Platelet Accumulation in Brain Microvessels in Fatal Pediatric Cerebral Malaria

Georges E. Grau,[1] Charles D. Mackenzie,[2] Richard A. Carr,[4] Mireille Redard,[6] Giampaolo Pizzolato,[6] Claude Allasia,[1] Claude Cataldo,[1] Terrie E. Taylor,[3,7] and Malcolm E. Molyneux[5,8]

[1]Experimental Parasitology Unit, IFR48, Université de la Méditerranée, Marseille, France; Colleges of [2]Veterinary and [3]Osteopathic Medicine, Michigan State University, East Lansing; [4]South Warwickshire General Hospitals, Warwick, and [5]School of Tropical Medicine, University of Liverpool, United Kingdom; [6]University of Geneva, Switzerland; [7]Blantyre Malaria Project, University of Malawi College of Medicine, and [8]Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi

The pathogenesis of fatal cerebral malaria (CM) is not well understood, in part because data from patients in whom a clinical diagnosis was established prior to death are rare. In a murine CM model, platelets accumulate in brain microvasculature, and antiplatelet therapy can improve outcome. We determined whether platelets are also found in cerebral vessels in human CM, and we performed immunohistopathology for platelet-specific glycoprotein, GPIIb-IIIa, on tissue from multiple brain sites in Malawian children whose fatal illness was severe malarial anemia, CM, or nonmalarial encephalopathy. Platelets were observed in 3 locations within microvessels: between malaria pigment and leukocytes, associated with malaria pigment, or alone. The mean surface area of platelet staining and the proportion of vessels showing platelet accumulation were significantly higher in patients with CM than in those without it. Platelet accumulation occurs in the microvasculature of patients with CM and may play a role in the pathogenesis of the disease.

Malaria remains a major threat to life, and the treatment of severe disease is unsatisfactory [1]. Progress in preventing and treating malaria is hampered by our ignorance of pathogenic mechanisms and the causes of death. None of the existing animal or in vitro models exactly mimic the human disease [2], and most information to date has come from autopsy studies of adults.

Ninety percent of malaria-associated mortality occurs in African children [3]. In the clinical manifestations of severe malaria, there are important differences between children and adults, so the findings in adult postmortem studies may not be entirely applicable to children [4]. None of the autopsy studies conducted to date has contained all of the elements required for description of malaria pathogenesis in African children [4].

Cerebral malaria (CM) is characterized by an accumulation of parasitized red blood cells (pRBCs) in brain microvessels. The mechanism of this accumulation has been extensively studied, but its role in the pathogenesis of fatal disease remains incompletely understood [5–7], in part because of the paucity of histopathologic studies, particularly in children. It is likely that the accumulation of pRBCs interferes with microcirculation, leading to ischemia, but other mechanisms may further contribute to pathogenesis. In addition to pRBCs [8], sequestered cells such as leukocytes (macrophages and

Received 29 April 2002; revised 13 September 2002; electronically published 24 January 2003.

Presented in part: 48th meeting of the American Society of Tropical Medicine and Hygiene, Washington, DC, December 1999 (abstract 780).

Informed consent was obtained from patients or their parents/guardians, and human experimentation guidelines of the US Department of Health and Human Services and/or the ethical review committees at the University of Malawi College of Medicine, Michigan State University, and the University of Liverpool were followed in the conduct of clinical research.

Financial support: National Institutes of Health (grant RO1-AI34969-01A1); Wellcome Trust (grant 058390/Z/99/Z).

Reprints or correspondence: Prof. Georges E. Grau, Experimental Parasitology Unit, IFR 48, Faculties of Medicine and Pharmacy, Université de la Méditerranée, 27, Boulevard Jean Moulin, F-13 385 Marseille Cedex 5, France (georges.grau@medecine.univ-mrs.fr).

The Journal of Infectious Diseases 2003;187:461–6

0022-1899/2003/18703-0014$15.00

monocytes) have been described in brain vessels in patients with CM [9–11].

In a mouse model of CM, platelet deposition appears to be a major contributor to death, given that platelets accumulate in microvessels and that antiplatelet therapy can improve outcome [12, 13]. In murine CM, however, pRBC accumulation is not a major histopathologic feature, although it has been described [14, 15]. In vitro, platelets have been shown to act as effectors of vascular damage, when endothelial cells have been prestimulated by tumor necrosis factor (TNF) [16]. In histopathologic studies of human CM, the presence of platelets in brain vessels has been described [17]. However, because no comparisons have been made between patients who were comatose before death and those who were not, the relationship to cerebral dysfunction has not been established.

The aims of the present study were to investigate whether platelets selectively accumulate in the cerebral microvessels in fatal human malaria, to ascertain whether platelets are associated with malaria pigment or leukocytes in these sites, and, finally, to determine whether the pattern or intensity of platelet deposition differs in children dying of CM compared with children dying of severe malarial anemia (SMA) and nonmalarial diseases.

PATIENTS AND METHODS

Patients

Postmortem sampling was performed during autopsies of children in whom the following diagnoses were determined during life.

Group 1: SMA. All patients with SMA ($n = 5$) had *Plasmodium falciparum* parasitemia and severe anemia (hematocrit, <12%) and were conscious until ⩽2 h before death. At the time of death, 2 were judged to have congestive cardiac failure, 1 was acidotic and hypoglycemic, and 1 had *Salmonella enteritidis* bacteremia. There was no evidence of renal failure, jaundice, bleeding tendency, pulmonary infection, or meningitis in any of this group.

Group 2: CM. Patients with CM ($n = 7$) were admitted to the hospital while in a coma (Blantyre coma score ⩽2/5) and had *P. falciparum* parasitemia and no other clinically evident cause of unconsciousness. The median duration of coma prior to death was 20 h (range, 14–56 h). In 3 patients, hypoglycemia was identified at some point during the illness and was corrected without an effect on the depth of coma. Two patients developed severe anemia (hematocrit, <12%) and received blood transfusions; 1 child became severely anemic (packed cell volume, 13%) and died before a blood transfusion could be given. Lumbar puncture yielded normal cerebrospinal fluid in 4 patients but was not performed in 3 others because of the presence of papilloedema (in 2 cases) or respiratory distress (in 1 case). All patients had hypertonicity, opisthotonos, seizures, or posturing of limbs. Several were clinically and/or biochemically acidemic. None had jaundice, renal failure, hemoglobinuria, or abnormal bleeding.

Group 3: nonmalarial encephalopathies (NMEs). Patients with NME ($n = 5$) had altered consciousness (Blantyre coma score, 0–3/5) in the absence of malaria parasitemia. Two were hypoglycemic and acidotic, with no clinical response to treatment and, at autopsy, no evident cause of death; Reye's syndrome was considered to be a possible diagnosis in these 2 cases. Another patient had the characteristic history and features of organophosphate poisoning, 1 had tuberculous meningitis, and 1 had *Haemophilus influenzae* meningitis.

Details of the autopsy procedure in the study have been described elsewhere [18]. The interval between death and sampling was recorded. In the present study, after macroscopic appearances of the intact and transected brain were noted, 2-mm cubes of brain tissue were placed in optimum-cutting-temperature medium (Tisse TEK; Leica) and immediately were immersed in liquid nitrogen. Three forebrain areas (frontal, parietal, and temporal lobes) and cerebellum were studied. Immunohistochemical analyses showed consistent results in these areas, and the data shown herein are from the parietal lobe. All vessels were analyzed: there was no selection. Both gray and white matter were studied, with identical results. Brain samples from the parietal lobe were also fixed in formalin and were processed for staining with hematoxylin and eosin. At least 100 brain capillaries from each patient were observed under oil, and the contents of each capillary were counted (unpigmented parasites, pigmented parasites, and extra-erythrocytic pigment globules). The proportion of capillaries containing any of these was included to determine one measure of sequestration, the percentage of vessels parasitized; the sum of unpigmented plus pigmented parasites was calculated and used as a second measure of sequestration (R.A.C., unpublished data). All histopathologic studies were performed blinded—that is, without knowledge of the clinical diagnosis.

Immunohistochemical Staining and Quantitative Image Analysis

Mouse anti-human GPIIb-IIIa monoclonal antibodies (mAbs) (anti-CD61 [DAKO] and anti-CD41 [Immunotech]) were used to stain cryopreserved brain samples, with each of the mAbs showing identical staining patterns. After a 30-min incubation at 10 μg/mL, sections were washed and revealed by a peroxidase-labeled goat anti-mouse Ig antibody, according to the manufacturer's recommendations (Vector). Herein, the term "malaria pigment" refers to all pigment, whether within or outside pRBC and white blood cells (WBCs). Mouse anti-human anti-CD36 (FA6 clone; Immunotech) was used. Controls for immunohistochemistry consisted of irrelevant, isotype-matched mAbs as a first step and of labeled secondary antibody

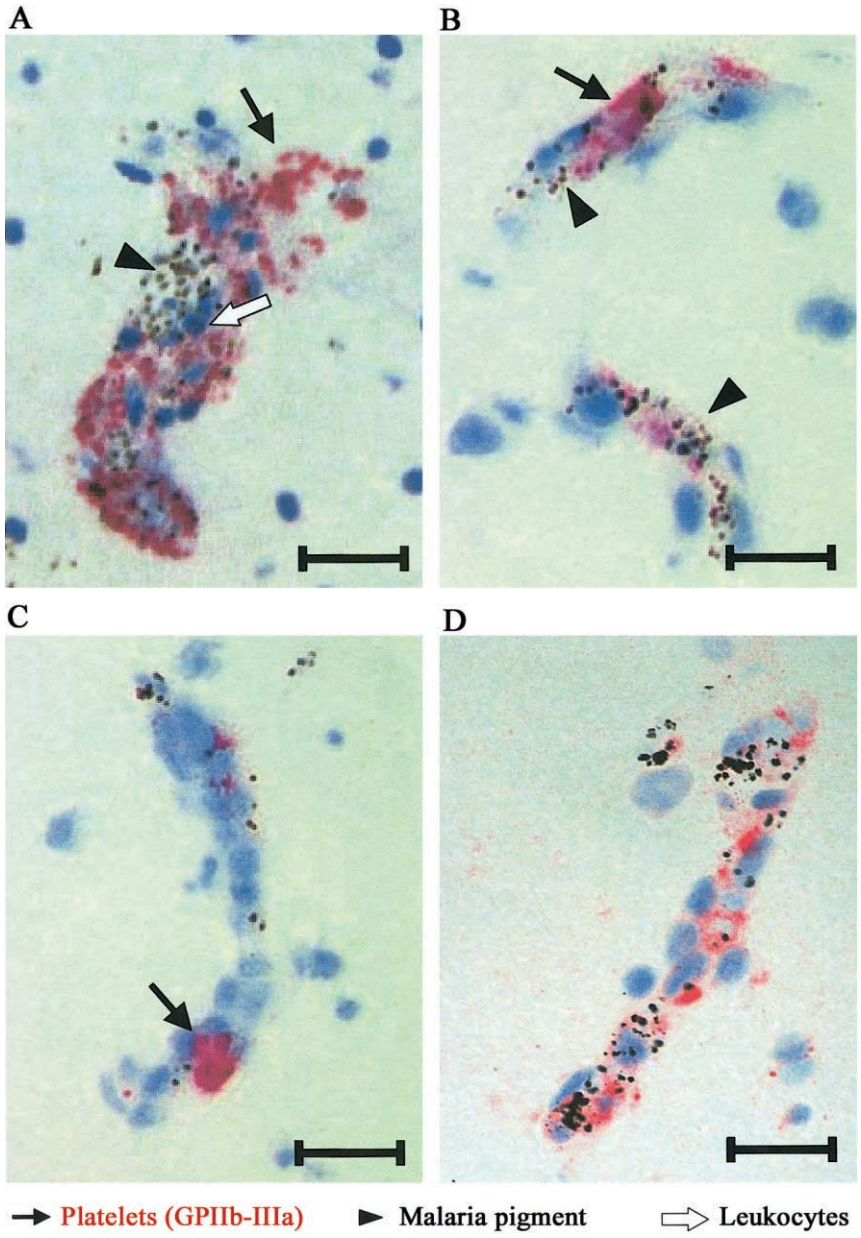

Figure 1. Platelet accumulation in brain vessels of patients with cerebral malaria: different patterns of distribution. *A–C,* Immunostaining with anti-CD41 (GPIIb-IIIa) monoclonal antibodies (mAbs), showing platelets between leukocytes and malaria pigment *(A),* platelets with malaria pigment *(B),* platelets alone *(C),* and staining with anti-CD36 mAb *(D).* Scale bars, 50 μm *(A)* and 25 μm (*B–D).*

alone. Control brain samples were from road-accident victims without brain injury. Samples were analyzed by a Zeiss Axiophot microscope coupled to a quantitative image-analysis device, SAMBA 2005 (Faculty of Medicine, Université de la Méditerranée).

Statistical Analyses

Groups were compared by the nonparametric Mann-Whitney *U* test. Correlations were evaluated by the nonparametric Spearman test.

RESULTS

In the children studied, malaria pigment was seen not just within pigmented trophozoites and intact schizonts but also seemingly free lying within vessels—or, perhaps more likely, within "pigmented ghosts" and aggregated within intravascular WBCs [19].

In brain capillaries and postcapillary venules of patients with CM, the lumen showed a marked staining with anti–GPIIb-IIIa mAb (figure 1). Three patterns of platelet accumulation were observed: platelets were clustered between malaria pig-

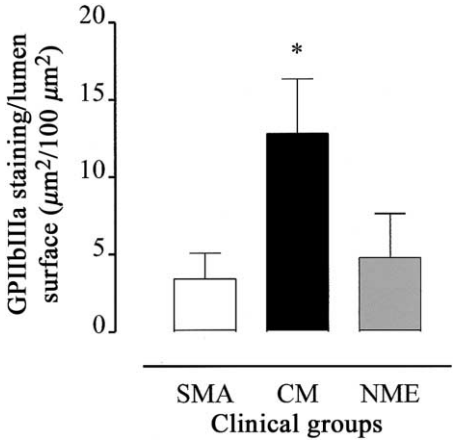

Figure 2. Quantitation of platelet sequestration in different clinical groups: surface of GPIIb-IIIa staining present in >30 vessels per brain site, normalized on the surface of the lumen (data normalized on the surface of CD31 are similar; not shown). Confidence intervals: cerebral malaria (CM) vs. severe malarial anemia (SMA), 0.92–17.79; CM vs. nonmalarial encephalopathy (NME), 17.33–1.35. *$P = .03$, vs. SMA.

ment and leukocytes (figure 1*A*), were associated with malaria pigment (figure 1*B*), or occurred alone (figure 1*C*). These patterns were not mutually exclusive, and there was no predominant pattern in any patient with CM. Staining with anti-CD36 mAb revealed a platelet pattern inside the vessel lumens that was similar to that obtained with anti–GPIIb-IIIa (figure 1*D*).

Prior to quantitative image analysis, we documented the proportions of vessels in each sample that were sectioned transversely, obliquely, and longitudinally—long:short axes <2:1, 2–4:1, and >4:1, respectively—to ensure comparability between patients. The mean ± SD of the transverse/longitudinal/oblique proportion was similar in the 3 groups of patients: $47.54 \pm 5.25/11.72 \pm 4.14/40.74 \pm 5.08$ in group 1, $47.74 \pm 4.26/11.27 \pm 3.52/40.97 \pm 3.03$ in group 2, and $50.42 \pm 8.65/11.36 \pm 4.69/38.24 \pm 9.52$ in group 3. We also quantitated the vessel density in each tissue section, using immunostaining for CD31 and, similarly, found no difference between the 3 groups of patients: the mean ± SD surface of CD31 staining, expressed in μm^2/100 μm^2 of vessel lumen surface, was 29.27 ± 4.32 in group 1, 27.67 ± 4.13 in group 2, and 32.83 ± 4.12 in group 3.

To evaluate the relationship between platelet accumulation and clinical syndrome, the GPIIb-IIIa immunostaining in the 3 groups of patients was quantitated and compared. Brain vessels were individually delineated. The surface area of immunostaining (red channel) and the area of the lumen surface were calculated by planimetry in each vessel. At least 30 microvessels (capillaries and postcapillary venules) were measured in each patient. Data are expressed as immunostained surface (in μm^2) per 100 μm^2 of lumen surface and as the number of vessels presenting a platelet immunostaining >5 μm^2 in their lumen, in the whole length of vessel visible. Intravascular leukocytes were counted in the same length of vessel. Intravascular malaria pigment was estimated by dividing the total pigment surface area (black channel) by the mean of individual pigment surface areas. Globules of malaria pigment were readily countable on frozen sections, whereas intact trophozoites and schizonts were not.

As shown in figure 2, platelet accumulation was significantly more abundant in patients with CM than in those who died from either SMA ($P = .03$) or NME ($P = .001$). In addition, the proportion of vessels with platelet accumulation, defined as an area >5 μm^2, was significantly higher in patients with CM (26.6% ± 6.5%) than in those with SMA (8.6% ± 2.5%) ($P = .022$) but was similar in patients with CM and in patients with NME (26.8 ± 5.8%).

Malaria-pigment sequestration was significantly higher in patients with CM (figure 3 and table 1). Because platelet accumulation was commonly accompanied by malaria pigment and/or leukocytes and because the distribution of these elements was not uniform between vessels in a given patient, the relationship between these elements was analyzed. In each brain, the densities of platelets, malaria pigment, and leukocytes were recorded for each of ⩾30 microvessels, and correlations were calculated to assess colocalization of platelets with the other cell types. Platelet accumulation was found to colocalize with malaria pigment, as determined by a significant ($P < .01$) correlation (Spearman test) in 6 of 7 patients in group 2 but in none of the patients in the other 2 groups.

In 5 of 7 patients with CM, the area of intravascular platelet staining was correlated significantly with the number of leukocytes, but this was not the case in the CM group as a whole. In patients with either SMA or NME, there was no correlation between either platelets and malaria pigment or platelets and

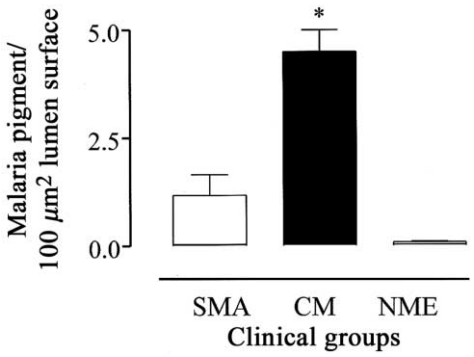

Figure 3. Quantitation of intravascular pigments in different clinical groups. Computerized image analysis of the number of malaria pigments, as described. Clinical groups are as in figure 2. Confidence intervals: cerebral malaria (CM) vs. severe malarial anemia (SMA), 1.58–4.49; CM vs. nonmalarial encephalopathy (NME), −5.50 to −3.31. *$P = .0007$, vs. SMA.

Table 1. Proportion of vessels with platelet accumulation in brain vessels of patients from the 3 clinical groups.

Cases	GPIIb-IIIa-positive vessels	upp + pp/100 cross-sectioned vessels	Total vessels (fever[a]) [coma[b]]	Percentage of vessels with	
				Platelets	Parasites
SMA					
MP2	12	0	199 (100) [0]	6	18
MP4	13	110	223 (12) [0]	6	67
MP7	33	10	176 (25) [0]	19	15
MP12	4	2	122 (74) [0]	3	23
MP19	46	0	1006 (76) [0]	5	3
CM					
MP5	52	588	632 (90) [30]	8	89
MP6	74	202	153 (73) [20]	48	86
MP11	96	209	248 (99) [56]	39	94
MP13	48	361	168 (96) [15]	29	96
MP15	42	206	214 (33) [19]	20	84
MP16	12	318	195 (30) [28]	6	77
MP21	134	697	368 (30) [14]	36	97
NME					
MP8	17	0	152 (54) [7]	11	1
MP10	83	8	182 (9) [9]	46	13
MP17	24	6	120 (32) [20]	20	8
MP18	58	0	158 (64) [14]	37	0
MP20	20	0	100 (90) [30]	20	0

NOTE. A vessel was defined as positive for platelets if it contained a surface of GPIIb-IIIa staining >5 μm^2 in its lumen. CM, cerebral malaria; NME, nonmalarial encephalopathies; pp, pigmented parasites; SMA, severe malarial anemia; upp, unpigmented parasites.

[a] Duration of fever (h) from reported onset until time of death.

[b] Duration of coma (h) from reported onset until time of death.

leukocytes, except patient 18. In no patient group was there any discernible relationship between the duration of either fever or coma and the presence or intensity of platelet staining in cerebral vessels.

DISCUSSION

We have shown that the degree of platelet accumulation in microvessels is significantly greater in patients with CM than in those with either SMA or NME and that, in most patients with CM, platelets are colocalized with malaria pigment and white cells. The proportion of vessels showing platelet accumulation is significantly higher in patients with CM than in those with SMA.

Platelet changes have long been known in malaria, thrombocytopenia being a usual feature of plasmodial infections. The pathogenesis of malaria-induced thrombocytopenia appears to involve immune mechanisms. This has been described in *P. falciparum* [20] and *P. vivax* [21] malaria, as well as in a murine CM model, *P. berghei ANKA* (PbA) infection [22]. In addition to immune-mediated damage, a direct interaction between platelets and the parasite has been observed—namely, merozoite engulfment by platelets [23, 24]. Fajardo [25] has reviewed evidence suggesting that platelets may play important roles, both beneficial and deleterious, in infections.

The presence of platelets in human CM has been described by electron microscopy [17], but its relation to different disease syndromes has not been defined. Platelet sequestration has been demonstrated in murine CM [12]. In this case, the presence of platelets is 1 of 3 arguments in favor of their role in the pathogenesis of the syndrome; the other 2 arguments include the capacity of anti–LFA-1 mAb to ablate platelet trapping in the brain and the beneficial effect of decreasing platelet counts in PbA-infected mice [12]. The patterns of platelet sequestration described herein are identical to those found in mice with CM, and the phenomenon is quantitatively comparable [26].

Platelets can bind to endothelial cells by various molecules [27]; among these is CD36, a counterreceptor for pRBCs [28, 29]. In our patients, CD36 was found on brain microvessels from all patients, including control subjects (data not shown), which confirms the data reported by Barnwell et al. [29]. In addition to endothelial cells and monocytes, human erythrocytes express CD36 [30] and therefore can bind platelets. In turn, CD36 may serve as a receptor for some pRBCs and, indirectly, may have a role in platelet and leukocyte arrest in brain vessels. It has been shown that pRBCs that bind to CD36 can activate platelets and monocytes [31]. Recently, platelets have been shown to mediate the clumping of pRBC, a phenomenon that is associated with severe malaria [32]. In addition to platelet-endothelial interactions, platelet-leukocyte interactions may be important in CM, as suggested by the correlation found between these 2 cell types in brain vessels.

Platelet accumulation may have deleterious effects on endothelial-cell viability. Although platelets exert a trophic role on endothelium in physiological conditions [33], it has been proposed that an amplification of this phenomenon, particularly when endothelial cells are prestimulated by TNF, can lead to endothelial injury [16]. A possible role of platelets in microvascular pathology is supported by data in an experimental CM model in vivo and by the numerous mechanisms by which platelets can alter endothelial functions [27]; these mechanisms include an induction of the adhesiveness of endothelial cells for leukocytes, an enhancement of TNF-induced killing, and the modulation of leukocyte functions, notably the induction of their chemokine synthesis.

Taken together, the data presented herein are the first demonstration of the presence of platelets in human CM in a controlled study. On the basis of a study of this kind we are unable to determine whether platelet deposition makes an important contribution to the pathogenesis of either coma or death in

severe malaria. An alternative possibility is that platelet deposition occurs as an end-stage consequence of endothelial damage, after the sequestration and eventual rupture of parasitized erythrocytes, and that other parasite-induced mechanisms or host responses are responsible for pathogenesis and clinical disease. Distinguishing among these possible mechanisms will be important in the search for new therapies for life-threatening malaria.

References

1. Beales PF, Brabin B, Dorman E, et al. Severe falciparum malaria. Trans R Soc Trop Med Hyg **2000**; 94:S1–90.
2. Lou J, Lucas R, Grau GE. Pathogenesis of cerebral malaria: recent experimental data and possible applications in man. Clin Microbiol Rev **2001**; 14:810–20.
3. Oaks SC, Mitchell VS, Pearson GW, Carpenter CCJ, eds. Malaria: obstacles and opportunities. Washington, DC: National Academy Press, **1991**.
4. World Health Organization, Communicable Diseases Cluster. Severe falciparum malaria. Trans R Soc Trop Med Hyg **2000**; 94(Suppl 1):S1–90.
5. Berendt AR, Turner GDH, Newbold CI. Cerebral malaria: the sequestration hypothesis. Parasitol Today **1994**; 10:412–4.
6. Clark IA, Rockett KA. The cytokine theory of human cerebral malaria. Parasitol Today **1994**; 10:410–2.
7. Grau GE, de Kossodo S. Cerebral malaria: mediators, mechanical obstruction or more? Parasitol Today **1994**; 10:408–9.
8. Silamut K, Phu NH, Whitty C, et al. A quantitative analysis of the microvascular sequestration of malaria parasites in the human brain. Am J Pathol **1999**; 155:395–410.
9. Oo MM, Aikawa M, Than T, et al. Human cerebral malaria: a pathological study. J Neuropathol Exp Neurol **1987**; 46:223–31.
10. Porta J, Carota A, Pizzolato GP, et al. Immunopathological changes in human cerebral malaria. Clin Neuropathol **1993**; 12:142–6.
11. Patnaik JK, Das BS, Mishra SK, Mohanty S, Satpathy SK, Mohanty D. Vascular clogging, mononuclear cell margination, and enhanced vascular permeability in the pathogenesis of human cerebral malaria. Am J Trop Med Hyg **1994**; 51:642–7.
12. Grau GE, Tacchini-Cottier F, Vesin C, et al. TNF-induced microvascular pathology: active role for platelets and importance of the LFA-1/ICAM-1 interaction. Eur Cytokine Netw **1993**; 4:415–9.
13. Grau GE, Lou J. TNF in vascular pathology: importance of platelet-endothelium interactions. Res Immunol **1993**; 144:355–63.
14. Jennings VM, Actor JK, Lal AA, Hunter RL. Cytokine profile suggesting that murine cerebral malaria is an encephalitis. Infect Immun **1997**; 65:4883–7.
15. Hearn J, Rayment N, Landon DN, Katz DR, Desouza JB. Immunopathology of cerebral malaria: morphological evidence of parasite sequestration in murine brain microvasculature. Infect Immun **2000**; 68: 5364–76.
16. Lou J, Donati YRA, Juillard P, et al. Platelets play an important role in TNF-induced microvascular endothelial cell pathology. Am J Pathol **1997**; 151:1397–405.
17. Pongponratn E, Riganti M, Harinasuta T, Bunnag D. Electron microscopy of the human brain in cerebral malaria. Southeast Asian J Trop Med Public Health **1985**; 16:219–27.
18. Brown H, Turner G, Rogerson S, et al. Cytokine expression in the brain in human cerebral malaria. J Infect Dis **1999**; 180:1742–6.
19. Chandak PB, Carr RA, Seed PT, et al. Fibrin thrombi in the brain in fatal pediatric malaria correlate with malarial pigment globules [abstract 297]. In: Program and abstracts of the 48th Annual Meeting of the American Society of Tropical Medicine and Hygiene (Washington, DC). Am J Trop Med Hyg **1999**; 61(3[Suppl]):272.
20. Kelton JG, Keystone J, Moore J, et al. Immune-mediated thrombocytopenia of malaria. J Clin Invest **1983**; 71:832–6.
21. Yamaguchi S, Kubota T, Yamagishi T, et al. Severe thrombocytopenia suggesting immunological mechanisms in two cases of vivax malaria. Am J Hematol **1997**; 56:183–6.
22. Grau GE, Piguet PF, Gretener D, Vesin C, Lambert PH. Immunopathology of thrombocytopenia in experimental malaria. Immunology **1988**; 65:501–6.
23. Fajardo LF, Tallent C. Malarial parasites within human platelets. JAMA **1974**; 229:1205.
24. Fajardo LF. Malarial parasites in mammalian platelets. Nature **1973**; 243: 298–9.
25. Fajardo LF. The role of platelets in infections. I. Observations in human and murine malaria. Arch Pathol Lab Med **1979**; 103:131–4.
26. Piguet PF, DaLaperrousaz C, Vesin C, Tacchinicottier F, Senaldi G, Grau GE. Delayed mortality and attenuated thrombocytopenia associated with severe malaria in urokinase- and urokinase receptor-deficient mice. Infect Immun **2000**; 68:3822–9.
27. Männel DN, Grau GE. Role of platelet adhesion in homeostasis and immunopathology. Mol Pathol **1997**; 50:175–85.
28. Ockenhouse CF, Tandon NN, Magowan C, Jamieson GA, Chulay JD. Identification of a platelet membrane glycoprotein as a falciparum malaria sequestration receptor. Science **1989**; 243:1469–71.
29. Barnwell JW, Asch AS, Nachman RL, Yamaya M, Aikawa M, Ingravallo P. A Human 88-kd membrane glycoprotein (CD36) functions in vitro as a receptor for a cytoadherence ligand on *Plasmodium falciparum*–infected erythrocytes. J Clin Invest **1989**; 84:765–72.
30. Vanschravendijk MR, Handunnetti SM, Barnwell JW, Howard RJ. Normal human erythrocytes express CD36, an adhesion molecule of monocytes, platelets, and endothelial cells. Blood **1992**; 80:2105–14.
31. Ockenhouse CF, Magowan C, Chulay JD. Activation of monocytes and platelets by monoclonal antibodies or malaria-infected erythrocytes binding to the CD36 surface receptor in vitro. J Clin Invest **1989**; 84: 468–75.
32. Pain A, Ferguson DJP, Kai O, et al. Platelet-mediated clumping of *Plasmodium falciparum*–infected erythrocytes is a common adhesive phenotype and is associated with severe malaria. Proc Natl Acad Sci USA **2001**; 98:1805–10.
33. Johnson SA, Balboa RS, Dessel BH, Monto RW, Siegesmund KA, Greenwalt TJ. The mechanism of the endothelial supporting function of intact platelets. Exp Mol Pathol **1964**; 3:115–27.