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Looking through a cranial window

Intravital microscopy for in vivo study of cerebral malaria

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Malaria is a potentially life-threatening disease with 200–500 million new infections and up to one million deaths annually.[1] Half the world's population is at risk of being infected by one of the five *Plasmodium* parasite species causing malaria in humans. *P. falciparum* is responsible for the most fatal outcomes occurring in sub-Saharan Africa.[2] Infection can progress very fast from a mild into a severe form featuring typical symptoms such as anemia, respiratory abnormalities, cerebral malaria (CM), and coma.[3] Children under the age of five are most susceptible to the development of CM and account for most deaths.[4,5] Patients who survive CM are likely to suffer long-standing neurocognitive impairment.[6-8] The underlying pathogenesis of CM is not well understood. A histological hallmark of CM is the sequestration of the parasite-infected red blood cells to the microvasculature of the brain.[9,10] Multiple factors such as microvascular obstruction, vascular inflammation, hemostasis dysfunction, reduced microcirculatory blood flow, and breakdown of the blood–brain barrier (BBB) are all considered to be important contributors to the development of CM.[11-13] In order to develop successful adjunctive therapy to anti-malarial treatment to prevent neurological impairment in patients, an improved understanding of CM pathogenesis is essential.

The study of CM in humans is restricted to the correlation of different pathologies to define and diagnose CM and to the examination of postmortem samples,[14-16] which reveal insight into histological patterns associated with the disease. The exact mechanisms leading to human CM are largely unknown and previous therapeutic attempts were based on the observed pathophysiology in humans.[17,18] For this reason, murine malaria models such as the *Plasmodium berghei* ANKA (PbA) model have been introduced to gain a better understanding of the mechanisms contributing to the development and protection of experimental cerebral malaria (ECM). While this model does not reflect the complexity of disease observed in humans, similarities in CM pathogenesis such as breakdown of the BBB and vascular damage make it to date the best available small animal model for severe malaria and ECM.[19-21] A major concern regarding the suitability of the ECM model has been based on observations that sequestration to endothelial cells of the brain during human CM is caused by *P. falciparum* infected RBCs, while in the ECM-susceptible C57BL/6 mouse strain infected with PbA leukocytes appear to take this role.[22] Important in this context, only recently has the first direct in vitro evidence been provided demonstrating sequestration of PbA infected RBCs to endothelial cells of mouse brain involving host receptor VCAM-1 as a potential mediator of adherence.[23]

One way to gain important information regarding underlying pathological features of the microvasculature contributing to ECM progression has been made feasible through recent implementation of intravital microscopy through a closed cranial window by Carvalho and colleagues.[24,25] This tool allows for the first time continuous in vivo analysis of the pial microcirculation in a singl e area of the brain, which reflects general brain circulation behavior and function including BBB properties.[26] Importantly, pathological changes during ECM progression in the pial vessels (pial arterioles and pial venules) appear similar to the ones observed in brain parenchym vessels. This includes hemodynamic and inflammatory changes due to leukocyte sequestration, vascular occlusion, hypoperfusion, leakage, and microhemorrhages.[22]

In previous studies, intravital microscopy revealed that vasoconstriction is caused by decreased cerebral blood flow and is closely associated with ECM.[22] A correlation of the number of sequestering leukocytes to the vessel endothelium and the severity of vasoconstriction was observed, causing decreased blood flow and cellular hypoxia, and potentially leading to vascular collapse. With the recognition that vasculopathy appears to be an important contributor to the pathogenesis of CM, the successful treatment of ECM with the calcium-channel blocker and vasodilator nimodipine combined with antimalarials was performed and monitored by intravital microscopy.[22] In another study, intravital microscopy was applied to demonstrate that supplementation of nitric oxide supported prevention of vasoconstriction, leukocyte sequestration, and development of brain hemorrhages.[27] This finding pointed to a role of nitric oxide synthases in cerebrovascular dysfunctions during ECM[28] and complemented previous observations that treatment of patients and mice with exogenous arginine increased nitric oxide production and resulted in reduced BBB perfusion during progression of CM.[29,30]

Vascular dysfunction is a major feature of human CM and alterations of cerebral microcirculation lead to cerebral hypoxia.[13,31]

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Whitening of the retina, hemorrhage, and changes in vessel color due to parasite sequestration and obstruction of blood flow have been interpreted as indirect indications of reduced cerebral oxygenation linked to CM.[32,33] Since sufficient brain oxygenation is vital to resolve clinical consequences of a *P. falciparum* infection, a better understanding of cerebral hypoxia associated with CM is essential for the development of novel interventions.

The article in this issue of *Virulence* by Cabrales et al.[34] describes for the first time a direct, quantitative, and dynamic in vivo measurement of hypoxia during ECM development using intravital microscopy through a closed cranial window. The technique is combined with phosphorescence-quenching microscopy to measure oxygen tensions and pH-sensitive fluorescence to assess pH values of perivascular tissue.[35] Individual vessels of ECM-susceptible C57BL/6 mice and ECM-resistant BALB/c mice infected with PbA were investigated for blood flow, oxygen tension, and transport. With the onset of ECM development, decreased pH values and drop of the core body temperature in C57BL/6 mice were observed, potentially affecting cell function and blood flow. PbA infection in the ECM-susceptible mice induced compromised oxygenation and impaired pial hemodynamics, while ECM-resistant mice had better oxygen delivery and extraction levels. Reduced oxygen delivery was correlated with the number of sequestering leukocytes during early and late ECM. Resulting vascular obstruction and vasoconstriction promoted anemia and hypoperfusion, which further impaired oxygenation. This led to increased acidosis, hypothermia with potential neurological damage developing.

In summary, Cabrales et al. present for the first time a quantitative analysis of fluctuations of oxygen transport and tension during ECM progression and its contribution to the severity of disease. The study highlights the pial tissue as highly sensitive to changes of blood flow, anemia, and low oxygen tension impacting sufficient oxygen delivery. Further, the findings herein complement previous observations of changes in energy metabolism and transcription factors marking the state of hypoxia.[36,37]

It becomes apparent that the use of intravital microscopy carries a huge potential for the mechanical dissection of microvascular changes as part of the underlying pathogenesis of CM. It also facilitates the development of new therapeutics for the prevention of CM by allowing long-term imaging with testing effects of new compounds on the microvasculature directly after administration. Intravital microscopy may also be applied for the analysis of the contribution of genes to the development of vasculopathy, which may be performed through gene deletion approaches. Cytoadherence to the cerebral endothelium mediated by infected RBCs and leukocytes may be studied by intravital microscopy, leading to identification of underlying mechanisms determining different vascular conditions during the progression of CM.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. World Health Organization. World malaria report 2010. Geneva, Switzerland: World Health Organization; 2010. Available at http://www.who.int/malaria/world_malaria_report_2010/en/index.html.
2. Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI. The global distribution of clinical episodes of Plasmodium falciparum malaria. Nature 2005; 434:214-7; PMID:15759000; http://dx.doi.org/10.1038/nature03342
3. Schofield L, Grau GE. Immunological processes in malaria pathogenesis. Nat Rev Immunol 2005; 5:722-35; PMID:16138104; http://dx.doi.org/10.1038/nri1686
4. Desruisseaux MS, Machado FS, Weiss LM, Tanowitz HB, Golightly LM. Cerebral malaria: a vasculopathy. Am J Pathol 2010; 176:1075-8; PMID:20093501; http://dx.doi.org/10.2353/ajpath.2010.091090
5. Dondorp AM, Lee SJ, Faiz MA, Mishra S, Price R, Tjitra E, Than M, Htut Y, Mohanty S, Yunus EB, et al. The relationship between age and the manifestations of and mortality associated with severe malaria. Clin Infect Dis 2008; 47:151-7; PMID:18533842; http://dx.doi.org/10.1086/589287
6. Boivin MJ. Effects of early cerebral malaria on cognitive ability in Senegalese children. J Dev Behav Pediatr 2002; 23:353-64; PMID:12394524; http://dx.doi.org/10.1097/00004703-200210000-00010
7. Boivin MJ, Gladstone MJ, Vokhiwa M, Birbeck GL, Magen JG, Page C, Semrud-Clikeman M, Kauye F, Taylor TE. Developmental outcomes in Malawian children with retinopathy-positive cerebral malaria. Trop Med Int Health 2011; 16:263-71; PMID:21143354; http://dx.doi.org/10.1111/j.1365-3156.2010.02704.x
8. Carter JA, Ross AJ, Neville BG, Obiero E, Katana K, Mung'ala-Odera V, Lees JA, Newton CR. Developmental impairments following severe falciparum malaria in children. Trop Med Int Health 2005; 10:3-10; PMID:15655008; http://dx.doi.org/10.1111/j.1365-3156.2004.01345.x
9. Aikawa M, Iseki M, Barnwell JW, Taylor D, Oo MM, Howard RJ. The pathology of human cerebral malaria. Am J Trop Med Hyg 1990; 43:30-7; PMID:2202227
10. Turner GD, Morrison H, Jones M, Davis TM, Looareesuwan S, Buley ID, Gatter KC, Newbold CI, Pukritayakamee S, Nagachinta B, et al. An immunohistochemical study of the pathology of fatal malaria. Evidence for widespread endothelial activation and a potential role for intercellular adhesion molecule-1 in cerebral sequestration. Am J Pathol 1994; 145:1057-69; PMID:7526692
11. Ahlqvist J. Decreased red cell deformability and vascular obstruction in falciparum malaria illustrated by a fatal case. Scand J Haematol 1985; 35:531-5; PMID:3911374; http://dx.doi.org/10.1111/j.1600-0609.1985.tb02824.x
12. Adams S, Brown H, Turner G. Breaking down the blood-brain barrier: signaling a path to cerebral malaria? Trends Parasitol 2002; 18:360-6; PMID:12377286; http://dx.doi.org/10.1016/S1471-4922(02)02353-X
13. Rénia L, Howland SW, Claser C, Charlotte Gruner A, Suwanarusk R, Hui Teo T, Russell B, Ng LF. Cerebral malaria: mysteries at the blood-brain barrier. Virulence 2012; 3:193-201; PMID:22460644; http://dx.doi.org/10.4161/viru.19013
14. Newton CR, Krishna S. Severe falciparum malaria in children: current understanding of pathophysiology and supportive treatment. Pharmacol Ther 1998; 79:1-53; PMID:9719344; http://dx.doi.org/10.1016/S0163-7258(98)00008-4
15. Medana IM, Day NP, Hien TT, Mai NT, Bethell D, Phu NH, Farrar J, Esiri MM, White NJ, Turner GD. Axonal injury in cerebral malaria. Am J Pathol 2002; 160:655-66; PMID:11839586; http://dx.doi.org/10.1016/S0002-9440(10)64885-7
16. Dorovini-Zis K, Schmidt K, Huynh H, Fu W, Whitten RO, Milner D, Kamiza S, Molyneux M, Taylor TE. The neuropathology of fatal cerebral malaria in malawian children. Am J Pathol 2011; 178:2146-58; PMID:21514429; http://dx.doi.org/10.1016/j.ajpath.2011.01.016
17. Kwiatkowski D, Molyneux ME, Stephens S, Curtis N, Klein N, Pointaire P, Smit M, Allan R, Brewster DR, Grau GE, et al. Anti-TNF therapy inhibits fever in cerebral malaria. Q J Med 1993; 86:91-8; PMID:8329024
18. Crawley J, Waruiru C, Mithwani S, Mwangi I, Watkins W, Ouma D, Winstanley P, Peto T, Marsh K. Effect of phenobarbital on seizure frequency and mortality in childhood cerebral malaria: a randomised, controlled intervention study. Lancet 2000; 355:701-6; PMID:10703801; http://dx.doi.org/10.1016/S0140-6736(99)07148-2

19. Langhorne J, Buffet P, Galinski M, Good M, Harty J, Leroy D, Mota MM, Pasini E, Renia L, Riley E, et al. The relevance of non-human primate and rodent malaria models for humans. Malar J 2011; 10:23; PMID:21288352; http://dx.doi.org/10.1186/1475-2875-10-23
20. Hunt NH, Grau GE. Cytokines: accelerators and brakes in the pathogenesis of cerebral malaria. Trends Immunol 2003; 24:491-9; PMID:12967673; http://dx.doi.org/10.1016/S1471-4906(03)00229-1
21. Hansen DS. Inflammatory responses associated with the induction of cerebral malaria: lessons from experimental murine models. PLoS Pathog 2012; 8:e1003045; PMID:23300435; http://dx.doi.org/10.1371/journal.ppat.1003045
22. Cabrales P, Zanini GM, Meays D, Frangos JA, Carvalho LJ. Murine cerebral malaria is associated with a vasospasm-like microcirculatory dysfunction, and survival upon rescue treatment is markedly increased by nimodipine. Am J Pathol 2010; 176:1306-15; PMID:20110412; http://dx.doi.org/10.2353/ajpath.2010.090691
23. El-Assaad F, Wheway J, Mitchell AJ, Lou J, Hunt NH, Combes V, Grau GE. Cytoadherence of Plasmodium berghei-infected red blood cells to murine brain and lung microvascular endothelial cells in vitro. Infect Immun 2013; 81:3984-91; PMID:23940206; http://dx.doi.org/10.1128/IAI.00428-13
24. Cabrales P, Carvalho LJ. Intravital microscopy of the mouse brain microcirculation using a closed cranial window. J Vis Exp 2010; 45:2184; PMID:21113121
25. Ong PK, Meays D, Frangos JA, Carvalho LJ. A chronic scheme of cranial window preparation to study pial vascular reactivity in murine cerebral malaria. Microcirculation 2013; 20:394-404; PMID:23279271; http://dx.doi.org/10.1111/micc.12034
26. Abbott NJ. Inflammatory mediators and modulation of blood-brain barrier permeability. Cell Mol Neurobiol 2000; 20:131-47; PMID:10696506; http://dx.doi.org/10.1023/A:1007074420772
27. Cabrales P, Zanini GM, Meays D, Frangos JA, Carvalho LJ. Nitric oxide protection against murine cerebral malaria is associated with improved cerebral microcirculatory physiology. J Infect Dis 2011; 203:1454-63; PMID:21415018; http://dx.doi.org/10.1093/infdis/jir058
28. Ong PK, Melchior B, Martins YC, Hofer A, Orjuela-Sánchez P, Cabrales P, Zanini GM, Frangos JA, Carvalho LJ. Nitric oxide synthase dysfunction contributes to impaired cerebroarteriolar reactivity in experimental cerebral malaria. PLoS Pathog 2013; 9:e1003444; PMID:23818850; http://dx.doi.org/10.1371/journal.ppat.1003444
29. Yeo TW, Lampah DA, Gitawati R, Tjitra E, Kenangalem E, McNeil YR, Darcy CJ, Granger DL, Weinberg JB, Lopansri BK, et al. Impaired nitric oxide bioavailability and L-arginine reversible endothelial dysfunction in adults with falciparum malaria. J Exp Med 2007; 204:2693-704; PMID:17954570; http://dx.doi.org/10.1084/jem.20070819
30. Zanini GM, Cabrales P, Barkho W, Frangos JA, Carvalho LJ. Exogenous nitric oxide decreases brain vascular inflammation, leakage and venular resistance during Plasmodium berghei ANKA infection in mice. J Neuroinflammation 2011; 8:66; PMID:21649904; http://dx.doi.org/10.1186/1742-2094-8-66
31. Penet MF, Viola A, Confort-Gouny S, Le Fur Y, Duhamel G, Kober F, Ibarrola D, Izquierdo M, Coltel N, Gharib B, et al. Imaging experimental cerebral malaria in vivo: significant role of ischemic brain edema. J Neurosci 2005; 25:7352-8; PMID:16093385; http://dx.doi.org/10.1523/JNEUROSCI.1002-05.2005
32. Beare NA, Lewis DK, Kublin JG, Harding SP, Zijlstra EE, Molyneux ME. Retinal changes in adults with cerebral malaria. Ann Trop Med Parasitol 2003; 97:313-5; PMID:12803862; http://dx.doi.org/10.1179/000349803235001859
33. Dondorp AM, Ince C, Charunwatthana P, Hanson J, van Kuijen A, Faiz MA, Rahman MR, Hasan M, Bin Yunus E, Ghose A, et al. Direct in vivo assessment of microcirculatory dysfunction in severe falciparum malaria. J Infect Dis 2008; 197:79-84; PMID:18171289; http://dx.doi.org/10.1086/523762
34. Cabrales P, Martins YC, Ong PK, Zanini GM, Frangos JA, Carvalho LJ. Cerebral tissue oxygenation impairment during experimental cerebral malaria. Virulence 2013; 4:686-97; PMID:24128424; http://dx.doi.org/10.4161/viru.26348
35. Cabrales P, Nacharaju P, Manjula BN, Tsai AG, Acharya SA, Intaglietta M. Early difference in tissue pH and microvascular hemodynamics in hemorrhagic shock resuscitation using polyethylene glycol-albumin- and hydroxyethyl starch-based plasma expanders. Shock 2005; 24:66-73; PMID:15988323; http://dx.doi.org/10.1097/01.shk.0000167111.80753.ef
36. Sanni LA, Rae C, Maitland A, Stocker R, Hunt NH. Is ischemia involved in the pathogenesis of murine cerebral malaria? Am J Pathol 2001; 159:1105-12; PMID:11549603; http://dx.doi.org/10.1016/S0002-9440(10)61786-5
37. Rae C, McQuillan JA, Parekh SB, Bubb WA, Weiser S, Balcar VJ, Hansen AM, Ball HJ, Hunt NH. Brain gene expression, metabolism, and bioenergetics: interrelationships in murine models of cerebral and noncerebral malaria. FASEB J 2004; 18:499-510; PMID:15003995; http://dx.doi.org/10.1096/fj.03-0543com