Original Article

Asian Pacific Journal of Tropical Biomedicine

apjtb.org

doi: 10.4103/2221−1691.391157

Impact Factor® 1.7

Interleukin−33 exerts pleiotropic immunoregulatory effects in response to *Plasmodium berghei* ANKA (PbA) infection in mice

Mohammad Faruq Abd Rachman Isnadi[1,8], Rusliza Basir[1]✉, Ramatu Bello Omenesa[1,2], Roslaini Abd Majid[3], Maizaton Atmadini Abdullah[4], Che Norma Mat Taib[1], Sivan Padma Priya[5], Yong Yean Kong[6], Chin Voon Kin[7], Gambo Lawal Mukhtar[1,9]

[1]*Department of Human Anatomy, Faculty of Medicine & Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia*

[2]*Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna, Nigeria*

[3]*Faculty of Medicine and Defence Health, National Defence University of Malaysia, Kem Sungai Besi, Kuala Lumpur, Malaysia*

[4]*Department of Pathology, Faculty of Medicine & Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia*

[5]*RAK College of Dental Sciences, Ras Al Khaimah Medical and Health Sciences University, Ras Al Khaimah, United Arab Emirates*

[6]*Laboratory Centre, Xiamen University Malaysia, Sepang, Selangor, Malaysia*

[7]*Segi University, Faculty of Medicine, Nursing and Health Sciences, 9, Jalan Tecknologi, PJu 5 Kota Damansara, Petaling, Selangor, Malaysia*

[8]*Department of Pathobiology and Medical Diagnostics, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Malaysia*

[9]*Department of Microbiology, Faculty of Natural & Applied Sciences, Umaru Musa Yar' adua University, Katsina, Nigeria*

ABSTRACT

Objective: To determine the involvement and the modulatory effects of IL-33 during *Plasmodium berghei* ANKA (PbA) infection.

Methods: PbA infection in male ICR mice was utilized as a model of malaria. Systemically circulating IL-33 levels were determined in blood plasma by enzyme-linked immunosorbent assay (ELISA). After 24 hours post-inoculation of PbA, recombinant IL-33 and ST2, and antibodies against IL-33 and IgG treatments were administered daily for 3 days. Tissue expression and localization of IL-33 were assessed in organs generally affected by malaria *via* immunohistochemistry. Moreover, histopathological examination was performed to assess the effects of the treatments.

Results: The levels of systemic IL-33 were elevated at the critical phase of PbA infection. Likewise, immunohistochemical analysis revealed a significant upregulation of IL-33 expression at the critical phase in the brain, lungs, and spleen of PbA-infected mice as compared to healthy controls. Treatment with IL-33 protected against experimental cerebral malaria development and reduced pathological features in the brain and lungs of the PbA-infected mice.

Conclusions: A potential critical role and involvement of IL-33 in PbA infection may hint at the resolution of immunopathological sequelae associated with malaria.

KEYWORDS: *Plasmodium berghei* ANKA; Malaria; IL-33; Immunology; Immunotherapy

Significance

IL-33 has been described as a pleiotropic cytokine and its role has been extensively studied in many inflammatory-associated disorders. However, the potential involvement and modulation of its activity in malaria is not well elucidated. The present study reveals the involvement and critical role of IL-33 in *Plasmodium berghei* ANKA (PbA) infection. Moreover, modulation of IL-33 release demonstrated pleiotropic effects as an immune-modulating cytokine, preventing the occurrence of cerebral pathology during the critical phase of PbA infection.

✉To whom correspondence may be addressed. E-mail: rusliza@upm.edu.my

How to cite this article: Abdul Rachman Isnadi MF, Basir R, Omenesa RB, Abd Majid R, Abdullah MA, Mat Taib CN, et al. Interleukin-33 exerts pleiotropic immunoregulatory effects in response to *Plasmodium berghei* ANKA (PbA) infection in mice. Asian Pac J Trop Biomed 2023; 13(12): 521-531.

Article history: Received 21 August 2023; Revision 7 September 2023; Accepted 10 December 2023; Available online 28 December 2023

1. Introduction

Malaria is a vector-borne disease widely distributed in tropical and sub-tropical regions worldwide[1]. Persistent infection with malaria typically carries considerable morbidity and mortality and exerts significant economic pressures in endemic regions[2]. It is among the deadliest diseases in the world, accounting for 627 000 fatalities globally in 2020 alone[3]. From the latest World Malaria report, there was an increase in malaria cases from 245 million in 2020 to 247 million in 2021[2,4]. The *Plasmodium berghei* ANKA (PbA) infection is a well-known experimental model for malaria[5]. PbA can cause an infection in mice similar to cerebral malaria in humans. The pathogenesis of PbA infection involves the development of high parasitemia accompanied by symptoms of severe anemia and enlargement of vital organs such as the spleen and liver. Additionally, recent research has shown that PbA infection can lead to overproduction of pro- and anti-inflammatory cytokines[6].

Decades of research have substantiated the role of cytokines as biomarkers and immune mediators that drive inflammatory cascades during the development of malaria. Atypical cytokine signaling in malaria has been associated with uncontrolled inflammation accompanied by deleterious consequences to the affected host[7].

Interleukin-33 (IL-33) is a member of the IL-1 cytokine superfamily[8]. It possesses multifarious roles as a classical extracellular cytokine or an intracellular nuclear factor and gene expression regulator[9], although the latter role perhaps has been debunked[10,11]. Depending upon the pathway of disease etiology, IL-33 demonstrates the capacity to function either as a pro-inflammatory or an anti-inflammatory cytokine[9]. Extracellular IL-33 has been described as an 'alarmin' cytokine *via* an endogenous danger signal as well as a ligand for the ST2-based receptors involved in the activation of responses towards tissue damage, trauma, stress or infection[12,13]. Meanwhile, intracellular IL-33 was thought to regulate gene expression and promote the transcription of multiple cytokines and chemokines[9,13] before the transcriptional role of the cytokine was most likely invalidated in more recent studies[10,11].

IL-33 plays prominent roles in the induction and effector phase of type 2 immune responses as opposed to other IL-1 family members[8,14]. Moreover, it renders protection against extracellular parasitic or helminth infections[15-17] and plays clinically significant roles in the pathogenesis of Th2-associated autoimmune diseases such as asthma[18,19] and atopic dermatitis[20,21]. Beneficial immunotherapeutic roles have been ascribed to IL-33 in Alzheimer's disease[22,23] and myocardial infarction[24,25]. Intriguingly, only a few studies report the effects of modulating IL-33 during malaria. IL-33 was demonstrated either to confer protection[26-28] or detrimental effects[29-32] for the experimental cerebral malaria (ECM) development and incriminated on promoting the production of pro-inflammatory cytokines during malaria[29,30] or vice versa[27]. Therefore, in this study, the time course of IL-33 secretion either systemically in the blood circulation or localized in the tissue, in addition to the effects of modulating IL-33 on the histopathological level during PbA infection, was investigated.

2. Materials and methods

2.1. Experimental animals

Male ICR mice initially weighing between 17-20 g were utilized in this study. All mice were procured from an authorized local supplier (Takrif Bistari Enterprise, Malaysia) and housed at the Animal House Facility, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia in standard polypropylene cages with free access to food and water and subjected to 12 hours of light-dark cycles.

2.2. Ethical statement

All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of University Putra Malaysia (Approval No: AUP: R038/2016) in accordance with the Animal Welfare Act 2015 (Laws of Malaysia). All efforts were made to minimize the discomfort, suffering, and number of animals utilized.

2.3. PbA infection

Plasmodium berghei parasite strain ANKA was originally acquired from the Institute of Medical Research, Kuala Lumpur, Malaysia, and subsequently maintained *via* alternation between *in vivo* passage in male ICR mice and cryo-preservation *ex vivo* at –80 ℃. A model of severe PbA infection was established *via* intraperitoneal inoculation of 200 μL of 2×10^7 parasitized blood into normal healthy ICR male mice. Following infection, mice were grouped based on the onset (day 1), middle (day 3), and critical (day 5) phase post *Plasmodium berghei* inoculation. The endpoint of day 5 was selected due to the high mortality rate (70%) of infected ICR mice recorded during the animal model's establishment (Figure 1). Parasitemia count was assessed daily as described by Basir *et al*.[33]. Parasitemia levels of ≥ 40% were uniformly utilized for each of the experimental protocols.

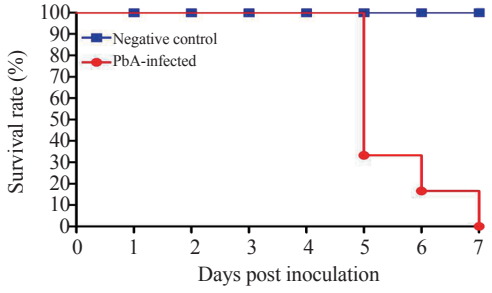

Figure 1. Survival graph after PbA infection. Survival graph analyzed from an animal model study and establishment of infection prior to the study was conducted. On day 5 of post-injection with *Plasmodium berghei* ANKA (PbA), 70% mortality rate with 30% survival rate was recorded, followed by 80% of mortality rate with 20% survival rate on day 6 and 100% mortality rate on day 7 compared to the negative control group.

2.4. Determination of IL–33 in the systemic circulation

Sample preparation and IL-33 measurement by enzyme-linked immunosorbent assay were performed according to the manufacturer's protocol. Blood samples for preparation of plasma from control and PbA-infected mice were collected from anesthetized mice *via* cardiac puncture (n = 24). The plasma was obtained by centrifugation of the blood samples at 2000 ×g for 20 min and stored at –80 ℃ before analysis. The concentration of IL-33 was measured using Quantikine® ELISA Mouse IL-33 Immunoassay (R&D Systems, USA). Plates were read at a wavelength of 450 nm with the aid of a microplate reader (VersaMax® Molecular Devices, China).

2.5. Tissue localization of IL–33 by immunohistochemistry

Organ samples consisting of the brain, heart, liver, lungs, spleen, and kidneys were collected from each anesthetized mouse with isoflurane (n = 6). Organ samples were fixed with 10% neutral buffered formalin and embedded with paraffin wax after 48 h. Tissue sections of 4 μm thickness at 40 μm intervals were obtained through a rotary microtome on Superfrost Plus adhesion microscope slides (Thermo, Germany). The sections were subsequently deparaffinized in a microwave oven set at 60 ℃ for 60 min, rehydrated in a series of graded alcohol concentrations, and depigmented with 10% ammonium hydroxide. Antigen retrieval was performed *via* microwave heating of the sections in sodium citrate buffer (10 mM sodium citrate, 0.05% Tween-20, pH 6.0) for 10 min, followed by immunohistochemistry staining with Anti-Goat HRP-DAB Cell & Tissue Staining Kit (R&D Systems, USA) according to the manufacturer's protocol.

2.6. Modulation of IL–33 release in control and PbA–infected mice

PbA-infected mice comprised of the following groups: recombinant IL-33 (rIL-33)-treated group, neutralizing IL-33 (nIL-33)-treated group, recombinant suppression of tumorigenicity 2 (rST2)-treated group, IgG antibody (IgGab)-treated group and phosphate-buffered saline (PBS)-treated group. Meanwhile, non-infected mice were comprised of IgGab treated mice and PBS-treated mice. Treatment was comprised of recombinant Mouse IL-33 Protein (rIL-33, R&D Systems) administered at a dose of 20 μg/mouse[34], Goat Anti-Mouse IL-33 Antigen Affinity-Purified Polyclonal Antibody or neutralizing IL-33 (nIL-33, R&D Systems) administered at a dose of 40 μg/mouse[35], recombinant Mouse ST2/IL-33R Fc Chimera Protein (rST2, R&D Systems) administered at a dose of 20 μg/mouse[35], Mouse IgG antibody (R&D Systems) of 100 μg/mouse[36] and 0.1 mM of PBS (Sigma-Aldrich). All treatments were intraperitoneally administered consecutively for 3 d in a total volume of 200 μL per treatment after 24 hours post-inoculation of PbA. Parasitemia progression was recorded daily post-PbA parasite inoculation before organ and sample collection on day 5 (120 hours post-inoculation).

2.7. Histological analysis

Organ samples were fixed with 10% formalin and embedded with paraffin wax after 72 hours of fixation. Tissue sections of 4 μm thickness were deparaffinized in a microwave oven set at 60 ℃ for 60 min before haematoxylin and eosin staining. Haematoxylin and eosin staining was performed following standard staining protocol. Arbitrary scales for the physical signs of illness, histopathological features, and post-mortem observations were observed and recorded according to methods described by Basir *et al.*[33] while histological scores were performed according to methods described by Fazalul Rahiman *et al.*[37].

2.8. Statistical analysis

Data were expressed as mean ± standard error of mean (SEM). The concentration of systemic IL-33 and its expression level in the brain and during parasitemia development were analyzed with one-way ANOVA and Tukey's multiple comparisons.

3. Results

3.1. Systemic release of IL–33 during PbA infection

A gradual elevation of plasma IL-33 levels was observed in PbA-infected mice from day 1 [(24.80 ± 5.52) pg/mL] to day 3 [(37.13 ± 7.11) pg/mL] and day 5 [(52.85 ± 14.16) pg/mL] of infection, unlike the negative control mice in which significantly lower and stable plasma IL-33 levels [(16.66 ± 2.59) pg/mL] remained unaltered throughout PbA infection (Figure 2). There was no significant difference observed in the levels of plasma IL-33 (P > 0.05) between PbA-infected mice [(24.80 ± 5.52) pg/mL] and the uninfected control mice [(16.66 ± 2.58) pg/mL] on day 1. However, on day 3 and day 5 of infection, a significant elevation of plasma IL-33 concentration was recorded (P < 0.05) in PbA-infected mice as compared to their respective control counterparts on similar days. Additional *post hoc* tests revealed significant differences (P < 0.01) in plasma IL-33 concentration between groups of PbA-infected mice on day 1 and day 3 or day 5 post-infection.

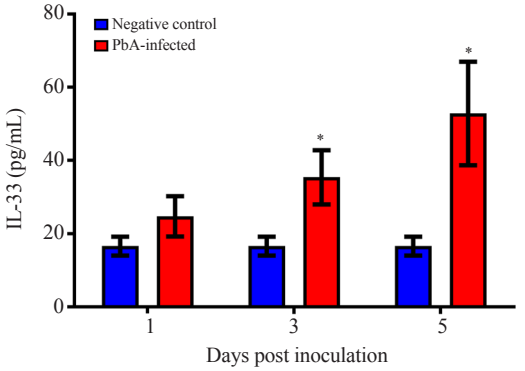

Figure 2. Release of extracellular IL-33 in PbA-infected mice. ICR male mice were infected *i.p.* with PbA (≥ 40% parasitized red blood cells). Results are expressed as mean ± SEM (n = 6). $^*P$ < 0.05 compared to the negative control group.

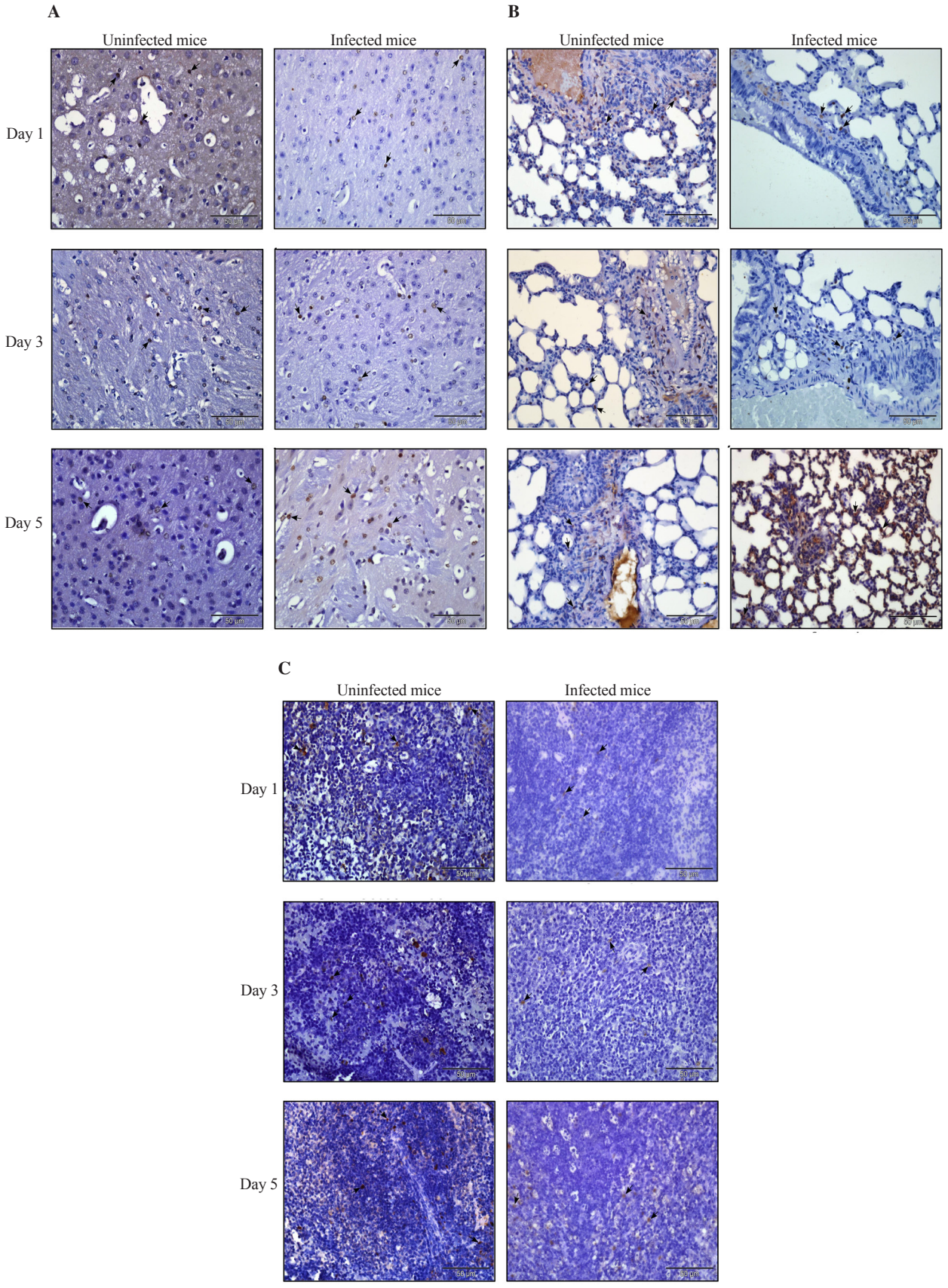

Figure 3. Expression of IL-33 in the (A) brain (in the nucleus of the glial cells), (B) lungs (in the nucleus and cytoplasm of the alveolar epithelial cells), and (C) spleen (in the nucleus and cytoplasm of the spleen lymphocytes) of PbA-infected mice, compared with negative control mice. IL-33 expression (black arrow) was determined by immunohistochemical staining ($n = 6$). All images were acquired at 400× magnification.

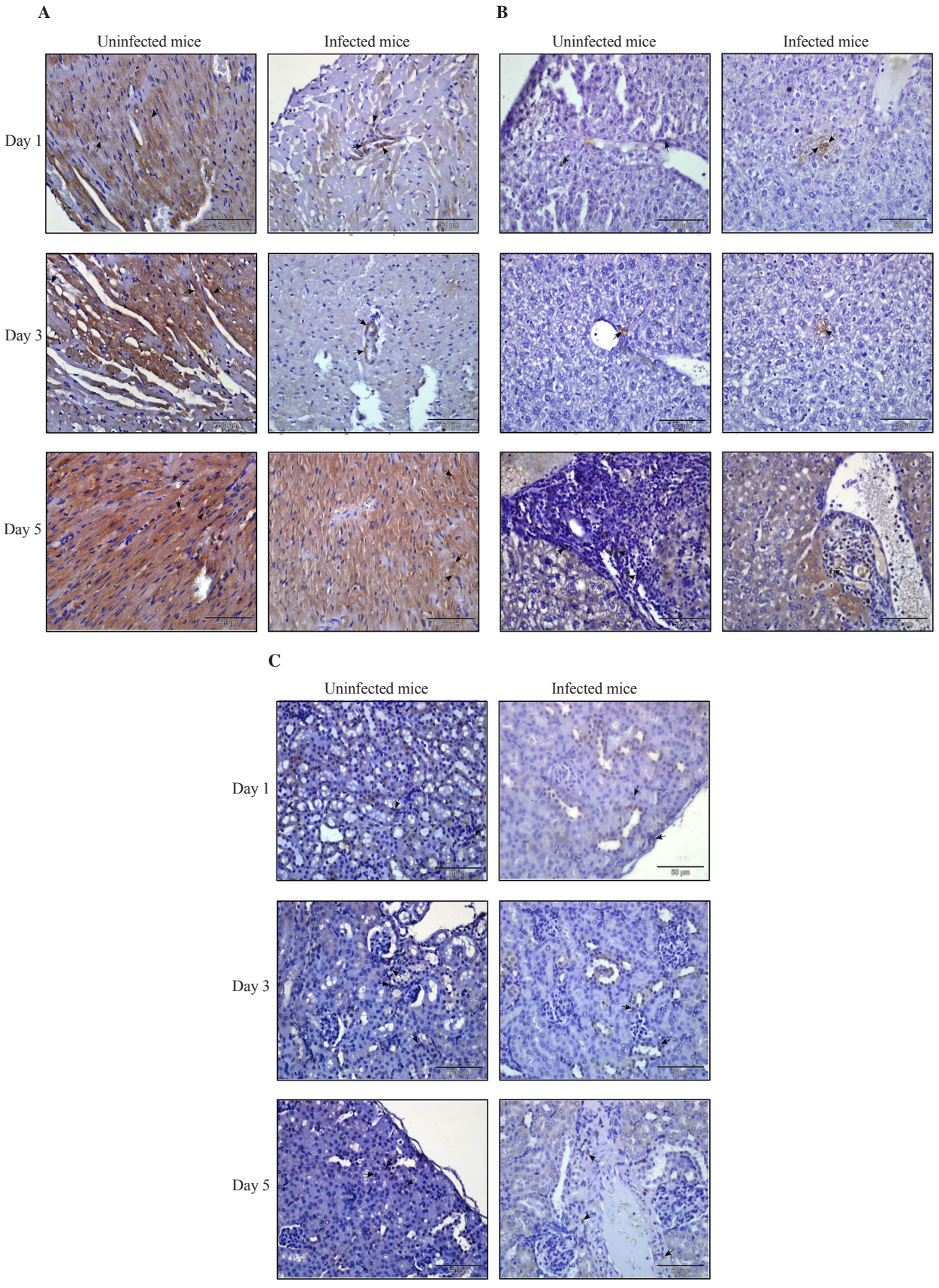

Figure 4. Constitutive expression of IL-33 in the (A) heart (the nuclei and cytoplasm of cardiac myocytes and endothelial cells), (B) liver (the nuclei and cytoplasm of hepatocytes and endothelial cells) and (C) kidney (primarily localized in the endothelial and epithelial cells of the kidney) of both malaria and negative control mice. IL-33 expression (black arrow) was determined by immunohistochemical staining (n = 6). All images were acquired at 400× magnification.

3.2. Local expression of IL-33 in major organs

Extensive distributions of densely stained IL-33$^+$ cells were observed in the brain, lungs, and spleen of PbA-infected mice, compared with negative control mice. Immunoreactivity for IL-33$^+$ was predominantly localized in the nucleus of endothelial and epithelial cells in all organs sampled from PbA-infected mice excluding epithelial cells of the spleen. In comparison to negative control mice, the frequency and intensity of IL-33$^+$ cells in brain sections from PbA-infected mice increased significantly ($P < 0.05$) from day 1 to day 5 of infection. In PbA-infected mouse brain, immunoreactivity for IL-33 was primarily expressed by glial cells in addition to the nucleus of neurons in the white matter and cerebral cortex (Figure 3A).

IL-33 expression was apparent in alveolar epithelial cells of lung sections from PbA-infected mice. Similarly, stain intensity appeared to increase progressively in the lungs of PbA-infected mice as infection progressed from day 1 to day 5. Unlike PbA-infected mice, negative control mice demonstrated mild constitutive expression of IL-33$^+$ immunopositive cells in the lungs (Figure 3B). The expression of IL-33 in the spleen was predominantly localized in lymphocytes and increased as PbA infection progressed (Figure 3C). However, IL-33 expression was barely observed in sections of the spleen from negative control mice. The constitutive expression of IL-33 was observed in the heart (Figure 4A), liver (Figure 4B), and kidney (Figure 4C) of both malaria and negative control mice.

3.3. Effects of modulating the release of IL-33 on parasitemia development during PbA infection

Administration of rIL-33 to PbA-infected mice was associated with a delay in parasitemia patency between the first and second day of infection (Figure 5). Mean parasitemia percentages of 1.23% and 7.42% were recorded for PbA-infected mice that received rIL-33 protein on day 1 and day 2, respectively as compared to mean parasitemia percentages monitored on similar days in nIL-33-treated mice (4.44% and 7.98%), rST2-treated mice (1.67% and 9.10%), IgG-treated mice (5.46% and 10.20%), and PBS treated mice (3.56% and 12.61%), respectively (Figure 5). Overall, daily estimates of parasitemia progression [% parasitized red blood cells (PRBC) increment per day] across groups of PbA-infected mice revealed delayed parasitemia development in mice that received rIL-33 protein [(14.68 ± 11.20)%/day] in comparison to sustained parasitemia progression observed in PBS-treated mice [(14.73 ± 11.37)%/day] and IgG treated mice [(16.14 ± 12.44)%/day]. Comparatively, daily estimates of parasitemia progression in mice that received PBS or IgG treatment were higher than those in mice treated with either nIL-33 [(12.67 ± 10.70)%/day] or rST2 [(13.31 ± 9.95)%/day]. Significantly different rates of parasitemia development ($P < 0.05$) were observed on day 4 of infection between the rST2 and PBS-treated mice.

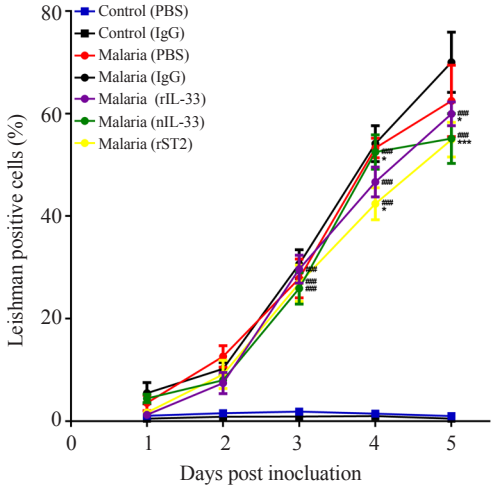

Figure 5. Effects of rIL-33, nIL-33, and rST2 treatments on the development of parasitemia. Parasitemia was measured based on the percentage of Leishman positive cells. Results are mean ± SEM ($n = 6$). $^{*}P < 0.05$, $^{***}P < 0.001$ compared to the PbA-infected group. $^{###}P < 0.001$ compared with the negative control group treated with PBS. PBS: phosphate-buffered saline; rIL-33: recombinant IL-33; nIL-33: neutralizing IL-33; rST2: recombinant ST2.

3.4. Effects of modulating the release of IL-33 on histopathological changes during PbA infection

After the modulation of IL-33 by administration of rIL-33, nIL-33, rST2, and IgG to PbA-infected mice, macroscopic observations of post-mortem tissues were shown in Supplementary Table. Macroscopically, all organs showed typical morphology (discoloration and enlargement) generally associated with PbA-infected tissues, however, microscopic examination of sections from harvested organs revealed marginal presence of pathological features associated with PbA infection in sections from the brain, lung, liver, and spleen of PbA-infected mice treated with rIL-33 protein compared to other treatment groups (Figures 6-9).

4. Discussion

Plasma IL-33 concentration was highly elevated during the critical phase of PbA infection (day 5) compared to the previous phases of infection (days 1 and 3); this observation is similar to severe falciparum malaria in humans[38]. Moreover, plasma levels of IL-33 were correlated positively and significantly with parasitemia development, hence supporting the presumption that IL-33 is involved and significantly activated during the development of malaria possibly as an endogenous 'alarmin' crucial to the innate immune response to malaria[13,38].

A study by Palomo *et al*. reported undetectable levels of IL-33 in serum from both PbA-infected and naïve mice, however, elevated levels of the cytokine (IL-33) were found following the development

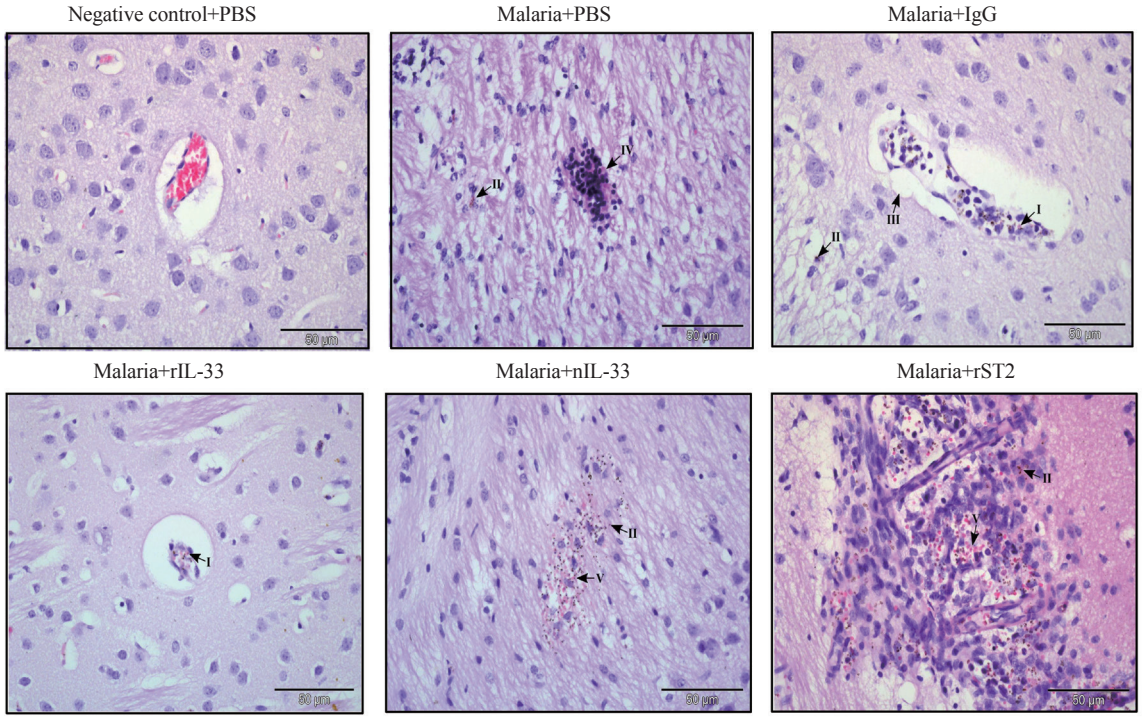

Figure 6. Effects of rIL-33, nIL-33, and rST2 treatments on the brain. All brain sections were stained with H&E (n = 6). Histopathological features (indicated with black arrow) are (Ⅰ) parasitized red blood cells, hemozoin-laden macrophages, perivascular activated macrophages, and intravascular lymphocytes inside the blood vessels, (Ⅱ) hemozoin deposition on the cerebral tissue, (Ⅲ) perivascular space enlargement, (Ⅳ) brain parenchymal hemorrhage with lymphocytic infiltration and (Ⅴ) petechial hemorrhage. Images were acquired at 400× magnification.

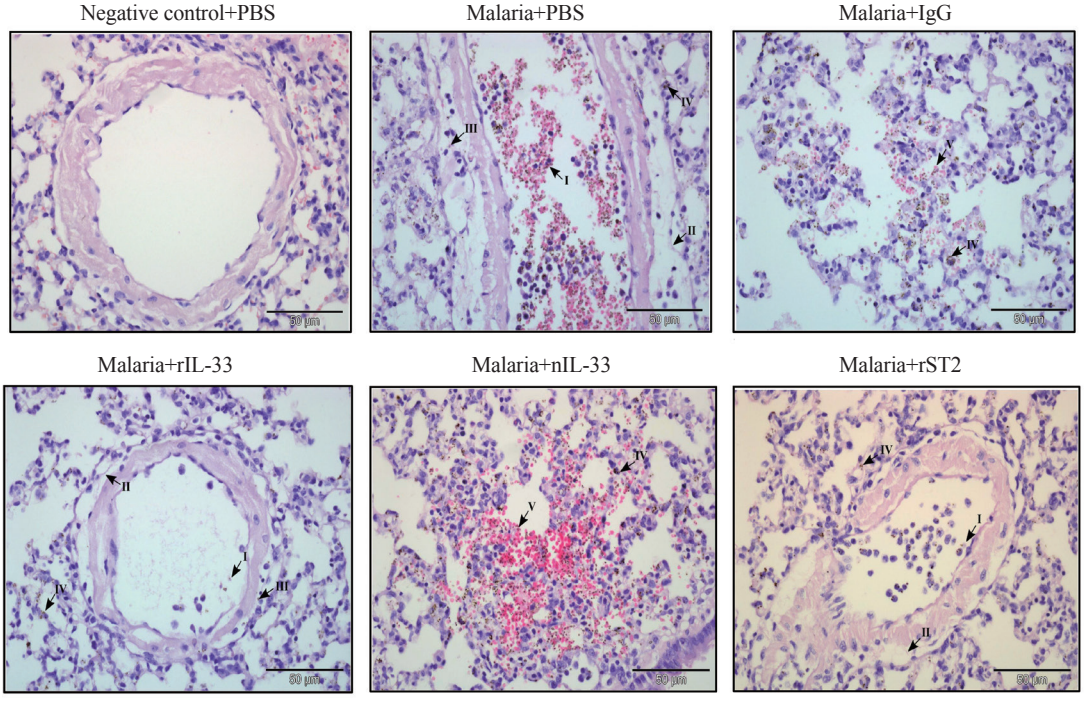

Figure 7. Effects of rIL-33, nIL-33, and rST2 treatments on the lungs. All lung sections were stained with H&E (n = 6). Histopathological features (indicated with black arrow) are (Ⅰ) parasitized red blood cells, hemozoin-laden macrophages, and lymphocytes, (Ⅱ) hyalinized membrane, (Ⅲ) hemozoin and parasitized red blood cells within a hyalinized membrane, (Ⅳ) hemozoin deposition in alveolar tissues and (Ⅴ) alveolar hemorrhage. Images were acquired at 400× magnification.

of malaria in tissue homogenates of the brain, lung, and spleen from infected subjects[32]. Aside from endothelial and epithelial cells[39], a plethora of leukocytes such as macrophages have demonstrated high-affinity binding capacity to extracellular IL-33, as well as

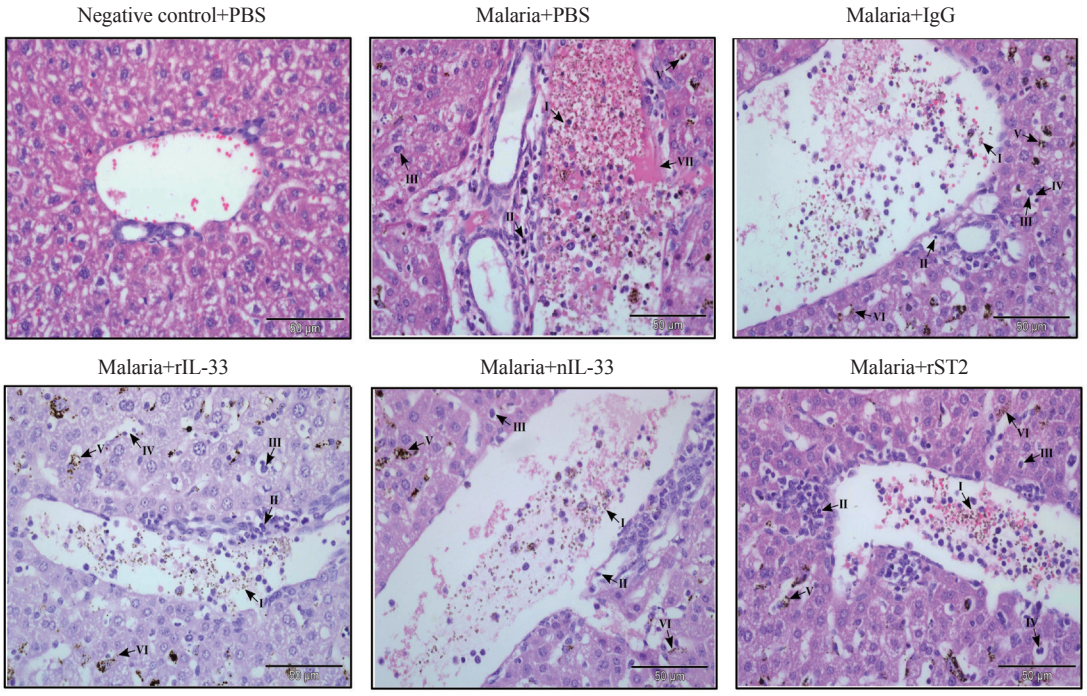

Figure 8. Effects of rIL-33, nIL-33, and rST2 treatments on the liver. All liver sections were stained with H&E (n = 6). Histopathological features (indicated with black arrow) are (Ⅰ) parasitized red blood cells, hemozoin-laden macrophages, and lymphocytes inside the blood vessels in the liver, (Ⅱ) infiltration of inflammatory cells towards hepatic vessel, (Ⅲ) hypertrophy and (Ⅳ) hyperplasia of Kupffer cells, (Ⅴ) hemozoin deposition in the sinusoid and (Ⅵ) hepatic cords and (Ⅶ) liver parenchymal hemorrhage with fibrin deposition. Images were acquired at 400× magnification.

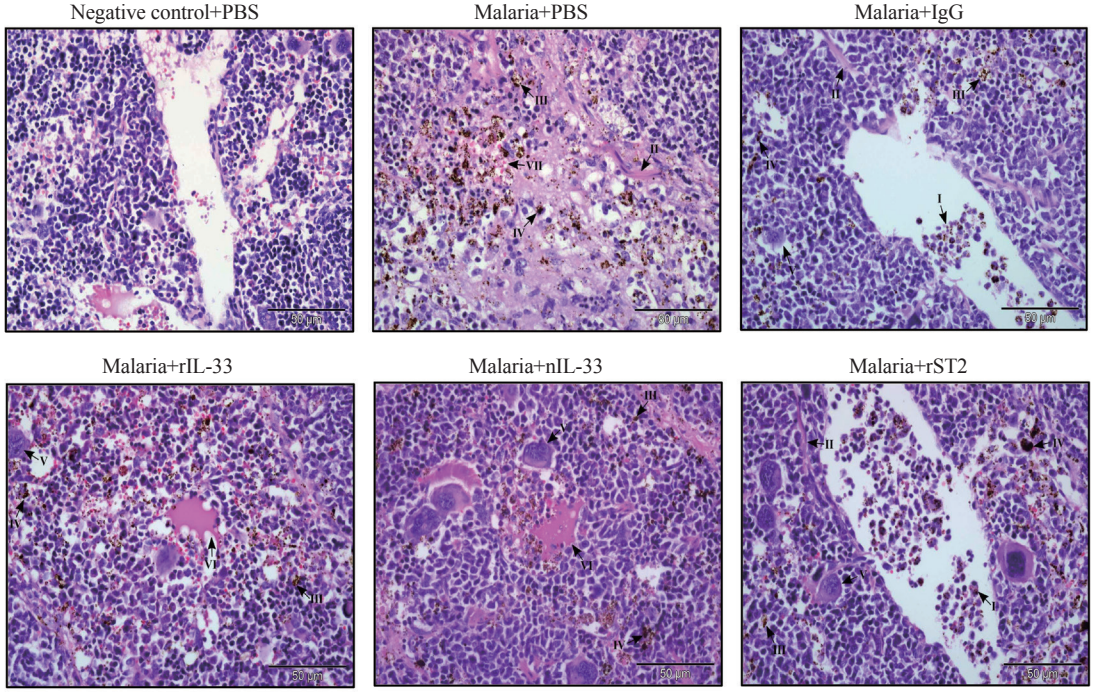

Figure 9. Effects of rIL-33, nIL-33, and rST2 treatments on the spleen. All spleen sections were stained with H&E (n = 6). Histopathological features (indicated with black arrow) are (Ⅰ) parasitized red blood cells, hemozoin-laden macrophages, and lymphocytes, (Ⅱ) thickened trabeculae, hemozoin deposition in (Ⅲ) splenic sinuses, and (Ⅳ) cords, (Ⅴ) multinucleated giant cells, (Ⅵ) fibrin deposition and (Ⅶ) splenic hemorrhage. Images were acquired at 400× magnification.

production of the cytokine during necrosis[38]. We thus inferred that the observed upregulation of systemic IL-33 levels in PbA-infected mice likely originated from the aforementioned cellular sources (leukocytes and/or macrophages) in response to danger-associated molecular patterns released upon the progression of malaria.

Immunohistochemistry revealed significant expression of IL-33 in the brain of PbA-infected mice during the critical phase (day 5) of malaria. This observation corroborates previous reports describing

the expression of IL-33 in the brain during experimental cerebral malaria[30,32,40].

The expression of IL-33 visibly increased as PbA infection progressed from the onset phase to the critical phase of infection. A progressive increase in the intensity and expanse of immunoreactivity for IL-33 was also noticeable in the lungs and spleen of PbA-infected mice compared to negative control mice. Similarly, a study reported increased IL-33 expression in the lungs during severe malaria associated with pulmonary edema[31] and in a model of allergic airway inflammation[41]. Furthermore, previous reports on elevated IL-33 protein[32] and mRNA[40] levels corroborate the observation of dense IL-33 expression that was observed in the spleen of PbA-infected mice. Unlike the brain, lungs, and spleen of PbA-infected mice, IL-33 was only marginally expressed and the levels of expression were unaltered throughout PbA infection in the heart, liver, and kidneys of the infected mice. The expression of IL-33 in sections from the heart of PbA-infected mice was similar to the constitutive expression of IL-33 in cardiac cells observed in certain studies during necrosis[42].

A delay in parasitemia progression was evident at the onset phase of PbA infection in mice that received rIL-33 treatment. However, similar to a study by Besnard *et al*.[27], parasitemia progression towards the critical phase (day 5) of infection was unremarkable in rIL-33-treated mice and assumed a similar pace of development with the ensuing hyperparasitemia bore striking similarity to parasitemia levels in infected mice that were treated with PBS. These observations on parasitemia progression between nIL-33 and PBS-treated mice were similar to previous reports by Shibui *et al*.[40], where the parasitemia development between IL-33 deficient (IL-33$^{-/-}$) mice and their wild type counterparts of either BALB/c or C57BL/6 background was comparable in a model of PbA infection.

Overall, pathological features typically associated with malaria such as PRBC sequestration, perivascular space enlargement, and hemozoin deposition if present were profoundly reduced in rIL-33 treated mice compared with their neutralizing antibody-treated counterparts that sustained a normal incidence of malaria pathology. Moreover, the absence of both petechial and parenchymal hemorrhage in the brain of the rIL-33-treated mice, but not in the nIL-33 and rST2-treated mice, indicated that treatment with rIL-33 conferred protection against brain pathology and concomitant ECM development. Similar findings have been demonstrated by Besnard *et al*.[27] and Strangward *et al*.[28]. Additionally, an earlier study documented similar rates of PRBC sequestration in the brain of PbA-infected, ST2$^{-/-}$ mice, and their wild type counterparts[27].

Minimal histopathological changes were observed in the lungs of rIL-33-treated mice including the absence of PRBC sequestration and reduction of hemozoin deposition. However, the presence of inflammatory cells was observed in lung tissue from certain rIL-33-treated mice. Our findings on the effects of IL-33 in the lungs of PbA-infected subjects were corroborated by Schmitz *et al*.[8].

Comparable to observations in the lungs, inflammatory cells were similarly visible albeit to a lesser degree in the liver, spleen, and kidneys of certain PbA-infected mice that received rIL-33. A study by Clark *et al*.[15] demonstrated a certain degree of cellular infiltrations and decreased necrotic foci in the livers of IL-33-treated mice infected with *Toxoplasma gondii*. Remarkably, while differences were observed in histopathological features of harvested organs between rIL-33-treated and other PbA-infected mice, variations in the extent of splenic pathology between treatment groups were negligible. Therefore, we surmised that the varying outcomes of IL-33 modulation observed in PbA-infected mice subsequent to rIL-33 treatment are dependent upon the pathway of disease etiology varying from infectious diseases to autoimmune disorders or oncogenic processes. Even though lesser pathological changes were observed in the kidney of the rIL-33-treated mice, features such as acute tubular necrosis and partial necrosis of glomeruli were still present. The inhibition of IL-33 signaling in the kidney through sST2 treatment perhaps reduced the development of acute tubular necrosis as well as acute renal injury as demonstrated by Akcay *et al*.[43]. Moreover, the progression of chronic kidney disease has been correlated with the increase of both IL-33 and ST2 levels[44].

We observed increased frequencies of macrophages in the spleen of rIL-33-treated mice, this is consistent with reports on the involvement of IL-33 in the spleen by Besnard *et al*.[27] and Schmitz *et al*.[8] who demonstrated increased macrophage population and polarization to an M2 phenotype during PbA infection. Macrophages are of crucial importance in arresting parasite development and restoration of tissue damage following pathological tissue remodeling in malaria.

Based on the histological examination of organs critically affected by malaria, IL-33 appeared to play both protective and damaging roles in PbA-infected mice. We surmised that IL-33 treatment produced ambiguous effects and its actions depend upon the context of disease etiology.

Overall, we demonstrated that IL-33 is significantly upregulated in serum and tissues of malaria subjects possibly due to increased transcription of IL-33 mRNA in response to PbA infection. IL-33, documented as 'alarmin' cytokine[13], released as a consequence of damage to the mechanical barriers of epithelial and endothelial cells[10], was highly expressed and correlated significantly with parasitemia development during critical phases of PbA infection. Administration of rIL-33 to PbA-infected mice was found to exhibit ambiguous effects. Howbeit the incidence of organ-related malaria pathology with concomitant protection of rIL-33-treated mice from ECM is suggestive of the possible action of IL-33 *via* various cell types and pathways which is not explored in this study. Furthermore, this study is mostly limited to the quantitative assessment of the effects of IL-33, anti-IL-33, and its decoy receptor ST2 in critical organs while IL-33 expression at the cellular level was not quantitatively measured. In conclusion, modulation of IL-33 releases demonstrated pleiotropic effects during the onset of malaria and as an immune-modulating cytokine could prevent the occurrence of

cerebral pathology during the critical phase of PbA infection.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgments

We would like to convey our gratitude to the Institutional Animal Care and Use Committee of Universiti Putra Malaysia for the animal handling facility provided by the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, and Malaysia Ministry of Higher Education for the research funding under the Fundamental Research Grant Scheme.

Funding

The research was supported by the Fundamental Research Grant Scheme (FRGS) from the Malaysia Ministry of Higher Education (FRGS/1/2016/SKK10/UPM/02/1).

Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

MFARI conceptualized the work, carried out literature search, experimental studies and data acquisition, and wrote the original draft. RB conceptualized the work, sought for funding, supervised the project and edited the manuscript. RBO, RAM, MAA, CNMT, SPP, YYK, and CVK reviewed and edited the manuscript. MGL made critical revision and contributed to the final version of the manuscript.

References

[1] Escalante AA, Pacheco MA. Malaria molecular epidemiology: An evolutionary genetics perspective. *Microbiology Spectrum* 2019; **7**(4). doi: 10.1128/microbiolspec.ame-0010-2019.

[2] World Health Organization. World malaria report 2022. [Online] Available from: https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2022 [accessed on 31 October 2023].

[3] Muhammad AB, Nor Azman E, Eddie NA, Azmi NI, Yee VCT, Idris ZM. The rise of *Plasmodium knowlesi* cases: Implication to Malaysia's malaria-free status. *Asian Pac J Trop Med* 2022; **15**(8): 337-338.

[4] Guo SY, Sai WB, Li M. Malaria vaccine development: State of the art and beyond. *One Health Bull* 2021; **1**(1): 12-16. doi: 10.4103/2773-0344.329028.

[5] Olatunde AC, Cornwall DH, Roedel M, Lamb TJ. Mouse models for unravelling immunology of blood stage malaria. *Vaccines (Basel)* 2022; **10**(9): 1525. doi: 10.3390/vaccines10091525.

[6] Junaid QO, Khaw LT, Mahmud R, Ong KC, Lau YL, Borade PU, et al. Pathogenesis of *Plasmodium berghei* ANKA infection in the gerbil (*Meriones unguiculatus*) as an experimental model for severe malaria. *Parasite* 2017; **24**: 38. doi: 10.1051/parasite/2017040.

[7] Pohl K, Cockburn IA. Innate immunity to malaria: The good, the bad and the unknown. *Front Immunol* 2022; **13**. doi: 10.3389/fimmu.2022.914598.

[8] Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals *via* the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 2005; **23**(5): 479-490. doi: 10.1016/j.immuni.2005.09.015.

[9] Guo H, Bossila EA, Ma X, Zhao C, Zhao Y. Dual immune regulatory roles of Interleukin-33 in pathological conditions. *Cells* 2022; **11**(20): 3237. doi: 10.3390/cells11203237.

[10] Cayrol C. IL-33, an alarmin of the IL-1 family involved in allergic and non allergic inflammation: Focus on the mechanisms of regulation of its activity. *Cells* 2022; **11**(1): 107. doi: 10.3390/cells11010107.

[11] He Z, Chen L, Furtado GC, Lira SA. Interleukin 33 regulates gene expression in intestinal epithelial cells independently of its nuclear localization. *Cytokine* 2018; **111**: 146-153. doi: 10.1016/j.cyto.2018.08.009.

[12] Cayrol C, Girard JP. Interleukin-33 (IL-33): A nuclear cytokine from the IL-1 family. *Immunol Rev* 2018; **281**(1): 154-168. doi: 10.1111/imr.12619.

[13] Oboki K, Ohno T, Kajiwara N, Arae K, Morita H, Ishii A, et al. IL-33 is a crucial amplifier of innate rather than acquired immunity. *Proc Natl Acad Sci U S A* 2010; **107**(43): 18581-18586. doi: 10.1073/pnas.1003059107.

[14] Vasanthakumar A, Kallies A. Interleukin (IL)-33 and the IL-1 family of cytokines-regulators of inflammation and tissue homeostasis. *Cold Spring Harb Perspect Biol* 2019; **11**(3). doi: 10.1101/cshperspect.a028506.

[15] Clark JT, Christian DA, Gullicksrud JA, Perry JA, Park J, Jacquet M, et al. IL-33 promotes innate lymphoid cell-dependent IFN-γ production required for innate immunity to *Toxoplasma gondii*. *Elife* 2021; **10**. doi: 10.7554/eLife.65614.

[16] Hung LY, Tanaka Y, Herbine K, Pastore C, Singh B, Ferguson A, et al. Cellular context of IL-33 expression dictates impact on anti-helminth immunity. *Sci Immunol* 2020; **5**(53). doi: 10.1126/sciimmunol.abc6259.

[17] Mahlakõiv T, Flamar AL, Johnston LK, Moriyama S, Putzel GG, Bryce PJ, et al. Stromal cells maintain immune cell homeostasis in adipose tissue *via* production of interleukin-33. *Sci Immunol* 2019; **4**(35). doi: 10.1126/sciimmunol.aax0416.

[18] Curren B, Ahmed T, Howard DR, Ashik Ullah M, Sebina I, Rashid RB, et al. IL-33-induced neutrophilic inflammation and NETosis underlie rhinovirus-triggered exacerbations of asthma. *Mucosal Immunol* 2023; **16**(5): 671-684. doi: 10.1016/j.mucimm.2023.07.002.

[19] van der Ploeg EK, Krabbendam L, Vroman H, van Nimwegen M, de

Bruijn MJW, de Boer GM, et al. Type-2 $CD8^+$ T-cell formation relies on interleukin-33 and is linked to asthma exacerbations. *Nat Commun* 2023; **14**(1): 5137. doi: 10.1038/s41467-023-40820-x.

[20]Hou T, Sun X, Zhu J, Hon KL, Jiang P, Chu IM, et al. IL-37 ameliorating allergic inflammation in atopic dermatitis through regulating microbiota and AMPK-mTOR signaling pathway-modulated autophagy mechanism. *Front Immunol* 2020; **11**. doi: 10.3389/fimmu.2020.00752.

[21]Guttman-Yassky E, Diaz A, Pavel AB, Fernandes M, Lefferdink R, Erickson T, et al. Use of tape strips to detect immune and barrier abnormalities in the skin of children with early-onset atopic dermatitis. *JAMA Dermatol* 2019; **155**(12): 1358-1370.

[22]Liang CS, Su KP, Tsai CL, Lee JT, Chu CS, Yeh TC, et al. The role of interleukin-33 in patients with mild cognitive impairment and Alzheimer's disease. *Alzheimers Res Ther* 2020; **12**(1): 86. doi: 10.1186/s13195-020-00652-z.

[23]Saresella M, Marventano I, Piancone F, La Rose F, Galimberti D, Fenoglio C, et al. IL-33 and its decoy sST2 in patients with Alzheimer's disease and mild cognitive impairment. *J Neuroinflammation* 2020; **17**(1): 174.

[24]Chen Y, Zuo J, Chen W, Yang Z, Zhang Y, Hua F, et al. The enhanced effect and underlying mechanisms of mesenchymal stem cells with IL-33 overexpression on myocardial infarction. *Stem Cell Res Ther* 2019; **10**(1): 295.

[25]Li, J, Shen D, Tang, J, Wang, Y, Wang B, Xiao Y, et al. IL33 attenuates ventricular remodeling after myocardial infarction through inducing alternatively activated macrophages ethical standards statement. *Eur J Pharmacol* 2019; **854**: 307-319.

[26]Fernander EM, Adogamhe P, Datta D, Bond C, Zhao Y, Bangirana P, et al. Elevated plasma soluble ST2 levels are associated with neuronal injury and neurocognitive impairment in children with cerebral malaria. *Pathog Immun* 2022; **7**(1): 60-80.

[27]Besnard AG, Guabiraba R, Niedbala W, Palomo J, Reverchon F, Shaw TN, et al. IL-33-mediated protection against experimental cerebral malaria is linked to induction of type 2 innate lymphoid cells, M2 macrophages and regulatory T cells. *PLoS Pathog* 2015; **11**. doi: 10.1371/journal.ppat.1004607.

[28]Strangward P, Haley MJ, Albornoz MG, Barrington J, Shaw T, Dookie R, et al. Targeting the IL33-NLRP3 axis improves therapy for experimental cerebral malaria. *Proc Natl Acad Sci U S A* 2018; **115**: 7404-7409.

[29]Seki T, Obata-Ninomiya K, Shimogawara-Furushima R, Arai T, Akao N, Hoshino T, et al. IL-33/ST2 contributes to severe symptoms in *Plasmodium chabaudi*-infected BALB/c mice. *Parasitol Int* 2018; **67**: 64-69.

[30]Reverchon F, Mortaud S, Sivoyon M, Maillet I, Laugeray A, Palomo J, et al. IL-33 receptor ST2 regulates the cognitive impairments associated with experimental cerebral malaria. *PLoS Pathog* 2017; **13**. doi: 10.1371/journal.ppat.1006322.

[31]Ampawong S, Chaisri U, Viriyavejakul P, Prapansilp P, Grau GE, Turner GDH, et al. A potential role for interleukin-33 and γ-epithelium sodium channel in the pathogenesis of human malaria associated lung injury. *Malar J* 2015; **14**: 1-15.

[32]Palomo J, Reverchon F, Piotet J, Besnard A, Couturier-Maillard A, Maillet I, et al. Critical role of IL-33 receptor ST2 in experimental cerebral malaria development. *Eur J Immunol* 2015; **45**: 1354-1365.

[33]Basir R, Rahiman SSF, Hasballah K, Chong WC, Talib H, Yam MF, et al. *Plasmodium berghei* ANKA infection in ICR mice as a model of cerebral malaria. *Iran J Parasitol* 2012; **7**: 62.

[34]Rizvi S, Mertens JC, Bronk SF, Hirsova P, Dai H, Roberts LR, et al. Platelet-derived growth factor primes cancer-associated fibroblasts for apoptosis. *J Biol Chem* 2014; **289**: 22835-22849.

[35]Liu T, Kanaoka Y, Barrett NA, Feng C, Garofalo D, Lai J, et al. Aspirin-exacerbated respiratory disease involves a cysteinyl leukotriene-driven IL-33-mediated mast cell activation pathway. *J Immunol* 2015; **195**: 3537-3545.

[36]Jaiswal MK, Katara GK, Mallers T, Chaouat G, Gilman-Sachs A, Beaman KD. Vacuolar-ATPase isoform a2 regulates macrophages and cytokine profile necessary for normal spermatogenesis in testis. *J Leukoc Biol* 2014; **96**: 337-347.

[37]Fazalul Rahiman SS, Basir R, Talib H, Tie TH, Chuah YK, Jabbarzare M, et al. Interleukin-27 exhibited anti-inflammatory activity during *Plasmodium berghei* infection in mice. *Trop Biomed* 2013; **30**: 663-680.

[38]Ayimba E, Hegewald J, Segbena AY, Gantin RG, Lechner CJ, Agosssou A, et al. Proinflammatory and regulatory cytokines and chemokines in infants with uncomplicated and severe *Plasmodium falciparum* malaria. *Clin Exp Immunol* 2011; **166**: 218-226.

[39]Moussion C, Ortega N, Girard JP. The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells *in vivo*: A novel 'alarmin'? *PLoS One* 2008; **3**. doi: 10.1371/journal.pone.0003331.

[40]Shibui A, Takamori A, Tolba MEM, Nambu A, Shimura E, Yamaguchi S, et al. IL-25, IL-33 and TSLP receptor are not critical for development of experimental murine malaria. *Biochem Biophys Rep* 2015; **5**: 191-195.

[41]Glineur C, Leleu I, Pied S. The IL-33/ST2 pathway in cerebral malaria. *Int J Mol Sci* 2022; **23**(21). doi: 10.3390/ijms232113457.

[42]Demyanets S, Kaun C, Pentz R, Krychtiuk KA, Rauscher S, Pfaffenberger S, et al. Components of the interleukin-33/ST2 system are differentially expressed and regulated in human cardiac cells and in cells of the cardiac vasculature. *J Mol Cell Cardiol* 2013; **60**: 16-26.

[43]Akcay A, Nguyen Q, He Z, Turkmen K, Lee DW, Hernando AA, et al. IL-33 exacerbates acute kidney injury. *J Am Soc Nephrol* 2011; **22**: 2057-2067.

[44]Gungor O, Unal HU, Guclu A, Gezer M, Eyileten T, Guzel FB, et al. IL-33 and ST2 levels in chronic kidney disease: Associations with inflammation, vascular abnormalities, cardiovascular events, and survival. *PLoS One* 2017; **12**. doi: 10.1371/journal.pone.0178939.

Publisher's note