In Vivo Switching between Variant Surface Antigens in Human Plasmodium falciparum Infection

ARTICLE *in* THE JOURNAL OF INFECTIOUS DISEASES · OCTOBER 2002

Impact Factor: 6 · DOI: 10.1086/342390 · Source: PubMed

6 AUTHORS, INCLUDING:

Lars Hviid
University of Copenhagen
179 PUBLICATIONS **6,485** CITATIONS
SEE PROFILE

Ibrahim M Elhassan
Jazan University
55 PUBLICATIONS **1,298** CITATIONS
SEE PROFILE

David E Arnot
The University of Edinburgh
110 PUBLICATIONS **4,152** CITATIONS
SEE PROFILE

Thor G Theander
University of Copenhagen
278 PUBLICATIONS **9,294** CITATIONS
SEE PROFILE

CONCISE COMMUNICATION

In Vivo Switching between Variant Surface Antigens in Human *Plasmodium falciparum* Infection

Trine Staalsoe,[1] Amel A. Hamad,[2] Lars Hviid,[1] Ibrahim M. Elhassan,[3] David E. Arnot,[4] and Thor G. Theander[1]

[1]Centre for Medical Parasitology, Copenhagen University Hospital (Rigshospitalet), and Institute for Medical Microbiology and Immunology, University of Copenhagen, Copenhagen, Denmark; [2]Malaria Research Unit, Ministry of Health, and [3]Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan; [4]Institute for Cell, Animal, and Population Biology, University of Edinburgh, Edinburgh, United Kingdom

A semi-immune individual was retrospectively found to have maintained an apparently monoclonal and genotypically stable asymptomatic infection for months after clinical cure of a *Plasmodium falciparum* malaria episode. Before the attack, the individual had no antibodies to variant surface antigens (VSAs) expressed by an isolate (isolate A) obtained at the time of the episode or by a genotypically identical isolate (isolate B) obtained from the same individual 3 months later. Six weeks after the attack, a strong isolate A–specific VSA antibody response had developed in the complete absence of isolate B–specific antibodies. In contrast, plasma obtained 7 months after the attack contained high levels of VSA antibodies recognizing both isolates. This is the first direct evidence of in vivo switching between VSAs in human *P. falciparum* infection. Our results suggest that VSA switching is an important survival strategy of *P. falciparum,* enabling the parasite to persist despite protective, parasite-specific immune responses.

Individuals living in areas of endemic *Plasmodium falciparum* transmission gradually acquire protective immunity against malaria. The targets and mechanisms of this protection are not fully understood, but an increasing body of evidence indicates that antibodies directed against parasite-encoded variant surface antigens (VSAs) on the surface of infected red blood cells (iRBCs) play an important role [1–3]. *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) is the dominant and best characterized VSA. These highly polymorphic proteins are encoded by the *var* gene family, which is present at 40–50 loci per genome [4]. *var* gene switching and ensuing changes in PfEMP1 expression have been demonstrated in vitro [5], and clonal antigenic variation with successive appearance of distinct VSA occurs in simian malaria parasites in vivo [6].

Individual clones of the *P. falciparum* parasite can maintain genotypically stable, asymptomatic blood-stage infections in human carriers for long periods in areas where mosquito transmission is essentially absent and clinical disease very rare [7, 8]. Precisely how the immune system can control parasite multiplication and keep parasitemia at a very low level for >1 year without eliminating the infection is not clear, but the phenomenon has been linked to the above-mentioned clonal antigenic variation [9]. However, so far, direct evidence of in vivo VSA switching in *P. falciparum*–infected humans has been lacking.

We studied a cohort of individuals living in an area of unstable, seasonal *P. falciparum* transmission in eastern Sudan. Among the cohort members, an individual was retrospectively found to have maintained a submicroscopic, apparently monoclonal, and genotypically stable *P. falciparum* infection for months after treatment of a malaria episode. We report here our analysis of antibodies specific for VSA expressed by 2 parasite isolates obtained from that individual at the time of clinical disease and 3 months later.

Methods

Study area and study population. This study was conducted in Daraweesh village, Gedaref State, Sudan, a study site used exten-

Received 20 March 2002; revised 20 April 2002; electronically published 5 August 2002.

Informed consent was obtained from all individuals studied. The study was approved by the Ethical Committee of the University of Khartoum, and the human experimentation guidelines of the Sudanese Ministry of Health were followed in conducting this research.

Financial support: Enhancement of Research Capacity in Developing Countries, Danish International Development Assistance (Danida; grant 104.Dan.8L/401); Danish Medical Research Council (Statens Sundhedsvidenskabelige Forskningsråd; grant 9702273); Danish Research Council for Development Research (Rådet for Ulandsforskning; grants 90839 and 90969); International Cooperation with Developing Countries, Commission of the European Communities (grant ERBIC 18CT970238); United Nations Development Programme/World Bank/World Health Organization (grant TDR960448).

Reprints or correspondence: Dr. Trine Staalsoe, Dept. of Infectious Diseases M7641, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen Ø, Denmark (tscmp@rh.dk).

The Journal of Infectious Diseases 2002; 186:719–22

0022-1899/2002/18605-0020$15.00

sively by us for more than a decade. The epidemiology of malaria in this area has been described in detail elsewhere [10]. In brief, the area is characterized by hypoendemic, unstable parasite transmission with marked seasonal variation, and essentially all malaria episodes occur during and shortly after the annual rains, between June and October. The remainder of the year is very dry, and the level of parasite transmission is undetectable.

Clinical surveillance and collection of blood samples. Thirty-seven individuals who contracted *P. falciparum* malaria during the 1996 transmission season were followed up regularly. Heparinized blood samples were collected from all of these individuals at the beginning of the study, before the 1996 transmission season. Additional samples were collected at the time that clinical *P. falciparum* malaria was diagnosed, 4–6 weeks after the attack, and at the end of the 1996 transmission season. Throughout the following dry season, venous blood samples were collected at monthly intervals, interspersed fortnightly by collection of filter-paper blood spots. For details, see [8].

Sample preparation. From all the heparinized samples, we separated plasma and RBCs by centrifugation (at 800 *g* for 10 min). Plasma was stored at −40°C, and the RBC pellet was snap-frozen and stored in N_2 as described elsewhere [2].

DNA extraction and polymerase chain reaction (PCR) analysis. All blood samples and filter-paper samples were genotyped by PCR at the *msp1, msp2,* and *glurp* loci and sequenced so that the number of parasite clones could be estimated, as described elsewhere [8].

Parasites, in vitro culture, and preparation of late-stage iRBCs. In vitro *P. falciparum* cultures were established from cryopreserved iRBC. Immediately before assays were done, we labeled the cultures with ethidium bromide (40 μg/mL) and purified late-stage iRBCs (hemozoin-containing trophozoites and schizonts) by exposure to a high-gradient magnetic field, as described elsewhere [11].

Measurement of plasma antibodies to VSAs by flow cytometry. Levels of plasma IgG with specificity for VSAs were measured by flow cytometry as described in detail elsewhere [11]. In brief, we sequentially incubated ethidium bromide–labeled iRBCs (2×10^5 RBCs) with 5 μL of plasma (30 min), 0.4 μL of goat anti–human IgG (Dako) (30 min), and 4 μL of fluorescein isothiocyanate–conjugated rabbit anti–goat IgG (Dako) (30 min). Data were collected with a FACScan flow cytometer (BD PharMingen), using a gate on ethidium bromide–positive (and thus infected) RBCs. Flow cytometry data were analyzed by means of WinMDI 2.8 software (Flow Cytometry Core Facility, Scripps Research Institute; http://facs.scripps.edu.software.html).

Results

Retrospective analysis by PCR showed evidence of submicroscopic infections that persisted for extended periods after curative drug treatment in 12 of 37 of the cohort members. In 5 of these 12 subjects, the chronic infection was monoclonal and was identical to the disease-inducing infection ([8]; data not shown). We succeeded in establishing in vitro parasite cultures from the blood samples collected from 1 subject (AE5, a 21-year-old man) at the time of the clinical attack (23 October 1996; isolate A) and 3 months later (31 January 1997; isolate B, when the infection was only detectable by PCR). Genotyping of these 2 isolates yielded the expected PCR profile (figure 1). Attempts to establish in vitro cultures from blood samples with submicroscopic infection obtained from the remaining 4 individuals were unsuccessful.

We next used flow cytometry to measure levels of VSA-specific IgG in plasma samples collected ~2 months (3 September 1996) before and 6 weeks (7 December 1996) and 7 months (1 June 1997) after the disease episode [11]. Before the malaria episode, we did not detect expression of IgG specific for VSA by the parasite isolate A (VSA_A), which was collected at the time of the attack (figure 2*A*). However, 6 weeks after the clinical episode, VSA_A IgG levels had increased several-fold (figure 2*B*), and they remained high 7 months later (figure 2*C*). This shows that the clinical episode was caused by a parasite expressing VSA to which the patient did not have preexisting IgG and that the malaria attack caused a rapid and sustained IgG response specifically recognizing the VSA (i.e., VSA_A) expressed by the infecting isolate. These results are in complete agreement with those of previous studies [1, 12]. In marked contrast, no IgG specific for the VSA expressed by the genotypically identical parasite isolate B (VSA_B), which was collected 3 months later, could be detected before the clinical episode (figure 2*D*), and only borderline reactivity was seen 6 weeks after the clinical

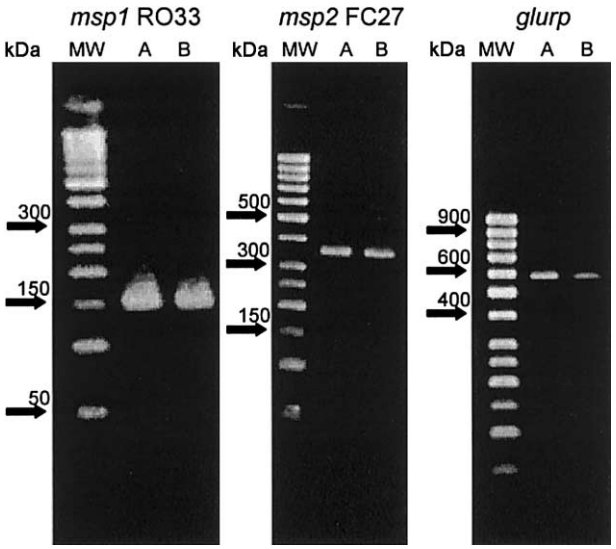

Figure 1. Genotyping by polymerase chain reaction, using primers designed to fit polymorphic regions of merozoite surface antigen (MSP)–1 (*msp1,* type RO33), MSP-2 (*msp2,* type FC27), and glutamate-rich protein *(glurp),* of *Plasmodium falciparum* isolates from patient AE5. For each antigen, molecular weight markers (MW; *left lanes*), size indications, and results that were identical for isolate A (obtained at the time of the clinical attack, on 23 October 1996; *center lanes*) and isolate B (obtained 3 months later, on 31 January 1997; *right lanes*) are shown. No other type-specific *msp1* or *msp2* bands were seen in either isolate (not shown).

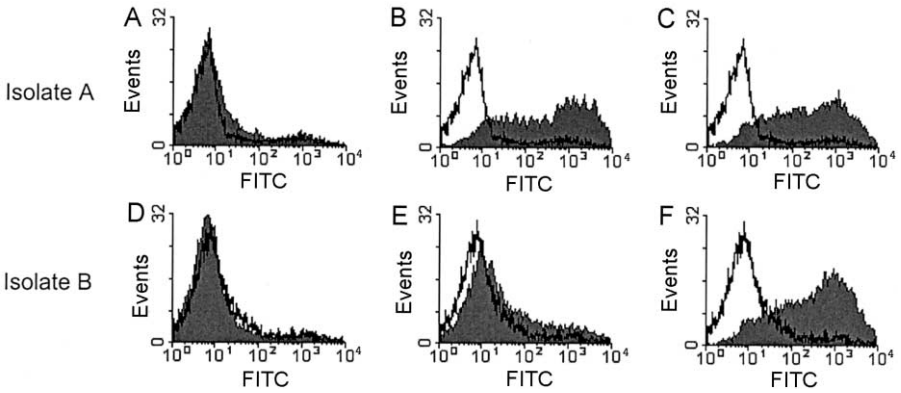

Figure 2. Plasma levels of IgG specific for variable surface antigens (VSAs) expressed by *Plasmodium falciparum* isolate A, which was obtained from individual AE5 at the time of malaria attack (23 October 1996; *A–C*), and isolate B, which was obtained 3 months later from the same individual (31 January 1997; *D–F*). *A* and *D,* IgG levels before the malaria attack (3 September 1996). *B* and *E,* IgG levels 6 weeks after the attack (7 December 1996). *C* and *F,* IgG levels 7 months after the malaria attack (1 June 1997). In each panel, the VSA antibody reactivity in plasma from patient AE5 *(shaded histograms)* and the corresponding reactivity in a plasma sample from a donor without *P. falciparum* exposure *(open histograms)* are shown. FITC, fluorescein isothiocyanate.

attack (figure 2*E*). However, high levels of VSA_B-specific IgG levels were found in the plasma sample collected 4 months after the collection of isolate B (figure 2*F*).

Discussion

Results of animal experiments have long indicated that antigenic variation of VSAs is the basis for the persistence of malaria parasitemia despite the presence of a continuing immunological attack [6, 13, 14]. Furthermore, recent studies of human *P. falciparum* infections have indicated that acquisition of VSA-specific immunity can shape the VSA-expression pattern, probably by gradually narrowing the repertoire of VSAs compatible with parasite survival in the semi-immune host, which leads to a decrease in the virulence of the infection [2, 15]. Thus, acquisition of VSA-specific antibodies appears to be central to acquisition of protective immunity in humans. However, although antigenic variation is known to occur in cloned *P. falciparum* in vitro, only circumstantial evidence has been available, until now, that this also occurs in vivo in *P. falciparum*–infected humans. By documenting the progression in plasma antibody levels with specificity for VSA expressed by *P. falciparum* isolates obtained at different time points, we show directly that the antigenic phenotype of a genotypically stable and monoclonal *P. falciparum* infection can change in the course of a human infection, and that these changes can induce production of VSA-specific antibodies. Our data are the first, to our knowledge, to formally prove that the VSA-expression pattern changes with time during chronic malaria infections in humans, probably as a mechanism developed by the parasite to survive successive waves of immunological attack on parasite-encoded VSAs, allowing the maintenance of long-term, subclinical infections, similar to those described elsewhere in experimental simian infections [6, 13, 14].

Acknowledgments

We gratefully acknowledge the continued support and collaboration of the people of Daraweesh and the research team at Gedaref Malaria Research Station. We thank Shadin Abdelrahim, Anne Corfitz, Gitte Grauert, Gamilla Ibrahim, Kirsten Pihl, and Jimmy Weng for excellent assistance in the field and laboratory.

References

1. Bull PC, Lowe BS, Kortok M, Molyneux CS, Newbold CI, Marsh K. Parasite antigens on the infected red cell are targets for naturally acquired immunity to malaria. Nature Med **1998**;4:358–60.
2. Nielsen MA, Staalsoe T, Kurtzhals JAL, et al. *Plasmodium falciparum* variant surface antigen expression varies between isolates causing severe and non-severe malaria and is modified by acquired immunity. J Immunol **2002**; 168:3444–50.
3. Dodoo D, Staalsoe T, Giha H, et al. Antibodies to variant antigens on the surfaces of infected erythrocytes are associated with protection from malaria in Ghanaian children. Infect Immun **2001**;69:3713–8.
4. Su X, Heatwole VM, Wertheimer SP, et al. The large diverse gene family *var* encodes proteins involved in cytoadherence and antigenic variation of *Plasmodium falciparum*–infected erythrocytes. Cell **1995**;82:89–100.
5. Roberts DJ, Craig AG, Berendt AR, et al. Rapid switching to multiple antigenic and adhesive phenotypes in malaria. Nature **1992**;357:689–92.
6. Handunnetti SM, Mendis KN, David PH. Antigenic variation of cloned *Plasmodium fragile* in its natural host *Macaca sinica:* sequential appearance of successive variant antigenic types. J Exp Med **1987**;165: 1269–83.
7. Roper C, Elhassan IM, Hviid L, et al. Detection of very low level *Plasmodium falciparum* infections using the nested polymerase chain reaction and a reassessment of the epidemiology of unstable malaria in Sudan. Am J Trop Med Hyg **1996**;54:325–31.
8. Hamad AA, El Hassan IM, El Khalifa AA, et al. Chronic *Plasmodium fal-*

ciparum infections in an area of low intensity malaria transmission in the Sudan. Parasitology **2000**;120:447–56.

9. Staalsoe T, Hviid L. The role of variant-specific immunity in asymptomatic malaria infections: maintaining a fine balance. Parasitol Today **1998**;14: 177–8.
10. Giha HA, Rosthoj S, Dodoo D, et al. The epidemiology of febrile malaria episodes in an area of unstable and seasonal transmission. Trans R Soc Trop Med Hyg **2000**;94:645–51.
11. Staalsoe T, Giha HA, Dodoo D, Theander TG, Hviid L. Detection of antibodies to variant antigens on *Plasmodium falciparum*–infected erythrocytes by flow cytometry. Cytometry **1999**;35:329–36.
12. Ofori MF, Dodoo D, Staalsoe T, et al. Malaria-induced acquisition of antibodies to *Plasmodium falciparum* variant surface antigens. Infect Immun **2002**;70:1982–8.
13. Brown KN, Brown IN. Immunity to malaria: antigenic variation in chronic infections of *Plasmodium knowlesi*. Nature **1965**;208:1286–8.
14. Butcher GA, Cohen S. Antigenic variation and protective immunity in *Plasmodium knowlesi* malaria. Immunology **1972**;23:503–21.
15. Bull PC, Kortok M, Kai O, et al. *Plasmodium falciparum*–infected erythrocytes: agglutination by diverse Kenyan plasma is associated with severe disease and young host age. J Infect Dis **2000**;182:252–9.