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Single episode of mild murine malaria induces neuroinflammation, alters microglial profile, impairs adult neurogenesis, and causes deficits in social and anxiety-like behavior

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ABSTRACT

Cerebral malaria is associated with cerebrovascular damage and neurological sequelae. However, the neurological consequences of uncomplicated malaria, the most prevalent form of the disease, remain uninvestigated. Here, using a mild malaria model, we show that a single *Plasmodium chabaudi adami* infection in adult mice induces neuroinflammation, neurogenic, and behavioral changes in the absence of a blood–brain barrier breach. Using cytokine arrays we show that the infection induces differential serum and brain cytokine profiles, both at peak parasitemia and 15 days post-parasite clearance. At the peak of infection, along with the serum, the brain also exhibited a definitive pro-inflammatory cytokine profile, and gene expression analysis revealed that pro-inflammatory cytokines were also produced locally in the hippocampus, an adult neurogenic niche. Hippocampal microglia numbers were enhanced, and we noted a shift to an activated profile at this time point, accompanied by a striking redistribution of the microglia to the subgranular zone adjacent to hippocampal neuronal progenitors. In the hippocampus, a distinct decline in progenitor turnover and survival was observed at peak parasitemia, accompanied by a shift from neuronal to glial fate specification. Studies in transgenic Nestin-GFP reporter mice demonstrated a decline in the Nestin-GFP$^+$/GFAP$^+$ quiescent neural stem cell pool at peak parasitemia. Although these cellular changes reverted to normal 15 days post-parasite clearance, specific brain cytokines continued to exhibit dysregulation. Behavioral analysis revealed selective deficits in social and anxiety-like behaviors, with no change observed in locomotor, cognitive, and depression-like behaviors, with a return to baseline at recovery. Collectively, these findings indicate that even a single episode of mild malaria results in alterations of the brain cytokine profile, causes specific behavioral dysfunction, is accompanied by hippocampal microglial activation and redistribution, and a definitive, but transient, suppression of adult hippocampal neurogenesis.

1. Introduction

Malaria, caused by protozoan parasites of the *Plasmodium* spp., results in 300–400 million clinical cases and 1–2 million deaths each year, with more than 40% of the world's population at risk (Kappe et al., 2010; Health Organization, 2011). Epidemiological evidence suggests that both non-cerebral and cerebral malaria are associated with long-term neurological impairments (Carter et al., 2005; John et al., 2008; Kariuki et al., 2011; Nankabirwa et al., 2013; Kihara et al., 2006). Given that the malarial parasite is non-neurotropic, neurological sequelae are a consequence of a neuroinflammatory response, with the specific case of cerebral malaria involving cytoadhesion and brain sequestration of infected red blood cells (Hansen, 2012; Grau and Craig, 2012; Gay et al., 2012). However, the majority of malarial infections are of the non-cerebral uncomplicated type (World Health Organization, 2011). While we have now gained insights into cerebral malaria and its effects on the brain, the mechanisms underlying the neurological sequelae

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of non-cerebral, uncomplicated malaria still remain poorly elucidated. Given that patients never develop sterile immunity to the infection and clinical immunity develops only after repeated exposures (Marsh and Kinyanjui, 2006), it is crucial that the neurological repercussions of non-cerebral uncomplicated malaria, and its mechanisms be better understood.

Experimental models of infection (Jurgens et al., 2012; Calsavara et al., 2013; Lieblein-Boff et al., 2013; Buenz et al., 2006; Granger et al., 2013; Evans et al., 2014; Vyas, 2013), including exposure to non-neurotropic infectious agents, have been linked to cognitive dysfunction and mood-related abnormalities, with specific inflammatory changes, structural and functional effects often reported within the hippocampus, a brain region associated with cognition (Eichenbaum, 2004) and mood (Nestler et al., 2002; Vaidya and Duman, 2001). In particular, mouse models of cerebral malaria have been reported to exhibit enhanced anxiety-like behavior and significant cognitive impairment associated with increased pro-inflammatory cytokines and alterations in hippocampal structure (Desruisseaux et al., 2008; Reis et al., 2010, 2012; de Miranda et al., 2011; Miranda et al., 2013). The hippocampus in addition to its important role in cognition and mood, also exhibits significant structural plasticity including the ability to generate new neurons throughout life (Ming and Song, 2005). This process of adult hippocampal neurogenesis is particularly responsive to environment and experience, including neuroimmune cues (Glasper et al., 2012; Ziv and Schwartz, 2008). Emerging evidence strongly suggests that the immune system contributes to the regulation of adult hippocampal neurogenesis, and it may serve to integrate peripheral cues and translate these into localized changes within the hippocampal neurogenic niche (Ziv and Schwartz, 2008; Rolls et al., 2007; Butovsky et al., 2006; Derecki et al., 2010). Here, using the *Plasmodium chabaudi adami* model which is one of the mildest murine models, and is closest to the human uncomplicated disease (Langhorne et al., 2011; Craig et al., 2012) we investigated the effects of mild malaria on the murine brain and specifically on the hippocampus, which is particularly vulnerable to neuroinflammatory insults (Eriksson et al., 1999).

2. Materials and methods

2.1. Mice

Wild type C57BL/6 male mice (6–8 weeks) and transgenic reporter male mice from a mixed C57BL/6 and CD2 background, expressing transgenic green fluorescent protein (GFP) under the Nestin promoter (Yu et al., 2005), a kind gift of Prof. S.G. Kernie (Columbia University, USA), were bred and housed in specific pathogen-free facility at the Tata Institute of Fundamental Research (TIFR). All experiments were approved by the TIFR animal ethics committee, and were in accordance with the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals; registration No. 56/1999/CPCSEA).

2.2. Parasites and induction of blood stage malarial infection

P. chabaudi adami parasites, a kind gift from Prof. Nirbhay Kumar (Department of Tropical Medicine, Tulane University School of Public Health and Tropical Medicine), were revived from frozen stocks. The infection was initiated by intraperitoneal (i.p.) injection of 10^6–10^7 parasitized erythrocytes as previously described (Sanni et al., 2001). The progress of infection was monitored by microscopic examination of Giemsa-stained thin blood smears where at least 1000 erythrocytes were counted per slide. The progress of infection was monitored by calculating percentage parasitemia {(number of infected erythrocytes/total number of erythrocytes) × 100} and the maximum parasitemia attained post infection was defined as the 'peak' (day 9); 'recovery' was day 25 post infection and 15 days post parasite clearance (Fig. 1A).

2.3. Splenic index and flow cytometry

Spleens were isolated from control and infected euthanized mice at the peak and recovery time points (n = 6–7 per group,

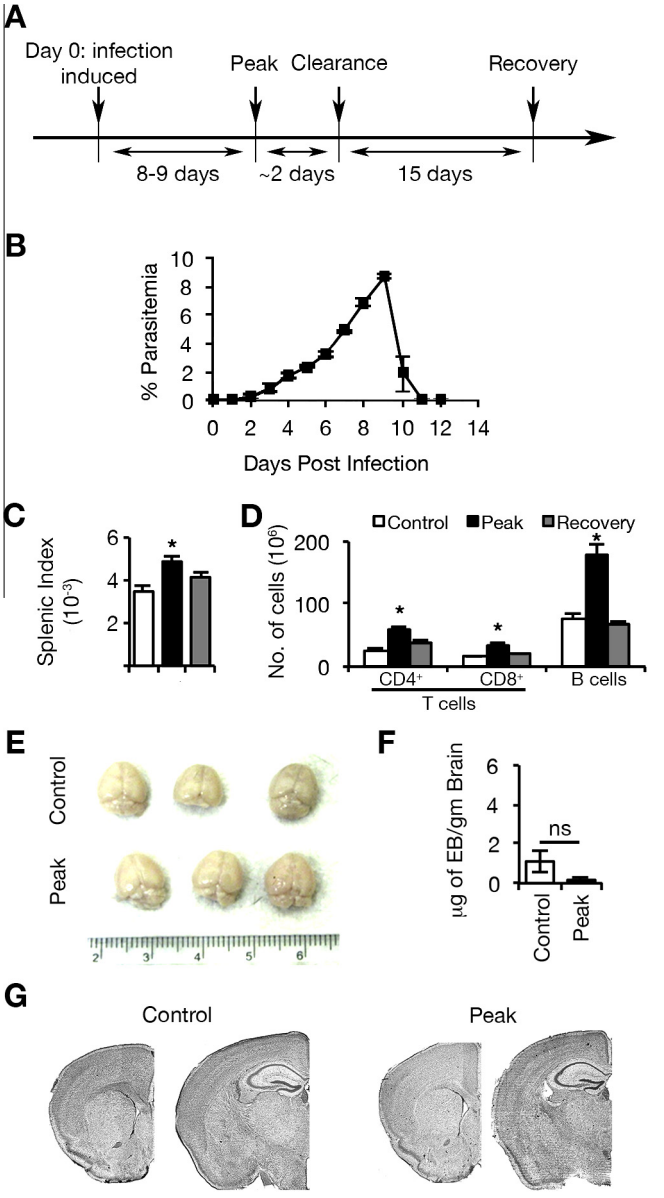

Fig. 1. Mild malaria induces a reversible splenic immune response and does not induce blood–brain barrier disruption or neuroanatomical changes. (A) Schematic representation of the parasite kinetics. (B) Typical parasitemia profile of a *P. chabaudi adami* infection (N = 3, n = 3) induced in C57BL/6 mice. (C, D) Splenic response to parasitic infection (C) represented as a mean splenic index (ratio of splenic weight to body weight) and (D) as average splenic $CD4^+$ T cells, $CD8^+$ T cells and $CD19^+$ B cell numbers of uninfected (Control), peak infection point (Peak), and 15 days post-parasite clearance (Recovery) mice (n = 6–7 mice per group). (E, F) Blood brain barrier integrity assessed by the absence of Evans Blue (EB) extravasation following its administration as revealed by (E) macroscopic images of the brain and from (F) colorimetric measurement of the dye per gram of brain tissue from uninfected (Control) and infected (Peak) mice and at peak of parasitemia (n = 3 mice per group). (G) Bright field photomicrographs of coronal brain sections revealing absence of major anatomical alterations in brain tissue of control and infected mice at peak. Individual 2.5× images were used to generate a single composite image. Results are expressed as mean ± SEM. $^*p < 0.05$ (Student's t test).

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