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Interleukin-27 exhibited anti-inflammatory activity during *Plasmodium berghei* infection in mice

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Abstract. Interleukin-27 (IL-27) has a pleiotropic role either as a pro-inflammatory or anti-inflammatory cytokine in inflammatory related diseases. The role and involvement of IL-27 during malaria was investigated and the effects of modulating its release on the production of major inflammatory cytokines and the histopathological consequences in major affected organs during the infection were evaluated. Results showed that IL-27 concentration was significantly elevated throughout the infection but no positive correlation with the parasitaemia development observed. Augmentation of IL-27 significantly elevated the release of anti-inflammatory cytokine, IL-10 whereas antagonising and neutralising IL-27 produced the opposite. A significant elevation of pro-inflammatory cytokines (IFN-γ and IL-6) was also observed, both during augmentation and inhibition of IL-27. Thus, it is suggested that IL-27 exerts an anti-inflammatory activity in the T_h1 type response by signalling the production of IL-10 during malaria. Histopathological examination showed sequestration of PRBC in the microvasculature of major organs in malarial mice. Other significant histopathological changes include hyperplasia and hypertrophy of the Kupffer cells in the liver, hyaline membrane formation in lung tissue, enlargement of the white and red pulp followed by the disappearance of germinal centre of the spleen, and tubular vacuolation of the kidney tissues. In conclusion, it is suggested that IL-27 may possibly acts as an anti-inflammatory cytokine during the infection. Modulation of its release produced a positive impact on inflammatory cytokine production during the infection, suggesting its potential in malaria immunotherapy, in which the host may benefit from its inhibition.

INTRODUCTION

Malaria remains a serious global threat to public health, attributing to nearly 216 million cases worldwide with almost one million deaths every year (WHO, 2011). It is responsible for 86% of deaths among African children under 5 years of age. Defense against malaria infection involves a series of complex immune response started with the initiation of T helper type 1 (T_h1) response leading to the elevation of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ) and interleukin-12 (IL-12) which are essential in providing protective immunity against the intracellular parasites and thus limiting the severity of the infection (Torre *et al.*, 2002). However, overproduction of the inflammatory mediators due to the hyperactivation of

immune cells in the systemic circulation can contribute towards life-threatening disease pathogenesis (Plebanski & Hill, 2000). T_h2 response is induced later on during the infection and anti-inflammatory cytokines, interleukin-4 (IL-4) and interleukin-10 (IL-10) are released to balance out the prior inflammatory activities and provide protection to the host by controlling the inflammation (Bakir *et al.*, 2011). Immunomodulation in malaria has been proven to be beneficial in limiting the disease severity. Previous studies have successfully proved that immunomodulatory drugs such as anti-TNF and anti-IFN-γ antibodies, thalidomide and recombinant IL-12 were capable of down-regulating the hyper-activated immune system hence reducing the morbimortality associated with malaria (Muniz-Junqueira, 2007).

Interleukin-27 (IL-27) is a recently discovered protein molecule that interacts with the immune cells and eventually activating the immune response. It is thought to exert both pro- and anti-inflammatory activities (Villarino *et al.*, 2004). Its pleiotropic effects had been demonstrated in regulating the interaction of immune cells in several parasitic infections (Yoshida *et al.*, 2006). For instance, IL-27 was shown to actively involve in inducing T_h1 response upon *Leishmania major* infection hence reducing the parasites burden and footpad swelling during the course of infection (Yoshimoto *et al.*, 2007). On the contrary, IL-27 negatively regulated the inflammatory response during *Trypanosoma cruzi* and *Toxoplasma gondii* infection in which the pro-inflammatory cytokines elevated significantly in IL-27R-deficient mice and led to fatality (Hunter *et al.*, 2004). In other inflammatory-related diseases, IL-27 showed its potential as a therapeutic target in reducing the severity of collagen-induced arthritis (Niedbala *et al.*, 2008), increased the bacterial clearance during septic peritonitis (Wirtz *et al.*, 2006) and preventing further liver injury in Concanavalin-A induced hepatitis (Siebler *et al.*, 2008). A recent study also showed that IL-27 signalling is essential in regulating the inflammatory response and eventually protecting the host from severe immunopathological reactions during malaria infection (Findlay *et al.*, 2010). IL-27 also triggers the release of IL-10 by T_h1 effector cells, which may be one of the possible underlying immunoprotection mechanisms for the malaria-infected host (Freitas do Rosario *et al.*, 2012).

In this study, we investigated the effects of modulating IL-27 on the course of malaria infection including parasitaemia development, survival, histopathological changes and cytokines production.

MATERIALS AND METHOD

Animals and parasite

Male ICR mice weighing initially between 17-20g were used throughout the study. Malaria was initiated in the animals using *Plasmodium berghei* ANKA strain. All animal-related procedures were conducted according to the rules and regulations set by The Animal Care and Use Committee (ACUC) of Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (Approval no.: UPM/FSPK/PADS/BR-UUH/00365).

Infection procedure and parasitaemia measurement

Infection was initiated by intraperitoneal (i.p) injection of 2×10^7 parasitized red blood cells (PRBC) (0.2ml). Control animals received an equivalent volume and dilution of normal red blood cells (RBC). Parasitaemia development was monitored daily by microscopic examination of Leishman-stained thin blood smears. Parasitaemia was determined by counting the Leishman positive cells with the aid of a graticule and hand counter. Five fields of approximately 200 cells each were counted and the parasitaemia was calculated as the percentage of the total red blood cells containing Leishman positive bodies.

Determination of plasma IL-27 concentration in malarial mice

For this experiment, blood were collected from control and malaria infected mice and plasma was prepared through centrifugation. IL-27 concentrations in the plasma were

determined by means of ELISA (R&D Systems, USA).

Investigating the effects of modulating IL-27 on parasitaemia development and survival of malaria-infected mice

Malaria infected mice were treated with either 1µg/ml recombinant murine IL-27 (rmIL-27), 5 µg/ml of WSX-1 Fc chimera (WSX-1Fc) or 25 µg/ml of WSX-1 monoclonal antibody (WSX-1mAb), all purchased from R&D Systems, USA. Treatments were carried out once daily for five consecutive days starting on day 1 post inoculation. The drugs were injected intravenously (i.v) through the tail vein. The control uninfected mice received an equivalent volume of sterile PBS and treated in similar manner as the malarial mice. One group of malarial mice received PBS and served as negative control as treated malarial mice. Parasitaemia development was monitored daily and mortality were observed and recorded throughout the experiment.

Investigating the effects of modulating IL-27 on the release of pro- and anti-inflammatory cytokines in malaria-infected mice

In this study, blood from control and malarial mice treated with either PBS, 1 µg/ml of rmIL-27, 5 µg/ml of WSX-1Fc or 25 µg/ml of WSX-1 mAb (clone numbers: 263517) were collected on day 5 post inoculation and treatment for plasma determination of pro- and antiinflammatory cytokines release which include IFN-γ, IL-6 and IL-10. Cytokines concentrations were measured using commercially available ELISA kit (R&D system, USA).

Investigating the effects of modulating IL-27 on the histopathology of malaria in mice

All control and treated malarial mice (either with PBS, 1 µg/ml of rmIL-27, 5 µg/ml of WSX-1Fc or 25 µg/ml of WSX-1 mAb) were sacrificed on day 5 post inoculation and treatment, and five major organs, i.e., the brain, liver, spleen, lungs and kidney were removed from the animals for histo-pathological study. All organs were fixed in 10% freshly prepared formalin, processed using an automated tissue processor (Leica, Germany), and embedded into melted paraffin wax using a histoembedder (Leica, Germany). Sections were sliced into 4 µm thick using a microtome (Leica, Germany), stained with H&E, and observed under a light microscope at 100x, 200x and 400x magnifications.

Histopathological analysis

Several important histopathological features commonly associated with malaria were analysed. Changes in the histopathological features in all five organ tissues were scored using a corresponding scale as follows: Architecture loss: nil (0), partial loss (1), moderate loss (2), total loss (3); Sequestration of PRBCs in microvessels: nil (0), mild (1), moderate (2), intense (3): Pigment deposition: nil (0), mild (1), moderate (2), intense (3); Inflammation: nil (0), mild (1), moderate (2), severe (3).

Statistical analysis

All data were expressed as the mean ± standard error of the mean (s.e.m). Comparisons between groups were analysed statistically using Graph Pad Prism 5 software. Two-tailed Student t-test and one way ANOVA followed by Tukey's test were employed where appropriate. For survival analysis, Kaplan-Meier plots were generated to compare the survival curves using a log-rank test. A p value <0.05 was considered as statistically significant.

RESULTS

Plasma IL-27 concentration in malarial mice

Significant elevation of plasma IL-27 concentrations was observed in malarial mice as compared to the control (Fig. 1). High levels of systemic IL-27 concentration were recorded as early as day 1 post inoculation and the level persisted throughout till the late stage of the infection. No positive correlation was found between the elevated levels of IL-27 and the percentage parasitaemia development (Fig. 2).

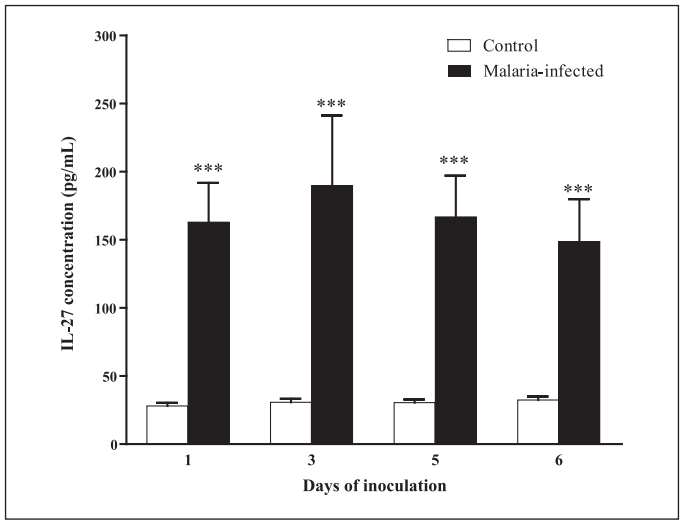

Figure 1. The systemic expression of plasma IL-27 in malaria-infected and control mice. Results are expressed as mean in pg/mL ± s.e.m. (n=8). *** indicates significant differences as compared to the control group at $p < 0.05$

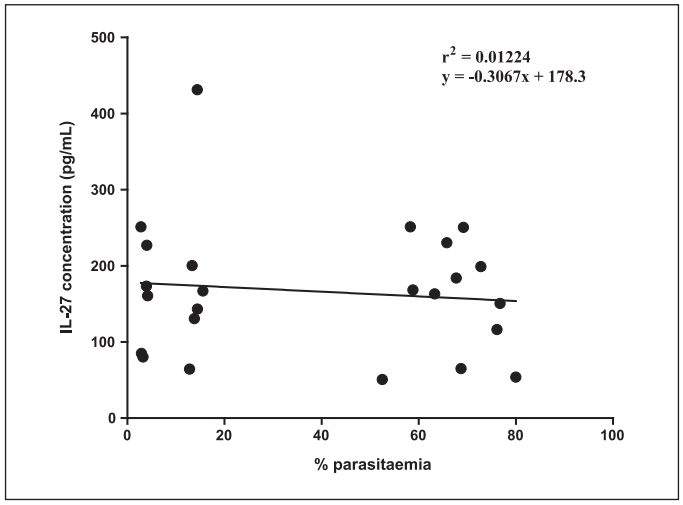

Figure 2. Scatter plot showed no positive correlation between IL-27 plasma concentrations and the percentage of parasitaemia development in malaria-infected mice throughout the infection period. Coefficient correlation was calculated using Spearman's rank test

Parasitaemia and survival rate in malarial mice treated with IL-27-related drugs

Figure 3 and 4 showed the effects on parasitaemia development and the survival rate respectively upon treatment of malarial mice with rmIL-27, WSX-1Fc and WSX-1mAb on the. On day 2 post inoculation and onwards, the results showed that the parasitaemia percentage in PBS-treated malarial mice was significantly higher as compared to the PBS-treated control mice. However, there were no significant difference in parasitaemia percentage ($p > 0.05$) measured between the PBS-treated and rmIL-27-treated malarial mice throughout the infection period (Fig. 3a). Survival rate recorded in the malarial

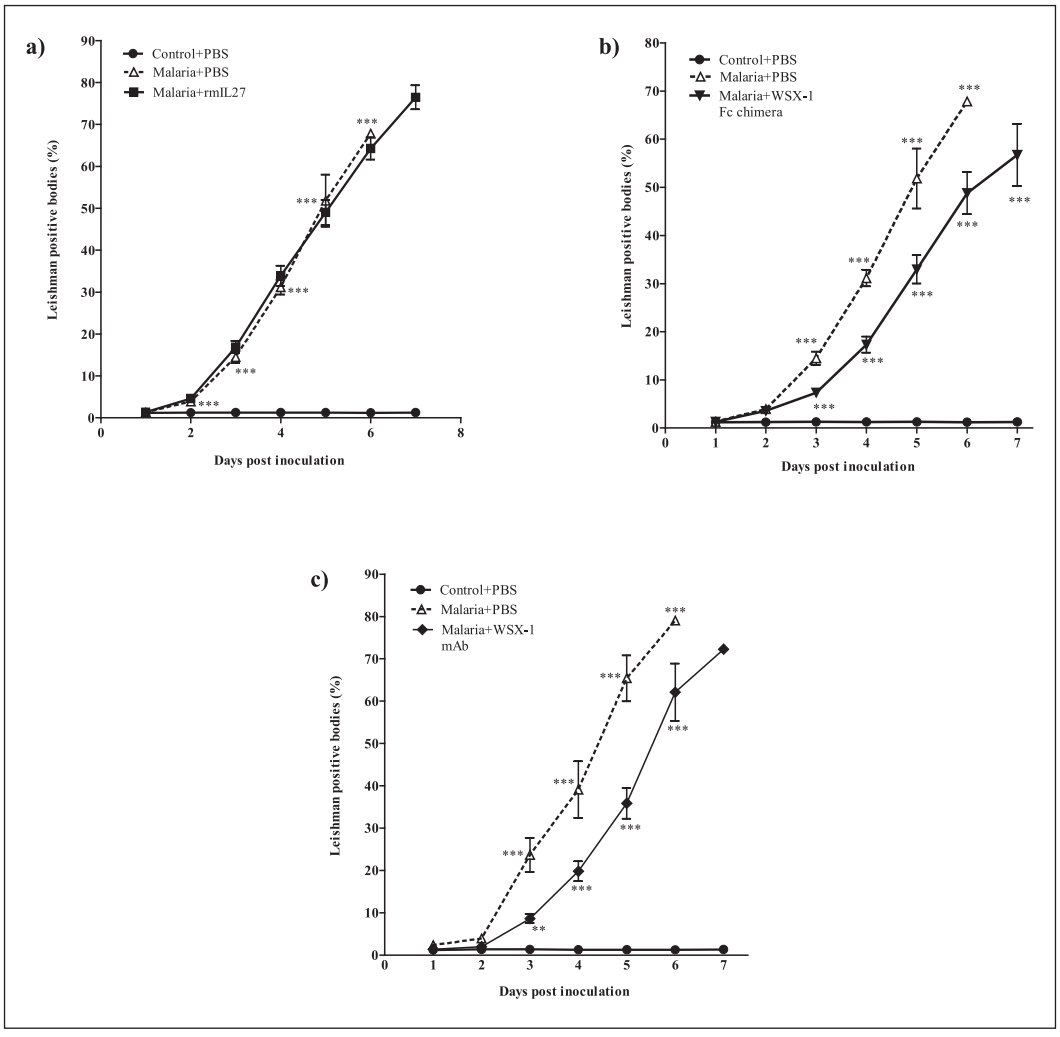

Figure 3(a-c). Effects of modulating IL-27 release on parasitaemia development. Percentage of Leishman positive cells measured in (a) rmIL-27-treated, (b) WSX-1mAb-treated and (c) WSX-1Fc-treated malarial mice in three separate experiments. Results are expressed as mean in percentage ± s.e.m. (n=8). **p<0.005 and ***p<0.0005 indicates significant differences between the treated malarial groups and malaria-PBS group on successive days post inoculation

mice treated with rmIL-27 from day 1 until day 5 post inoculation showed 100% survival (Fig. 4a). Survival rate were reduced to 62% on day 6 and 37% on day 7. By day 8, 100% mortality was observed in the rmIL-27-treated malarial mice. This is comparable to malarial mice treated with PBS which recorded a survival rate of 62% on day 5 of infection and down to 25% on day 6, followed by 100% mortality by day 7.

A significant increase in parasitaemia levels between PBS-treated malarial mice and PBS-treated control mice was observed starting from day 3 of infection until the end of the treatment period (Fig. 3b). The results also demonstrated that WSX-1Fc-treated malarial mice were presented with a significantly slower parasitaemia development on day 3, 4 and 5 of infection as compared to PBS-treated malarial mice. On day 6, all PBS-treated malarial mice succumbed to the infection and died from hyperparasitaemia (67%) whilst WSX-1 Fc chimera-treated malarial mice survived with parasitaemia percentage of around 49% and then increased to 57% on day 7 of infection. Ultimately, all malarial mice treated with WSX-1 Fc chimera died on day 8. No mortality

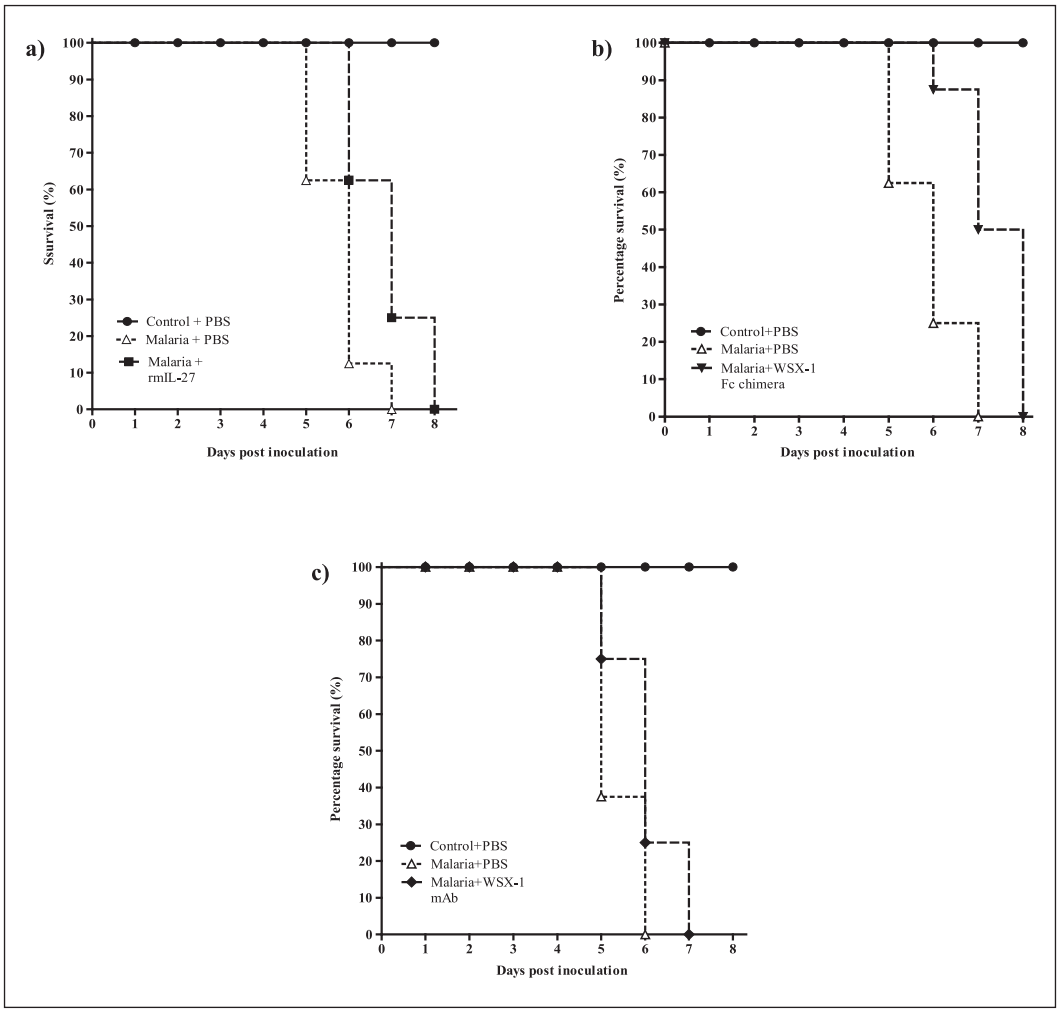

Figure 4 (a-c). Effects of modulating IL-27 release on survival rate of treated malaria-infected mice. The survival rate of (a) rmIL-27-treated, (b) WSX-1Fc-treated and (c) WSX-1mAb-treated malarial mice were monitored (n=8) in three separate experiments

was recorded in control mice treated with PBS throughout the experiment. All PBS-treated malarial mice were able to survive against the parasites up to day 4 post inoculation but the survival rate of this group reduced to 62.50% on day 5 post inoculation. As for the WSX-1Fc-treated malarial mice, they survived 100% up to day 5 of infection (Fig. 4b). The percentage survival of WSX-1Fc chimera-treated mice was reduced to 87% on day 6 post inoculation but this is still significantly higher than PBS-treated malarial mice on the same day. As the infection progressed to day 7, all of the PBS-treated malarial mice died, as opposed to the WSX-1Fc-treated malarial group in which 50% still surviving before the 100% mortality on day 8.

Neutralization of IL-27/WSX-1 following treatment with WSX-1mAb in malarial mice showed a significant reduction in parasitaemia development as shown in Figure 3c. A significantly lower parasitaemia percentage was observed in the WSX-1 mAb treated mice compared to the PBS-treated malarial mice. On day 6, all PBS-treated malarial mice died with high level of parasitaemia, while WSX-1 mAb-treated mice continued to survive despite the high level of parasitaemia (62%). WSX-1mAb-treated malarial mice showed 100% survival until day 5 post infection as compared to only

37% survival rate of PBS-treated malarial mice on the same day (Fig. 4c). However, the survival rate of WSX-1mAb-treated malarial mice dropped to 75% on day 6 and further reduced to only 25% on day 7 and 100% mortality was observed on day 8. This finding is comparable with the PBS-treated malarial mice, which showed 100% mortality as early as day 6 of infection.

Pro- and anti-inflammatory cytokines release during IL-27 modulation in malarial mice

Plasma IL-10 concentrations were found to be significantly elevated upon treatment with rmIL-27, whereas treatment with WSX-1Fc and WSX-1mAb significantly decreased the plasma IL-10 concentrations as compared to the plasma IL-10 concentration in the PBS-treated malarial mice (Fig. 5a). A significant increase in plasma IFN-γ was observed in all the rmIL-27, WSX-1Fc- and WSX-1mAb-treated malarial mice as compared to the malarial group treated with PBS with the highest concentration of plasma IFN-γ measured during treatment with rmIL-27 (Fig. 5b). For plasma IL-6 concentrations, treatment of malarial mice with all the IL-27-related drugs has significantly increased the levels as compared to the PBS-treated malarial group (Fig. 5c). The highest plasma IL-6 concentration was recorded in the WSX-1Fc-treated malarial group (191.60 ± 39.53 pg/mL).

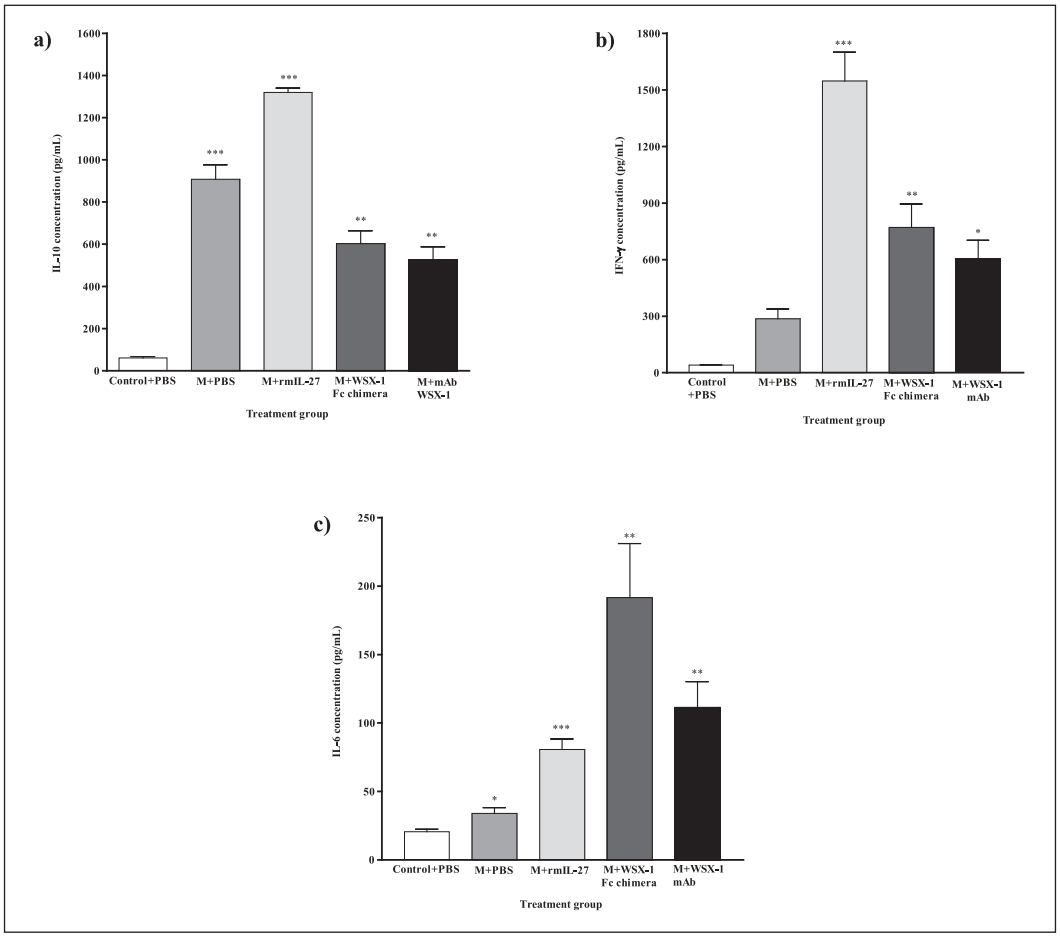

Figure 5 (a-c). Modulatory effects of IL-27 on pro- and anti-inflammatory cytokines release. Upon modulation of IL-27 related drugs, the effect of the treatments on (a) IL-10, (b) IFN-γ and (c) IL-6 expression during malaria infection were measured. Results are expressed as mean in concentration (pg/mL) ± s.e.m. (n=8). *p<0.05, **p<0.005 and ***p<0.0005 indicates significant differences between treatments groups and malaria-PBS group on day 5 post inoculation. Keynote: C – control, M – malaria

Histopathological conditions of malaria-infected mice upon IL-27 modulation

Histopathological analysis in the brain found that all malarial mice in both rmIL-27-treated and WSX-1Fc-treated groups exhibited moderate sequestration of PRBC in the microvasculature, similar to PBS-treated malarial mice (Fig. 6a and Fig. 7). Lungs tissues revealed abundant PRBC congestion in the interalveolar spaces of PBS- and rmIL-27-treated malarial mice whereas WSX-1Fc-treated malarial mice showed minimal congestion of PRBC in the lungs. There was hyaline membrane formation in the alveolar

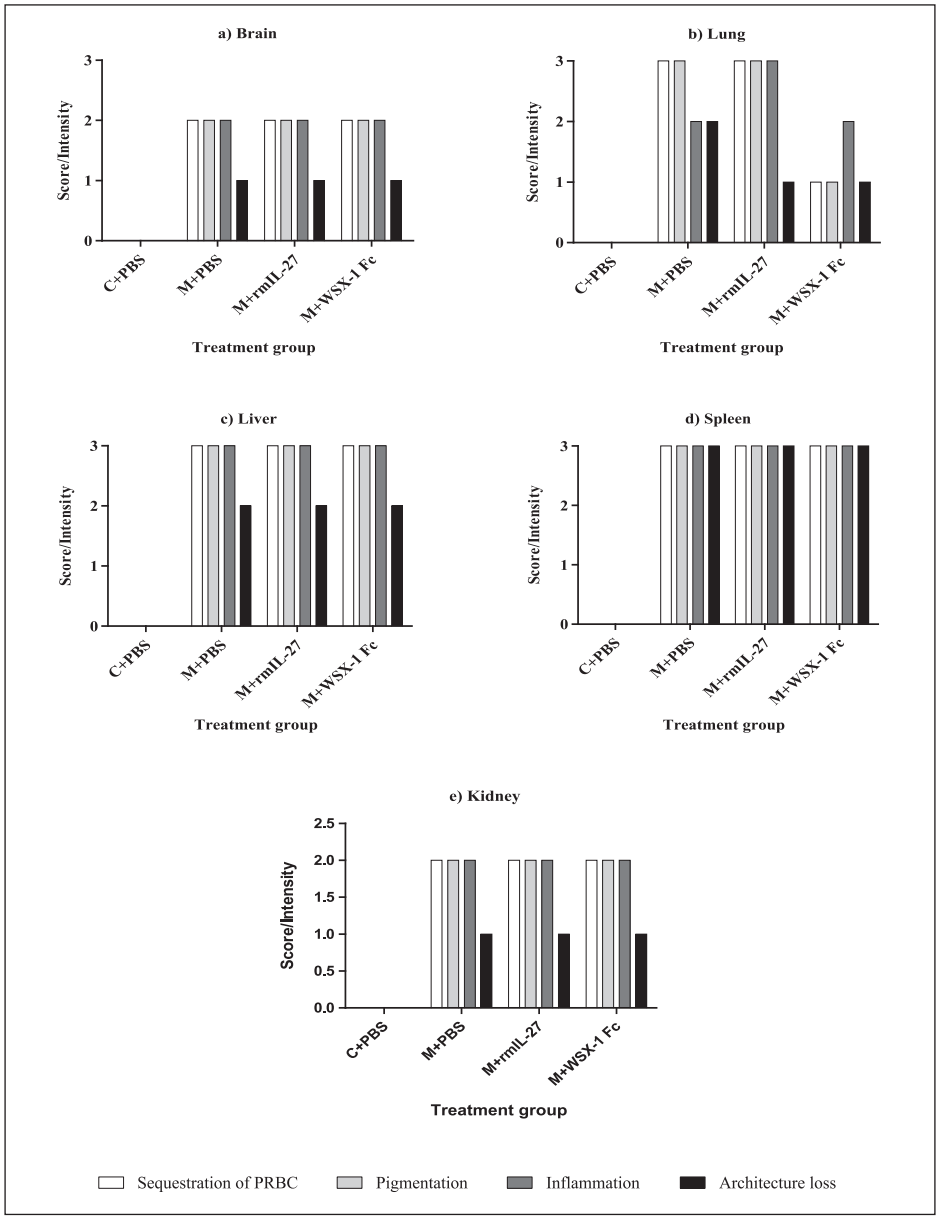

Figure 6 (a-e). Histological changes examined in the a) Brain, b) Lung, c) Liver, d) Spleen and e) Kidney of control and malarial mice treated with either PBS, rmIL-27, or WSX-1Fc. The observation was scored accordingly, sequestration of PRBC: nil (0), mild (1), moderate (2), intense (3); pigmentation: nil (0), mild (1), moderate (2), intense (3); inammation: nil (0), mild (1), moderate (2), extensive (3); architecture loss: maintained (0), partial loss (1), moderate loss (2), total loss (3). Keynote: C – control, M – malaria

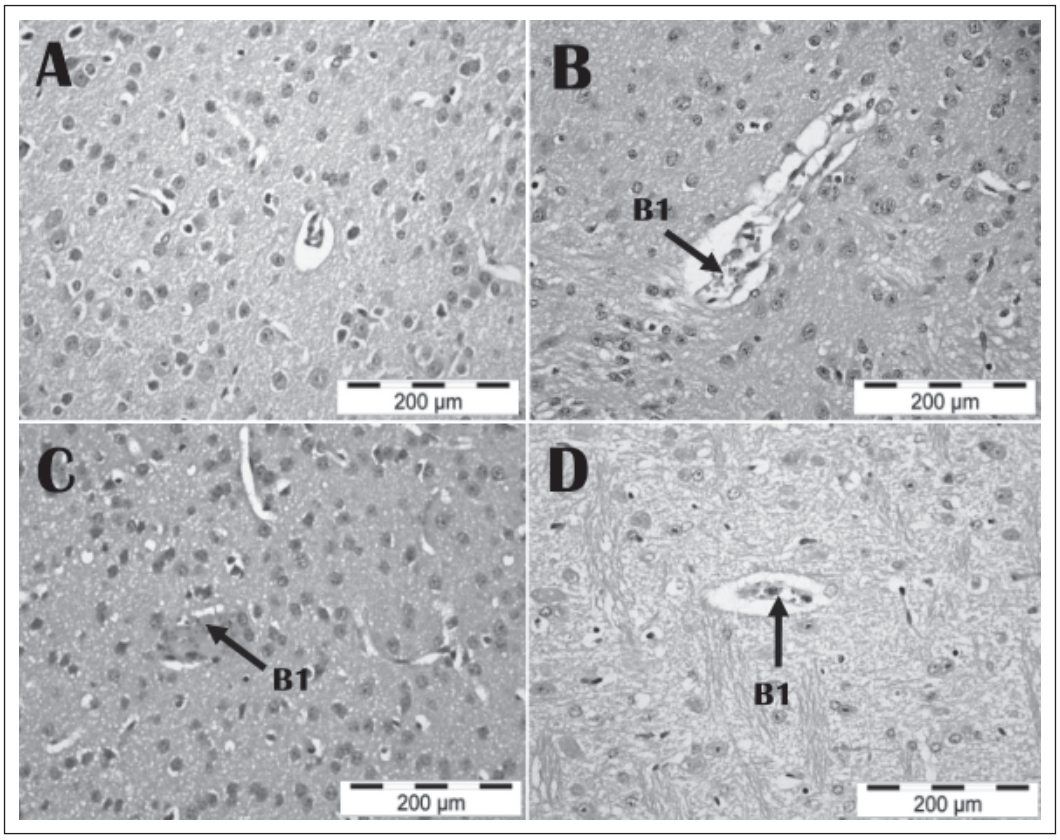

Figure 7. H&E staining of brain tissue in the (A) PBS-treated control mice, (B) PBS-treated, (C) rmIL-27-treated and (D) WSX-1Fc-treated malarial mice. There were sequestrations of PRBC (B1), found in the brain blood vessels of (B), (C) and (D). The specimens were examined under light microscopy at 400x magnification

wall of PBS-treated malarial mice, but none was observed in malarial mice treated with rmIL-27 or WSX-1Fc. A moderate inflammation in the lung tissues was examined in all malaria-infected mice (Fig. 6b and Fig. 8). Extensive inflammation around centrilobular vein was observed in liver tissues of malarial mice treated with rmIL-27 whereas both PBS-treated and WSX-1Fc-treated malarial mice displayed only moderate inflammation (Fig. 6c and Fig. 9). Other hepatopathological changes include hypertrophy and hyperplasia of Kupffer cells with haemozoins accumulation, vacuolar degeneration and hyperraemia that were accompanied with dilatation of the sinusoids found in malarial mice following treatment with PBS, rmIL-27 and WSX-1Fc chimera (Fig. 9). No significant histopathological changes were observed in the spleen and kidney of malarial mice treated with all the IL-27-related drugs as compared to the malarial mice treated with PBS (Fig 6d and 6e). The spleen of malaria-infected mice following PBS, rmIL-27 and WSX-1Fc chimera treatments showed loss of typical architecture as shown by the enlargement of red and white pulps (Fig. 10) with plentiful of haemozoin accumulation in the red pulp histiocytes, sinusoidal lining areas and occasionally in the microvasculature. In kidney tissue, congestion of the tubular cells with haemozoins and PRBC together with the presence of cytoplasmic vacuolation were observed in malarial mice treated with PBS, rmIL-27 and WSX-1Fc chimera (Fig. 11).

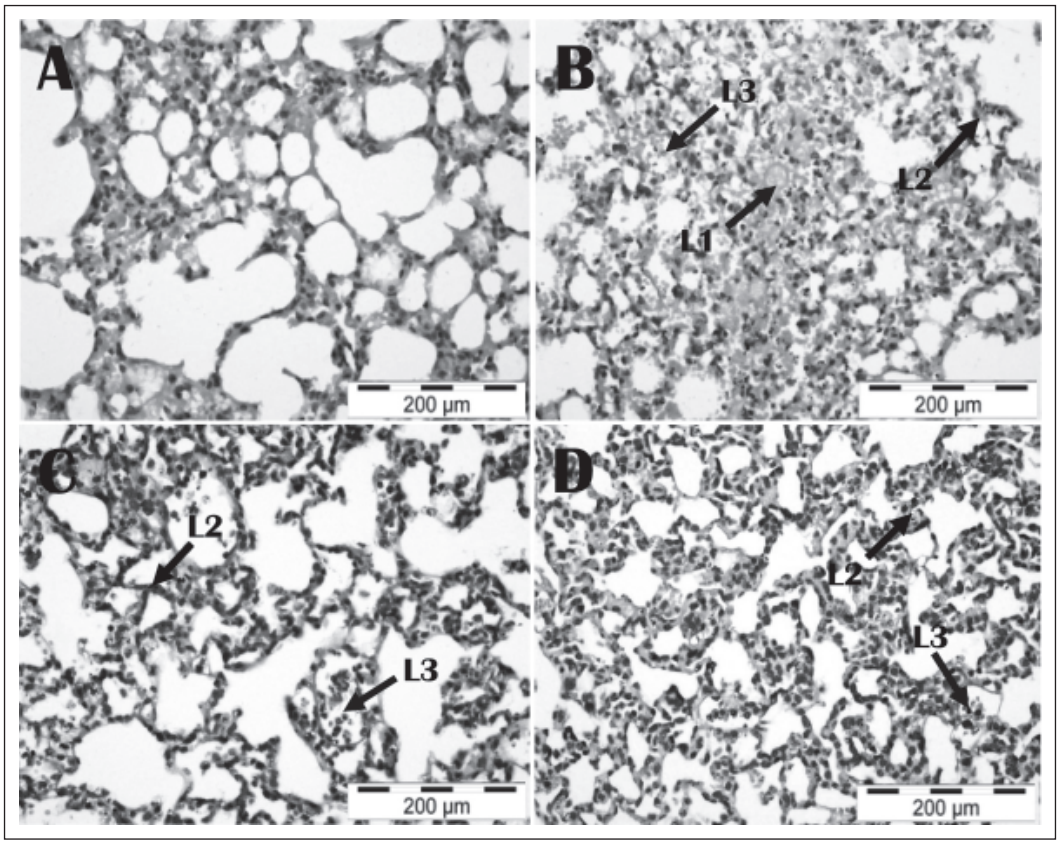

Figure 8. H&E staining of lung tissue in the (A) PBS-treated control mice, (B) PBS-treated, (C) rmIL-27-treated and (D) WSX-1Fc-treated malarial mice. In (B), a feature like hyaline membrane formation at the alveolar walls (L1) was observed. It was absent in the lungs tissues of (C) and (D). Congestion of PRBC and haemozoins in the interstitium (L2) and interalveolar spaces (L3) of lungs tissues were observed in (B), (C) and (D). The specimens were examined under light microscopy at 400x magnification

DISCUSSION

Previous studies have shown that IL-27 can exert a pleiotropic role either as pro-inflammatory or anti-inflammatory cytokine in regulating the immune response during parasitic infection (Villarino *et al.*, 2004; Yoshida *et al.*, 2006; Carl & Bai, 2008). In *L. major* infection, IL-27 was shown to actively induce T_h1 response that results in the reduction of parasites burden and footpad swelling during the course of the infection (Yoshimoto *et al.*, 2007). This T_h1 response was claimed to be governed by the presence of IL-4 (Artis *et al.*, 2004). Furthermore, IL-27 also up-regulates the pro-inflammatory cytokine secretion, the IFN-γ, besides enhancing the proliferation of $CD4^+$ and $CD8^+$ T cells as demonstrated during *T. gondii* (Villarino *et al.*, 2003) and *T. cruzi* infections (Hamano *et al.*, 2003). As for type 2 immunity, IL-27 was thought to act as a negative regulator during *Trichuris muris* infection as shown by the elevated levels of T_h2 cytokines followed by accelerated expulsion of larval parasites in WSX-1- deficient mice (Artis *et al.*, 2004).

In the present study, we report that IL-27 concentrations are highly elevated in the plasma of malarial mice right from the early stages of the infection and persisted throughout till the late critical stage of infection. The elevated level of IL-27 do not correlate with the parasitaemia development, which may suggests that the release of IL-27 during malaria infection is

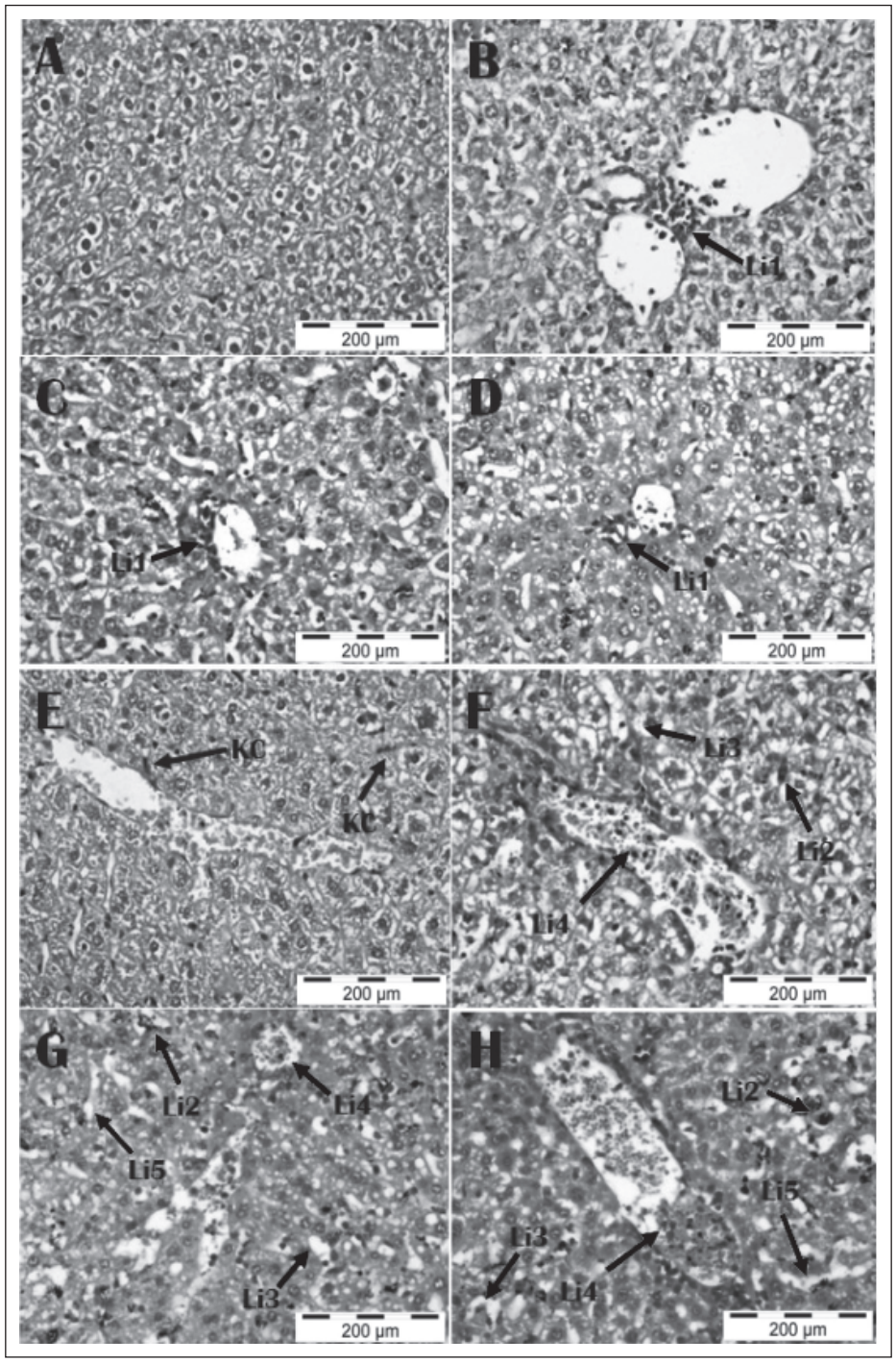

Figure 9. H&E staining of liver tissue in the PBS-treated control mice (A, E), PBS-treated (B, F), rmIL-27-treated (C, G) and WSX-1Fc-treated (D, H) malarial mice. There were lymphocytic infiltrations observed around the centrilobular veins (Li1), hyperplasia and hypertrophy of Kupffer cells (Li2) with vacuolar degeneration and atrophy of hepatocytes (Li3) observed in the malarial mice. Congestion of blood vessels with PRBC was occasionally seen (Li4). Hyperaemia accompanied with the dilatation of the vascular channels (sinusoids) (Li4) was also observed in the infected mice. The specimens were viewed under light microscopy at 400x magnification. Keynote: KC – Kupffer cells

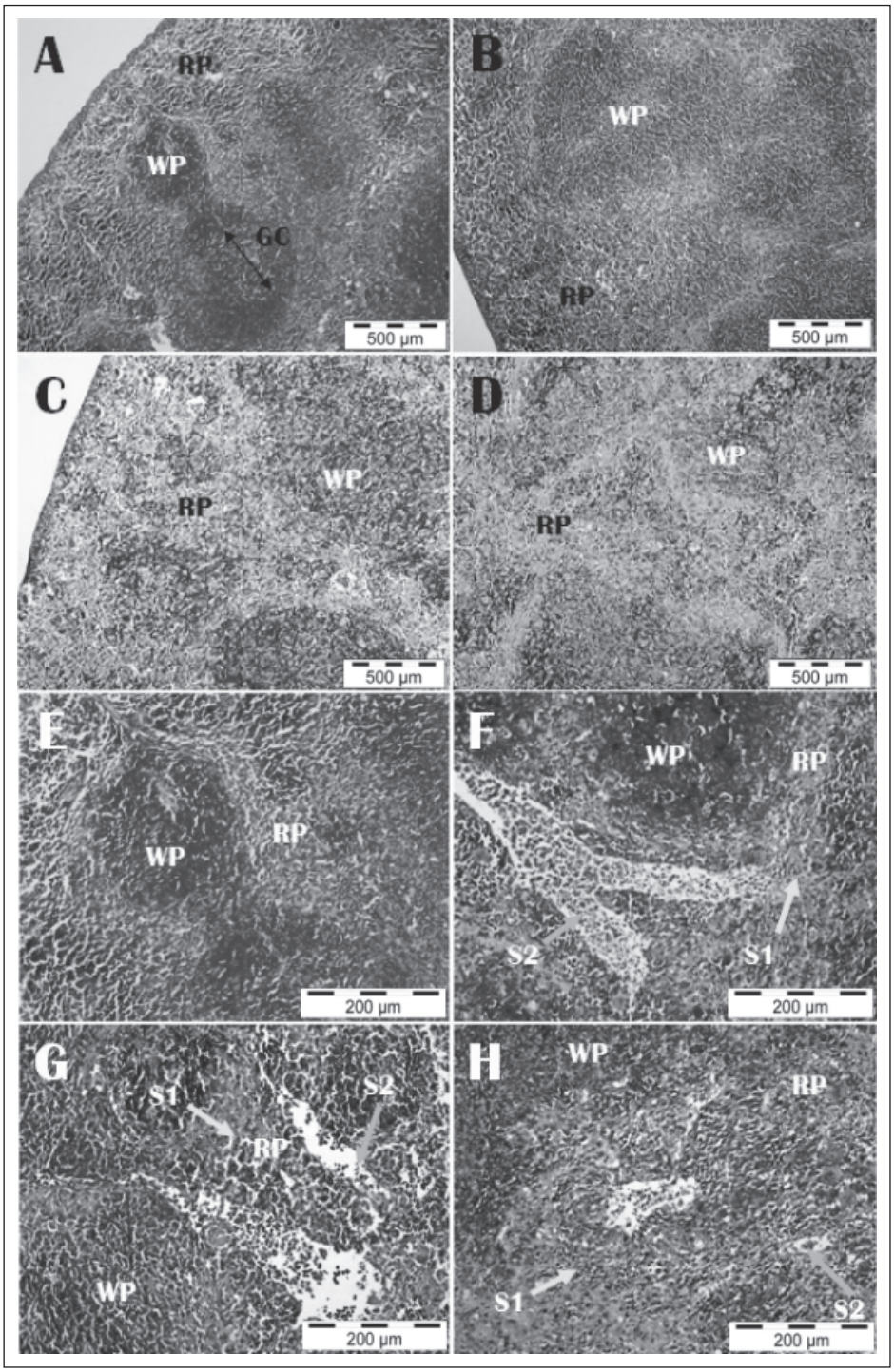

Figure 10. H&E staining of spleen tissue in the PBS-treated control mice (A, E), PBS-treated (B, F) rmIL-27-treated (C, G) and WSX-1Fc-treated (D, H) malarial mice. The red pulp and white pulp in (B), (C) and (D) were enlarged accompanied with the disappearance of germinal centre typical structure. The malarial pigments were deposited abundantly in the red pulp histiocytes, sinusoidal lining areas (S1) and the microvascular (S2) as observed in (F), (G) and (H). The specimens were viewed under light microscopy at 100x and 400x magnification. Keynote: RP – red pulp, WP – white pulp, GC – germinal centre

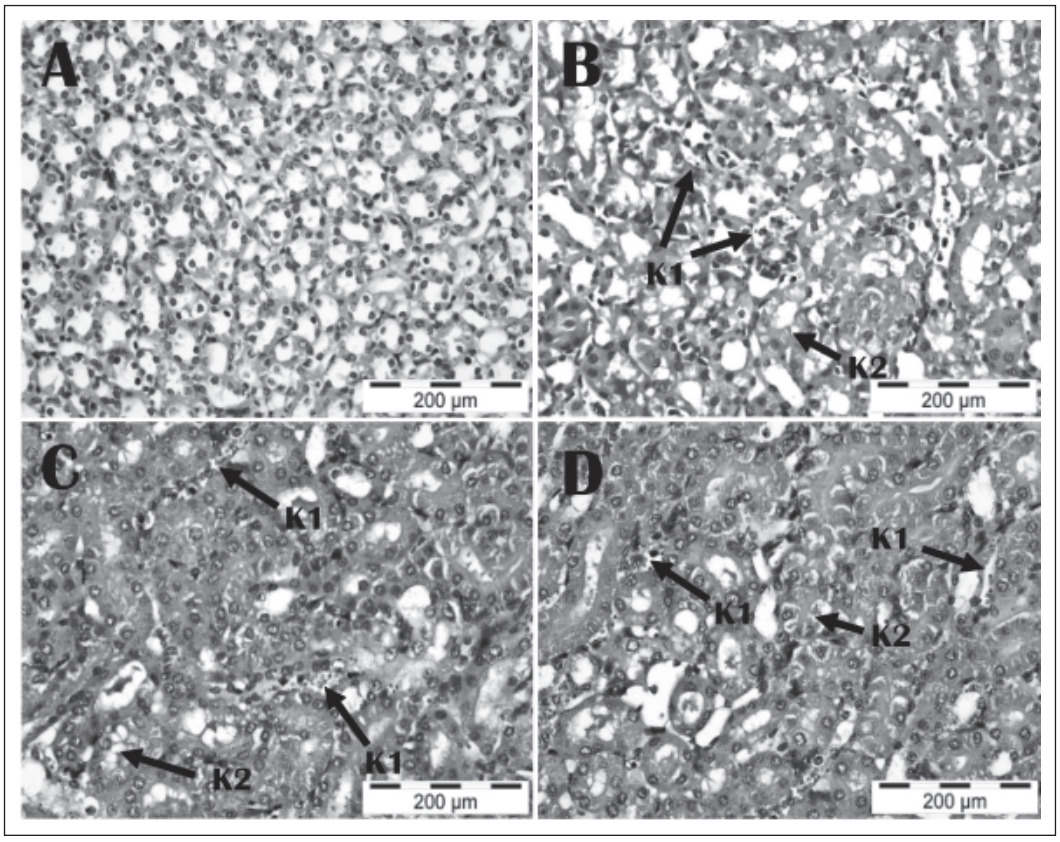

Figure 11. H&E staining of kidney tissue in the (A) PBS-treated control mice, (B) PBS-treated, (C) rmIL-27-treated and (D) WSX-1Fc-treated malarial mice. Widespread of malarial pigments deposition was observed in the microvascular and in the interstitial tissues (K1) in (B), (C) and (D). Vacuolation of the tubules (K2) were exhibited as well. The specimens were viewed under light microscopy at 400x magnification

independent of the degree of severity of the infection. From the *in vivo* study, we found that following induction of IL-27 with recombinant mouse IL-27 during malaria, the circulating levels of pro-inflammatory (IFN-γ and IL-6) and anti-inflammatory cytokines (IL-10) are intensified. Blockade of IL-27/WSX-1 ligation in malarial mice by means of antagonism with WSX-1Fc chimera and neutralising WSX-1 monoclonal antibody during the treatment period result in an increase in production of IFN-γ and IL-6 but a decrease in IL-10 concentration as compared to the PBS-treated malarial mice. Thus, it can be postulated that IL-27 plays a vital role in the production of IL-10, suggesting its suppressive role in an inflammatory response towards malaria infection.

A plausible explanation for this could be that IL-27 promotes its anti-inflammatory action during T_h1 response independently with the elevated IFN-γ and IL-6 levels upon induction and blockade of IL-27/WSX-1 ligation during the course of malaria. This is supported by study proposing the correlation of IL-27 and IL-10 in regulating and balancing the inflammatory cascade despite their distinct functions (Awasthi *et al.*, 2007). IL-10 is initially described as anti-inflammatory cytokine (Kossodo *et al.*, 1997) while IL-27 was first identified to develop T_h1 response following immune stimulation (Chen *et al.*, 2000). Other reports mentioned that the release of IL-10 and IFN-γ is due to the increase of IFN-γ^+IL-10^+-producing T cells in T_h1 condition upon induction of IL-27

(Stumhofer *et al.*, 2007; Batten *et al.*, 2008). Furthermore, IL-10 produced by T_h1 cells is shown to contribute in suppressing the immune response *in vivo* during *T. gondii* and *L. major* infections (Anderson *et al.*, 2007; Jankovic *et al.*, 2007). IL-10 is originally released by T_h2 cells, but now is thought to be produced by other types of cells as well including B cells, macrophages and T_h1 cells (O'Garra & Vieira, 2007). Recent evidence also disclosed that IL-27 mediated the production of IL-10 by inducible regulatory helper T cells (Tr1 cells) but at a transient duration. Combination of IL-27 with TGF-β is found to produce synergistic effect in expanding and maintaining the release of IL-10 by Tr1 cells (Awasthi *et al.*, 2007). Furthermore, addition of IL-27 to $CD4^+$ T cells *in vitro* generated an abundant number of IL-10 cells, indicating that IL-27 helped to induce IL-10 release in the plasma (Stumhofer *et al.*, 2007). This supports the findings in the present study which showed significantly high level of IL-10 released in the WSX-1Fc chimera-treated and WSX-1 mAb-treated malarial mice when compared to control group even when IL-27 receptors were blocked by an antagonist or neutralised by an antibody. This indicates the existence of other mechanism or signalling pathway responsible for the IL-10 release during the infection.

Our observations are consistent with several corroborating reports that confirmed the anti-inflammatory property of IL-27 in several protozoan-induced infections (Hamano *et al.*, 2003; Artis *et al.*, 2004). For instance, during *T. cruzi* infection, WSX-1-deficient mice elicited higher level of IFN-γ concurrently with hyperproduction of other pro-inflammatory cytokines from $CD4^+$ T cells such as IL-6 and TNF-α as compared to the wild type. The production of IFN-γ provided protective immunity to the infected host despite causing liver damage due to excessive release of inflammatory mediators in the latter stage of infection (Hamano *et al.*, 2003). Additional *in vivo* studies revealed the reduction of IL-10 level upon parasite invasion in WSX-1-deficient mice during toxoplasmosis infection (Stumhofer *et al.*, 2007). The immunosuppressive function of IL-27 is further demonstrated during tuberculosis in which the WSX-$1^{-/-}$ mice were likely to succumb to *Mycobacterium tuberculosis* and presented with chronic inflammation in the liver and the lungs (Hölscher *et al.*, 2005; Robinson & Nau, 2008). Recently, several researches have investigated the involvement of IL-27 during malaria infection. A study by Freitas do Rosário *et al.* (2012) demonstrated that IL-27 signalling is indispensable for optimal IL-10 production by IFN-γ^+ T_h1 cells and eventually provide protection against severe immune-mediated pathology in *Plasmodium chabaudi chabaudi* AS malaria infection. Moreover, IL-27/WSX-1 ligation is found to be important in limiting the liver damage caused by severe inflammatory response during malaria as shown in *P. berghei* NK65 infection. The protection against disease pathology however is thought to be mediated by another additional, IL-10-independent pathway (Findlay *et al.*, 2010).

In term of parasite clearance and resolvement of malaria infection successfully, a sufficient timing and adequate amount of pro- and anti-inflammatory cytokines are required to reduce the parasite burden within the systemic circulation (Angulo & Fresno, 2002; Couper *et al.*, 2008). The outcome of this study demonstrated that blocking and antagonising WSX-1 receptor site governed the parasite clearance as denoted by slower parasitaemia percentage pattern, hence prolonged the survival of treated hosts as compared to the PBS-treated malarial mice. Therefore, we suggest that the control of parasite replication among the malarial mice treated with WSX-1-related drugs is mediated by the IFN-γ at its optimal concentration. This is supported by earlier studies revealing that IFN-γ is capable of provoking intracellular parasite killing upon macrophage activation by release of reactive oxygen and nitrogen intermediates. Subsequently, the parasite loads in the circulation decreased and thus improve the survival of the infected hosts (Hölscher *et al.*, 1998; Pearl *et al.*, 2001).

Our observations showed that the recombinant IL-27-treated malarial mice presented with unexpected insignificant

parasitaemia percentage despite the elevation of IFN-γ, IL-6 and IL-10. Based on the finding, we suggest that the significant abundant release of IL-10 following the treatment period may explain the insignificant pattern of parasitaemia curves between PBS-treated and recombinant IL-27-treated malarial mice. Initial inflammatory response by T_h1 cells is essential in parasite removal from the circulation and followed by T_h2 response that is necessary to counteract the excessive inflammation. The early production of IL-10 hinders the control of parasite replication by inflammatory mediators and eventually increases the parasite burden in the infected host (Couper *et al.*, 2008). A study conducted by Roque *et al.* (2007) revealed that IL-10 production was correlated with reduced parasite control during *Mycobacterium avium* infection in which early release of IL-10 failed to terminate parasites growth in BALB/c mice. In addition to that, several previous studies revealed that severe malarial anaemia and hyperparasitaemia among African children were associated with high levels of IL-10. This suggests that the release of IL-10 during human and rodent malaria infection is somehow detrimental to the infected host (Ouma *et al.*, 2008). Contrarily, another previous study has concluded that IL-10 exerts an anti-parasitic effect and inhibits the production of nitric oxide by IFN-γ-activated macrophages (Othoro *et al.*, 1999). The association of the elevated IL-10 level with increased parasites burden is however poorly understood (Couper *et al.*, 2008).

Previous histological studies have shown that the malarial parasites can be found accumulating in the microvasculature of organs such as the brain (Franke-Fayard *et al.*, 2005), lungs (Smith *et al.*, 1982), liver (Meis *et al.*, 1983), spleen (Coquelin *et al.*, 1999) and kidneys (Rajapurkar, 1994) besides residing in the peripheral circulation. The sequestration of these malarial parasites and PRBC are causing the disease pathogenesis such as cerebral malaria, pulmonary oedema, splenomegaly and kidney injury (Mackintosh *et al.*, 2004). Histological analysis in this study showed significant deposition of malarial parasites and PRBC in the brain, lungs, liver, spleen and kidneys of malaria-infected mice. Following treatments with rmIL-27 and WSX-1Fc chimera, the treated malarial mice displayed similar histopathological changes with PBS-treated malarial group, except for minor differences in the lungs and liver. Therefore, it can be suggested that both induction and inhibition of IL-27 cause severe immunopathological consequences in *P. berghei*-infected ICR mice. This could be due to insufficient IL-27 concentration at the local level. Further investigation is needed to be carried out in elucidating the expression of IL-27 signals in the respective organs during malaria infection.

Based on the findings in this study, IL-27 may possess a potential role of balancing the immunological response by activating both anti-inflammatory cytokine, IL-10 and pro-inflammatory cytokines (IFN-γ and IL-6) during malaria infection. This study shows that modulating the releases of IL-27 does not benefit the malaria-infected host in limiting the immunopathological consequences in major organs. The positive modulatory effects of IL-27 on cytokines production during the infection may suggest its potential in malaria immunotherapy in which the host may benefit from its inhibition. Future investigation on the dynamics of activation and inhibition of IL-27 during malaria infection is necessary since both experiments mimicking the two processes showed detrimental effects histologically.

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REFERENCES

Anderson, C.F., Oukka, M., Kuchroo, V.K. & Sacks, D. (2007). $CD4^+CD25^-Foxp3^-$ T_h1 cells are the source of IL-10-mediated immune suppression in chronic cutaneous leishmaniasis. *Journal of Experimental Medicine* **204**: 285-297.

Angulo, I. & Fresno, M. (2002). Cytokines in the pathogenesis of and protection against malaria. *Clinical and Diagnostic Laboratory Immunology* **9**: 1145-1152.

Artis, D., Johnson, L.M., Joyce, K., Saris, C., Villarino, A., Hunter, C.A. & Scott, P. (2004). Early IL-4 production governs the requirement for IL-27-WSX-1 signalling in the development of protective T_h1 cytokine responses following *Leishmania major* infection. *The Journal of Immunology* **172**: 4672-4675.

Artis, D., Villarino, A., Silverman, M., He, W., Thornton, E.M., Mu, S., Summer, S., Covey, T.M., Huang, E., Yoshida, H., Koretzky, G., Goldschmidt, M., Wu, G.D., de Sauvage, F., Miller, H.R.P., Saris, C.J.M., Scott, P. & Hunter, C.A. (2004). The IL-27 receptor (WSX-1) is an inhibitor of innate and adaptive elements of type 2 immunity. *The Journal of Immunology* **173**: 5626-5634.

Awasthi, A., Carrier, Y., Peron, J.P.S., Bettelli, E., Kamanaka, M., Flavell, R.A., Kuchroo, V.K., Oukka, M. & Weiner, H.L. (2007). A dominant function for interleukin-27 in generating interleukin 10-producing anti-inflammatory T cells. *Nature Immunology* **8**: 1380-1389.

Bakir, H.Y., Tomiyama, C. & Abo, T. (2011). Cytokine profile of murine malaria: stage-related production of inflammatory and anti-inflammatory cytokines. *Biomedical Research* **32**: 203-208.

Batten, M., Kljavin, N.M., Li, J., Walter, M.J., de Sauvage, F.J. & Ghilardi, N. (2008). IL-27 is a potent inducer of IL-10 but not Foxp3 in murine T cells. *The Journal of Immunology* **180**: 2752-2756.

Carl, J.W. & Bai, X. (2008). IL27: Its roles in the induction and inhibition of inflammation. *International Journal of Clinical and Experimental Pathology* **1**: 117-123.

Chen, Q., Ghilardi, N., Wang, H., Baker, T., Xie, M.H., Gurney, A., Grewal, I.S. & de Sauvage, F.J. (2000). Development of Th1-type immune responses requires the type I cytokine receptor TCCR. *Nature* **407**: 916-920.

Coquelin, F., Boulard, Y., Mora-Silvera, E., Richard, F., Chabaud, A.G. & Landau, I. (1999). Final stage of maturation of the erythrocytic schizonts of rodent *Plasmodium* in the lungs. *Comptes Rendus de l'Académie des Sciences – Series III - Sciences de la Vie* **322**: 55-62.

Couper, K.N., Blount, D.G. & Riley, E.M. (2008). IL-10: The master regulator of immunity to infection. *The Journal of Immunology* **180**: 5771-5777.

Findlay, E.G., Greig, R., Stumhofer, J.S., Hafalla, J.C.R., de Souza, J.B., Saris, C.J., Hunter, C.A., Riley, E.M. & Couper, K.N. (2010). Essential role for IL-27 receptor signalling in prevention of T_h1-mediated immunopathology during malaria infection. *The Journal of Immunology* **185**: 2482-2492.

Franke-Fayard, B., Janse, C.J., Cunha-Rodrigues, M., Ramesar, J., Buscher, P., Que, I., Lowik, C., Voshol, P.J., den Boer, M.A., van Duinen, S.G., Febbraio, M., Mota, M.M. & Waters, A.P. (2005). Murine malaria parasite sequestration: CD36 is the major receptor, but cerebral pathology is unlinked to sequestration. *Proceedings of the National Academy of Sciences* **102**: 11468-11473.

Freitas do Rosário, A.P., Lamb, T., Spence, P., Stephens, R., Lang, A., Roers, A., Muller, W., O'Garra, A. & Langhorne, J. (2012). IL-27 promotes IL-10 production by effector T_h1 $CD4^+$ T cells: a critical mechanism for protection from severe immunopathology during malaria infection. *The Journal of Immunology* **188**: 1178-1190.

Hamano, S., Himeno, K., Miyazaki, Y., Ishii, K., Yamanaka, A., Takeda, A., Zhang, M., Hisaeda, H., Mak, T.W., Yoshimura, A. & Yoshida, H. (2003). WSX-1 is required for resistance to *Trypanosoma cruzi* infection by regulation of proinflammatory cytokine production. *Immunity* **19**: 657-667.

Hölscher, C., Hölscher, A., Rückerl, D., Yoshimoto, T., Yoshida, H., Mak, T., Saris, C. & Ehlers, S. (2005). The IL-27 receptor chain WSX-1 differentially regulates antibacterial immunity and survival during experimental tuberculosis. *The Journal of Immunology* **174**: 3534-3544.

Holscher, C., Kohler, G., Muller, U., Mossman, H., Schaub, G.A. & Brombacher, F. (1998). Defective nitric oxide effector functions leas to extreme susceptibility of *Trypanosoma cruzi*-infected mice deficient in gamma-interferon receptor or inducible nitric oxide synthase. *Infection and Immunity* **66**: 208-1215.

Hunter, C.A., Villarino, A.V., Artis, D. & Scott, P. (2004). The role of IL-27 in the development of T-cell responses during parasitic infection. *Immunology Reviews* **202**: 106-114.

Jankovic, D., Kullberg, M.C., Feng, C.G., Goldszmid, R.S., Collazo, C.M., Wilson, M., Wynn, T.A., Kamanaka, M., Flavell, R.A. & Sher, A. (2007). Conventional T-bet^{+}Foxp3^{-} T_h1 cells are the major source of host-protective regulatory IL-10 during intracellular protozoan infection. *The Journal of Experimental Medicine* **204**: 273-283.

Kossodo, S., Monso, C., Juillard, P., Velu, T., Goldman, M. & Grau, G.E. (1997). Interleukin-10 modulates susceptibility in experimental cerebral malaria. *Immunology* **91**: 536-540.

Mackintosh, C.L., Beeson, J.G. & Marsh, K. (2004). Clinical features and pathogenesis of severe malaria. *Trends in Parasitology* **20**: 597-603.

Meis, J.F., Verhave, J.P., Jap, P.H., Sinden, R.E. & Meuwissen, J.H. (1983). Malaria parasites - discovery of the early liver form. *Nature* **302**: 424-426.

Muniz-Junqueira, M.I. (2007). Immunomodulatory therapy associated to anti-parasite drugs as a way to prevent severe forms of malaria. *Current Clinical Pharmacology* **2**: 59-73.

Niedbala, W., Cai, B., Wei, X., Patakas, A., Leung, B.P., McInnes, I.B. & Liew, F.Y. (2008). Interleukin-27 attenuates collagen-induced arthritis. *Annals of the Rheumatic Diseases* **67**: 1474-1479.

O'Garra, A. & Vieira, P. (2007). T_h1 cells control themselves by producing interleukin-10. *Nature Review Immunology* **7**: 425-428.

Othoro, C., Lal, A.A., Nahlen, B., Koech, D., Orago, A.S. & Udhayakumar, V. (1999). A low interleukin-10 tumor necrosis factor-alpha ratio is associated with malaria anemia in children residing in a holoendemic malaria region in western Kenya. *The Journal of Infectious Diseases* **179**: 279-282.

Ouma, C., Davenport, G.C., Were, T., Otieno, M.F., Hittner, J.B., Vulule, J.M., Martinson, J., Ong'echa, J.M., Ferrell, R.E. & Perkins, D. J. (2008). Haplotypes of IL-10 promoter variants are associated with susceptibility to severe malarial anaemia and functional changes in IL-10 production. *Human Genetics* **124**: 515-524.

Pearl, J.E., Saunders, B., Ehlers, S., Orme, I.M. & Coope, A.M. (2001). Inflammation and lymphocyte activation during mycobacterial infection in the interferon-β-deficient mouse. *Cell Immunology* **211**: 43-50.

Plebanski, M. & Hill, A.V.S. (2000). The immunology of malaria infection. *Current Opinion in Immunology* **12**: 437-441.

Rajapurkar, M.M. (1994). Renal involvement in malaria. *Journal of Postgraduate Medicine* **40**: 132-134.

Robinson, C.M. & Nau, G.J. (2008). Interleukin-12 and interleukin-27 regulate macrophage control of *Mycobacterium tuberculosis*. *The Journal of Infectious Diseases* **198**: 359-366.

Roque, S., Nobrega, C., Appelberg, R. & Correia-Neves, M. (2007). IL-10 underlies distinct susceptibility of BALB/c and C57BL/6 mice to *Mycobacterium avium* infection and influences efficacy of antibiotic therapy. *The Journal of Immunology* **178**: 8028-8035.

Siebler, J., Wirtz, S., Frenzel, C., Schuchmann, M., Lohse, A.W., Galle, P.R. & Neurath M.F. (2008). A key pathogenic role of IL-27 in T cell-mediated hepatitis. *The Journal of Immunology* **180**: 30-33.

Smith, L.P., Hunter, K.W., Oldfield, E.C. & Strickland, G.T. (1982). Murine malaria: blood clearance and organ sequestration of *Plasmodium yoelii*-infected erythrocytes. *Infection and Immunity* **38**: 162-167.

Stumhofer, J.S., Silver, J.S., Laurence, A., Porrett, P.M., Harris, T.H., Turka, L.A., Ernst, M., Saris, C.J.M., O'Shea, J.J. & Hunter, C.A. (2007). Interleukins-27 and 6 induce STAT3-mediated T cell production of interleukin-10. *Nature Immunology* **8**: 1363-1371.

Torre, D., Speranza, F. & Martegani, R. (2002). Role of pro-inflammatory and anti-inflammatory cytokines in the immune response to *Plasmodium falciparum* malaria. *The Lancet Infectious Diseases* **2**: 719-720.

Villarino, A., Hibbert, L., Lieberman, L., Wilson, E., Mak, T., Yoshida, H., Kastelein, R.A., Saris, C. & Hunter, C.A. (2003). The IL-27R (WSX-1) is required to suppress T cell hyperactivity during infection. *Immunity* **19**: 645-655.

Villarino, A.V., Huang, E. & Hunter, C.A. (2004). Understanding the pro- and anti-inflammatory properties of IL-27. *The Journal of Immunology* **173**: 715-720.

WHO (2011). World Malaria report 2011 fact sheet. Retrieved December 13, 2011, from http://www.who.int/malaria/world_malaria_report_2011/WMR2011_factsheet.pdf

Wirtz, S., Tubbe, I., Galle, P.R., Schild, H.J., Birkenbach, M., Blumberg, R.S. & Neurath, M.F. (2006). Protection from lethal septic peritonitis by neutralizing the biological function of interleukin-27. *The Journal of Experimental Medicine* **208**: 1875-1881.

Yoshida, H., Seng, W. & Miyazaki, Y. (2006). Yin and yang of IL-27 in immune regulation during parasitic infection. *Current Trends in Microbiology* **2**: 85-93.

Yoshimoto, T., Yoshimoto, T., Yasuda, K., Mizuguchi, J. & Nakanishi, K. (2007). IL-27 suppresses Th2 cell development and T_h2 cytokines production from polarized T_h2 cells: a novel therapeutic way for T_h2-mediated allergic inflammation. *The Journal of Immunology* **179**: 4415-4423.