A Single Human Cerebral Malaria Histopathologic Study Can Be Worth a Thousand Experiments

David J. Sullivan, Jr.

W. Harry Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA

ABSTRACT Severe malaria is a density-dependent disease that comprises infected-erythrocyte sequestration, with or without monocytic infiltration, as seen in renal, placental, and lung tissues from severe malaria patients. HIV induces a chronic proinflammatory state with higher numbers of inflammasome-activated monocytes and platelets circulating. The epidemiological and pathological study of S. E. Hochman et al. that was published in a recent issue of *mBio* (Hochman SE, Madaline TF, Wassmer SC, Mbale E, Choi N, et al., mBio 6:e01390-15, 2015, doi:10.1128/mBio.01390-15) analyzes a large cohort of Malawian children and shows that cerebral malaria in younger HIV-negative children presents as an acute disease predominated by sequestered infected erythrocytes. In contrast, they show that case presentation in older HIV-positive children is as a more lethal acute on chronic disease marked by double the monocytic infiltrates and 5 times as many platelets. This study suggests that cerebral involvement in severe malaria is a pathology similar to that of other organ involvement of severe malaria, with a bias in HIV-positive individuals toward more monocytic infiltrates. The study also addresses the important association of severe malaria and HIV prevalence.

Uncomplicated malaria is a red cell disease that is unaffected by white cell immunosuppression in HIV. Early in the HIV epidemic, large numbers of uncomplicated malaria subjects showed that malaria was not worse in HIV-positive patients and that HIV-positive patients did not have more incidence of uncomplicated malaria. The numbers were never large enough to make conclusions about severe malaria, mainly because severe malaria occurs in less than 1% of malaria cases and correlation requires thousands of malaria patients intersecting with HIV. The retrospective review by Chandramohan and Greenwood found no change in uncomplicated malaria in hundreds of cases of HIV and malaria with only a dozen total severe malaria cases, which were thus insufficient for analysis (1).

The first measured impact of HIV on malaria was observed in placental malaria, where it was demonstrated that HIV-positive mothers lost the malaria immune protection acquired from a previous pregnancy (2, 3). The study of S. E. Hochman et al., published in a recent issue of *mBio* (4), accumulated more than 2,000 severe malaria cases over 12 years and established that in HIV-positive patients, cerebral malaria presented later in life and was more lethal. The prevalence of HIV among this large collection of well-documented cerebral malaria cases was 15%, with a baseline HIV population rate of 2%. A separate study by this group showed that severe malaria anemia actually has a 20% HIV rate within the same population (5). The current study is important both for adding solid epidemiological numbers on severe malaria and HIV prevalence and for making an important histopathologic association of monocytes and platelets in severe cerebral malaria.

Sophie Spitz, a military surgeon, reported on fifty autopsy cases of lethal malaria after WWII, noting higher localization of adherent organisms in the spleen, liver, and bone marrow but uniform distribution in other organs, including the brain (6). In heavy infections, there were equal numbers of adherent parasites in the brain, lungs, heart, and intestines, even though the clinical symptoms pointed to a single organ. Light infections were similarly uniformly distributed. Selective localization of adherent infected erythrocytes did not account for the often organ-specific clinical presentation of fatal malaria. She also noted thrombotic lesions in the cerebral vasculature alone and not in other organs. More recently, Milner et al. confirmed these uniform organ distribution findings in the lungs of 55 children who had died of cerebral malaria and in the lungs of 45 who died of noncerebral malaria (7). In those with cerebral malaria, the lungs had similar large numbers of adherent parasites, along with large amounts of extracellular hemozoin deposits, compared to the lower numbers of parasites and amounts of hemozoin in noncerebral malaria cases. The numbers of pulmonary macrophages did not differ between those with cerebral malaria, noncerebral malaria, and no malaria diagnosis. Adherent parasites and monocytes have been a frequent pathological finding in malaria patients, but the significance of monocytes and platelets has remained a point of debate.

An important solid addition of this new study is not just the demonstration of increased numbers of monocytes in a few cerebral malaria autopsies but also the contribution of a large data set with statistical proof of the association of monocytes and platelets with cerebral malaria, regardless of CD4 levels. This report examined 30 (15 HIV-positive and 15 HIV-negative) random autopsies out of nearly 100 and observed twice as much monocytic infiltrate accumulation and 5 times as many platelets in the brains of HIV-positive than of HIV-negative cases. The monocyte and platelet infiltrations were highly correlated with the amount of hemozoin (free, intraparasitic, or intramonocytic) in the brain. HIV-positive patients also had statistically higher levels of hemozoin in the brain. Peripheral blood measurements of *Plasmodium falciparum* histidine-rich protein 2, a biomarker of total infected parasite biomass, were similar in HIV-positive and HIV-negative cases.

The monocyte infiltrations observed in placental malaria have also been characterized pathologically into the following four cat-

Published 17 November 2015

Citation Sullivan DJ, Jr. 2015. A single human cerebral malaria histopathologic study can be worth a thousand experiments. mBio 6(6):e01818-15. doi:10.1128/mBio.01818-15.

Address correspondence to David J. Sullivan, Jr., dsulliv7@jhmi.edu.

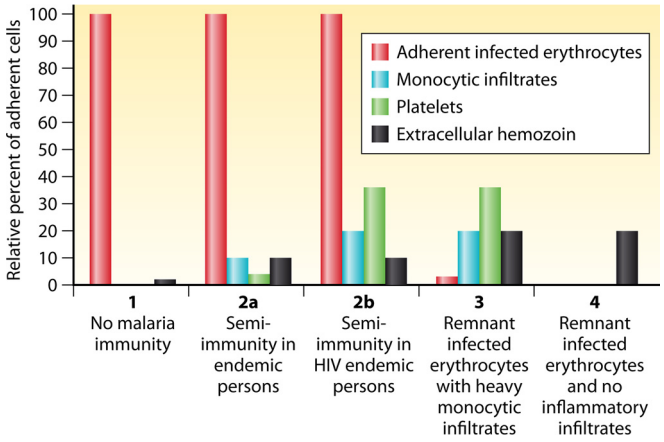

FIG 1 Four severe malaria pathology categories for placental, cerebral, or severe anemia malaria or other organ involvement. (1) Acute severe malaria manifests with organ dysfunction with only a high number of adherent infected erythrocytes in postcapillary venules in persons with no malaria immunity. (2a) In persons with partial immunity in areas where malaria is endemic, organs in severe malaria are associated with both monocytic and platelet infiltrates. (2b) In HIV-positive persons with partial immunity in areas where malaria is endemic, the numbers of monocytes and platelets increase by 2- and 5-fold, respectively, in the affected organ. (3) Placental malaria (and possibly all severe malaria) after a treated infection can have pathology comprising abundant extracellular hemozoin, no infected erythrocytes, and heavy monocytic infiltrates. This is also seen in the mouse model of experimental cerebral malaria, which occurs only in a proinflammatory C57BL/6 black mouse with pathologically abundant monocytes/leukocytes, few adherent infected erythrocytes, and limited hemozoin. (4) Organs can contain extracellular hemozoin with no adherent parasites or inflammatory infiltrates, indicative of past malaria disease.

egories (Fig. 1): (1) an acute phase with newly abundant sequestered parasites adhered to chondroitin sulfate A by the var2CSA *P. falciparum* erythrocyte membrane protein, (2) a combination of adherent parasites and inflammatory infiltrates of monocytes, (3) hemozoin and inflammatory monocytic infiltrates, or (4) hemozoin only, with neither parasites nor monocytes (8). In renal disease of severe malaria, monocytic infiltration has also been seen (9). Severe pulmonary pathology also has a component of monocytic infiltration (10). Rather than providing evidence that the cerebral malaria pathology is distinct from other severe malaria organ presentations, this study points to a common pathological process in line with that of placental malaria. Acute rapid-onset malaria with sequestration of high numbers of infected erythrocytes can result in severe disease in any organ at any time. However, an additional pathological process is severe disease in the setting of monocytic infiltrates, with or without associated platelets, along with high numbers of adherent infected erythrocytes. This reflects a chronic pathology of monocytes recruited to the site of adherent parasites and hemozoin. While there is still debate on whether the monocytic infiltrate is protective or exacerbates disease, the monocyte and platelet association in this pathological study is statistically clear. This study establishes cerebral malaria presented in older HIV-positive patients as a chronic disease with the co-occurrence of monocytes, platelets, and parasites to effect individual organ dysfunction. The four pathological entities descriptive of placental malaria serve also to describe cerebral malaria or severe malaria anemia. Severe malaria can therefore present either as an acute, rapidly progressive pathology or as a subacute on chronic pathology, with the implication of a more lethal outcome in the latter.

REFERENCES

1. **Chandramohan D, Greenwood BM.** 1998. Is there an interaction between human immunodeficiency virus and *Plasmodium falciparum*? Int J Epidemiol **27:**296–301. http://dx.doi.org/10.1093/ije/27.2.296.
2. **Bloland PB, Wirima JJ, Steketee RW, Chilima B, Hightower A, Breman JG.** 1995. Maternal HIV infection and infant mortality in Malawi: evidence for increased mortality due to placental malaria infection. AIDS **9:**721–726. http://dx.doi.org/10.1097/00002030-199507000-00009.
3. **Steketee RW, Wirima JJ, Bloland PB, Chilima B, Mermin JH, Chitsulo L, Breman JG.** 1996. Impairment of a pregnant woman's acquired ability to limit *Plasmodium falciparum* by infection with human immunodeficiency virus type-1. Am J Trop Med Hyg **55:**42–49.
4. **Hochman SE, Madaline TF, Wassmer SC, Mbale E, Choi N, Seydel KB, Whitten RO, Varughese J, Grau GER, Kamiza S, Molyneux ME, Taylor TE, Lee S, Milner DA, Jr., Kim K.** 2015. Fatal pediatric cerebral malaria is associated with intravascular monocytes and platelets that are increased with HIV coinfection. mBio **6:**e01390-15. http://dx.doi.org/10.1128/mBio.01390-15.
5. **Bronzan R, Taylor T, Mwenechanya J, Tembo M, Kayira K, Bwanaisa L, Njobvu A, Kondowe W, Chalira C, Walsh A, Phiri A, Wilson L, Molyneux M, Graham S.** 2007. Bacteremia in Malawian children with severe malaria: prevalence, etiology, HIV coinfection, and outcome. J Infect Dis **195:**895–904. http://dx.doi.org/10.1086/511437.
6. **Spitz S.** 1946. The pathology of acute falciparum malaria. Mil Surg **99:**555–572.
7. **Milner D, Jr., Factor R, Whitten R, Carr RA, Kamiza S, Pinkus G, Molyneux M, Taylor T.** 2013. Pulmonary pathology in pediatric cerebral malaria. Hum Pathol **44:**2719–2726. http://dx.doi.org/10.1016/j.humpath.2013.07.018.
8. **Rogerson SJ, Hviid L, Duffy PE, Leke RF, Taylor DW.** 2007. Malaria in pregnancy: pathogenesis and immunity. Lancet Infect Dis **7:**105–117. http://dx.doi.org/10.1016/S1473-3099(07)70022-1.
9. **Nguansangiam S, Day NPJ, Hien TT, Mai NTH, Chaisri U, Riganti M, Dondorp AM, Lee SJ, Phu NH, Turner GDH, White NJ, Ferguson DJP, Pongponratn E.** 2007. A quantitative ultrastructural study of renal pathology in fatal *Plasmodium falciparum* malaria. Trop Med Int Health **12:**1037–1050. http://dx.doi.org/10.1111/j.1365-3156.2007.01881.x.
10. **Chua CLL, Brown G, Hamilton JA, Rogerson S, Boeuf P.** 2013. Monocytes and macrophages in malaria: protection or pathology? Trends Parasitol **29:**26–34. http://dx.doi.org/10.1016/j.pt.2012.10.002.

The views expressed in this Commentary do not necessarily reflect the views of this journal or of ASM.