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Ependymal damage in a *Plasmodium yoelii yoelii* lethal murine malaria model

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Summary. Malaria continues to be a major global health problem, and over 40% of the world's population is at risk. Severe or complicated malaria is defined by clinical or laboratory evidence of vital organ dysfunction, including dysfunction of the central nervous system (CNS). The pathogenesis of complicated malaria has not been completely elucidated; however, the development of the multiorgan affection seems to play an important role in the disruption of the blood brain barrier (BBB) that protects the CNS against chemical insults. Historically, the BBB has received more attention in the pathogenesis of malaria than have the cerebrospinal fluid-brain barrier (CSFBB) and ependymal cells. This perspective may be misguided because, in the context of disease or toxicity, the CSFBB is more vulnerable to many foreign invaders than are the capillaries. Given the lack on studies of the damage to the CSFBB and ependymal epithelium in experimental murine malaria, the present study evaluated morphological changes in the ependymal cells of CD-1 male mice infected with lethal *Plasmodium yoelii yoelii* (*Pyy*) via histopathology and scanning electron microscopy (SEM). Samples were taken two, four and six days post-infection (PI). No lesions were observed upon the initial infection. By the fourth day PI, fourth ventricle ependymal samples exhibited disruptions and roughened epithelia. More severe injuries were observed at six days PI and included thickened cilia and deep separations between the ependymal intercellular spaces. In some of the analyzed areas, the absence of microvilli and cell layer detachment were observed, and some areas exhibited blebbing surfaces. The ependymal cell lesions observed in the CD1 male mice infected with lethal *Pyy* seemed to facilitate the paracellular permeability of the CSFBB and consequently promote the access of inflammatory mediators and toxic molecules through the barrier, which resulted in damage to the brain tissue. Understanding the mechanism of ependymal disruption during lethal murine malaria could help to elucidate the local and systemic factors that are involved in the pathogenesis of the disease and may provide essential clues for the prevention and treatment of complicated human malaria.

Key words: *Plasmodium yoelii yoelii*, Ependymal cells, Cerebrospinal fluid-brain barrier, Electron microscopy.

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Introduction

The recognition that severe malaria, which is predominantly caused by *Plasmodium falciparum* (*Pf*), is a complex multi-system disorder that presents with a range of clinical features has been major change in recent years (Mackintosh et al., 2004). The pathological expression of malaria infection depends largely on the immunopathologic response induced by the parasite (Hayder et al., 2008). Inappropriate immunoresponses to some malarial antigens can generate major complications of malaria, particularly neurovascular lesions (Grau et al., 1992). Cerebral malaria (CM) is the most severe neurological complication of infection with

Pf, and children in sub-Sahara Africa are the most affected (Idro et al., 2011). CM is usually secondary to *Pf* infection; however, there are infrequent reports of cerebral malaria associated with *P. vivax* infection (Ozen et al., 2006). In malaria, the sequestration of falciparum-non-infected and -infected red blood cells (iRBC) into endothelial cells leads to profound endothelial alterations that trigger immunopathological changes, varying degrees of brain edema and blood flow alterations (Grau et al., 2013). Hypotheses that imply that only the parasitized red cells are altered do not explain why CM does not occur in all individuals with massive infections. In the mouse model, the sequestration of parasitized red cells is absent or marginal, while hemorrhagic necrosis is fully developed, which indicates that the sequestration of red cells may not be necessary for the development of CM (Grau et al., 1992). The impermeable BBB and CSFBB act together to protect the CNS from potentially injurious agents in the blood (Cammer et al., 1989; Zheng et al., 2003; Johanson et al., 2011; Masocha and Kristensson, 2012). Malaria parasites can damage these barriers nervous structures increase their permeability and allow access to an exacerbated amount of proinflammatory cytokines that results in damage to the brain tissues and CM (Angulo and Fresno, 2002). Animal models of malaria have provided convincing evidence of the important role of inflammatory processes in the development of CM. Monkey, rat and mouse models have been used, although none of these models completely duplicate the situation in humans (de Souza and Riley, 2002). The susceptibility to CM in murine models is highly dependent on the genetic background of the mouse and on the genetic differences in the parasite strains that determine their virulence (Amani et al., 1998). Historically, the BBB has received more attention than the CSFBB in malaria pathology. Given the lack of information about the damage to the CSFBB in experimental malaria, the present study evaluated the effects of the *Pyy* lethal strain on the ependymal cells of CD1 male mice to obtain evidence regarding CSFBB damage during infection in the absence of parasite sequestration within the brain.

Materials and methods

Animals

Animals were obtained from the Faculty of Medicine of the UNAM vivarium. Thirty-five male CD1 mice weighing 30 g were used for the experiments. The animals were maintained in polyethylene cages in a temperature and humidity controlled environment with filtered air. The mice were managed under the guidelines of the Faculty of Medicine Ethics Commission and according to the Mexican official norm NOM-062-ZOO-1999 for the production, care, and use of laboratory animals in compliance with international rules. The mice were divided into seven groups of five mice each. The mice were under the expert care of laboratory animal technicians.

Parasite maintenance

Pyy lethal strain was obtained from the London School of Hygiene and Tropical Medicine and maintained by serial mouse-to-mouse passages in CD1 mice.

Experiment

The mice were infected via the receipt of 25×10^3 parasitized erythrocytes per mouse via the intraperitoneal route. Five mice remained as healthy controls. The infections were followed by the preparation of tail blood smears and calculations of the individual percent of parasitemia in 2,000 cell samples from random fields (Rivera et al., 2013a). The animals were scarified in groups of ten mice each at two, four and six days PI. The animals were anesthetized with sodium pentobarbital and perfused via the aorta with saline containing 2% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer. The brain was removed and placed in fixative solution for 2 h and processed for histopathology according to Rivera et al., (2013b) and for scanning electron microscopy (SEM) evaluation according to Avila et al. (2005). The fourth ventricles were analyzed with SEM and light microscopy. Histological examinations were performed via observations of not less than 10 fields per sample, and images were obtained with Motic Image Advanced 3.1 software for Windows. SEM samples were analyzed with a Zeiss SEM DSM-950 electron microscope.

Cell count analysis

To determine the average numbers of sloughed cells during the *Pyy* infections in the mice, five ventricular segments of 0.5 cm each were obtained from each mouse and placed on a flat-end pin for SEM analysis. Cell counting was performed in 50 randomly selected ependymal cells at 3000×, and counts were performed directly on the screen (Avila et al., 2005). The average numbers of sloughed cells ± the S.E. (standard deviation) were compared against those from the controls. One-way ANOVA was used to analyze differences between groups. A Spearman's correlation was performed between the percentages of parasitemia, sloughing, and ependymal damage. Statistical analyses were performed using SigmaPlot® (v. 12.0). The samples were evaluated in a double-blind manner.

Results

Clinical signs

No clinical signs were observed in the animals that had been infected for two days. The mice that had been infected with *Pyy* for four and six days developed

clinical signs of malaria infection that included the appearance of ruffled fur, asthenia, pale mucous membranes, deep breathing, dehydration and weight loss. At six days PI, the mice developed shivering and hematuria. The infected mice did not develop classic signs of cerebral pathology such as ataxia, paralysis, convulsions or seizures and coma.

Histopathology

No macroscopic or microscopic changes were observed in the control or the two-day infected mouse samples. No lesions or structural macroscopic changes were observed in the infected animals. The histopathology lesions observed in the brains of the infected animals included congestion, edema, hemorrhage and gliosis.

Midbrains of the two-day infected mice. The ependymal channels appeared to be coated by ependymocyte layers without pathological changes (Fig. 1a).

Midbrains of the four-day infected mice. Below the ependymal channel, a moderate acute inflammatory infiltrate that was mainly composed of lymphocytes and macrophages was observed. The ependymocytes appeared swollen and exhibited mild hyperplasia and discrete exfoliation (Fig. 1b).

Midbrains of the six-day infected mice. Moderate edema and hemorrhage below the ependymal canal were observed (Fig. 1c). The ependymocytes appeared very swollen and exhibited severe exfoliation and hypertrophy.

Scanning electron microscopy

The controls and two-day infected mice exhibited no structural changes. The ependymal cells exhibited healthy epithelia (Fig. 2a) with microvilli and cilia covering the ependymal surface. The cilia were disposed in a regular orientation. At four days PI, the ependymal epithelia of the infected mice exhibited disrupted structures compared to those of the controls; i.e., the cilia tufts appeared less regularly orientated (Fig. 2b). At this time, the numbers of cilia tufts seemed to be reduced, and morphological abnormalities were observed. In some areas, the cilia seemed conglomerated (Fig. 2c). In some of the analyzed regions, microvilli were absent, and detached cells were observed (data not shown). At six days PI, the cilia were completely conglomerated and enlarged, and the tufts could not be distinguished, neither the surface epithelia nor the microvilli were distinguishable (Fig. 2d).

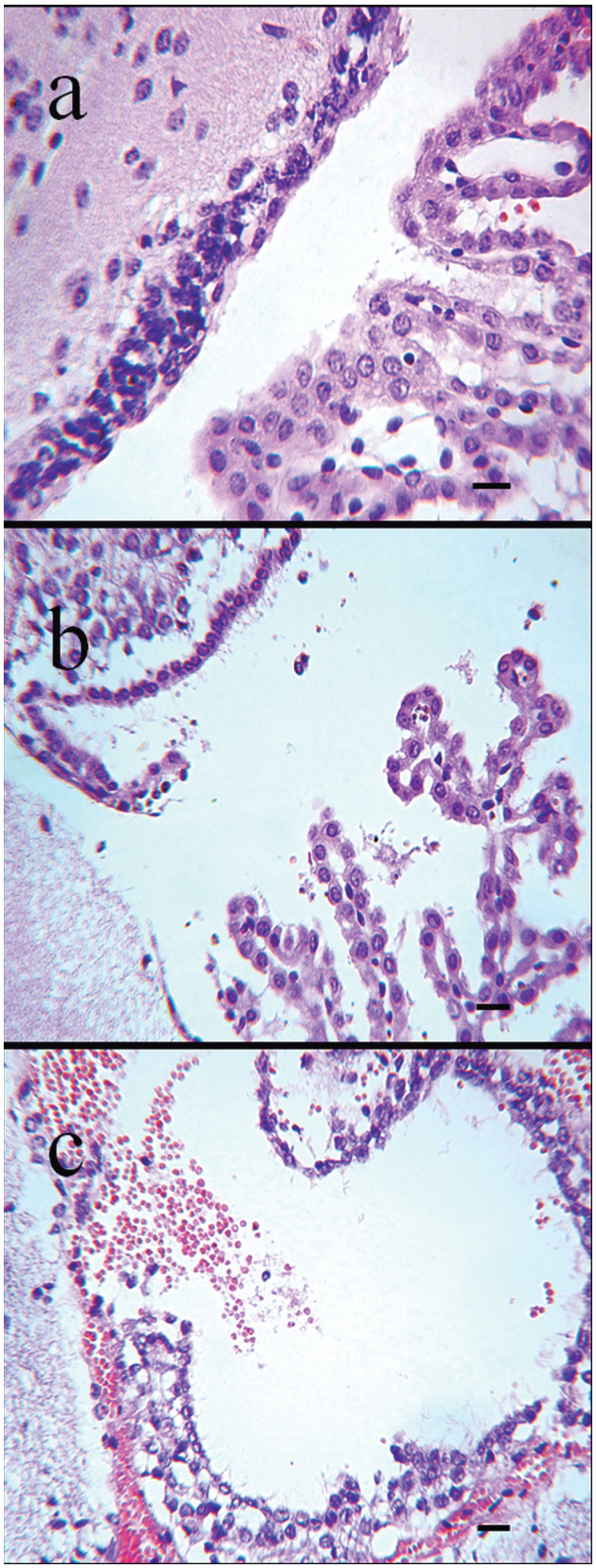

Fig. 1. Histological images of fourth ventricle ependymal cells. **a.** Healthy control. **b.** Fourth day PI; where a discrete cell exfoliation was observed. **c.** Sixth day PI; hemorrhage, severe exfoliation and hypertrophy was reported. Bars: 20 µm.

In the control and two-day infected mice, the surfaces of the modified ependymal cells of the fourth ventricle were constituted by cubic simple epithelia with normal junctions. The presence of microvilli on the surfaces of each cell was visible (Fig. 3a). At four days PI, the cells appeared swollen with deep furrows between them, and a lack of microvilli was observed on the surfaces of some cells (Fig. 3b).

Blebs of different sizes were observed on the surfaces in some of the analyzed areas (Fig. 3c). The cell surfaces of some of the samples exhibited deep depressions and flat blebs (Fig. 3d). At six days PI, the analyzed areas exhibited complete surface disarrangement and high numbers of flattened blebs. The majority of the observed cells lacked microvilli (Fig. 3e). In some of the evaluated areas, the damage was so severe that the epithelial structure was completely destroyed (Fig. 3f).

Cell counting analysis

The altered surface structure of the ependymal cells was characterized by the loss of cilia and microvilli cell sloughing and, in some cases, cell layer detachment from

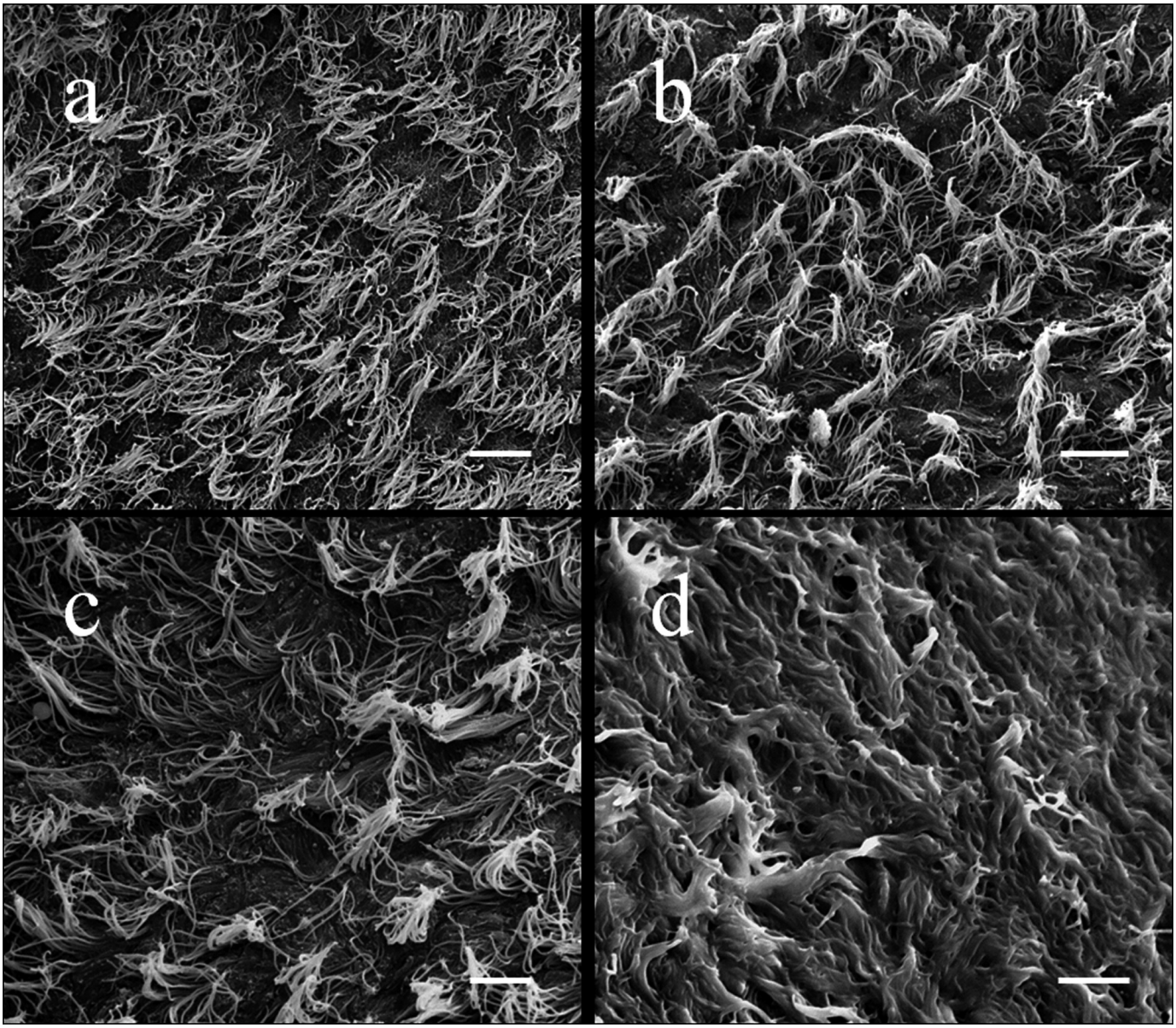

Fig. 2. Scanning electron micrographs of coronal sectioned fourth ventricle. **a.** Control ependymal cell layer with an orderly arrangement of ciliary clusters. **b and c.** Micrographs of fourth day PI showing a disrupted arrangement. **d.** At sixth day PI cilia were completely entangled and swollen. Bars: a, 10 μm; b-d, 5 μm.

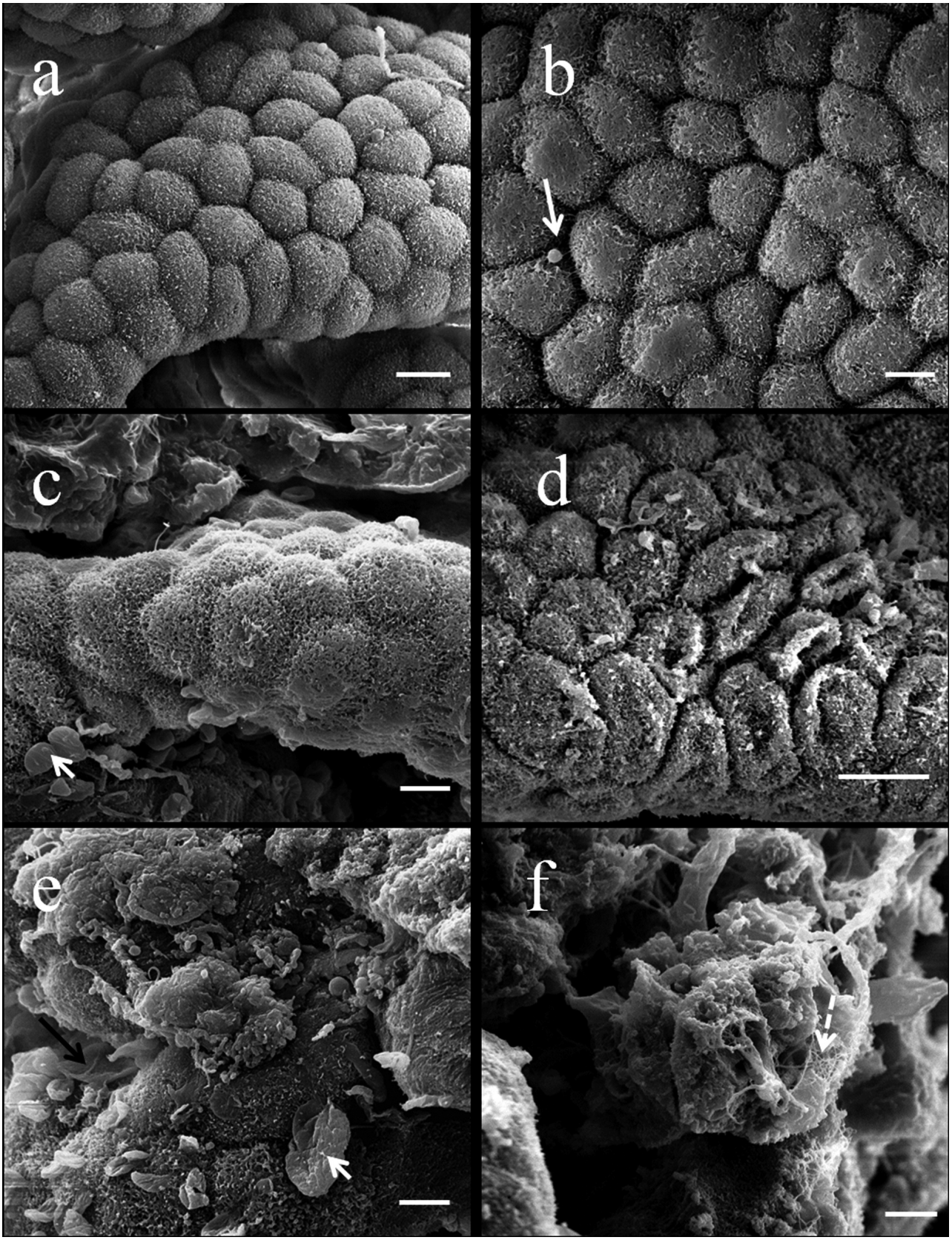

Fig. 3. Fourth ventricle coronal sections of ependymal cells. **a.** Control mice. Cuboidal ciliated ependyma lining the fourth ventricle. **b and c.** At fourth day PI, the cells appeared swollen with deep furrows between them (large white arrow) and surface blebbing (short white arrow). **d-f.** Progressive damage at sixth day PI; in some areas the cells showed thick furrows between them **(d)** as well as flat blebs (**e**; white short arrow). **f.** Cytoplasmic debris were observed in some destroyed cells (dotted white arrow). Bars: a, d, 10 μm; b, c, e, f, 5 μm.

the basal membrane that caused evident disruptions of the BCSFB. The assessment of the cell sloughing data with Spearman rank correlation tests revealed a significant correlation between ependymal cell sloughing and parasitemia percentage (r=0.87, P<001) (Fig. 4). Thus, the animals with more sloughed ependymal cells had greater degrees of parasitemia (defined as the percentage of red blood cells that harbored parasites relative to the total number of red blood cells).

Discussion

There are many similarities between human and murine complicated malaria, particularly regarding pathological lesions (de Souza and Riley, 2002); thus, the use of rodent malaria parasites in mice is the model of choice for the study of the pathogenesis of severe malaria (de Souza and Riley, 2002; Engwerda et al., 2005). One percent of world-wide symptomatic malaria infections may become complicated and develop into severe malaria. The terms "severe" and "cerebral", when used in conjunction with the term "malaria", have been used interchangeably; however, severe malaria involves a range of clinical syndromes, several of which do not involve the CNS (Craig et al., 2012). Human adults with complicated malaria exhibit signs of profound metabolic derangement. The adults may have CM, placental malaria and (unlike children) multi-organ failure that includes the kidney and liver. In children, severe disease includes metabolic derangement, CM and severe anemia (Craig et al., 2012).

The genetic background of the host and genetic differences between the parasites appear to be equally important determinants of virulence in the development of severe malaria (Amani et al., 1998). In our experience, *Pyy* murine malaria infection in CD1 male mice is a severe, complicated and lethal disease that does not involve any clinical signs of cerebral involvement. Pathology studies of our rodents (Rivera et al., 2013b) have demonstrated that *Pyy*-infected CD1 male mice die with high parasitemia (>80%), severe anemia, hemoglobinuria and hypoglycemia six to seven days PI, which seems to be typical of most complicated malaria infections in mice (Cross and Langhorne, 1998). Our *Pyy*-infected male mice always exhibit multi-organic involvement with more severe damage to the kidneys, liver, spleen, and CNS. More than 30% of our infected terminal male mice develop acute renal failure and a blackwater fever-like syndrome (Rivera et al., 2013b); in contrast, female *Pyy*-infected mice survive for more than 15 days and never develop hematuria or CNS lesions (unpublished data). An important feature of mouse malaria infection is that different strains of mice are differentially susceptible to lethal infections and that related strains of parasites have different virulence in the same strains of mice. Lethal and non-lethal variants of *P. yoelii* give rise to lethal infections only in some strains of mice, and the mortalities of the susceptible strains are highest in the male mice (Stevenson et al., 1982). In the present study, the animals were not left to die due to the natural course of the infection; rather, they were sacrificed to observe the brain lesions that resulted from the infection and not from postmortem changes.

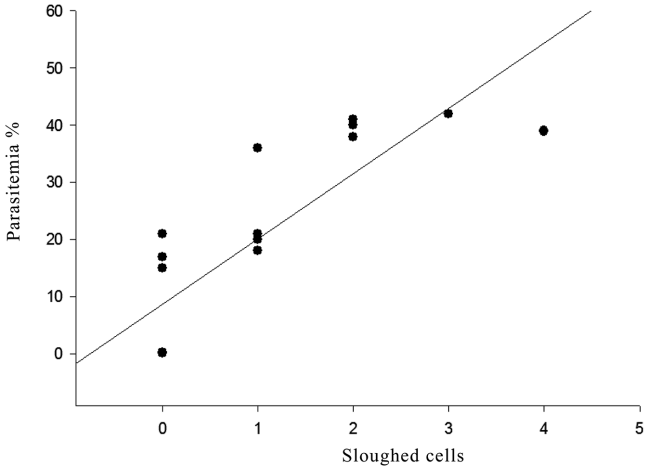

Fig. 4. Spearman's correlation between parasitemia percentage and sloughed cells. r = 0.87, P<001.

In the present study, the kinetic damage to the CSFBBs and ependymal epithelia of CD1 *Pyy*-infected male mice was evaluated at two, four and six days PI. The ependyma is a simple ciliated epithelium that lines the ventricular surface of the central nervous system and extends from the lateral ventricles to the filum terminale. Ependymal cells and their epithelial derivatives of the choroid plexus (CP) have several important functions that include the production and movement of cerebrospinal fluid (CSF) (Del Bigio, 2010). The CP consists of modified ependymal cells with fenestrated capillaries that form the CSFBB. CSF is the main secretion of the choroid plexus, and the CSF rapidly and widely disseminates the various substances that have penetrated a breached CSFBB through the central nervous system (Johanson et al., 2011). The CSFBB, the ependymal cells and the BBB tight junctions are the first impediments that parasites encounter when they reach the CNS. Additionally, these structures play important roles in the neuropathogenesis of infections with *Plasmodium* parasites that do not enter the brain (Combes et al., 2010).

In the present study, no macroscopic lesions were observed in the infected mice. The histopathological samples of the brain tissues and fourth ventricles of the infected animals exhibited mild leukocyte infiltration, edema, hemorrhage and gliosis. SEM micrographs of the fourth ventricles revealed morphological damage to the CSFBB and ependymal cells that included epithelial disruption, cilia enlargement and conglomeration, cilia loss, cell sloughing, surface blebbing and, in the worst cases, ependymal cell layer detachment. These results

agree with those that have been reported for humans with CM by Medana and Turner, (2001) and are similar to those obtained by Thumwood et al (1988), who examined *P. berghei* ANKA infection in mice. Normally, the impermeable CSFBB and BBB act together to protect neuronal networks from potentially injurious agents in the blood; however, historically, the BBB has received more pathologic attention than the CSFBB. This perspective may be erroneous because, in the contexts of disease or toxicity, the CSFBB is more vulnerable than are the capillaries to many foreign invaders (Levine, 1987; Johanson et al., 2011). The BBB refers to the widespread, impermeable capillaries in the CNS, and the CSFBB is most commonly defined as the tight junctions in the CP epithelium and the CNS-inward influx across the arachnoid membrane into the subarachnoid space. According to Johanson et al. (2011), the midbrain edema and hemorrhage observed in the *Pyy*-infected mice in our study may have been due to the leakage of CSF through the ependymal layer that would have been a direct effect of the elevated ventricular fluid pressure and be enhanced by the ependymal cell sloughing and detachment. Disruption of the CSFBB greatly increases the permeability of the CSFBB and promotes the penetration of greater numbers of macromolecules, which makes the ependymal cells more accessible to plasma proteins, cytokines and immunoglobulins, and more vulnerable to suffering damage in systemic diseases, which in turn weakens the BBB and damages the brain (Smith et al., 1981). Prendergast and Anderton (2009) reported that the upregulation of CP chemokines, integrins, selectins, and matrix metalloproteinases during pathologic or toxicologic stress renders the CSFBB more penetrable.

The ependyma ciliary disruptions observed in our infected animals are in agreement with observations made by different authors on ependymal damage due to viral, bacterial or toxicologic factors (Oliveira et al. 2003; Toskala et al., 1995). There are at least eight categories of cilia in the human body, and the malfunctioning of each of these types of cilia will have different consequences for the patient (Afzelius, 2004). Ependymal cells are multiciliated epithelial cells that are polarized in the epithelial plane, and this planar polarity is essential for propelling the CSF that helps to redistribute and dilute local concentrations of toxins and metabolites (Del Bigio, 2010). The disruption of ependymal ciliary beating results in the accumulation of CSF in the brain ventricles and damage to the brain (Mirzadeh et al., 2010). In the present study, the degeneration of the ependymal cilia could be argued to be a response to an adaptive control process of CSF osmoregulation in response to a disrupted CSFBB. The conglomerated cilia may not have been able to effectively move the CSF, which would have allowed accumulated leucocytes, soluble toxic factors from the iRBC and inflammatory mediators to remain in these tissues for longer times and thus exacerbated the ependymal damage. The ciliary conglomeration may have been due to the secondary ingrowth of new ependymal cell processes. The pathogenesis of malaria can be initiated or amplified by factors that are secreted directly into the host blood and are subsequently released into the CSF; elevated levels of picolinic acid (PIC) in the CSF of patients with CM have been reported by Medana et al. (2001). Similar studies of *P. berghei* in mice have shown increased CSF PIC levels during malaria infection (Clark et al., 2005). It has been hypothesized that high brain levels of PIC may play a significant role in the neurological signs and death that are associated with *P. berghei* ANKA infection in mice (Clark et al., 1983; Greve et al., 2000; Nahrevanian, 2006). It would be interesting to further assess the flow and composition of the CSF during *Pyy* infection in mice.

In the present study, the ependymal modified cell specimens from the fourth ventricle included a small proportion of cells with a different type of surface; these cells had blebs that occurred either singly or in clumps, were rounded or collapsed and did not have a regular distribution pattern. Bleb-covered choroid plexus cells have been reported to be a normal feature of the surfaces of choroid plexus cells in healthy rats; Collins and Morris (1975) reported an average of 20 blebs per animal. Evidence presented by some authors suggests that ependymal blebbing represents a physiologically significant mechanism by which proteins and other molecules enter the CSF (Agnew et al., 1980). In our study, no blebs were observed in the control mice, and we regard the increase in the surface blebbing of the ependymal cells as a result of inflammatory reaction and the swelling of the microvilli. Increases in the numbers of blebs on the surface of the adult mouse choroid plexus have been observed after chronic exposure to Cd^{2+} in drinking water; these blebs may be the result of hyper-peroxidation of the membrane. Oxidative stress to the membranes of nervous tissue can produce damage by several interacting mechanisms that include elevations in intracellular Ca^{2+} (Wang and Du, 2013).

The clinical manifestations of malaria and experimental malaria depend on the degree of parasitosis, virulence factors and tissue damage. In the present study, despite the observations of lesions to the ependymal epithelia of the infected mice, neither hydrocephaly nor nervous signs were observed. Patients with severe malaria who present with anemia, acidosis, hypoxia or severe renal or hepatic insufficiency may have associated alteration in BBB function that are caused by these systemic metabolic disturbances and are independent of local events in the cerebral vasculature (Papadopolus et al., 2000; Green et al 2004; Clark et al., 2005; Medana and Turner, 2006). We have previously reported that our *Pyy*-infected male CD1 mice develop tubular necrosis and that some of them die with blackwater fever-like syndrome (Rivera et al., 2013b); these animals present with damage to the CNS and no clinical signs of cerebral involvement.

Conclusion

Here, we confirmed that CD1 male mice infected with *Pyy* lethal strain exhibit damage to the CSFBB and ependymal cells. The severity of the damage to the epithelium was observed to be dependent on the extent of parasitemia. Many factors influence the pathological challenges to the ependymal epithelium and CSFBB function in complicated experimental malaria; nevertheless, the direct damage to and dysfunction of the CSFBB may not lead to neurological features. The determination of whether the CSFBB is altered in animal models of complicated malaria constitutes an important area of study that may provide insight into manipulations of the host immune system that could aid the development of novel adjuvant treatments for severe and cerebral human malaria.

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References

Afzelius B.A. (2004). Cilia-related diseases. J. Pathol. 204, 470-477.

Agnew W.F., Yuen T.G. and Achtyl T.R. (1980). Ultrastructural observations suggesting apocrine secretion in the choroid plexus: a comparative study. Neurol. Res. 1, 313-32.

Amani V., Boubou M.I., Pied S., Marussig M., Walliker D., Mazier D. and Reina L. (1998). Cloned lines of Plasmodium berghei ANKA differ in their abilities to induce experimental cerebral malaria. Infect. Immun. 66, 4093-4099.

Angulo I. and Fresno M. (2002). Cytokines in the pathogenesis of and protection against malaria. Clin. Diagn. Lab. Immunol. 9, 1145-1152.

Avila C.M.R., Barenque C.L., Zepeda R.A., Antuna B.S., Saldivar O.L., Espejel M.G., Mussali G.P., Reyes O.A., Anaya M.V. and Fortoul T.I. (2005). Ependymal epithelium disruption after vanadium pentoxide inhalation. A mice experimental model. Neurosci. Lett. 381, 21-25.

Cammer W., Tansey F., Abramovitz M., Ishigaki S. and Listowsky I. (1989). Differential localization of glutathione-s-transferase Yp and Yb subunits in oligodendrocytes and astrocytes of rat brain. J. Neurochem. 52, 876-883.

Clark I.A., Cowden W.B., Butcher G.A. and Hunter N.H. (1983). Free oxygen radicals in malaria. Lancet 1, 358-360.

Clark C.J., Mackay G.M., Smythe G.A., Bustamante S., Stone T.W. and Philips R.S. (2005). Prolonged survival of a murine model of cerebral malaria by Kynurenine pathway inhibition. Infect. Immun. 73, 5249-51.

Collins P. and Morris M.G. (1975). Changes in the surface features of choroid plexus of the rat following the administration of acetazolamide and other drugs wich affects CSF secretion. J. Anat. 120, 571-579.

Combes V., El-Assad Faille D., Jambou R., Hunt N.H. and Grau G.E. (2010). Microvesiculation and cell interaction at the brain-endothelial interface in cerebral malaria pathogenesis. Prog. Neurobiol. 91, 140-51.

Craig A.G., Grau G.E., Janse C., Kazura J.W., Milner D., Barnwell J.W., Turner G. and Langhorne J. (2012). The role of animal models for research on severe malaria. Plos. Path. 8, 1-9.

Cross C.E. and Langhorne J. (1998). *Plasmodium chabaudi chabaudi* (AS): Inflammatory cytokines and pathology in an erythrocytic-stage infection in mice. Exp. Parasitol. 90, 220-229.

de Souza B.J. and Riley E.M. (2002). Cerebral malaria: the contribution of studies in animal models to our understanding of immunopathogenesis. Microbes Infect. 4, 291-300.

Del Bigio M.R. (2010). Ependymal cells: biology and pathology. Acta. Neuropathol. 119, 55-73.

Engwerda C.R., Beattie L. and Amante F.H. (2005). The importance of the spleen in Malaria. Trends Parasitol. 21, 75-80.

Grau G.E., Piguet P.F. and Lambert P.H. (1992). Immunopathology of malaria: role of cytokine production and adhesion molecules. Mem. Inst. Oswaldo. Cruz. 87, 95-100.

Grau G.E., El-Assad F. and Combes V. (2013). Experimental models of microvascular immunopathology: The example of cerebral malaria. J. Neuroinfect. Dis. 4, 1-11.

Green R., Scott L.K., Minagar A. and Conrad S. (2004). Sepsis associated encelopathy (SAE): a review. Front. Biosci. 9, 1637-1641.

Greve B., Kremsner P.G., Lell B., Luckner D. and Schmid D. (2000). Malaria anemia in African children associated with high oxygen-radical production. Lancet 355,40-41.

Hayder A.G., Mustafa I.E., Ishraga E.A., Thoraya M.E. and Gadir E.L. (2008). Biomodal transmission of cerebral malaria and severe malaria anemia and reciprocal co-existence of sexual and asexual parasitemia in an area of seasonal malaria transmission. Parasitol. Res. 103, 81-85.

Idro R., Marsh K., John C.C. and Newton C.R. (2011). Cerebral malaria: mechanisms of brain injury and strategies for improved neurocognitive outcome. Pediatr. Res. 68, 267-74.

Johanson C., Stopa E., McMillan P., Roth D., Funk J. and Krinke G. (2011). The distributional nexus of choroid plexus to cerebrospinal fluid, ependyma and brain: Toxicology/pathologic phenomena, periventricular destabilization, and lesion spread. Tox. Pathol. 39, 186-212.

Levine S. (1987). Choroid plexus: Target for systemic disease and pathway to the brain. Lab. Invest. 56,231-33.

Mackintosh C.L., Beeson J.G. and Marsh K. (2004). Clinical features and pathogenesis of severe malaria. Tends. Parasitol. 20, 597-603.

Masocha W. and Kristensson K. (2012). Passage of parasites across the blood-brain barrier. Virulence 3, 202-212.

Medana I.M. and Turner D.H.G. (2006). Human cerebral malaria and the blood-brain barrier. Int. J. Parasitol. 36, 555-568.

Medana I.M., Chaudhri G., Chan-Ling T. and Hunt N. (2001). Central nervous system in malaria: Innocent bystander or active participant in the induction of immunopatology? Immun. Cell. Biol. 79, 101-120.

Mirzadeh Z., Han Y.G., Soriano N.M., García V.J.M. and Álvarez B.A. (2010). Cilia organize ependymal planar polarity. J. Neurosci. 30, 2600-2610.

Nahrevanian H. (2006). Immune effectors mechanisms of the nitric oxide pathway in malaria: Cytotoxicity versus cytoprotection. Braz. J. Infect. Dis. 10, 283-292.

Olivieira M.J., Pereira A.S., Guimaraes N.R., Grande N.R., Moreira C. and Aguas A.P. (2003). Zonation of ciliated cells on the epithelium of

the rat trachea. Lung 181, 275-282.

Ozen M., Gungor S., Atambay M. and Daldal N. (2006). Cerebral malaria owing to *Plasmodium vivax*: case report. Ann. J. Pediatr. 26, 141-144.

Papadopolus M.C., Davies D.C., Moss R.F., Tighe D. and Bennett E.D. (2000). Pathophysiology of septic encelopathy: a review. Crit. Care Med. 28, 3019-3024.

Prendergast C.T. and Anderton S.M. (2009). Immune cell entry central nervous system-Current understanding and perspective therapeutics targets. Endocr. Metab. Immune Disord. Drug Targets 9, 315-327.

Rivera N., Marrero P.Y., Aran V.J., Martinez C. and Malagón F. (2013a). Biological assay of a novel quinoxalinone with antimalarial efficacy on *Plasmodium yoelii yoelii*. Parasitol. Res. 112, 1523-1527.

Rivera N., Samanta E.R., Menchaca A., Zepeda A., García L.E., Salas G., Romero L.P. and Malagón F. (2013b). Blackwater fever like in murine malaria. Parasitol. Res. 112, 1021-1029.

Smith Q.R., Pershing L.K. and Johanson C.E. (1981). A comparative analysis of extracellular fluid volume of several tissue as determined by six different markers. Life Sci. 29, 449-56.

Stevenson M.M., Lyanga J.J. and Skamene E. (1982). Murine malaria: Genetic control of resistance to *Plasmodium chabaudi*. Infect. Immun. 38, 80-88.

Thumwood C.M., Hunt N.H., Clark I.A. and Cowden W.B. (1988). Breakdown of the blood-brain barrier in murine cerebral malaria. Parsitology 96, 579-589.

Toskala E., Nuutinem J. and Rautianen M. (1995). Scanning electron microscopy findings of human respiratory cilia in chronic sinusitis and in recurrent respiratory infections. J. Larryngol. Otol. 109, 509-514.

Wang B. and Due Y. (2013). Cadmium and its neurotoxic effects. Oxid. Med. Cell. Longev. 2013, 898034.

Zheng W., Aschner M. and Ghersi-Egeac J.F. (2003). Brain barrier systems: a new frontier in metal neurotoxicological research. Toxicol. Appl. Pharmacol. 192, 1-11.

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