J Parasit Dis (Jan-Mar 2019) 43(1):139–153
https://doi.org/10.1007/s12639-018-1070-3

ORIGINAL ARTICLE

TREM-1 modulation produces positive outcome on the histopathology and cytokines release profile of *Plasmodium berghei*-infected mice

Voon Kin Chin[2] · Afiq Mohd Yusof Asyran[1] · Zainul Amiruddin Zakaria[4] · Wan Omar Abdullah[5] · Pei Pei Chong[2] · Norshariza Nordin[4] · Zaid Osamah Ibraheem[1] · Roslaini Abdul Majid[3] · Rusliza Basir[1]

Received: 3 September 2018 / Accepted: 3 December 2018 / Published online: 15 December 2018

Abstract Triggering receptor expressed on myeloid cells 1 (TREM-1) is a potential molecular therapeutic target for various inflammatory diseases. Despite that, the role of TREM-1 during malaria pathogenesis remains obscure with present literature suggesting a link between TREM-1 with severe malaria development. Therefore, this study aims to investigate the role of TREM-1 and TREM-1 related drugs during severe malaria infection in *Plasmodium berghei*-infected mice model. Our findings revealed that TREM-1 concentration was significantly increased throughout the infection periods and TREM-1 was positively correlated with malaria parasitemia development. This suggests a positive involvement of TREM-1 in severe malaria development. Meanwhile, blocking of TREM-1 activation using rmTREM-1/Fc and TREM-1 clearance by mTREM-1/Ab had significantly reduced malaria parasitemia and suppressed the production of pro- inflammatory cytokines (TNF-α, IL-6 and IFN-γ) and anti-inflammatory cytokine (IL-10). Furthermore, histopathological analysis of TREM-1 related drug treatments, in particular rmTREM-1/Fc showed significant improvements in the histological conditions of major organs (kidneys, spleen, lungs, liver and brain) of *Plasmodium berghei*-infected mice. This study showed that modulation of TREM-1 released during malaria infection produces a positive outcome on malaria infection through inhibition of pro-inflammatory cytokines secretion and alleviation of histopathological conditions of affected organs. Nevertheless, further investigation on its optimal dosage and dose dependant study should be carried out to maximise its full potential as immunomodulatory or as an adjuvant in line with current antimalarial agents.

Keywords TREM-1 · Experimental malaria infection · Cytokines · Histopathology · TREM-1 modulation

✉ Rusliza Basir
rusliza@upm.edu.my

1 Pharmacology Unit, Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

2 School of Biosciences, Faculty of Health and Medical Sciences, Taylor's University, No 1, Jalan Taylor's, 47500 Subang Jaya, Selangor, Malaysia

3 Department of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

4 Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

5 Department of Medical Sciences, Faculty of Medicine and Health Sciences, Universiti Sains Islam Malaysia, 55100 Pandan Indah, Kuala Lumpur, Malaysia

Introduction

Malaria is the most widespread parasitic infection accounting for an estimated 216 million cases and 445,000 deaths annually. Most of the malaria cases occur in African regions, followed by South-East Asia and Eastern Mediterranean regions (WHO 2016). The high incidence rate indicates that malaria remains a serious public health problem which requires attention worldwide to tackle or mitigate the impact of this infection.

In human malaria, cytokine network has a critical role in the development of a potent antimalarial immunity in the host. Pro-inflammatory cytokines are required to initiate the inflammatory responses and to kill the malaria parasites whereas anti-inflammatory cytokines are required to counteract excessive pro-inflammatory cytokine response

to prevent possible cytopathic effects. Most of the malaria-related cytokines including IL-4, TNF-α, IL-10 and IFN-γ, play a double-edged role during the infection. Overproduction or deficiency of these mediators can lead to severe pathology in malaria. Furthermore, it is well documented that the outcome of malaria infection is largely dependent on the balance between pro-inflammatory and anti-inflammatory cytokines (Malaguarnera and Musumeci 2002).

Although first-line anti-malarial drugs such as chloroquine, primaquine and artemisinin-based combination therapy (ACT) are useful in reducing malaria parasitemia, these drugs have minimal effects in controlling the inflammation provoked by the host immune system. This will eventually lead to seizures, coma, and long-term sequelae in patients with severe malaria (Achtman et al. 2012). Therefore, there is a constant need to search for alternatives to treat malaria infections. One of the ideal solutions to lower the mortality rate and to relieve malaria-associated symptoms is through manipulating immune system by inflammatory cytokines (Dondorp et al. 2009; Achtman et al. 2012).

Triggering receptor expressed on myeloid cells 1 (TREM-1) is a cell surface activating receptor belonging to the immunoglobulin superfamily. TREM-1 is involved in various immunological events such as monocytic activation, modulation of inflammatory response and contributes to septic shock caused by microbial infection (Bouchon et al. 2000). TREM-1 mediates inflammatory response via three main mechanisms (1) triggers the pro-inflammatory cytokines and chemokines release, (2) upregulates the surface expression of cell activation markers and (3) initiates the myeloperoxidase, monocyte and neutrophil release at the site of inflammation (Bouchon et al. 2000, 2001a, b). Meanwhile, previous study has also demonstrated the role of TREM-1 in inflammation where blocking of TREM-1 signalling partially protects the animals from death in a mouse model of septic shock (Bouchon et al. 2001a, b; Gibot et al. 2004). Additionally, exposure to infectious agents also increased the TREM-1 expression on blood monocytes and neutrophils, which are the primary mediators for host innate immunity (Bouchon et al. 2000). Taken together, TREM-1 could be a crucial key regulator for innate immunity and acts as a potential therapeutic candidate for inflammatory diseases.

In malaria context, we have performed Pubmed literature search by using keyword "TREM-1 and Malaria". To our surprise, there are only a few literature documenting the association between TREM-1 and malaria infection. Most of the studies suggest that TREM-1 is involved in malaria pathogenesis. For example, Chimma et al. (2009) reported that monocyte TREM-1 level increased in uncomplicated malaria cases when compared to healthy individuals. Meanwhile, another study by Erdman et al. (2011) demonstrated that plasma sTREM-1 levels were associated with disease severity in children with severe malaria. In addition, Adukpo et al. (2016) also reported that sTREM-1 levels were significantly higher in children with severe malaria than uncomplicated malaria. However, the exact mechanism on the involvement of TREM-1 in malaria pathogenesis remains elusive.

Since activation of TREM-1 has been associated with triggering of pro-inflammatory cytokines release and severe malaria development. Therefore, in this study, we employed *Plasmodium berghei* ANKA infection in ICR mice as a cerebral malaria model (Basir et al. 2012) to investigate the involvement of TREM-1 during malaria infection and to examine the effect of modulating TREM-1 release on the cytokine profiles and histopathological changes during malaria infection.

Materials and methods

Animals and malarial infection

Inbred ICR mice of weights around 20–25 g and *Plasmodium berghei* ANKA malaria parasite obtained from Institute for Medical Research (IMR), Malaysia were used throughout the study. The establishment of malaria infection in ICR mice was adopted from Basir et al. (2012). Briefly, ICR mice were injected with 2×10^7 parasitized red blood cells (PRBC) in a 0.2 mL volume via intraperitoneal route (Basir et al. 2012) while control ICR mice received the same volume and dilution of normal mouse red blood cells. Food and water were given to mice ad libitum. The study was approved and was carried out in accordance with the relevant guidelines and regulations by The Animal Care and Use Committee of the Faculty Medicine and Health Science, Universiti Putra Malaysia (UPM/FPSK/PADS/BR-UUH/00,300).

Parasitemia measurement

Parasitemia levels in mice were monitored at day 1, 2, 3, 4, 5 and 6 post inoculation with malaria parasite. A drop of blood was obtained from the mice via venesection of the tail and the blood was placed at the edge of a microscopic slide to make a thin blood film. Leishman's stain was then used to stain the slide and the slide was viewed under light microscope with oil immersion at 1000× magnification. A total of five different fields of thin blood film containing 200 red blood cells were counted. The parasitemia level was taken as the percentage of total red blood cells consisting of Leishman-positive bodies. The final percentage of parasitemia was based on the average of five different fields consisting of Leishman-positive bodies.

Determination of plasma TREM-1 levels in malarial mice

Control normal mice and malaria-infected mice were divided into three groups consisting of 8 mice each. Blood were collected via cardiac puncture, centrifuged at 2500 rpm for 15 min to separate the plasma from the red blood cells and stored at − 70 °C before use. Plasma TREM-1 concentration was determined through ELISA Quantikine Immunoassay Kit (R&D, USA) as per manufacturer's instructions at day 1, 2, 3, 4, 5 and 6 post inoculation.

Preparation of mouse TREM-1 polyclonal antibody (mTREM-1/Ab) and recombinant mouse TREM-1 Fc chimera (rmTREM-1/Fc)

Mouse TREM-1 polyclonal antibody (mTREM-1/Ab) (R&D Systems Inc. Minneapollis, US) was reconstituted at 0.5 mg/mL in sterile PBS and stored at − 70 °C. Meanwhile, recombinant mouse TREM-1 Fc Chimera (rmTREM-1/Fc) (R&D Systems Inc. Minneapollis, US) used in this study was a mouse recombinant fusion protein that protects the mice against LPS-induced shock. rmTREM-1/Fc was supplied in lyophilized form in 108 µL of a filtered solution in phosphate-buffer saline (PBS) with pH 7.4. It was reconstituted in 100 µg/mL of sterile PBS and was further aliquoted at 6.25 µg/1 mL and stored at − 80 °C before use.

Effects of TREM-1 modulation on parasitemia development and survival of malaria-infected mice

Twenty- four ICR mice with weight between 20 and 25 g were inoculated with 2×10^7 PRBC in 0.2 mL intravenously (i.v) from a donor mouse infected with *P. berghei* ANKA. On day 2 following post inoculation, mice were divided into four groups and each group was injected intravenously with normal saline, Mouse TREM-1 polyclonal antibody (mTREM-1/Ab) and Recombinant Mouse TREM-1 Fc Chimera (rmTREM-1/Fc). The dosage for mTREM-1/Ab and rmTREM-1/Fc in this study were adapted from study by Bouchon et al. (2001a, b).

The groupings were as follow:

Group 1: Control mice (C) + 0.9% normal saline (NS) (0.1 mL, i.v) (C + NS).
Group 2: Malaria (M) + Saline (S) (0.1 mL, i.v) (M + NS).
Group 3: Malaria (M) + mTREM-1/Ab (10 µg/0.2 mL/mouse, i.v) (M + Mtrem-1/Ab).
Group 4: Malaria (M) + rmTREM-1/Fc (1.25 µg/0.2 mL/mouse, i.v) (M + rmTREM-1/Fc).

One dose of each treatment was given at midday on day 2, 3, 4, and 5 of the infection. The animals received four doses of each treatment during the infection until day 5. Mortality and parasitemia development in all groups were closely monitored and recorded throughout the study.

Effects of TREM-1 modulation on cytokines release and histopathological changes during malaria infection

Mice grouping

Thirty-two ICR male mice were used in the study and were assigned to groups of eight mice each. The dosage for mTREM-1/Ab and rmTREM-1/Fc were adapted from study by Bouchon et al. (2001a, b).

The groupings were as follow:

Group 1: Control mice (C) + 0.9% normal saline (NS) (0.1 mL, i.v) (C + NS).
Group 2: Malaria (M) + Saline (S) (0.1 mL, i.v) (M + NS).
Group 3: Malaria (M) + mTREM-1/Ab (10 µg/0.2 mL/mouse, i.v) (M + Mtrem-1/Ab).
Group 4: Malaria (M) + rmTREM-1/Fc (1.25 µg/0.2 mL/mouse, i.v) (M + rmTREM-1/Fc).

Cytokine analysis

Blood were collected by cardiac puncture while the mice were under terminal general anaesthesia via inhalation of diethyl ether. Plasma samples were prepared from all groups at day 3, 4 and 5 post infection by centrifuging the whole blood at 2500 rpm for 15 min. Plasma samples were then stored at − 70 °C prior analysis. TNF-α, IFN-γ, IL-6 and IL-10 concentration at day 3, 4 and 5 post infections were measured using commercial ELISA kits Quantikine (R&D Systems, USA) as per manufacturer's instructions.

Histopathological analysis

At day 5 of post infection, all the mice in the study were anesthetized with diethyl ether 1.9% and sacrificed for histological analysis. Liver, brain, spleen, kidney and lung organs were retrieved as these organs were mainly affected during malaria infection (Basir et al. 2012). Perfusion was carried out for 5 min using PBS intracardially prior to analysis. All the organs retrieved (liver, brain, spleen, kidney and lung) were preserved in 10% buffered formalin for a few days and the samples were dehydrated in ascending series of alcohol dehydration, clearing with xylene and wax impregnation with paraffin wax for 14 h in an automated tissue processor (Leica, Germany). The

tissues were then embedded in paraffin wax, sectioned by using the microtome machine at 4.0 µm and stained with hematoxylin and eosin (H&E) with an autostainer (Leica, Germany). The morphological changes of the infected and normal tissues were observed under light microscopy at 100× and 400× magnifications.

Data analysis

All statistical analyses in this study were performed by using GraphPad Prism version 5.0 software (GraphPad Prism Inc., USA) and the results obtained were expressed as mean ± standard error of the means (SEM). Unpaired student *t* test was used to analyse the changes in plasma TREM-1 levels between control and malaria-infected mice. Correlation analysis between parasitemia level and plasma TREM-1 was performed using Spearman's rank-order correlation. Kaplan–Meier plots were used to compare the survival curves under different treatments using a log-rank test. Normality test was performed and the comparison of group mean values among at least three independent groups was performed via one-way analysis of variance (ANOVA) followed by Tukey's test as a single post hoc test. The level of statistical significance was set at $P < 0.05$.

Results

Plasma TREM-1 concentrations in malarial mice

High levels of systemic TREM-1 concentration were recorded since day 1 post inoculation in malarial mice as compared to normal mice. In addition, significant increase in the plasma TREM-1 concentrations from day 3 until day 6 post inoculation were recorded (Fig. 1). On the other hand, positive correlation between plasma TREM-1 level and percentage of parasitemia development was obtained (Fig. 2). The positive correlation implies that mice with low parasitemia levels have low concentrations of plasma TREM-1 and vice versa ($R = 0.567$).

Parasitemia and survival rate in malarial mice treated with TREM-1 related drugs

Effects of TREM-1 related drugs on parasitemia development and survival rate are depicted in Figs. 3 and 4. mTREM-1/Ab treated malarial mice showed significant reduction in parasitemia levels from day 4 to day 6 post infection as compared to malaria- infected mice. Meanwhile, rmTREM-1/Fc treated malarial mice also showed significant reduction in the parasitemia levels on day 2, day 4 to day 6 post-infection as compared to malaria- infected mice.

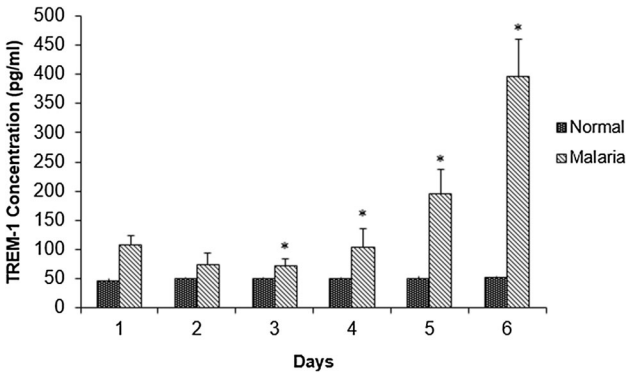

Fig. 1 TREM-1 concentrations in the plasma of control and malarial mice. The infected mice were inoculated with 2×10^7 PRBC (i.v.) from a donor mouse previously infected with *P. berghei* ANKA. Control mice received an equivalent volume and dilution (0.2 mL/animal) of normal mouse red blood cells. TREM-1 concentrations were determined in the plasma of control and malaria-infected mice. Results were expressed as the mean ± SEM (N = 8). $*P < 0.05$ indicates significant differences in parasitemia levels between control and malaria groups were determined using unpaired Student's *t*-test

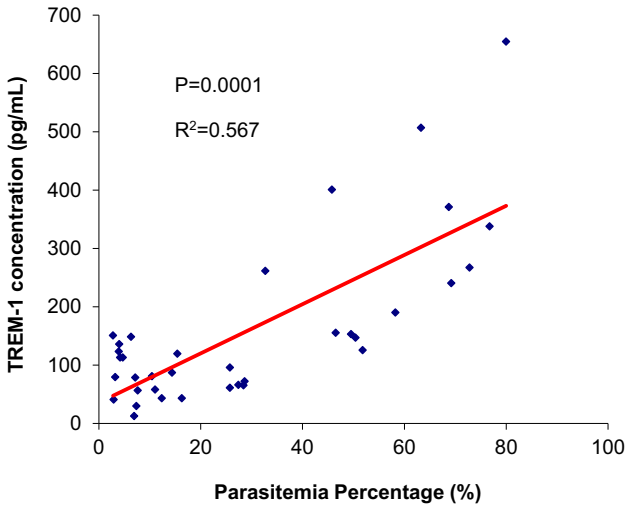

Fig. 2 Correlation between the increase in percentage parasitemia and plasma TREM-1 concentration (pg/mL) in malaria-infected mice. The infected animals were inoculated with 2×10^7 PRBC (i.v.) from a donor mouse previously infected with *P. berghei* ANKA. The correlation coefficient was analyzed by using linear regression of Pearson's rank order correlation coefficient

Meanwhile, 100% survival rates were recorded from day 1 until day 4 post infection in malaria-infected mice, mTREM-1/Ab treated malarial mice and rmTREM-1/Fc treated malarial mice. Both treatment groups showed improved survival rates, which was 87.5% at day 5 post infection as compared to 81.25% of survival rate as seen in malaria-infected mice. However, 100% mortality rates were recorded in all groups at day 6 post infection (Fig. 4).

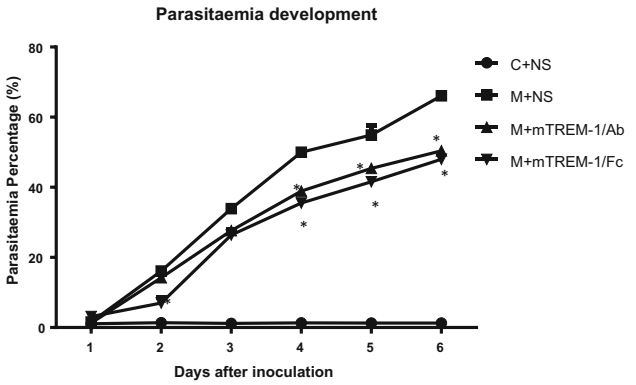

Fig. 3 Percentage parasitemia of control, malarial with saline, and malaria-infected mice with TREM-1 antibody treatments. The infected animals were inoculated with 2×10^7 PRBC (i.v) from a donor mouse previously infected with *P. berghei* ANKA. The treatment animals were injected with TREM-1 antibody, 10 µg per animal in 0.2 mL volume. Control animals received an equivalent volume and dilution (0.2 mL/animal) of normal mouse red blood cells. Results were expressed as the mean ± SEM (N = 8). $*P < 0.005$ significant differences in parasitemia levels between control, malaria and mouse TREM-1 antibody treatment groups. Results were analysed by using one-way analysis of variance (ANOVA) followed by Tukey's test

Pro- and anti-inflammatory cytokines release during TREM-1 modulation in malarial mice

As shown in Fig. 5, all malaria-infected mice expressed significant high levels of pro-inflammatory cytokines (TNF-α, IFNγ, and IL-6) and anti-inflammatory cytokine (IL-10) from day 3 to day 5 post infection. Meanwhile, TNF-α, IFNγ, IL-6, and IL-10 levels were suppressed in mTREM-1/Ab treated malarial on day 5 post infection. On the other hand, significant reduction of pro-inflammatory cytokines (TNF-α, IFNγ, and IL-6) on day 4 and day 5 post infection, and significant reduction of IL-10 level on day 5 post infection were observed in rmTREM-1/Fc treated malarial mice. These findings indicate that TREM-1 possesses modulatory effect on both pro-inflammatory cytokines and anti-inflammatory cytokine in this study.

Histopathology analysis

Pathological changes of uninfected control mice and malaria-infected mice (saline, mTREM-1/Ab and rmTREM-1/Fc treatment) were observed in five major organs (brain, liver, kidney, lung and spleen). In malaria-infected mice, congestion of malarial pigments, hemozoins were observed in brain tissues. The appearance of hemozoin pigments suggest the sequestration of PRBCs in the cerebral microvasculature (Fig. 6b). Sequestration of PRBCs in micro vessels were also observed in mTREM-1/Ab treated malarial mice (Fig. 6c) and rmTREM-1/Fc treated malarial mice (Fig. 6d). Meanwhile in control mice (Fig. 6a), no haemozoin or sequestration was observed.

Histologically, liver tissues exhibited intact features of sinusoids and kuppfer cells in uninfected mice (Fig. 7a). Meanwhile, hypertrophy and hyperplasia of kuppfer cells in malaria-infected mice (M + NS) (Fig. 7b, red arrow) were observed. In addition, microvascular congestion with PRBCs (Fig. 7b, black arrow) was observed in malaria-infected mice (M + NS). Moreover, liver tissues of malaria-infected mice appeared to be tainted with malaria pigments, hemozoin (Fig. 7b, yellow arrow). However, no significant difference was observed between malaria-infected mice and mTREM-1/Ab treated malarial mice (M + mTREM-1/Ab) (Fig. 7c). M + mTREM-1/Ab exhibited all pathological features of malarial mice in liver tissues. rmTREM-1/Fc treated malarial mice (M + rmTREM-1/Fc) (Fig. 7d) showed the absence of microvascular congestion with PRBCs, less hyperlasia and hypertrophy of Kupffer cells and hemozoin accumulation was reduced.

Kidneys of uninfected mice in cortex (Fig. 8a) and medulla area (Fig. 9a) showed a clear and normal renal

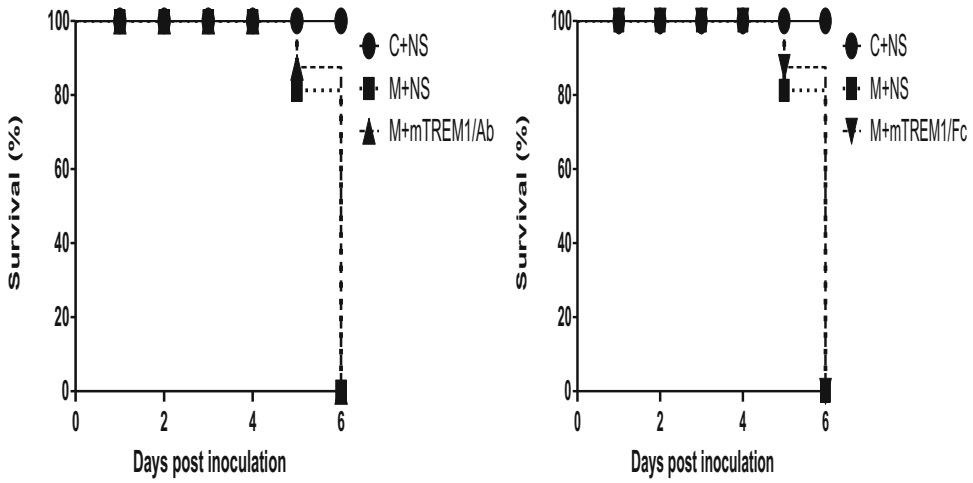

Fig. 4 Effects of modulating TREM-1 release on survival rate of treated malaria-infected mice with mTREM-1/Ab and mTREM-1/Fc (n = 8) in three separate experiments. Data were analysed by Kaplan–Meier survival estimator. Keynote: C = control, NS = normal saline, M = malaria, mTREM-1/Ab = mouse TREM-1 polyclonal antibody, mTREM-1/Fc = recombinant mouse TREM-1 Fc chimera

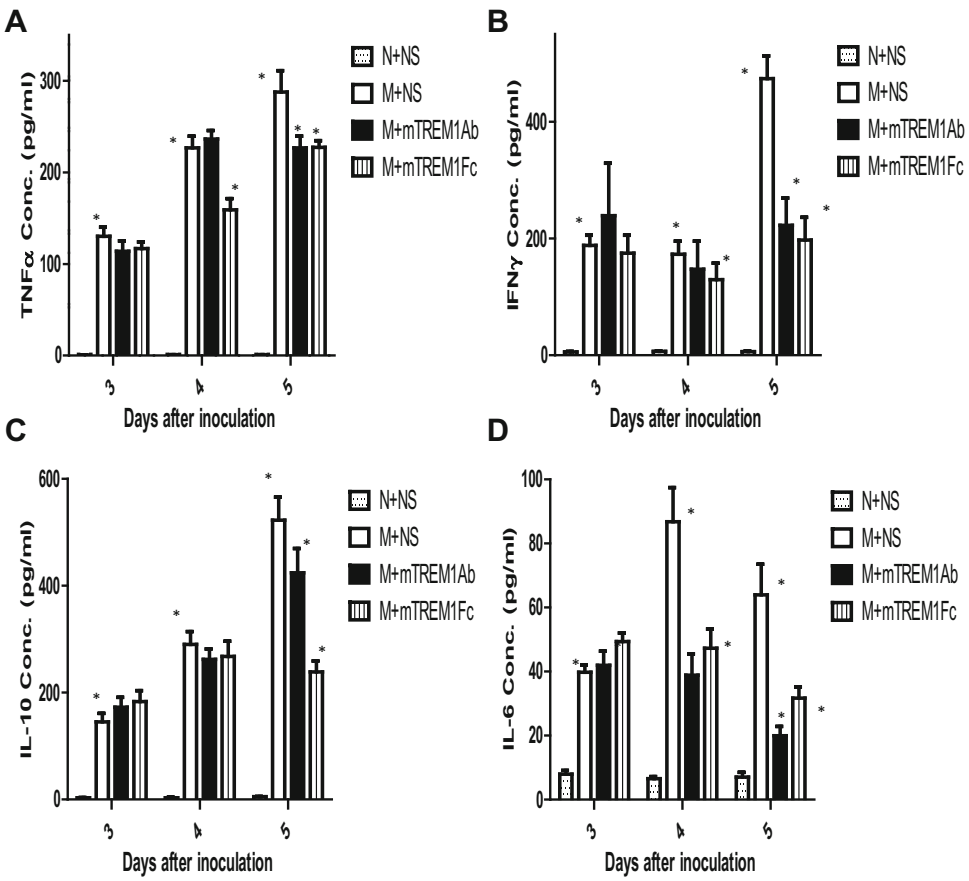

Fig. 5 **a–d** TNF-α, IFN-γ, IL-10 and IL-6 concentrations of control and treated malarial mice and effect of mTREM-1/Ab and rmTREM-1/Fc on the production of TNF-α, IFN-γ, IL-10 and IL-6 in malaria-infected mice. Each column represents the mean ± SEM of 8 mice.
C + NS = control + normal saline;
M + NS = malaria + normal saline; M + mTREM-1/Ab = malaria + mouse TREM-1 antibody; M + rmTREM-1/Fc = malaria + recombinant TREM-1 Fc. *$P < 0.05$ denotes the significant differences between treatment groups and malaria-normal saline group on day 3, 4 and 5 post inoculation. The data was analysed by using one-way analysis of variance (ANOVA) followed by Tukey's test

cell. Hemozoins accumulations, microvascular congestion with PRBC in cortex area were observed in malaria-infected mice (M + NS) (Fig. 8b), mTREM-1/Ab treated malarial mice (M + mTREM-1/Ab) (Fig. 8c) and rmTREM-1/Fc treated malarial mice (M + rmTREM-1/Fc) (Fig. 8d) as compared to uninfected mice. Malaria pigments, hemozoins were also observed in the cortex and medulla areas. There were no significant changes resulted from the treatment of rmTREM-1/Ab in both cortex and medulla area. As for the treatment with rmTREM-1/Fc, the treatment mostly improved the histopathological features in cortex (Fig. 8d) and medulla area (Fig. 9d) including decrease in hemozoins accumulations, microvascular congestions and portal infiltration.

In the spleen of malaria-infected mice (M + NS), mTREM-1/Ab treated malarial mice (M + mTREM-1/Ab) and rmTREM-1/Fc treated malarial mice (M + rmTREM-1/Fc), red and white pulp elements were expanded accompanied by the loss of typical structure of germinal centre (Fig. 10b, c, d) as compared to normal mice (N + NS) (Fig. 10a). The sequestration of PRBCs in the microvasculature and accumulations of hemozoins were also observed in all malaria-infected mice and this pathological feature was less significant in the malarial mice treated with rmTREM-1/Fc (M + rmTREM-1/Fc) (Fig. 11c).

Hyaline membrane formations due to alveolar hemorrhage were observed in the lung tissue of malarial mice (M + NS; Fig. 12b, black arrow) as compared to control mice (Fig. 12a). No hyaline membrane formation was observed in mTREM-1/Ab treated malarial mice (M + mTREM-1/Ab) and rmTREM-1/Fc treated malarial mice (M + rmTREM-1/Fc) (Fig. 12d, e). In the lung of malaria-infected mice (M + NS) and in rmTREM-1/Fc treated malarial mice (M + mTREM-1/Ab) (Fig. 12c, d: red arrow), microvascular congestions with PRBCs were observed. However, significantly lower numbers of microvascular congestions were recorded in rmTREM-1/Fc treated malarial mice (M + rmTREM-1/Fc) (Fig. 12e). Histological sections of all malaria-infected group (Fig. 12b–e: yellow arrow) were also tainted with malaria pigments, hemozoins.

Discussion

The evidence on the role of TREM and TREM- like receptors in regulating the acute infectious inflammatory, non-infectious and chronic inflammatory conditions have rapidly accumulated (Ford and McVicar 2009; Tammaro et al. 2017). Nonetheless, the role of TREM-1 during

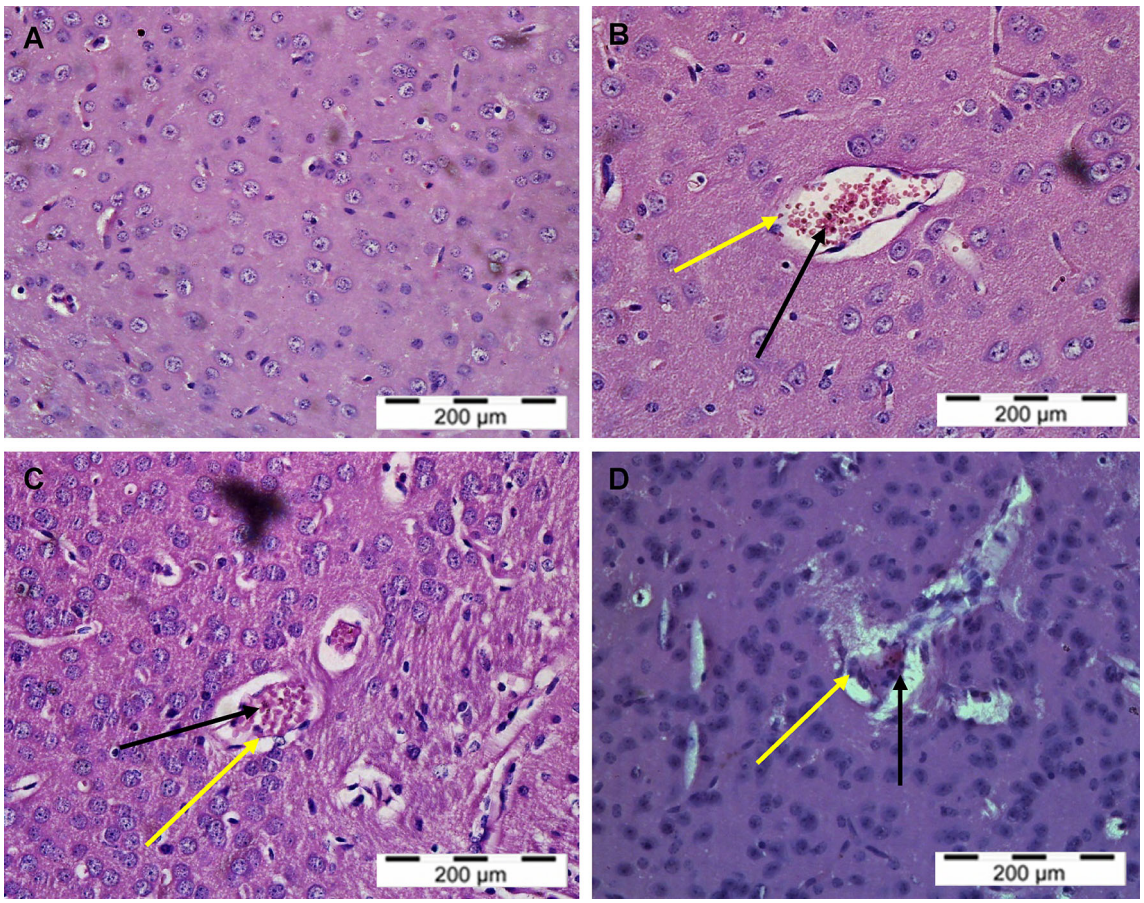

Fig. 6 Light microscopy of brain tissue in normal uninfected mice (N + NS, **a**), malaria-infected mice treated with normal saline (M + NS, **b**), mTREM-1/Ab (M + mTREM-1/Ab, **c**) and rmTREM-1/Fc (M + mTREM-1/Fc, **d**). Figures indicate the sequestration of PRBCs in the microvasculature of cerebral tissue (yellow arrow) and accumulation of hemozoins (black arrow) as compared to healthy control mice. No significant differences were observed between M + mTREM-1/Ab (**c**) and M + NS. However, this pathological feature was less significant in M + rmTREM-1/Fc as compared with control group of M + NS (H&E; n = 6; ×400) (color figure online)

parasitic infection, such as malaria, remains elusive. Therefore, this study was undertaken to elucidate the role of TREM-1 and the effects of TREM-1 modulation during malaria infection in *Plasmodium berghei*-infected mice model.

Our study showed that high plasma TREM-1 levels were recorded from day 2 until day 6 post infection in mice infected with *P. berghei*. The rapid accumulation of TREM-1 concentration in the circulation since day 2 post-infection suggests that TREM-1 is involved in the pathogenesis of malaria infection. Findings from Adukpo et al. (2016) also demonstrated that high sTREM-1 level is associated with severe malaria development. In addition, positive correlation between plasma TREM-1 levels and parasitemia levels further consolidate our hypothesis that TREM-1 might be involved in severe pathology associated with *P. berghei* infection.

In this study, mTREM-1/Ab was used to neutralize the release of TREM-1, while rmTREM-1/Fc served as an antagonist to TREM-1 ligands. Treatment with both mTREM-1/Ab and rmTREM-1/Fc reduced malaria parasitemia. Though no specific documentation was reported on the link between TREM-1 and parasitemia levels, we speculate that the involvement of TREM-1 in balancing the cytokine levels have given way to the host immune system to fight the parasite. In contrast, the survival of malaria mice treated with both mTREM-1/Ab and rmTREM-1/Fc drugs in this study were not improved despite significant inhibition on parasite growths. All mice were succumbed to death at day 6 post infection. This could be due to an irreversible pathological sequelae that has occurred during the acute infection and TREM-1 related drugs could not reverse the pathological damage. Another possible explanation is the effects of TREM-1 blockage is dependent on the pathogen. Several studies have shown positive outcome of blockage of TREM-1 on sepsis induced by other pathogens and vice versa (Gibot et al. 2006; Wang et al. 2012; Horst et al. 2013; Yang et al. 2015).

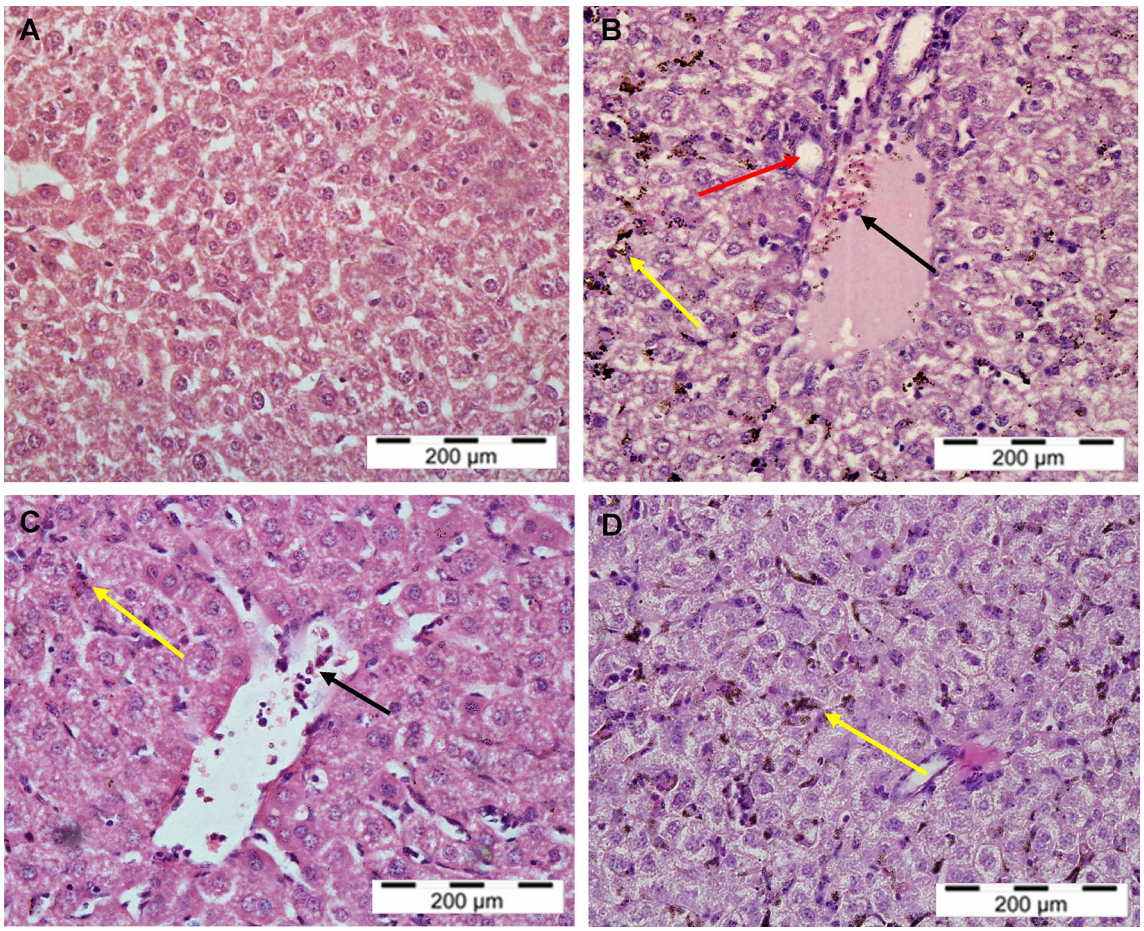

Fig. 7 Light microscopy of liver tissue in in normal uninfected mice (N + NS, **a**), malaria-infected mice treated with normal saline (M + NS, **b**), rmTREM-1/Ab (M + rmTREM-1/Ab, **c**) and rmTREM-1/Fc (M + mTREM-1/Fc, **d**). Figures indicate liver tissues were congested with PRBC. Hyperplasia and hypertrophy of Kuppfer cells (red arrow) accumulation of malaria pigment hemozoin (yellow arrow), microvascular congestion with PRBC (black arrow) (H&E; n = 6; ×400) (color figure online)

High levels of the parasitemia are usually associated with increased levels of pro-inflammatory cytokines such as TNF-α, IL-6 and IFN-γ and anti-inflammatory cytokine, IL-10 (Clark and Schofield 2000). Also, TNF-α, IL-6, and IFN-γ are often responsible for the immunopathological reactions and severity during malaria infection (Dodoo et al. 2002). Additionally, TNF-α, IL-6 and IFN-γ may have indispensable roles during malaria parasitemia development (Jason et al. 2001). In contrast, IL-10 modulates pro-inflammatory cytokines production associated with the development of T-helper 1 (Th1) type response (Warrell and Gilles 2002). Previous study has shown that IL-10 prevents severe pathology during malaria infection by inhibiting the macrophage production of IL-6, IL-12 and TNF-α (Poe et al. 1997).

In accordance with previous findings, our study also recorded high plasma levels of TNF-α, IFN-γ, IL-10 and IL-6 in malaria-infected mice. These high levels of cytokines release indicate their prominent roles in malaria pathogenesis. On the other hand, upon TREM-1 blockage with rmTREM-1/Fc, IL-6, TNF-α, IFN-γ and IL-10 levels were reduced throughout the infection periods. Similar suppression effects were also observed upon TREM-1 neutralisation with mTREM-1/Ab. Furthermore, we noticed that the effects of TREM-1 signalling blockage produced better suppression effect on pro-inflammatory cytokines. This is because TREM-1 signalling is able to intensify TLR signals to augment pro-inflammatory cytokine synthesis (Dower et al. 2008). In addition, pro-inflammatory cytokine production and lethality in mouse models of septic shock can be reduced by blocking TREM-1 with a recombinant TREM-1 fusion protein (mTREM-1-hFcγ1) (Bouchon et al. 2001a, b). Taken together, we speculate that TREM-1 posed a pro-inflammatory activity, which favour type 1 T helper cells (Th1) response during malaria infection, where this positive modulatory effect on pro-inflammatory cytokines could be potential immunotherapeutic target in malaria therapy.

During malaria infection, liver is the first organ where the parasites invade and evade the immune system (Sturm

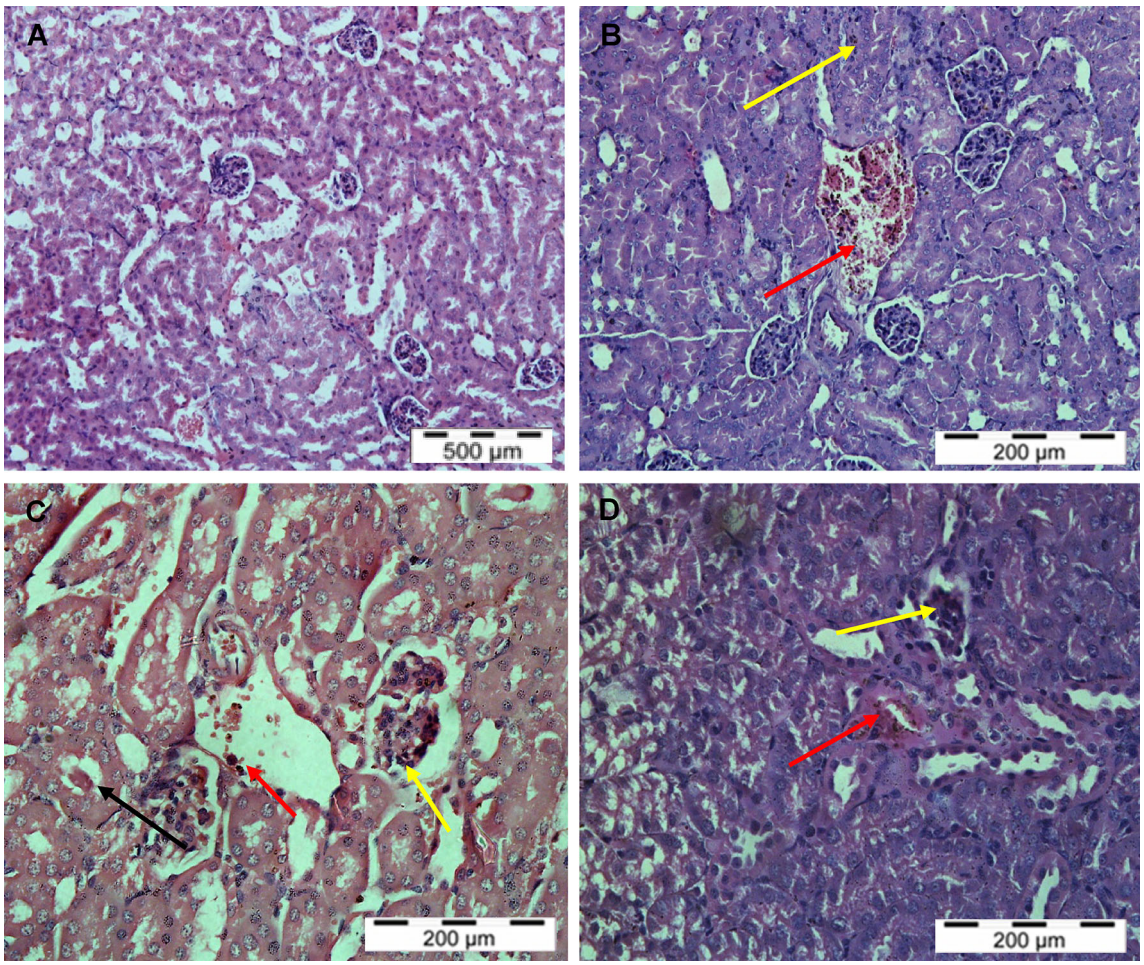

Fig. 8 Light microscopy of kidney tissue, cortex area in normal uninfected mice (N + NS) (**a**), malaria-infected mice treated with normal saline (M + NS, **b**), mTREM-1/Ab (M + mTREM-1/Ab, **c**) and rmTREM-1/Fc (M + mTREM-1/Fc, **d**). Microvascular congestion with PRBCs in cortex area (red arrow), hyaline cast in distal tubule (black arrow) and dark malarial pigment, hemozoin (yellow). Treatment with rmTREM-1/Fc (**d**) mostly improved the histopathological features in cortex area (H&E; n = 6; ×400) (color figure online)

et al. 2006). In this study, pathological features including hypertrophy, hyperplasia of kupffer cells with parasitized or unparasitized RBC, congestion of microvascular with packed red blood cells (PRBCs) and accumulation of haemozoin pigmentations were observed in the liver of all malaria-infected mice. All these symptoms mimic the symptoms found in fatal clinical case of human malaria infections (Baheti et al. 2003; Rupani and Amarapurkar 2009; Singh et al. 2010). Meanwhile, in the brain of malaria-infected mice, congestion of PRBCs accompanied by the accumulation of hemozoins were observed in the cerebral microvasculature. Cerebral microvasculature is characterized by the presence of brownish small spots around PRBCs in the microvasculature and this feature suggests that the sequestration of PRBCs were associated with cerebral malaria (Ponsford et al. 2011).

Malarial nephropathy is a life-threatening renal-related disorder caused by *Plasmodium* spp. (most commonly seen in *P. falciparum*) and is associated with cytoadherence and sequestration of PRBCs (Miller et al. 2002). Congestion of microvascular with PRBCs and appearance of hyaline casts within distal tubules in cortex areas and accumulations of malaria pigments hemozoins in both cortex and medulla areas were observed in malarial mice in this study. The findings in this study suggest that the sequestrations of PRBC's and cytoadherence (both in medulla and cortex areas) were involved in the malarial nephropathy of *P. berghei* in ICR mice.

Hemozoin accumulations are believed to exacerbate the cytoadherence through complex pro-inflammatory reactions, which would increase the obstruction of small vessel and reduced microvascular blood flow (ishaemic nephropathy) to the renal tissue (Dondorp 2005). Elias et al. (2012) also concluded that malarial nephropathy were caused by parasite adhesion and oxidative stress released from toxic heme from ruptured PRBCs during the

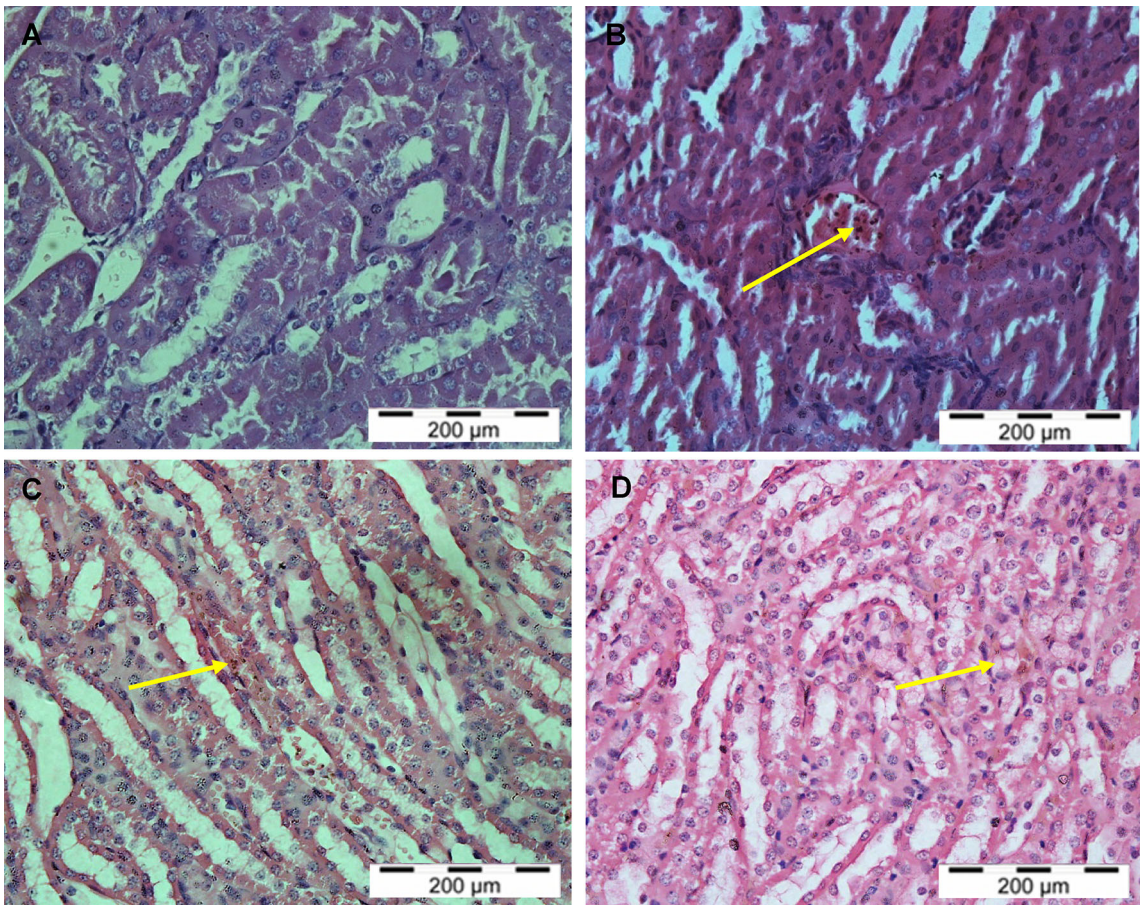

Fig. 9 Light microscopy of kidney tissue, medulla area in normal uninfected mice (N + NS) (**a**), malaria-infected mice treated with normal saline(M + NS, **b**), mTREM-1Ab (M + mTREM-1/Ab, **c**) and rmTREM-1/Fc (M + rmTREM-1/Fc, **d**). Dark malarial pigment, hemozoin (yellow arrow) were observed in all groups except in control uninfected mice. Treatment with rmTREM-1/Fc mostly improved the histopathological features in medulla area (H&E; n = 6; ×400) (color figure online)

infection. The sequestration of PRBCs also induces other severe malaria symptoms such as cerebral malaria, hyproglycemia and respiratory distress (Miller et al. 2002). Ischaemic nephropathy with combinations of dehydration, appearance of hyaline casts within distal tubules and black color urine due to increase destruction of RBC by malaria parasite (observed throughout the study) may suggest the infected mice suffered from Blackwater-like fever. Blackwater-like fever in animals also was possible as demonstrated by previous research (Rivera et al. 2013).

Spleen is crucial during malaria infection. It helps to filter out the altered red blood cells, particularly PRBCs. Spleen is usually the first organ to show morphological changes, as early as 2 days after the infection (Buffet et al. 2011). In this study, splenomegaly was observed in malaria-infected mice. Histologically, red and white pulp elements were enlarged accompanied by the loss of typical structure of germinal centre caused by the increase activity of macrophage to clear the malaria parasite in microenvironment of spleen. Sequestrations of PRBCs in the microvasculature, and accumulation of malarial pigments, hemozoins in the red pulp and sinusoidal lining areas were also observed in malaria-infected mice. Other than inducing the paroxysm as a malarial toxin, hemozoins also induce the macrophages to release cytokines, which would lead to imbalances of cytokines levels. These observations were overlapped with post-mortem study of malaria infection as demonstrated (Pongponratn et al. 1987; Urban et al. 1999).

In malaria-infected mice, the early sign of diffuse alveolar damage by hyaline membrane formation in the lung tissues could be an indication of acute respiratory distress syndrome (ARDS) (Valecha et al. 2009). Microvascular congestion with PRBC's along with accumulation of hemozoins with mononuclear cells were observed in alveolar capillaries and interstitium of lung tissues. This may suggest the involvement of adhesions (such as ICAM-1) and inflammatory markers in upregulating the sequestration of PRBCs around lung's microvasculatures (Lovegrove et al. 2008). Along with

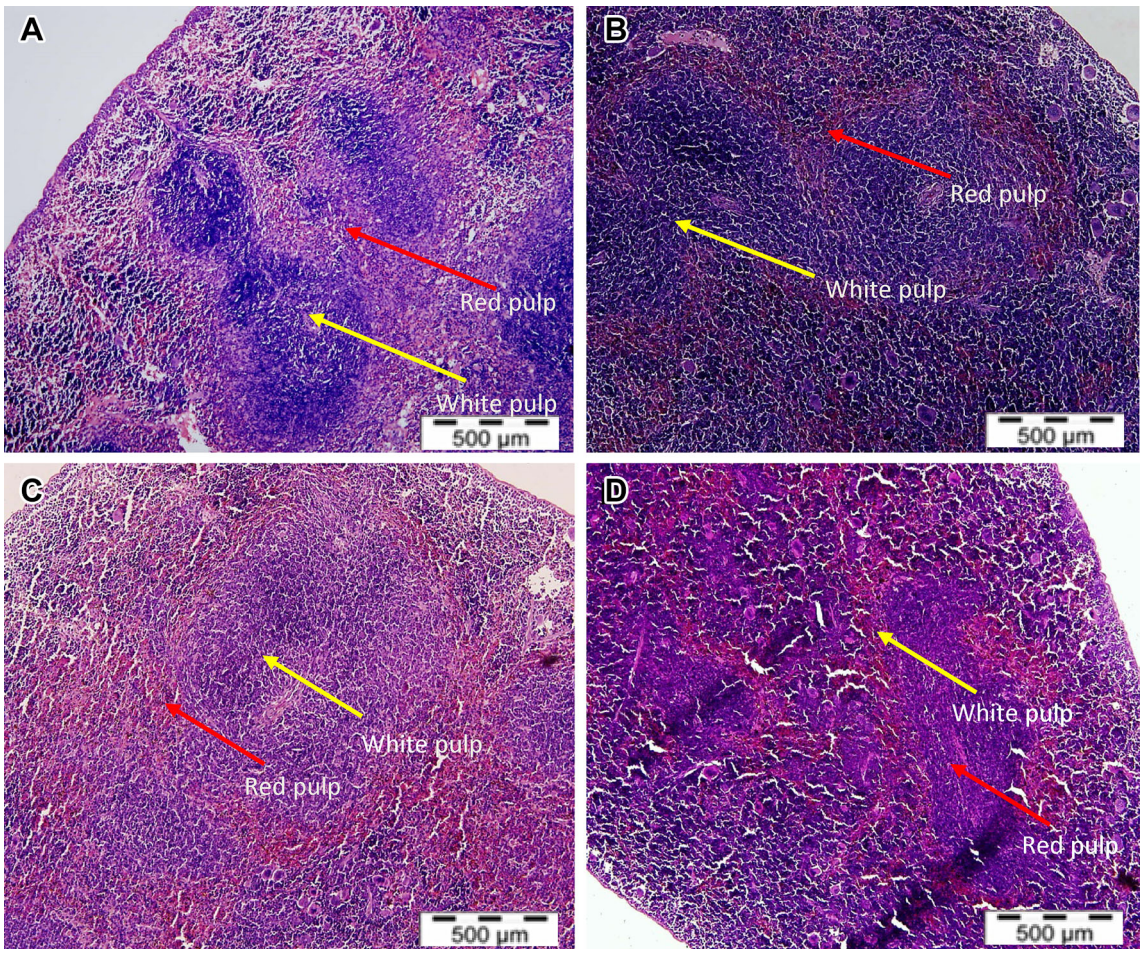

Fig. 10 Light microscopy of spleen tissue in normal uninfected mice (N + NS, **a**), malaria-infected mice treated with normal saline (M + NS, **b**), mTREM-1/Ab (M + mTREM-1/Ab, **c**) and rmTREM-1/Fc (M + rmTREM-1/Fc, **d**), spleen tissue. Red pulp (red arrow), white pulp (yellow arrow). Red and white pulp elements of M + NS (**b**), M + mTREM-1/Ab (**c**) and M + rmTREM-1/Fc (**d**) were enlarged accompanied by the loss of typical structure of germinal centre compared to normal mice N + NS (**a**) (H&E; n = 6; ×100) (color figure online)

metabolic acidosis, sequestration of PRBCs in the lung's microvasculatures will cause blockage of blood pathways. These combinations would eventually lead to clinical symptoms of respiratory distress such as tachypnea and labored breathing (Taylor et al. 2012). These histopathological observations in lung tissues were also in line with various case studies of respiratory distress in malaria infection (Valecha et al. 2009).

Hemozoin pigmentations were the common histopathological features among organs. The finding was consistent with previous clinical case study on *P.falciparum* (Baheti et al. 2003; Singh et al. 2010). Hemozoins are black pigmentation, which usually occurs as byproducts of haemoglobin metabolism by the parasites, and the appearances of these pigmentations in hepatocytes are caused by the ingestion of malaria sporozoites by Kuppfer cells, which give the black pigmentation to the organs. Malaria sporozoites eventually survive, replicate and metabolize within the Kuppfer cells (Pradel and Frevert 2001; Nobes et al. 2002). This explains on how malaria parasites escape the immune system, through specifically recognized and invade Kuppfer cells, avoid phagosomal acidification, and safely passage through these phagocytes and develop into exoerythrocytic schizonts (Pradel and Frevert 2001). Moreover, hemozoins are also responsible for severe malarial anemia (SMA) by triggering the bone marrow macrophages to release cytokines, chemokines and lipoperoxides, which further inhibit the erythropoiesis (Lamikanra et al. 2009).

This study also demonstrated the effects of modulating TREM-1 production on the histopathological changes of malaria-infected mice in five major organs (brain, liver, kidney, spleen and kidney). There was no previous study done on the effects of TREM-1 modulation on the major organs during malaria infection. These preliminary histopathological findings will perhaps provide new

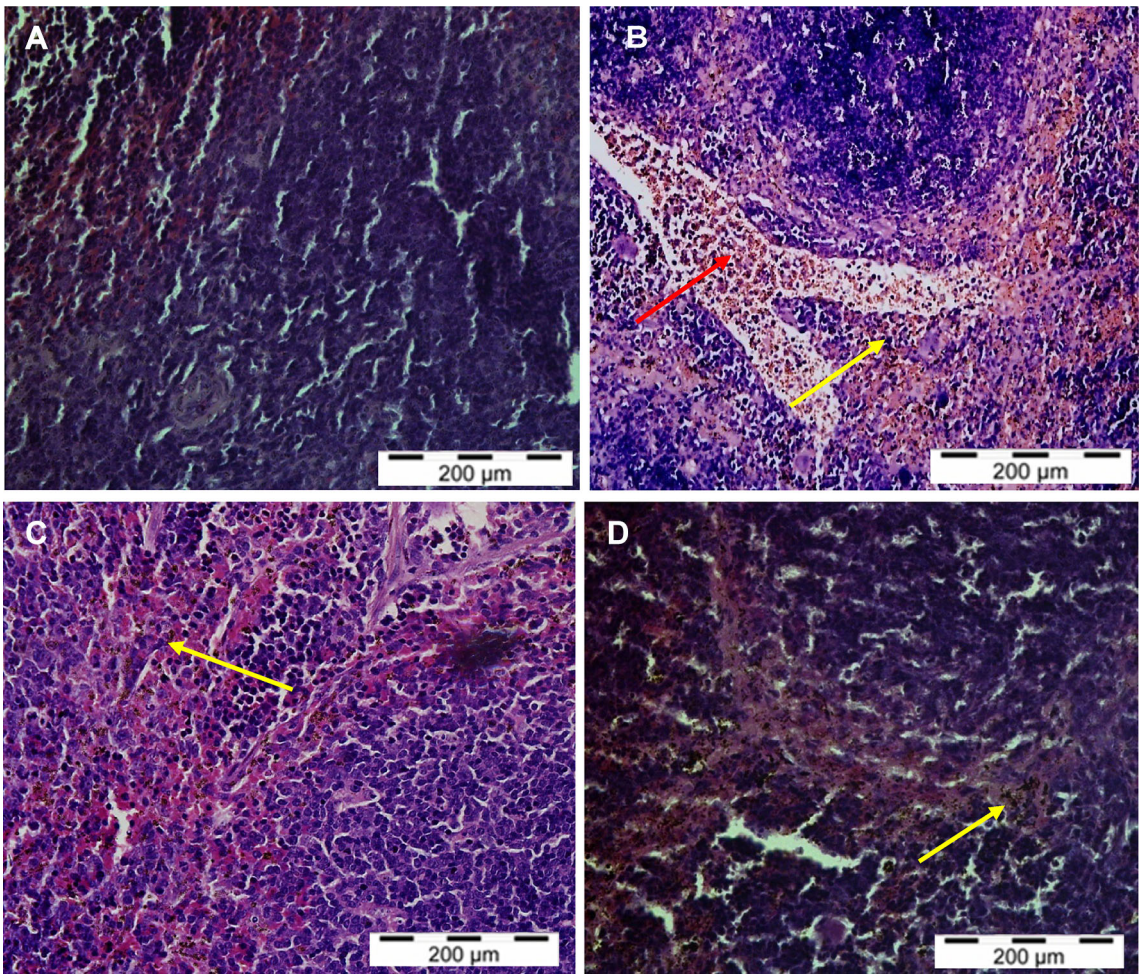

Fig. 11 Light microscopy of spleen tissue in normal uninfected mice (N + NS, **a**), malaria-infected mice treated with normal saline (M + NS, **b**), mTREM-1/Ab (M + mTREM-1/Ab, **c**) and rmTREM-1/Fc (M + rmTREM-1/Fc, **d**). No hemozoin and PRBC sequestration were observed in N + NS. The sequestration of PRBCs in the microvasculature was only observed in M + NS (red arrow). The accumulations of malarial pigments, hemozoin were observed in all groups of mice except control uninfected mice (**b**, **c**, **d**: yellow arrow). However this pathological feature was less significant in M + rmTREM-1/Fc (H&E; n = 6; ×400) (color figure online)

insights of TREM-1 in mediating immune response at the cellular level. Our study revealed that, especially by blocking the TREM-1 production using rmTREM-1/Fc, all malaria-infected organs showed significant improvement on overall histopathological conditions. These include (1) sequestration of microvessels and accumulation of hemozoins (observed in all major organs), (2) hypertrophy and hyperplasia of Kuppfer cells (liver), (3) appearance of hyaline cast in distal tubules (kidney), (4) hyaline membrane formations (lung) and (5) enlargement of red and white pulp (spleen). Meanwhile, treatment of malarial mice with mTREM-1/Ab shown minimal or no improvements on the pathology of the organs.

Overall, this study has some limitations. We did not perform any neurological study on the malaria-infected mice as the neurological syndrome is usually observed between 5–14 days post infection where all our mice in this study succumbed to death at day 6 post infection (Lou et al. 2001; Carroll et al. 2010). Although this study showed that treatment with TREM-1 decreases some pro-inflammatory cytokines production and alleviates the pathological changes in the organs studied, the mice treated with TREM-1 related drugs in our study still died on day 6 post infection, indicating that the decrease in pro-inflammatory cytokines production and alleviation of pathological changes in the infected organs were not sufficient to prolong the survival of the malaria-infected mice. However, we believe that adjusting the dosage for TREM-1 treatments during malaria infection might improve the integrity of the result in this study as the dosage for TREM-1 related drugs employed in this study were adapted from Bouchon et al. (2001a, b). Additionally, the optimal capabilities of TREM-1 in down-regulating the key inflammatory networks associated with fatality such as seizures, coma and

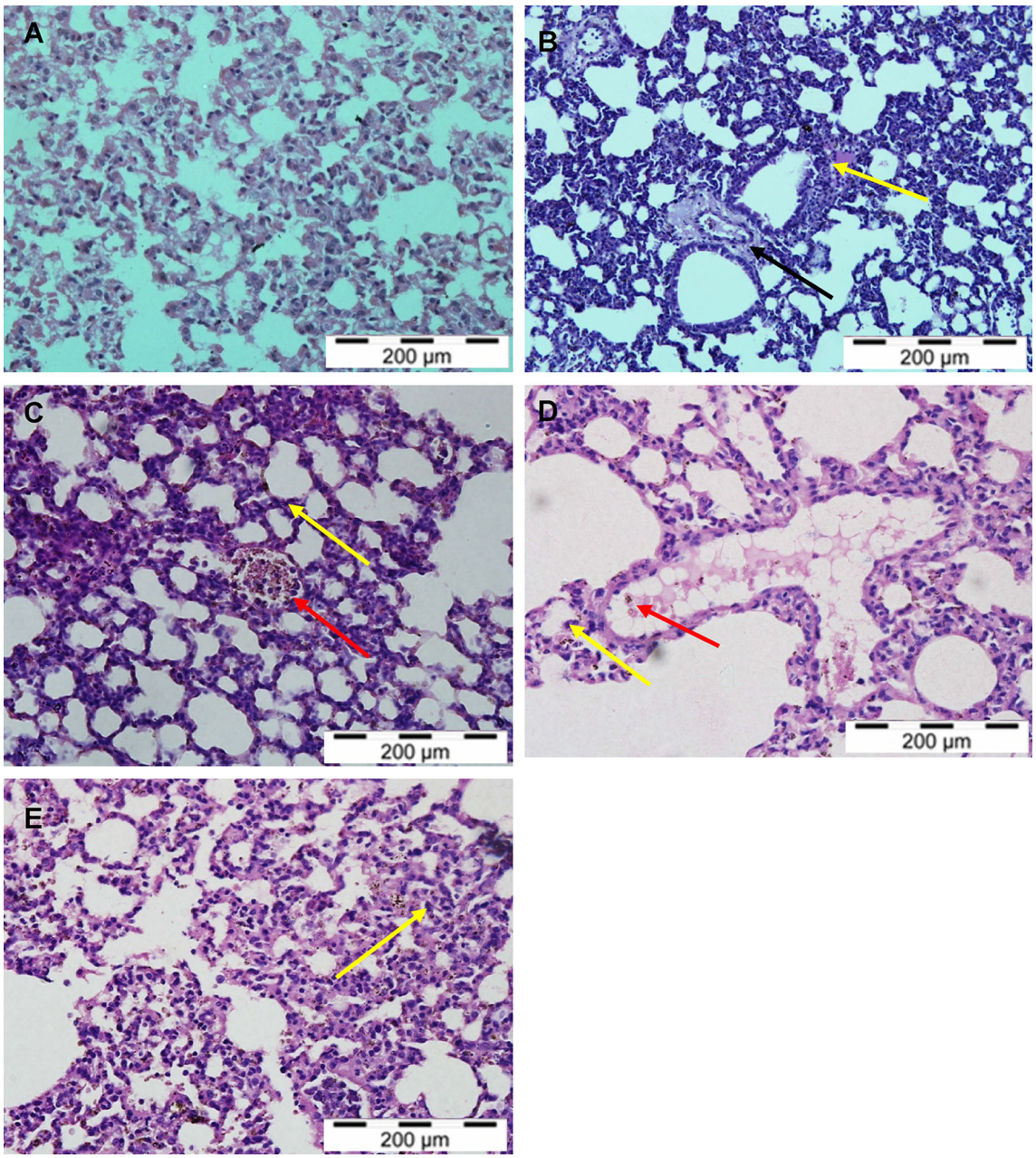

Fig. 12 Hematoxylin-eosin (H&E) staining of lung tissue in normal uninfected mice (N + NS, **a**), malaria-infected mice treated with normal saline (M + NS, **b**), mTREM-1/Ab (M + mTREM-1/Ab, **c**) and rmTREM-1/Fc (M + rmTREM-1/Fc, **d**). Hyaline membrane formations due to alveolar haemorrhage (black arrow) were observed in malaria-infected mice (M + NS; **b**). All malaria-infected mice were also tainted with malaria pigments, haemozoins, microvascular congestion with PRBCs (H&E; n = 6; ×400)

cerebral malaria must be extensively studied in different approaches.

Conclusion

This study highlights the involvement of TREM-1 during malaria infection. TREM-1 is believed to play important pro-inflammatory roles during malaria infection and may be one of the key mediator of the disease's severity. Modulation of TREM-1 released during malaria infection produces a better disease outcome in the context of cytokines release and histopathological improvements. Further investigations are warranted to evaluate TREM-1 as a potential immunotherapeutic target for malaria and there is a need to gather more information especially on finding the right dosage and duration of the treatment so that the full potential of TREM-1 can be discovered.

Acknowledgements We would like to acknowledge Universiti Putra Malaysia, Malaysia and Ministry of Education, Malaysia under Fundamental Research Grant Scheme (FRGS) (04-11-08-629FR) for providing financial and infrastructure support for the conduction of the research study.

Author contributions AAMY conducted the experiments and analyzed the data in this study. CVK analyzed the data, wrote and revised the manuscript. RB conceptualized the idea and experimental design in this study. RB, WOA, CPP, NN, ZOI and RAM provide technical supports in the experiments and revised the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Achtman AH, Pilat S, Law CW, Lynn DJ, Janot L, Mayer ML, Ma S, Kindrachuk J, Finlay BB, Brinkman FS, Smyth GK (2012) Effective adjunctive therapy by an innate defense regulatory peptide in a preclinical model of severe malaria. Sci Transl Med 4(135):135ra64

Adukpo S, Gyan BA, Ofori MF, Dodoo D, Velavan TP, Meyer CG (2016) Triggering receptor expressed on myeloid cells 1 (TREM-1) and cytokine gene variants in complicated and uncomplicated malaria. Trop Med Int Health 21(12):1592–1601

Baheti R, Laddha P, Gehlot RS (2003) Liver involvement in falciparum malaria—a histo-pathological analysis. J Indian Acad Clin Med 4(1):34–38

Basir R, Rahiman SF, Hasballah K, Chong WC, Talib H, Yam MF, Jabbarzare M, Tie TH, Othman F, Moklas MA, Abdullah WO (2012) Plasmodium berghei ANKA infection in ICR mice as a model of cerebral malaria. Iran J Parasitol 7(4):62

Bouchon A, Dietrich J, Colonna M (2000) Inflammatory responses can be triggered by TREM-1, a novel receptor expressed on neutrophils and monocytes. J Immunol 164:4991–4995

Bouchon A, Facchetti F, Weigand MA, Colonna M (2001a) TREM-1 amplifies inflammation and is a crucial mediator of septic shock. Nature 410:1103–1107

Bouchon A, Hernández-Munain C, Cella M, Colonna M (2001b) A DAP12-mediated pathway regulates expression of CC chemokine receptor 7 and maturation of human dendritic cells. J Exp Med 194(8):1111–1122

Buffet PA, Safeukui I, Deplaine G, Brousse V, Prendki V, Thellier M, Turner GD, Mercereau-Puijalon O (2011) The pathogenesis of *Plasmodium falciparum* malaria in humans: insights from splenic physiology. Blood 117(2):381–392

Carroll RW, Wainwright MS, Kim KY, Kidambi T, Gomez ND, Taylor T, Haldar K (2010) A rapid murine coma and behavior scale for quantitative assessment of murine cerebral malaria. PloS One 5(10):e13124

Chimma P, Roussilhon C, Sratongno P, Ruangveerayuth R, Pattanapanyasat K, Pérignon JL, Roberts DJ, Druilhe P (2009) A distinct peripheral blood monocyte phenotype is associated with parasite inhibitory activity in acute uncomplicated *Plasmodium falciparum* malaria. PLoS Pathog 5(10):e1000631

Clark IA, Schofield L (2000) Pathogenesis of malaria. Parasitol Today 16(10):451–454

Dodoo D, Omer FM, Todd J, Akanmori BD, Koram KA, Riley EM (2002) Absolute levels and ratios of proinflammatory and anti-inflammatory cytokine production in vitro predict clinical immunity to *Plasmodium falciparum* malaria. J Infect Dis 185(7):971–979

Dondorp AM (2005) Pathophysiology, clinical presentation and treatment of cerebral malaria. Neurol Asia 10:67–77

Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, Lwin KM, Ariey F, Hanpithakpong W, Lee SJ, Ringwald P (2009) Artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med 361(5):455–467

Dower K, Ellis DK, Saraf K, Jelinsky SA, Lin LL (2008) Innate immune responses to TREM-1 activation: overlap, divergence, and positive and negative cross-talk with bacterial lipopolysaccharide. J Immunol 180:3520–3534

Elias RM, Correa-Costa M, Barreto CR, Silva RC, Hayashida CY, Castoldi A, Goncalves GM, Braga TT, Barboza R, Rios FJ, Keller AC (2012) Oxidative stress and modification of renal vascular permeability are associated with acute kidney injury during *P. berghei* ANKA infection. PLoS ONE 7(8):e44004

Erdman LK, Dhabangi A, Musoke C, Conroy AL, Hawkes M, Higgins S, Rajwans N, Wolofsky KT, Streiner DL, Liles WC, Cserti-Gazdewich CM (2011) Combinations of host biomarkers predict mortality among Ugandan children with severe malaria: a retrospective case-control study. PLoS ONE 6(2):e17440

Ford JW, McVicar DW (2009) TREM and TREM-like receptors in inflammation and disease. Curr Opin Immunol 21(1):38–46

Gibot S, Kolopp-Sarda MN, Béné MC, Bollaert PE, Lozniewski A, Mory F, Levy B, Faure GC (2004) A soluble form of the triggering receptor expressed on myeloid cells-1 modulates the inflammatory response in murine sepsis. J Exp Med 200(11):1419–1426

Gibot S, Buonsanti C, Massin F, Romano M, Kolopp-Sarda MN, Benigni F, Faure GC, Béné MC, Panina-Bordignon P, Passini N, Lévy B (2006) Modulation of the triggering receptor expressed on the myeloid cell type 1 pathway in murine septic shock. Infect Immun 74(5):2823–2830

Horst SA, Linnér A, Beineke A, Lehne S, Höltje C, Hecht A, Norrby-Teglund A, Medina E, Goldmann O (2013) Prognostic value and therapeutic potential of TREM-1 in *Streptococcus pyogenes*-induced sepsis. J Innate Immun 5(6):581–590

Jason J, Archibald LK, Nwanyanwu OC, Bell M, Buchanan I, Larned J, Kazembe PN, Dobbie H, Parekh B, Byrd MG, Eick A (2001) Cytokines and malaria parasitemia. Clin Immunol 100(2):208–218

Lamikanra AA, Theron M, Kooij TW, Roberts DJ (2009) Hemozoin (malarial pigment) directly promotes apoptosis of erythroid precursors. PLoS ONE 4(12):e8446

Lou J, Lucas R, Grau GE (2001) Pathogenesis of cerebral malaria: recent experimental data and possible applications for humans. Clin Microbiol Rev 14(4):810–820

Lovegrove FE, Gharib SA, Peña-Castillo L, Patel SN, Ruzinski JT, Hughes TR, Liles WC, Kain KC (2008) Parasite burden and CD36-mediated sequestration are determinants of acute lung injury in an experimental malaria model. PLoS Pathog 4(5):e1000068

Malaguarnera L, Musumeci S (2002) The immune response to *Plasmodium falciparum* malaria. Lancet Infect Dis 2(8):472–478

Miller LH, Baruch DI, Marsh K, Doumbo OK (2002) The pathogenic basis of malaria. Nature 415(6872):673

Nobes MS, Ghabrial H, Simms KM, Smallwood RA, Morgan DJ, Sewell RB (2002) Hepatic Kupffer cell phagocytotic function in rats with erythrocytic-stage malaria. J Gastroenterol Hepatol 17(5):598–605

Poe JC, Wagner DH, Miller RW, Stout RD, Suttles J (1997) IL-4 and IL-10 modulation of CD40-mediated signaling of monocyte IL-1beta synthesis and rescue from apoptosis. J Immunol 159(2):846–852

Pongponratn E, Riganti M, Bunnag D, Harinasuta T (1987) Spleen in falciparum malaria: ultrastructural study. Southeast Asian J Trop Med Public Health 18(4):491–501

Ponsford MJ, Medana IM, Prapansilp P, Hien TT, Lee SJ, Dondorp AM, Esiri MM, Day NP, White NJ, Turner GD (2011) Sequestration and microvascular congestion are associated with coma in human cerebral malaria. J Infect Dis 205(4):663–671

Pradel G, Frevert U (2001) Malaria sporozoites actively enter and pass through rat Kupffer cells prior to hepatocyte invasion. Hepatology 33(5):1154–1165

Rivera N, Romero SE, Menchaca Á, Zepeda A, García LE, Salas G, Romero L, Malagón F (2013) Blackwater fever like in murine malaria. Parasitol Res 112(3):1021–1029

Rupani AB, Amarapurkar AD (2009) Hepatic changes in fatal malaria: an emerging problem. Ann Trop Med Parasitol 103(2):119–127

Singh R, Kaur M, Arora DA (2010) Prospective study of hepatic involvement in *Plasmodium falciparum* malaria. J Clin Diagn Res 4(2):2190–2197

Sturm A, Amino R, Van de Sand C, Regen T, Retzlaff S, Rennenberg A, Krueger A, Pollok JM, Menard R, Heussler VT (2006) Manipulation of host hepatocytes by the malaria parasite for delivery into liver sinusoids. Science 313(5791):1287–1290

Tammaro A, Derive M, Gibot S, Leemans JC, Florquin S, Dessing MC (2017) TREM-1 and its potential ligands in non-infectious diseases: from biology to clinical perspectives. Pharmacol Ther 177:81–95

Taylor WR, Hanson J, Turner GD, White NJ, Dondorp AM (2012) Respiratory manifestations of malaria. Chest 142(2):492–505

Urban BC, Ferguson DJ, Pain A, Willcox N, Plebanski M, Austyn JM, Roberts DJ (1999) *Plasmodium falciparum*-infected erythrocytes modulate the maturation of dendritic cells. Nature 400(6739):73

Valecha N, Pinto RG, Turner GD, Kumar A, Rodrigues S, Dubhashi NG, Rodrigues E, Banaulikar SS, Singh R, Dash AP, Baird JK (2009) Histopathology of fatal respiratory distress caused by *Plasmodium vivax* malaria. Am J Trop Med Hyg 81(5):758–762

Wang F, Liu S, Wu S, Zhu Q, Ou G, Liu C, Wang Y, Liao Y, Sun Z (2012) Blocking TREM-1 signaling prolongs survival of mice with *Pseudomonas aeruginosa* induced sepsis. Cell Immunol 272(2):251–258

Warrell DA, Gilles HM (2002) Essential malariology, 4th edn. Arnold, London, pp 8–20

WHO (2016) World malaria report 2016. World Health Organization, Geneva

Yang C, Chen B, Zhao J, Lin L, Han L, Pan S, Fu L, Jin M, Chen H, Zhang A (2015) TREM-1 signaling promotes host defense during the early stage of infection with the highly pathogenic *Streptococcus suis*. Infect Immun 83:3293–3301