Short Report: Divergent Roles of IRAK4-Mediated Innate Immune Responses in Two Experimental Models of Severe Malaria

Constance A. M. Finney, Ziyue Lu, Michael Hawkes, Wen-Chen Yeh, W. Conrad Liles, and Kevin C. Kain*

McLaughlin–Rotman Centre for Global Health, McLaughlin Centre for Molecular Medicine, UHN and University of Toronto, Ontario, Canada; Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada; Ontario Cancer Institute, Princess Margaret Hospital, Toronto, Ontario, Canada; Tropical Disease Unit, Division of Infectious Diseases, Department of Medicine, Toronto General Hospital, Toronto, Ontario, Canada

Abstract. Severe malaria represents a clinical spectrum of disease. We propose that innate immune inflammatory responses to malaria play key roles in the pathogenesis and clinical outcomes of distinct severe malaria syndromes. To investigate this hypothesis, mice deficient in IRAK4, central to Toll-like receptor (TLR)-mediated signaling, were studied in two experimental models of malaria: *Plasmodium berghei* (PbA) and *Plasmodium chabaudi* (PccAS). *Irak4*^{−/−} mice had decreased pro-inflammatory cytokine production during infection in both models. However, animals were relatively protected from PbA-associated symptoms compared with wild-type mice, whereas *Irak4*^{−/−} animals were more susceptible to PccAS-associated disease. These results show that IRAK4-mediated innate immune inflammatory responses play critical roles in divergent clinical outcomes in murine malaria models. As such, integrated approaches, using more than one model, are required to fully understand the parasite/host interactions that characterize severe malaria, and more importantly, to fully assess the effect of adjunctive therapies targeting innate immune responses to malaria.

The clinical presentation of a number of bacterial (e.g., leprosy¹) and parasitic diseases (e.g., leishmaniasis²), manifest across a broad clinical spectrum defined by the host immune response to infection. The poles of this spectrum are defined by either an excessive or a deficient inflammatory response to the infecting pathogen. Appropriate management of affected individuals varies according to the patient's position along this clinical and immunological continuum.

Of the estimated one million deaths from malaria each year, most are attributable to severe malaria caused by *Plasmodium falciparum* infection.³ Severe malaria has been increasingly recognized to be a multi-system disorder with a broad spectrum of clinical manifestations that include overlapping, evolving, or complex presentations of disease.⁴ Severe malaria primarily occurs in non-immune individuals where host defense is dependent, at least partly, on innate immune responses to infection.⁵

We propose that malaria may also present along a “clinical-immunological” spectrum with differential host innate immune responses to infection, resulting in divergent clinical outcomes.^{6,7} Individuals with an early deficient inflammatory response that fails to clear infection, define one pole of this spectrum. Whereas “hyper-responsive” hosts generate excess inflammation and cellular activation, which can ultimately contribute to the cerebral complications associated with cerebral malaria (CM),⁸ define the opposite pole.⁶ Representing the mid-range of immunological response are those with regulated responses sufficient to control parasite replication without invoking immunopathological tissue injury. In actual human infection, individuals may have complex responses and resultant mixed clinical syndromes. However, we propose a conceptual framework by which the clinical spectrum may be extended to murine models. *Plasmodium berghei* ANKA (PbA), a model of cerebral malaria where inflammation ex-

acerbates disease^{9,10} and *Plasmodium chabaudi chabaudi* AS (PccAS), a model of malaria infection where pro-inflammatory responses are required for parasite clearance and survival^{11,12} represent the framework's divergent poles, which correspond to differing host inflammatory responses to infection.

The PbA infection of susceptible mice (e.g., C57BL/6) is characterized by excessive early inflammation associated with high levels of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)/lymphotoxin- α and interferon- γ (IFN- γ),^{13,14} contributing to host immunopathology and death within 6–10 days of infection caused by cerebral complications.¹⁵ The PccAS infection is commonly used as a murine malaria model of blood stage parasite replication without cerebral complications.⁵ Susceptible mice lack strong pro-inflammatory responses, resulting in higher parasite loads and ultimately death.¹⁶ Resistant mice show symptoms of disease, usually peaking 10–12 days post-infection, but survive through a controlled inflammatory response.¹⁷ As such, strong inflammatory immune responses to malaria are linked with mortality in the PbA model, but necessary for survival in the PccAS model.

Based on the hypothesis that the innate immune-mediated inflammation plays a key role in defining pathogenesis and determining clinical outcome in severe malaria, we investigated mice deficient in inflammatory signaling caused by genetic deletion of the interleukin-1 receptor-associated kinase 4 (IRAK4) in both the PbA and PccAS murine models of malaria. IRAK4 integrates signals from all three Toll-like receptors (TLRs) so far implicated in the pathogenesis of malaria¹⁸ and from IL-1 and IL-18 cytokine signaling.¹⁹ Unlike TLRs, IL-1 and IL-18 signaling do not appear to be necessary for the development of CM.^{20–22} TLRs, a family of pattern recognition receptors, are essential components of the innate immune response to a variety of microbial pathogens. After ligand binding, TLRs interact with adaptor proteins to activate transcription factors, induce inflammatory responses, and activate adaptive immunity.²³ Mutations in the TLR adaptor protein IRAK4 have been shown to cause

* Address correspondence to Kevin C. Kain, Toronto General Hospital, University Health Network, 200 Elizabeth Street, EN 13-214, Toronto, Ontario, Canada M5G 2C4. E-mail: Kevin.Kain@uhn.on.ca

a primary immunodeficiency syndrome characterized by increased susceptibility to pyogenic bacterial infections in humans.²⁴

The role of TLRs in the host inflammatory response to malaria has been intensely studied. Several pro-inflammatory *Plasmodium* components are recognized by TLRs *in vitro*. The malaria toxin glycosylphosphatidylinositol,²⁵ PbA peroxiredoxin,²⁶ and malarial DNA complexed to parasite hemozoin^{27,28} have been shown to signal through TLR2, 4, and 9, respectively. However, the degree of stimulation and the contribution of each receptor to the overall host inflammatory response and outcome of infection remain controversial.^{20,21,29} Furthermore, human studies, have implicated polymorphisms in *TLR4*, *TLR9*, and *MAL* (an adaptor protein in MyD88-dependent signaling), but not *TLR2*, with disease severity.^{30–32} Precisely how these polymorphisms affect the clinical manifestations of malaria infection remains unclear.

Our experiments show that the survival of *Irak4*^{−/−} animals was enhanced in PbA-infected mice, but worsened in PccAS-infected animals; altering early host inflammatory responses had opposite results at either pole of the proposed conceptual framework. The *Irak4*^{−/−} mice showed marked differences to wild-type (WT) mice during infection in both experimental malaria models. During PccAS infection, mortality was significantly increased in *Irak4*^{−/−} mice compared with WT controls (Fisher's exact test, $P < 0.0001$, $N = 54$, Figure 1A). Parasitemia, the control of which has been linked to host immune responses,^{33–35} was increased in *Irak4*^{−/−} mice ($P < 0.0001$, $N = 75$, Figure 1B), whereas inflammatory cytokine levels were markedly decreased (Figure 1C). Elevated levels of IFN- γ ^{2,36} and TNF^{11,17} are necessary for resolving PccAS infection. The peaks of plasma IFN- γ and TNF (347.7 pg/mL and 94.5 pg/mL, respectively, $N = 40$, Figure 1C) observed in WT mice on Day 10 of PccAS infection, coincided with peak parasitemia and were reduced to near-background levels in *Irak4*^{−/−} mice (IFN- γ : 48.4 pg/mL, $P < 0.0001$; and TNF: 35 pg/mL, $P = 0.01$; $N = 45$, Figure 1C). Levels on measured on Day 1 or post-peak parasitemia (d15 and d18) showed no such difference. Interestingly, levels of the anti-inflammatory cytokine IL-10, which has been implicated as a regulator of inflammation in the PccAS model³⁷ and shown to impede parasite clearance during *Plasmodium yoelii* infection in mice,³⁸ were similar in *Irak4*^{−/−} and WT animals over the course of infection (Figure 1C). Collectively, these results show that IRAK4 plays a key role in regulating pro-inflammatory responses required for parasite clearance and survival during PccAS infection. It was recently demonstrated that macrophages from *Irak4*^{−/−} mice are impaired in their ability to phagocytose malaria-infected red blood cells.³⁹ Because this mechanism has been shown to significantly contribute to parasite clearance in the PccAS model,⁴⁰ impaired parasite clearance may contribute to the increased mortality observed in the *Irak4*^{−/−} animals infected with PccAS.

In contrast, *Irak4*^{−/−} mice survived significantly longer ($P < 0.0001$, $N = 66$, Figure 1D) than WT mice following infection with PbA, with little evidence of cerebral mononuclear cell infiltration (Figure 1F, left and right panels). During PbA infection, increased production of TNF⁹ and IFN- γ ⁴⁰ is associated with appearance of neurological signs of CM. On Day 5 of PbA infection, levels of pro-inflammatory cytokines in plasma were markedly reduced in *Irak4*^{−/−} versus

WT mice (IFN- γ : 253.4 pg/mL versus 44.8 pg/mL, $P = 0.03$, $N = 34$ and TNF: 33.1 pg/mL versus 19.5 pg/mL, $P = 0.01$, $N = 34$, Figure 1G). These data implicate IRAK4 in mediating early inflammatory responses during PbA infection, which are deleterious to the host. The prolonged survival observed in *Irak4*^{−/−} mice, despite equivalent parasitemia, compared with CM-susceptible WT controls (Figure 1E), highlights the critical role of the inflammatory response, rather than parasite burden, in mediating immunopathology. During PbA infection in susceptible C57BL/6 animals, the expansion of regulatory T cells is limited.²⁰ Cerebral malaria development can be inhibited through regulatory T cell expansion,⁴¹ although this remains controversial,⁴² and may be limited in the *Irak4*^{−/−} mice as a result of increases in regulatory T cells.

Our studies show a non-redundant role for the TLR-adaptor molecule IRAK4 as an essential regulator of inflammatory response and disease outcome in two experimental models of severe malaria (Figure 1). As such, our data may in part reconcile the currently discordant observations regarding the role of TLRs in malaria,^{21,43,44} by confirming the need to disrupt a common adaptor to impart a functional difference. Loss of a common TLR-adaptor such as IRAK4 would overcome any presumed functional redundancy that might exist between the individual TLRs previously implicated in malaria pathogenesis (i.e., TLR2, 4, and 9).

Suppressed inflammation conferred by IRAK4 deficiency improved survival in the “hyper-responsive” model, but conferred a worse clinical outcome in the “hypo-responsive” model (Figure 2). Interestingly, work by other groups supports this hypothesis. Griffith and others²⁰ reported that MyD88 deficiency on a C57BL/6 background conferred protection to experimental CM, whereas it conferred susceptibility on a Balb/c background. As with our results, modulating TLR signaling led to divergent outcomes, emphasizing the need to consider the spectrum of host response to infection and the use of integrated models of disease pathogenesis. Importantly, in human infection, complex responses to infection will result in mixed clinical syndromes, rendering prediction of disease outcome even more challenging.

Treatment with potent anti-malarials such as artesunate has decreased mortality in severe malaria, however the case-fatality rate remains high.^{45,46} Modulating the host immune response during malaria infection^{47–52} has been suggested as potential adjunctive therapy in conjunction with anti-malarials to further improve outcome in severe malaria.⁵³ However, our data highlight possible limitations of this approach and the potential to exacerbate disease manifestations at other points along the clinical spectrum, because inhibiting TLR-associated responses may prove beneficial in CM, but may potentially aggravate other clinical manifestations of malaria. Before initiating clinical trials of putative disease immunomodulators, an integrated approach that examines models representing the clinically relevant spectrum of disease may be informative in mitigating harmful effects that might result from unanticipated manipulation of protective host responses to infection, especially in light of the ever-increasing resistance to all anti-malarials.⁵⁴ Future studies of adjunctive therapies for malaria may need to consider individualized assessment of patient's clinical-immunological response to infection before instituting immunomodulatory therapies for severe malaria.

FIGURE 1. Divergent outcomes of PbA and PccAS infections in *Irak4*^{-/-} animals compared with wild-type (WT) controls. **(A)** Unlike in WT controls (8–12 week-old C57BL/6 mice), survival was significantly decreased in *Irak4*^{-/-} animals infected with 1×10^6 PccAS parasites by intraperitoneal injection (Fisher's exact test, $P < 0.0001$, $N = 54$). **(B)** Parasitemia, measured every 2 days by thin blood smear, was significantly increased during PccAS infection (4.9% mean increase over time course (mixed linear model [SPSS 16.0] of the response variable as a function of time and genotype [WT, *Irak4*^{-/-}] as fixed effects, with animal number and experiment identifiers as random effects, $P < 0.0001$, $N = 75$). **(C)** Median levels of plasma IL-10 (top panel), IFN- γ (mid panel), and TNF (bottom panel) collected on Days 5 and 10 and measured by Cytometric Bead Array (CBA) (BD Biosciences, Canada). Day 10 levels of TNF and IFN- γ were decreased in *Irak4*^{-/-} animals compared with WT mice (Mann-Whitney test, $P = 0.01$, and $P < 0.0001$, respectively, $N = 40$). **(D)** Survival was significantly increased (Fisher's exact test, $P < 0.0001$, $N = 66$) in *Irak4*^{-/-} compared with WT animals after infection with 1×10^6 PbA parasites by intraperitoneal injection **(E)** Cerebral mononuclear cell infiltration during PbA infection was not apparent in *Irak4*^{-/-} animals. Representative images from histopathological examinations of brains from WT and *Irak4*^{-/-} mice, isolated on Day 6 post-infection. Brains were formalin fixed, prepared into 5- μ m sections and stained with hematoxylin and eosin. Cerebral malaria (CM)-susceptible mice (WT) displayed characteristic histological evidence of CM. **(F)** Parasitemia, measured every 2 days by thin blood smear, was similar between both groups during PbA infection ($P = 0.1$, $N = 66$). **(G)** Decreases in TNF levels (33.1 pg/mL vs. 19.5 pg/mL, Mann-Whitney test, $P = 0.01$, $N = 34$ [bottom panel], data represented as median and individual values) were observed at Day 5 of PbA infection as were decreases in IFN- γ levels (253.4 pg/mL vs. 44.8 pg/mL, Mann-Whitney test, $P = 0.03$, $N = 34$ [top panel], data represented as median and individual values). Statistical details for all panels can be found in Supplementary Table 1. This figure appears in color at www.ajtmh.org.

FIGURE 2: Summary of findings. Infection with PccAS or PbA malaria parasites in C57BL/6 mice leads to divergent outcomes, which can be reversed by disrupting inflammatory responses. The C57BL/6 mice are resistant to PccAS infection (left panel); the mice show symptoms of malaria but control parasite burden and survive. However, in *Irak4*^{-/-} animals, inflammatory responses to malaria are decreased while parasite loads are increased, a process which may be caused by impaired macrophage function, resulting in reduced survival. When infected with PbA (right panel), C57BL/6 mice generate an excessive inflammatory response, leading to immunopathology and ultimately death of the host. *Irak4*^{-/-} animals, by contrast, have ablated malaria inflammatory responses resulting in decreased cerebral pathology, possibly through the expansion of regulatory T cells, and improved survival. Lower levels of inflammatory cytokines generated during malaria infection in *Irak4*^{-/-} animals reverses the outcomes of both PbA and PccAS infections. Animals lacking IRAK4 become susceptible to PccAS, but resistant to PbA, supporting our hypothesis that PbA and PccAS reflect a clinical spectrum of disease defined by host response to infection. This figure appears in color at www.ajtmh.org.

Received December 13, 2009. Accepted for publication March 12, 2010.

Note: A supplemental table appears at www.ajtmh.org.

Acknowledgments: We thank James Wasmuth for help with data analyses and Laura Erdman for her comments.

Financial support: This study was funded in part by a Canadian Institutes of Health Research (CIHR) Team Grant in Malaria (KCK), CIHR MOP-13721 (KCK), Genome Canada through the Ontario Genomics Institute (KCK), CIHR Canada Research Chairs (WCL, KCK), and the McLaughlin Centre for Molecular Medicine (WCL, KCK). The funding agencies had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors' addresses: Constance Finney, Ziyue Lu, and Michael Hawkes, McLaughlin-Rotman Centre, MaRS Centre, TMDT, Toronto, ON, Canada. E-mails: constance.finney@utoronto.ca, z.lu@utoronto.ca, and michael.hawkes@utoronto.ca. Wen-Chen Yeh, Amgen Inc., South San Francisco, CA. E-mail: weny@amgen.com. Conrad Liles and Kevin Kain, Toronto General Hospital, Toronto, ON, Canada. E-mails: Conrad.Liles@uhn.on.ca and Kevin.Kain@uhn.on.ca.

REFERENCES

- Matos DS, Azeredo-Coutinho RB, Schubach A, Conceicao-Silva F, Baptista C, Moreira JS, Mendonca SC, 2005. Differential interferon-gamma production characterizes the cytokine responses to *Leishmania* and *Mycobacterium leprae* antigens in concomitant mucocutaneous leishmaniasis and lepromatous leprosy. *Clin Infect Dis* 40: e5–e12.
- Walker SL, Lockwood DN, 2007. Leprosy. *Clin Dermatol* 25: 165–172.
- World Health Organization, 2000. Communicable diseases cluster: severe falciparum malaria. *Trans R Soc Trop Med Hyg* 94 (Suppl 1): S1–S90.
- Mackintosh CL, Beeson JG, Marsh K, 2004. Clinical features and pathogenesis of severe malaria. *Trends Parasitol* 20: 597–603.
- Stevenson MM, Riley EM, 2004. Innate immunity to malaria. *Nat Rev Immunol* 4: 169–180.
- Finney CA, Liles WC, Kain KC, 2008. Severe malaria and host paradigm shift in therapeutic strategies to improve clinical outcome. *Drug Discov Today Dis Mech* 4: 207–214.
- Walther M, Woodruff J, Edele F, Jeffries D, Tongren JE, King E, Andrews L, Bejon P, Gilbert SC, De Souza JB, Sinden R, Hill AV, Riley EM, 2006. Innate immune responses to human malaria: heterogeneous cytokine responses to blood-stage *Plasmodium falciparum* correlate with parasitological and clinical outcomes. *J Immunol* 177: 5736–5745.
- Hunt NH, Grau GE, 2003. Cytokines: accelerators and brakes in the pathogenesis of cerebral malaria. *Trends Immunol* 24: 491–499.
- Grau GE, Fajardo LF, Piguet PF, Allet B, Lambert PH, Vassalli P, 1987. Tumor necrosis factor (cachectin) as an essential mediator in murine cerebral malaria. *Science* 237: 1210–1212.
- Rudin W, Favre N, Bordmann G, Ryffel B, 1997. Interferon-gamma is essential for the development of cerebral malaria. *Eur J Immunol* 27: 810–815.
- Hernandez-Valladares M, Naessens J, Musoke AJ, Sekikawa K, Rihet P, Ole-Moiyoi OK, Busher P, Iraqi FA, 2006. Pathology of Tnf-deficient mice infected with *Plasmodium chabaudi adami* 408XZ. *Exp Parasitol* 114: 271–278.
- Su Z, Stevenson MM, 2000. Central role of endogenous gamma interferon in protective immunity against blood-stage *Plasmodium chabaudi* AS infection. *Infect Immun* 68: 4399–4406.
- de Kossodo S, Grau GE, 1993. Role of cytokines and adhesion molecules in malaria immunopathology. *Stem Cells* 11: 41–48.
- Grau GE, Piguet PF, Vassalli P, Lambert PH, 1989. Tumor-necrosis factor and other cytokines in cerebral malaria: experimental and clinical data. *Immunol Rev* 112: 49–70.
- Rest JR, 1982. Cerebral malaria in inbred mice. I. A new model and its pathology. *Trans R Soc Trop Med Hyg* 76: 410–415.
- Cross CE, Langhorne J, 1998. *Plasmodium chabaudi chabaudi* (AS): inflammatory cytokines and pathology in an erythrocytic-stage infection in mice. *Exp Parasitol* 90: 220–229.

17. Jacobs P, Radzioch D, Stevenson MM, 1996. A Th1-associated increase in tumor necrosis factor alpha expression in the spleen correlates with resistance to blood-stage malaria in mice. *Infect Immun* 64: 535–541.
18. Kim TW, Staschke K, Bulek K, Yao J, Peters K, Oh KH, Vandenburg Y, Xiao H, Qian W, Hamilton T, Min B, Sen G, Gilmour R, Li X, 2007. A critical role for IRAK4 kinase activity in Toll-like receptor-mediated innate immunity. *J Exp Med* 204: 1025–1036.
19. Suzuki N, Chen NJ, Millar DG, Suzuki S, Horacek T, Hara H, Bouchard D, Nakanishi K, Penninger JM, Ohashi PS, Yeh WC, 2003. IL-1 receptor-associated kinase 4 is essential for IL-18-mediated NK and Th1 cell responses. *J Immunol* 170: 4031–4035.
20. Griffith JW, O'Connor C, Bernard K, Town T, Goldstein DR, Bucala R, 2007. Toll-like receptor modulation of murine cerebral malaria is dependent on the genetic background of the host. *J Infect Dis* 196: 1553–1564.
21. Togbe D, Schofield L, Grau GE, Schnyder B, Boissay V, Charron S, Rose S, Beutler B, Quesniaux VF, Ryffel B, 2007. Murine cerebral malaria development is independent of toll-like receptor signaling. *Am J Pathol* 170: 1640–1648.
22. Cramer JP, Lepenies B, Kamena F, Holscher C, Freudenberg MA, Burchard GD, Wagner H, Kirschning CJ, Liu X, Seeberger PH, Jacobs T, 2008. MyD88/IL-18-dependent pathways rather than TLRs control early parasitaemia in non-lethal *Plasmodium yoelii* infection. *Microbes Infect* 10: 1259–1265.
23. Medzhitov R, 2001. Toll-like receptors and innate immunity. *Nat Rev Immunol* 1: 135–145.
24. Picard C, Puel A, Bonnet M, Ku CL, Bustamante J, Yang K, Soudais C, Dupuis S, Feinberg J, Fieschi C, Elbim C, Hitchcock R, Lammas D, Davies G, Al-Ghonaia A, Al-Rayes H, Al-Jumaah S, Al-Hajjar S, Al-Mohsen IZ, Frayha HH, Rucker R, Hawn TR, Aderem A, Tufenkeji H, Haraguchi S, Day NK, Good RA, Gougerot-Pocidalo MA, Ozinsky A, Casanova JL, 2003. Pyogenic bacterial infections in humans with IRAK-4 deficiency. *Science* 299: 2076–2079.
25. Krishnegowda G, Hajjar AM, Zhu J, Douglass EJ, Uematsu S, Akira S, Woods AS, Gowda DC, 2005. Induction of proinflammatory responses in macrophages by the glycosylphosphatidylinositols of *Plasmodium falciparum*: cell signaling receptors, glycosylphosphatidylinositol (GPI) structural requirement, and regulation of GPI activity. *J Biol Chem* 280: 8606–8616.
26. Furuta T, Imajo-Ohmi S, Fukuda H, Kano S, Miyake K, Watanabe N, 2008. Mast cell-mediated immune responses through IgE antibody and Toll-like receptor 4 by malarial peroxiredoxin. *Eur J Immunol* 38: 1341–1350.
27. Coban C, Ishii KJ, Kawai T, Hemmi H, Sato S, Uematsu S, Yamamoto M, Takeuchi O, Itagaki S, Kumar N, Horii T, Akira S, 2005. Toll-like receptor 9 mediates innate immune activation by the malaria pigment hemozoin. *J Exp Med* 201: 19–25.
28. Parroche P, Lauw FN, Goutagny N, Latz E, Monks BG, Visintin A, Halmen KA, Lamphier M, Olivier M, Bartholomeu DC, Gazzinelli RT, Golenbock DT, 2007. Malaria hemozoin is immunologically inert but radically enhances innate responses by presenting malaria DNA to Toll-like receptor 9. *Proc Natl Acad Sci USA* 104: 1919–1924.
29. Lepenies B, Cramer JP, Burchard GD, Wagner H, Kirschning CJ, Jacobs T, 2008. Induction of experimental cerebral malaria is independent of TLR2/4/9. *Med Microbiol Immunol (Berl)* 197: 39–44.
30. Mockenhaupt FP, Cramer JP, Hamann L, Stegemann MS, Eckert J, Oh NR, Otchwemah RN, Dietz E, Ehrhardt S, Schroder NW, Bienle U, Schumann RR, 2006. Toll-like receptor (TLR) polymorphisms in African children: common TLR-4 variants predispose to severe malaria. *Proc Natl Acad Sci USA* 103: 177–182.
31. Mockenhaupt FP, Hamann L, von Gaertner C, Bedu-Addo G, von Kleinsorgen C, Schumann RR, Bienle U, 2006. Common polymorphisms of toll-like receptors 4 and 9 are associated with the clinical manifestation of malaria during pregnancy. *J Infect Dis* 194: 184–188.
32. Khor CC, Chapman SJ, Vannberg FO, Dunne A, Murphy C, Ling EY, Frodsham AJ, Walley AJ, Kyrieles O, Khan A, Aucan C, Segal S, Moore CE, Knox K, Campbell SJ, Lienhardt C, Scott A, Aaby P, Sow OY, Grignani RT, Sillah J, Sirugo G, Peshu N, Williams TN, Maitland K, Davies RJ, Kwiatkowski DP, Day NP, Yala D, Crook DW, Marsh K, Berkley JA, O'Neill LA, Hill AV, 2007. A Mal functional variant is associated with protection against invasive pneumococcal disease, bacteremia, malaria and tuberculosis. *Nat Genet* 39: 523–528.
33. Cadman ET, Abdallah AY, Voisine C, Sponaas AM, Corran P, Lamb T, Brown D, Ndungu F, Langhorne J, 2008. Alterations of splenic architecture in malaria are induced independently of Toll-like receptors 2, 4, and 9 or MyD88 and may affect antibody affinity. *Infect Immun* 76: 3924–3931.
34. Su Z, Stevenson MM, 2002. IL-12 is required for antibody-mediated protective immunity against blood-stage *Plasmodium chabaudi* AS malaria infection in mice. *J Immunol* 168: 1348–1355.
35. van der Heyde HC, Pepper B, Batchelder J, Cigel F, Weidanz WP, 1997. The time course of selected malarial infections in cytokine-deficient mice. *Exp Parasitol* 85: 206–213.
36. Stevenson MM, Tam MF, 1993. Differential induction of helper T cell subsets during blood-stage *Plasmodium chabaudi* AS infection in resistant and susceptible mice. *Clin Exp Immunol* 92: 77–83.
37. Li C, Sanni LA, Omer F, Riley E, Langhorne J, 2003. Pathology of *Plasmodium chabaudi chabaudi* infection and mortality in interleukin-10-deficient mice are ameliorated by anti-tumor necrosis factor alpha and exacerbated by anti-transforming growth factor beta antibodies. *Infect Immun* 71: 4850–4856.
38. de Souza JB, Hafalla JC, Riley EM, Couper KN, 2009. Cerebral malaria: why experimental murine models are required to understand the pathogenesis of disease. *Parasitology* 137: 755–772.
39. Erdman LL, Cosio G, Helmers AJ, Gowda DC, Grinstein S, Kain KC, 2009. CD36 and TLR interactions in inflammation and phagocytosis: implications for malaria. *J Immunol* 183: 6452–6459.
40. Sponaas AM, Freitas do Rosario AP, Voisine C, Mastelic B, Thompson J, Koernig S, Jarra W, Renia L, Mauduit M, Potocnik AJ, Langhorne J, 2009. Migrating monocytes recruited to the spleen play an important role in control of blood stage malaria. *Blood* 114: 5522–5531.
41. Nie CQ, Bernard NJ, Schofield L, Hansen DS, 2007. CD4+ CD25+ regulatory T cells suppress CD4+ T-cell function and inhibit the development of *Plasmodium berghei*-specific TH1 responses involved in cerebral malaria pathogenesis. *Infect Immun* 75: 2275–2282.
42. Steeg C, Adler G, Sparwasser T, Fleischer B, Jacobs T, 2009. Limited role of CD4+Foxp3+ regulatory T cells in the control of experimental cerebral malaria. *J Immunol* 183: 7014–7022.
43. Coban C, Ishii KJ, Uematsu S, Arisue N, Sato S, Yamamoto M, Kawai T, Takeuchi O, Hisaeda H, Horii T, Akira S, 2007. Pathological role of Toll-like receptor signaling in cerebral malaria. *Int Immunol* 19: 67–79.
44. Franklin BS, Rodrigues SO, Antonelli LR, Oliveira RV, Goncalves AM, Sales-Junior PA, Valente EP, Alvarez-Leite JI, Ropert C, Golenbock DT, Gazzinelli RT, 2007. MyD88-dependent activation of dendritic cells and CD4(+) T lymphocytes mediates symptoms, but is not required for the immunological control of parasites during rodent malaria. *Microbes Infect* 9: 881–890.
45. Dondorp A, Nosten F, Stepniewska K, Day N, White N, 2005. Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. *Lancet* 366: 717–725.
46. Maude RJ, Pontavornpinyo W, Saralamba S, Aguas R, Yeung S, Dondorp AM, Day NP, White NJ, White LJ, 2009. The last man standing is the most resistant: eliminating artemisinin-resistant malaria in Cambodia. *Malar J* 8: 31.
47. Kwiatkowski D, Molyneux ME, Stephens S, Curtis N, Klein N, Pointaire P, Smit M, Allan R, Brewster DR, Grau GE, et al., 1993. Anti-TNF therapy inhibits fever in cerebral malaria. *Q J Med* 86: 91–98.
48. Parquet V, Briolant S, Torrentino-Madamet M, Henry M, Almeras L, Amalvict R, Baret E, Fusai T, Rogier C, Pradines B, 2009. Atorvastatin is a promising partner for antimalarial drugs in treatment of *Plasmodium falciparum* malaria. *Antimicrob Agents Chemother* 53: 2248–2252.

49. Prasad K, Garner P, 2000. Steroids for treating cerebral malaria. *Cochrane Database Syst Rev* CD000972.
50. Schofield L, Hewitt MC, Evans K, Siomos MA, Seeberger PH, 2002. Synthetic GPI as a candidate anti-toxic vaccine in a model of malaria. *Nature* 418: 785–789.
51. Tsutsui N, Kamiyama T, 1998. Suppression of *in vitro* IFN-gamma production by spleen cells of *Plasmodium chabaudi*-infected C57BL/10 mice exposed to dexamethasone at a low dose. *Int J Immunopharmacol* 20: 141–152.
52. Wassmer SC, Cianciolo GJ, Combes V, Grau GE, 2006. LMP-420, a new therapeutic approach for cerebral malaria? *Med Sci (Paris)* 22: 343–345.
53. Coban C, Ishii KJ, Horii T, Akira S, 2007. Manipulation of host innate immune responses by the malaria parasite. *Trends Microbiol* 15: 271–278.
54. Hayton K, Su XZ, 2004. Genetic and biochemical aspects of drug resistance in malaria parasites. *Curr Drug Targets Infect Disord* 4: 1–10.