 (cumulative parasitemia times days) for group 1 animals and 15370, 164674, and 12460 (cumulative parasitemia times days) for group 2 monkeys. The analysis yielded a P value of 0.46, thus the native antibody level lacked any significant effect on parasitemia.

Discussion

This study examined whether the levels of native anti-$MSP1_{19}$ antibody during initial infection of *Aotus* monkeys with malaria affected their response after rechallenge. The most notable finding was that none of the monkeys that previously had made high antibody titers against $MSP1_{19}$ became anemic or required treatment to survive subsequent malaria infection. All 3 monkeys that had previously been infected with malaria and had generated high anti-$MSP1_{19}$ antibody titers went on to clear their infections spontaneously. In contrast, all 3 monkeys that previously were infected with malaria but that produced little or no anti-$MSP1_{19}$ antibody developed a severe, life-threatening anemia and required treatment within 3 wk after infection.

Interestingly, 1 monkey (no. 471) from group 2 developed an antibody titer against $MSP1_{19}$ comparable to that of group 1 monkeys after rechallenge, but this animal was not protected from developing severe anemia. We hypothesize that the antibody response against MSP1 in monkey 471 was delayed compared with that of monkeys in group 1, which had made high antibody titers against $MSP1_{19}$ after exposure to *P. falciparum* during the first chal-

Table 3. Hematologic data acquired during days 4 through 29 of the follow-up period after challenge of *Aotus* monkeys with 10,000 *P. falciparum*-infected erythrocytes

Monkey no.	Group	Hematocrit			Day on which minimum hematocrit occurred	% decrease relative to baseline
		Baseline	Mean	Minimum		
477	1	53	52.7	46	29	13
489	1	54	48.1	39	22	28
496	1	50	42.7	32	22	36
471	2	49	41.6	24	22	51
547	2	45	38.7	21	22	53
579	2	48	40.7	15	18	69
581	3	41	40.6	38	11	7
1045	3	52	51.8	50	8	4

lenge 3 y before. At rechallenge, anti-$MSP1_{19}$ antibody titers most likely increased rapidly in group 1 monkeys, protecting them from developing high parasitemia and severe anemia. Because we did not measure antibody titers to $MSP1_{19}$ immediately after rechallenge, we cannot verify this hypothesis. However, low antibody titers against an infectious agent do not necessarily provide protection at challenge, thus necessitating multiple booster immunizations to achieve protective antibody titers in some cases.[20] In addition, once the immune system is primed against a particular antigen, the immune response to rechallenge is much faster, with the development of high antibody titers in a shorter period of time compared with those of a naïve immune system.

Thought to be due to partial immunity, anemia has occurred in *Aotus* monkeys repeatedly challenged with *P. falciparum* in experimental malaria vaccine trials.[6,13–15] Anemia is a common finding in humans with malaria and could be caused by any of 3 mechanisms: lysis of infected erythrocytes, lysis or sequestration of noninfected erythrocytes, and suppression of hematopoiesis.[15] Work by others has made it clear that more than one of these mechanisms is involved.[1,17,27] In monkey studies, Jones and collaborators[15] failed to find either antibody or complement on the surface of erythrocytes harvested while the animals were infected, indicating that neither antibody-mediated nor C3d-mediated lysis occurs. Similar studies in human volunteers in malarious areas have not been entirely consistent.[2,7,19] According to Jakeman and collaborators,[11] in human malaria, dyserythropoiesis plays an unimportant role in the resulting anemia, and the anemia occurs before a substantial antibody response to parasites or erythrocytes is generated. Those authors[11] suggest that uninfected erythrocyte destruction occurs through phagocytosis of erythrocytes bound to merozoites killed due to accompanying malaria paroxysms. The presence of rhoptry-associated protein and ring surface protein 2 on the surface of uninfected erythrocytes[5] and on erythroid precursor cells in the bone marrow[18] recently have been suggested to be responsible for the malaria-associated anemia in humans. These 2 proteins are deposited on the surface of uninfected erythrocytes during failed attempts of malaria parasites to invade the red cells, marking them for sequestration and destruction by the host immune system. A similar mechanism may occur in owl monkeys with partial immunity to falciparum malaria.

In mice, high titers of maternally derived $MSP1_{19}$ interfere with the production of an $MSP1_{19}$-specific antibody response in pups after vaccination.[25] The level of inhibition was influenced by the antibody titer. In adult mice, the presence of specific antibodies affected the number of B cells producing $MSP1_{19}$-specific antibodies in response to $MSP1_{19}$ vaccination.[25] In that study,[25] the authors concluded that if high levels of maternally derived antibody specific for a malaria subunit vaccine candidate are present in the infant, successful immunization of the infant with the same vaccine may not be efficient. This mechanism was most likely responsible for the lack of development of native antibodies against $MSP1_{19}$ in the *Aotus* monkeys passively immunized with serum containing high titers of $MSP1_{19}$ antibodies and challenged with *P. falciparum* FVO in our previous study.[8]

MSP1 apparently harbors multiple epitopes capable of eliciting humoral and cellular responses that efficiently inhibit parasite multiplication. According to a recent study,[28] even antibodies elicited by the apparently least-immunogenic regions of MSP1, as delineated by p30 and p38, were highly effective in inhibiting parasite multiplication. Because all combinations of antibodies examined showed additive inhibitory effects on parasite growth, the entire MSP1 molecule should be considered for purposes of vaccine production.[28] These findings are consistent with the results from the present study, in which monkeys that developed antibodies against a broad repertoire of *P. falciparum* antigens were protected against high parasitemia and severe anemia. However, antibodies specifically directed against $MSP1_{19}$ appear to be necessary for protection against anemia.

John and collaborators[12] found that residents of a highland malaria mesoendemic area in Africa with high levels of $MSP1_{19}$-specific invasion-inhibitory antibodies had substantially reduced risk of blood stage infection after treatment with antimalarial drugs and clearance of any prior parasitemia. In contrast, IgG or IgG subclass antibodies to recombinant $MSP1_{19}$ detected by serology did not correlate with invasion-inhibition activity or risk of infection. The authors[12] suggested that the protective epitopes of the native protein may be poorly represented in some recombinant constructs of MSP1. In addition, $MSP1_{19}$ recombinant proteins expressed in various systems do not react identically to the human antibodies elicited by natural infection.[12] This difference would explain why despite high antibody titers against $MSP1_{19}$ after immunization with experimental vaccine candidates, little or no protection against infection is noted in some human and monkey malaria challenge studies.

The monkeys we used in this study were all captive-born, from the same source, young adults, clinically healthy, and had the same environmental exposure. In our experience, captive-born, malaria-naïve *Aotus* monkeys are very reliable models for the study of falciparum malaria and show highly predictable parasitemia curves after experimental infection with the FVO strain. Therefore, small numbers of these valuable animals provide consistent results with biologic and statistical significance, allowing

reduction in the numbers of animals required for these studies. In contrast, wild-caught *Aotus* monkeys have no known clinical history and usually are infected with microfilarias that potentially can interfere with malaria infection.[23] In addition, wild-caught monkeys may have been exposed to *Plasmodium falciparum* in the wild[9] and their antibody titers may have waned over time, but when these animals are infected in the laboratory, their immune systems may be primed already, thus interfering with study objectives to evaluate the immunogenicity and protective efficacy of malaria candidate vaccines. Moreover, wild-caught monkeys can be infected with a variety of parasitic, bacterial, viral, and fungal agents whose effect on vaccine research is unknown at best. For all the stated reasons, wild-caught monkeys are less than desirable for malaria vaccine studies.

As established by others and as evident from the current study, prior exposure to malaria parasites results in reduced infection as measured by the time of the first appearance of parasites and the level of parasitemia. In addition, partial immunity to malaria parasites may lead to life-threatening anemia. The results of this study show that antibody to the 19-kDa carboxy-terminal region of MSP1 may play an important role in preventing the development of anemia. The mechanism by which $MSP1_{19}$ might prevent the development of anemia is unknown, but this finding agrees with previous studies that suggest that protection against malaria infection is only achieved when an antibody response is mounted against a broad repertoire of merozoite surface antigens. As shown in the present study, the *Aotus* monkey is an excellent model not only for testing vaccine candidates but also for studying the development of anemia associated with immunity to malaria in humans.

Acknowledgments

We thank Juan Rodas for excellent animal husbandry, and Trevor Jones, David Narum, and Dean Follmann for critical review and helpful suggestions during the preparation of this manuscript. This research was supported in part by the US Naval Medical Research and Development Command NNMC (Bethesda, MD), the Walter Reed Army Institute of Research (Washington, DC; work unit no., 627897A 870 F 8611), and the Intramural Research Program of the National Institutes of Health, National Institute of Allergy and Infectious Diseases. The opinions and assertions contained herein are not to be construed as official or as reflecting the views of the US Navy, US Army, US Department of Defense, or the Peruvian Government.

References

1. **Abdalla SH.** 1990. Hematopoiesis in human malaria. Blood Cells **16:**401–416.
2. **Abdalla S, Weatherall DJ.** 1982. The direct antiglobulin test in *P. falciparum* malaria. Brit J Haem **51:**415–425.
3. **Chang SP, Case SE, Gosnell WL, Hashimoto A, Kramer KJ, Tam LO, Hashiro CO, Nikaido CM, Gibson HL, Lee-Ng CT, Barr PJ, Yokota BT, Hut GS.** 1996. A recombinant baculovirus 42-kilodalton C-terminal fragment of *Plasmodium falciparum* merozoite surface protein 1 protects *Aotus* monkeys against malaria. Infect Immun **64:**253–261.
4. **Darko CA, Angov E, Collins WE, Bergmann-Leitner ES, Girouard AS, Hitt SL, McBride JS, Diggs CL, Holder AA, Long CA, Barnwell JW, Lyon JA.** 2005. The clinical-grade 42-kilodalton fragment of merozoite surface protein 1 of *Plasmodium falciparum* strain FVO expressed in *Escherichia coli* protects *Aotus nancymai* against challenge with homologous erythrocytic-stage parasites. Infect Immun **73:**287–297.
5. **Douki JBL, Sterkers Y, Lépolard C, Boubacar T, Costa FTM, Scherf A, Gysin J.** 2003. Adhesion of normal and *Plasmodium falciparum* ring-infected erythrocytes to endothelial cells and the placenta involves the rhoptry-derived ring surface protein-2. Blood **101:**5025–5032.
6. **Egan AF, Blackman MJ, Kaslow DC.** 2000. Vaccine efficacy of recombinant *Plasmodium falciparum* merozoite surface protein 1 in malaria-naive, -exposed and/or -rechallenged *Aotus vociferans* monkeys. Infect Immun **68:**1418–1427.
7. **Facer CA, Bray RS, Brown J.** 1979. Direct Coombs antiglobulin reactions in Gambian children with *Plasmodium falciparum* malaria. Clin Exp Immunol **35:**119–127.
8. **Gozalo A, Lucas C, Cachay M, Wellde B, Hall T, Bell B, Wood J, Watts D, Wooster M, Lyon J, Moch K, Haynes D, Williams J, Holland C, Watson E, Kester K, Kaslow D, Ballou WR.** 1998. Passive transfer of growth-inhibitory antibodies raised against yeast-expressed recombinant *Plasmodium falciparum* merozoite surface protein-1 19kD. Am J Trop Med Hyg **59:**991–997.
9. **Gozalo A, Lucas C, Montoya E, Ballou WR, Wooster M, Watts D.** 1997. Prevalence of antibody to *Plasmodium falciparum* antigens among feral *Saimiri* monkeys in the Amazon basin region of Peru. J Med Primatol **26:**204–206.
10. **Holder AA, Freeman RR.** 1984. The three major antigens on the surface of *Plasmodium falciparum* merozoites are derived from a single high molecular weight precursor. J Exp Med **160:**624–629.
11. **Jakeman GN, Saul A, Hogarth WL, Collins WE.** 1999. Anaemia of acute malaria infections in non-immune patients primarily results from destruction of uninfected erythrocytes. Parasitology **119(Pt 2):**127-133.
12. **John CC, O'Donnell RA, Sumba PO, Moormann AM, deKoning-Ward TF, King CL, Kazura JW, Crabb BS.** 2004. Evidence that invasion-inhibitory antibodies specific for the 19-kDa fragment of merozoite surface protein-1 (MSP-1 19) can play a protective role against blood-stage *Plasmodium falciparum* infection in individuals in a malaria endemic area of Africa. J Immunol **173:**666–672.
13. **Jones TR, Narum DL, Gozalo AS, Aguiar J, Fuhrmann SR, Liang H, Haynes JD, Moch JK, Lucas C, Luu T, Magill AJ, Hoffman SL, Sim BK.** 2001. Protection of *Aotus* monkeys by *Plasmodium falciparum* EBA-175 region II DNA prime-protein boost immunization regimen. J Inf Dis **183:**303–312.
14. **Jones TR, Obaldia N, Gramzinski RA, Hoffman SL.** 2000. Repeated infection of *Aotus* monkeys with *Plasmodium falciparum* induces protection against subsequent challenge with homologous and heterologous strains of parasite. Am J Trop Med Hyg **62:**675–680.
15. **Jones TR, Stroncek DF, Gozalo AS, Obaldia N, Andersen EM, Lucas C, Narum DL, Magill AJ, Sim BKL, Hoffman SL.** 2002. Anemia in parasite-and recombinant protein-immunized *Aotus* monkeys infected with *Plasmodium falciparum*. Am J Trop Med Hyg **66:**672–679.
16. **Kumar S, Yadava A, Keister DB, Tian JH, Ohl M, Perdue-Greenfield KA, Miller LH, Kaslow DC.** 1995. Immunogenicity and in vivo efficacy of recombinant *Plasmodium falciparum* merozoite surface protein-1 in *Aotus* monkeys. Mol Med **1:**325–332.
17. **Kurtzhals JAL, Rodrigues O, Addae M, Commey JOO, Nkrumah FK, Hviid L.** 1997. Reversible suppression of bone marrow response to erythropoietin in *Plasmodium falciparum* malaria. Brit J Haem **97:**169–174.
18. **Layez C, Nogueira P, Combes V, Costa FTM, Juhan-Vague I, Pereira da Silva L, Gysin J.** 2005. *Plasmodium falciparum* rhoptry protein RSP2 triggers destruction of the erythroid lineage. Blood **106:**3632–3638.

19. **Merry AH, Looareesuwan S, Phillips RE, Chanthavanich P, Supanaranond W, Warrell DA, Weatherall DJ.** 1986. Evidence against immune haemolysis in falciparum malaria in Thailand. Brit J Haem **64:**187–194.
20. **Myagkikh M, Alipanah S, Markham PD, Tartaglia J, Paoletti E, Gallo RC, Franchini G, Robert-Guroff M.** 1996. Multiple immunizations with attenuated poxvirus HIV type 2 recombinants and subunit boosts required for protection of rhesus macaques. AIDS Res Hum Retroviruses **12:**985-992.
21. **National Research Council.** 1996. Guide for the care and use of laboratory animals. Washington (DC): National Academy Press.
22. **Schmidt LH.** 1972. The course of *P. falciparum* (Vietnam Oak Knoll strain) in *Aotus trivirgatus*. Trans Royal Soc Trop Med Hyg **66:**521.
23. **Schmidt LH, Esslinger JH.** 1981. Courses of infections with *Plasmodium falciparum* in owl monkeys displaying a microfilaremia. Am J Trop Med Hyg **30:**5-11.
24. **Singh S, Miura K, Zhou H, Muratova O, Keegan B, Miles A, Martin LB, Saul AJ, Miller LH, Long CA.** 2006. Immunity to recombinant *Plasmodium falciparum* merozoite surface protein 1 (MSP1): protection in *Aotus nancymai* monkeys strongly correlates with anti-MSP1 antibody titer and in vitro parasite-inhibitory activity. Infect Immun **74:**4573–4580.
25. **Stanisic DI, Martin LB, Gatton ML, Good MF.** 2004. Inhibition of 19-kDa C-terminal region of merozoite surface protein -1-specific antibody responses in neonatal pups by maternally derived 19-kDa C-terminal region of merozoite surface protein -1-specific antibodies but not whole parasite-specific antibodies. J Immunology **172:**5570–5581.
26. **Stowers AW, Cioce V, Shimp RL, Lawson M, Hui G, Muratova O, Kaslow DC, Robinson R, Long CA, Miller LH.** 2001. Efficacy of two alternate vaccines based on *Plasmodium falciparum* merozoite surface protein 1 in an *Aotus* challenge trial. Infect Immun **69:**1536–1546.
27. **Weatherall DJ, Abdalla SH, Pippard MJ.** 1983. The anaemia of *Plasmodium falciparum* malaria. In: Evered D, Whelan J, editors. Malaria and the red cell. London: Pitman. p 74–99.
28. **Woehlbier U, Epp C, Kauth CW, Lutz R, Long CA, Coulibaly B, Kouyaté B, Arevalo-Herrera M, Herrera S, Bujard H.** 2006. Analysis of antibodies directed against merozoite surface protein 1 of the human malaria parasite *Plasmodium falciparum*. Infect Imm **74:**1313-1322.