International Immunopharmacology 76 (2019) 105882

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Modulation of anti-malaria immunity by vitamin A in C57BL/6J mice infected with heterogenic *plasmodium*

Guang Chen[a,b], Yun-ting Du[b,d], Jian-hua Liu[c], Ying Li[c], Li Zheng[b], Xiao-song Qin[c,*], Ya-ming Cao[b,*]

[a] Department of Basic Medical Sciences, Taizhou University Hospital, Taizhou University, No 1139 Shifu Road, Jiaojiang District, Taizhou 318000, China
[b] Department of Immunology, College of Basic Medical Sciences, China Medical University, Shenyang 110013, China
[c] Department of Laboratory Medicine, Shengjing Hospital of China Medical University, Shenyang 110004, China
[d] Department of Clinical Lab, Cancer Hospital of China Medical University, Liaoning Cancer Hospital & Institute, Number 44 Xiaoheyan Road, Dadong District, Shenyang 110042, China

ARTICLE INFO

Keywords:
Protection
Vitamin A
Heterogenic plasmodium
Plasmodium yoelii 17XL
Plasmodium berghei ANKA

ABSTRACT

Vitamin A (VA) is an anti-inflammatory agent that is important in modulating and balancing the immune system. The present study aimed to investigate the immunoregulatory effects of vitamin A supplement (VAS) in C57BL/6J mice infected with *Plasmodium yoelii* 17XL (*P.y*17XL) or *Plasmodium berghei* ANKA (*P.b*ANKA). Following VA treatment, parasitaemia decreased, but survival rate did not significantly change during *P.y*17XL infection. However, in *P.b*ANKA infected C57BL/6J mice, VA pretreatment decreased parasitaemia, and a lag in cerebral malaria (CM) was observed during the early stages of infection. Furthermore, VA pretreatment was also demonstrated to upregulate MHCII expression in dendritic cells (DCs), downregulate Th1 and Tregs, and downregulate TNF-α and IFN-γ production. The results of the current study indicated that VAS downregulated the inflammation response in CM, but did not exhibit an immunoregulatory effect against *P.y*17XL infection. VAS protected the onset of CM by reducing inflammation, and was also correlated with the downregulation of Th1 by modifying the function of DCs and Tregs. However, no significant effect was observed during *P.y*17XL infection.

1. Introduction

Malaria remains to be one of the most common parasitic infections worldwide. According to recent WHO statistics, 87 countries have reported the ongoing transmission of malaria, with Africa experiencing disproportionately high malaria cases (92% of the total), and the African Region accounting for 93% of all malaria associated deaths [1]. Children aged < 5 years account for 61% (266,000) of all malaria-associated deaths worldwide and are the most vulnerable group that are affected by malaria [1]. Despite the use of fast acting anti-malarials, including artemisinins, mortality rates in patients exhibiting severe malaria remain high [2,3]. WHO advocate the requirement for improved access to effective malarial interventions to prevent further increase in malaria associated morbidity and mortality. Therefore, an urgent requirement for an adjunctive therapy for malaria exists [4].

Recently, it has been widely demonstrated that host immune responses contribute to the pathogenesis of malaria, and overaggressive inflammation mediated Th1 pathway that is reported to incur host damage. Protection/pathology in malaria is directly correlated with immunology, especially inflammation (TNF-α, IFN-γ) and immune response (T cells response, Tregs, and DCs) [5–8]. Vitamin A (VA) is an anti-inflammatory agent and is important in the modulation and balance of the immune system [9,10]. Vitamin A, together with its derivatives, regulates a variety of difference life processes, including reproduction, embryogenesis, vision, growth, cellular differentiation, cell proliferation, maintenance of epithelial cellular integrity and immune function [11]. Vitamin A deficiency (VAD) is a common nutritional deficiency, which is present worldwide, and has been indicated to be associated with an increasing susceptibility to infectious diseases in human and animal models [10]. Additionally, in African children, malnutrition is a common and serious public health problem [12]. Recent estimates indicate that > 2 billion people are at risk of vitamin A, zinc and iron deficiencies worldwide [13]. An accumulation of data has revealed that children who inhabit malaria endemic areas are susceptible to *plasmodium*, and this may be associated with VAD. A recent study has demonstrated that vitamin A supplements (VAS)

* Corresponding authors at: Department of Immunology, College of Basic Medical Sciences, China Medical University, No. 77 Puhe Road, Shenbei New District, Shenyang 110013, China.
E-mail address: ymcao@mail.cmu.edu.cn (Y.-m. Cao).

https://doi.org/10.1016/j.intimp.2019.105882
Received 18 June 2019; Received in revised form 2 September 2019; Accepted 4 September 2019
Available online 11 September 2019

decrease the severity of malaria infection in children ages 6 months-5 years and reduces the number of febrile episodes, parasite density, and the proportion of patients exhibiting spleen enlargement [14]. However, recent research has indicated that infection can also cause VAD [15]. Infectious diseases have been demonstrated to induce micronutrient deficiency by decreasing food intake (anorexia), impairing nutrient absorption, causing direct nutrient loss, increasing metabolic requirements or catabolic losses and impairing utilization (by impairing transport to target tissues) [16–18]. Therefore, micronutrient deficiencies are associated with a number of infections due to specific micronutrients serving key roles in the function of the immune system. Specific deficiencies may also predispose patients to malaria [19]. Malaria has been associated with malnutrition [20] and has been indicated to be associated with micronutrient deficiencies [21].

Studies have also indicated that the outcome of malaria infection is associated with a variety of factors, including host status, socio-cultural conditions and the parasite condition [20]. Among these, the host nutritional status is a key outcome in malarial infection [22–24]. A total of 60% of malaria-associated deaths in young children are attributable to malnutrition [25]. However, the relationship between malaria incidence and nutritional condition still remains controversial.

Based on this evidence, the purpose of the current study was to determine the effect of VAS on the outcome of *plasmodium* infection, especially the immune modulatory role of VAS in heterogenic *plasmodium* infected C57BL/6J mice. A model of rodent malaria infection provides an available model for understanding the immune response of human *Plasmodium* infections. Therefore, VA treatment, and following *Plasmodium yoelii* 17XL (*P.y*17XL) or *Plasmodium berghei* ANKA (*P.b*ANKA) infected C57BL/6J mice were used, comparative analysis was performed on the condition of inflammation (TNF-α, IFN-γ) and immune response (Th1, Tregs and DCs), in order to assess the practical value of VAS use in malaria.

2. Methods

2.1. Mice, parasite and P.y17XL/P.bANKA infection

Female C57BL/6J mice (weight 18–25 g; 6–8 weeks old) were purchased from Beijing Animal Institute. The animals were housed in a 12 h light-dark cycle and fed under experimental conditions with a temperature of 22–24 °C and a humidity of 50 ± 5%. *P.y*17XL and *P.b*ANKA were provided by Dr. Motomi Torii (Department of Molecular Parasitology, Ehime University Graduate School of Medicine).

Mice were randomly divided into five groups: Mice that were uninfected (uninfected group, 0 day), mice that were infected with *P.y*17XL (*P.y*17XL group), mice that were infected with *P.b*ANKA (*P.b*ANKA group) and infected with *P.y*17XL (*P.y*17XL + VA group) or *P.b*ANKA (*P.b*ANKA + VA group). The construction of the *plasmodium*-infected mice model was performed according to a previously described method [26,27]. Infections were initiated using an IP injection of 1×10^6 *P.y*17XL/*P.b*ANKA parasitized erythrocytes in C57BL/6J mice. Parasitaemia ($n = 10$) was monitored using a light microscope, which was used to examine Giemsa-stained, thin (tail) blood smears [28]. Mortality (n = 10) was monitored daily. All experiments were performed in compliance with the China Medical University animal ethics committee.

2.2. Vitamin A treatment

VA was purchased from Sigma-Aldrich; Merck KGaA (St. Louis, MO, USA). VA was dissolved in soybean oil prior to use. For animal experiments, the VA pretreatment group were orally administered 50 mg/kg VA prior to infection with *P.y*17XL/*P.b*ANKA once per day for four successive days. The control group received an equal volume of soybean oil at the same time points.

2.3. Detection of cytokines by ELISA

For the quantification of cytokines, splenocytes were harvested from mice at the indicated time points (day 0, 3 and 5). Spleen cells were adjusted to a final concentration of 10^7 cells/ml in RPMI-1640 supplemented with 10% heat-inactivated FCS. Aliquots (500 μl/well) of the cell suspensions were incubated in 24-well flat-bottom tissue culture plates (Falcon®) in triplicate for 48 h at 37 °C in a humidified 5% CO_2 incubator. Supernatant fractions were collected and stored at −80 °C for use when detecting cytokines.

IFN-γ and TNF-α expression was measured using commercial enzyme linked immunosorbent assay (ELISA) kits, according to the manufacturer's protocol (R&D Systems, Inc. Minneapolis, MN). The OD values were measured using a microplate reader at 450 nm. Cytokine concentrations of each sample were calculated using a standard curve generated using recombinant cytokines.

2.4. Flow cytometry analysis

To detect the subsets of splenic DCs [the number of myeloid DCs (mDCs) and plasmacytoid DCs (pDCs)], the expression of major histocompatibility complex class II (MHCII), the numbers of $CD4^+$–T-bet^+–T-bet^+ T cells (IFN-f CD4atibility complex class II (MHCII) ombinaTregs), splenocytes were harvested from mice at the indicated time points (day 0, 3 and 5). Spleen cells were adjusted to a 10^7 cells/ml final concentration.

To assess DCs, cells were co-stained with FITC-conjugated CD11c mAb (clone HL-3), PE-conjugated anti-CD11b (clone M1/70), PerCP-conjugated CD45R/B220 (clone RA3-6B2) and APC-conjugated anti-MHC II (clone M5/114.15.2).

To assess $CD4^+$-T-bet^+-IFN-γ^+ T cells, fresh splenocytes were stained using fluorescein isothiocyanate- (FITC-) conjugated anti-CD4 (clone GK1.5) antibodies. Following fixation and permeabilization using staining buffer reagents, according to the manufacturer's protocol, cells were incubated with peridinin chlorophyll- (PerCP-) conjugated anti-T-bet (clone eGio4B10) and phycoerythrin- (PE-) conjugated anti-IFN-γ monoclonal antibodies (mAb; XMG1.2).

To measure the percentage of Tregs, FITC-conjugated anti-CD4 (clone GK1.5) and PE-conjugated anti-CD25 (clone PC61) antibodies were added to spleen cells, which were resuspended in 100 μl phosphate-buffered saline (PBS), and supplemented with 1% FCS for surface staining. Cells were subsequently fixed, permeabilized, and intracytoplasmic staining was performed using allophycocyanin- (APC-) conjugated anti-FOXP3 (clone FJK16s, eBioscience; Thermo Fisher Scientific, Inc. San Diego, CA) antibodies. Unless otherwise indicated, antibodies were purchased from BD Biosciences (San Jose, CA). Cells were then washed twice with PBS containing 1% FCS and suspended in 300 μl PBS. The cells were analyzed in a FACS Calibur™ fluorocytometer using CellQuest software. Viable cells were gated using forward and side scattering patterns.

2.5. Statistical analysis

Data were presented as the mean ± standard error of the mean (SEM). The statistical significance of each dataset was analyzed using a one-way ANOVA or *t*-test, as appropriate. Time-to-event data were statistically analyzed using the Kaplan–Meier (K–M) approach to survival analysis (SPSS 17.0; SPSS, Inc.). *P*-values were calibrated using Bonferroni correction and $P < 0.05$ was considered to indicate a statistically significant result.

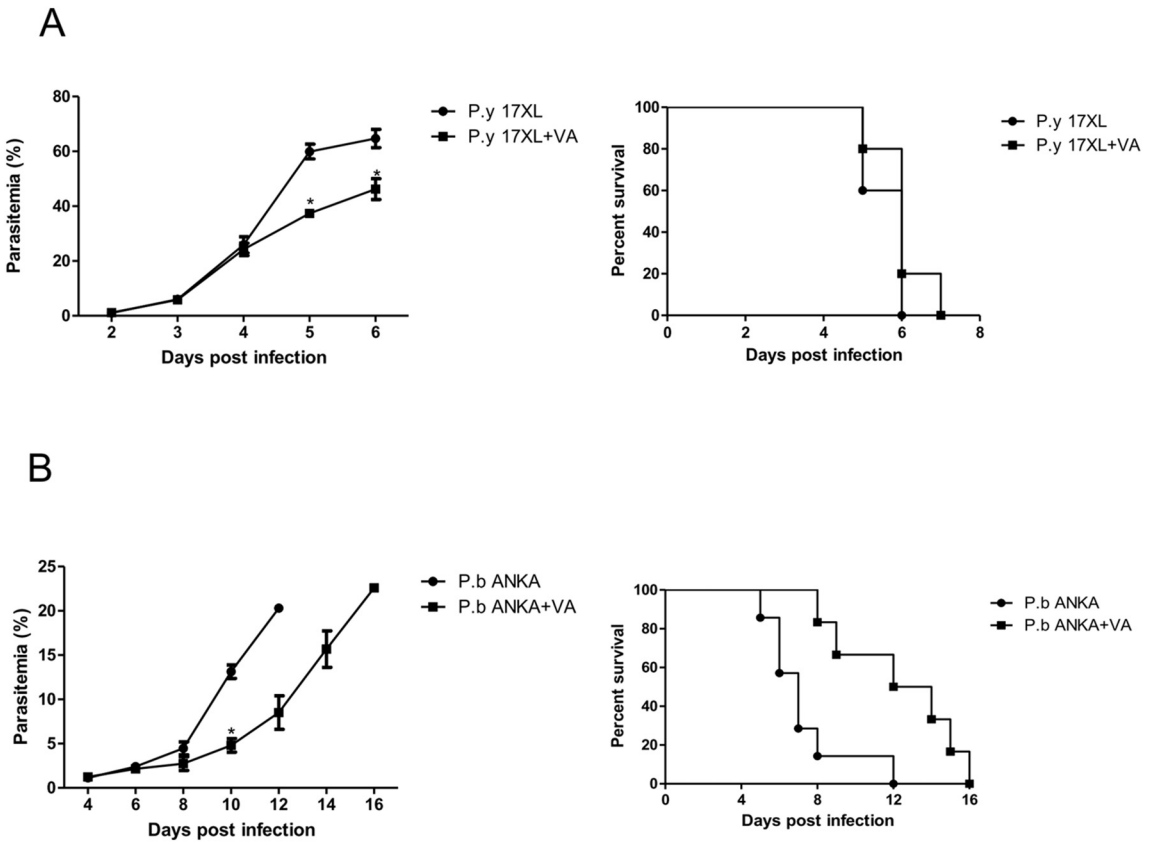

Fig. 1. Parasitaemia and survival rate of (A) *P.y*17XL and (B) *P.b*ANKA infection in control and VA treated C57BL/6J mice ($n = 10$). Parasitaemia was calculated by counting the number of parasite-infected erythrocytes per 1000 erythrocytes. Mortality was monitored daily. The data were representative of three separate experiments. *P.y*17XL, *Plasmodium yoelii* 17XL; *P.b*ANKA, *Plasmodium berghei* ANKA; VA, Vitamin A.

3. Results

3.1. VA supplementation effects the infection process in P.y17XL/P.bANKA infected C57BL/6J mice

To determine whether VA treatments could affect the outcome of *P.y*17XL/*P.b*ANKA infected C57BL/6J mice, parasitaemia and survival rate were assessed (Fig. 1A and B). VA decreased parasitaemia in the *P.y*17XL infected C57BL/6J mice compared with the normal infection group ($P < 0.05$). However, VA did not increase survival rate, and the mortality rate was 46.25% at 6 days post-infection (p.i.), with mice exhibiting very high parasitaemia. However, in *P.b*ANKA infected C57BL/6J mice, VA decreased the mean level of parasitaemia from 6 days p.i. (2.28%) to 12 days p.i. (8.5%) when compared with the normal infection group. Mortality rate of mice with VA pretreatment started to increase from 8 days p.i. The onset of mortality was postponed by 3 days compared with the normal infection group. Mice exhibited 100% mortality 16 days p.i.

3.2. VA supplementation regulates the number and activation of DCs in P.y17XL/P.bANKA infected C57BL/6J mice

DCs are a link between innate and adaptive immunity and serve a key function in cytokine production and the initiation of antigen presentation. To assess whether VA treatment can adjust the differentiation and function of DCs in malaria infected hosts, the population change of DCs and the expression of surface molecular on DCs were compared between the VA supplementation and normal infection group in C57BL/6J mice exhibiting *P.y*17XL/*P.b*ANKA infection. As presented in Fig. 2A–D, the proportion of DCs subsets ($CD11c^+CD11b^+$ and $CD11c^+B220^+$) derived from *P.y*17XL/*P.b*ANKA infected mice was higher compared with the normal infection group on day 3 p.i. This was followed by a sharp decrease in DCs subsets on day 5 p.i., which almost recovered to normal levels. Additionally, compared with normal infection mice, VAS DC1 did not differ in *P.y*17XL infected mice.

In a previous study, it was demonstrated that the function and maturity of DCs was impaired during malaria infection. Therefore, to investigate the relationship between VA and DCs in malaria, the expression of MHCII on DCs in C57BL/6J mice infected with *P.y*17XL/*P.b*ANKA was evaluated. As presented in Fig. 2E–F, compared with the normal infection group, VA pretreatment was indicated to reverse the impaired maturity rate of DCs (the expression of MHC II on DCs) during *P.b*ANKA infection, but not during *P.y*17XL infection.

3.3. VA supplementation inhibits the Th1 response in P.y17XL/P.bANKA infected C57BL/6J mice

VAD is associated with reduced effector T cell responses [28]. To determine whether the observed differences in mortality during the early stages of *P.y*17XL/*P.b*ANKA infection were due to the Th1 host immune responses, the proportion and absolute cell numbers of $CD4^+$T-bet^+ cells were compared prior to parasite infection at day 0 and at days 3 and 5 p.i. The results demonstrated that VA pretreatment decreased the number of Th1 cells in *P.y*17XL/*P.b*ANKA infected C57BL/6 mice on day 5 p.i. (Fig. 3A and B). These results have also been indicated in the Th1 cell numbers of a variety of different rodent malaria models.

To determine whether the observed differences in parasitaemia during the early stages of *P.y*17XL/*P.b*ANKA infection was due to pro-inflammatory cytokines production, TNF-α and IFN-γ expression was compared prior to parasite infection at day 0 and at day 3 and 5 p.i. The results indicated that VA pretreatment decreased TNF-α and IFN-γ

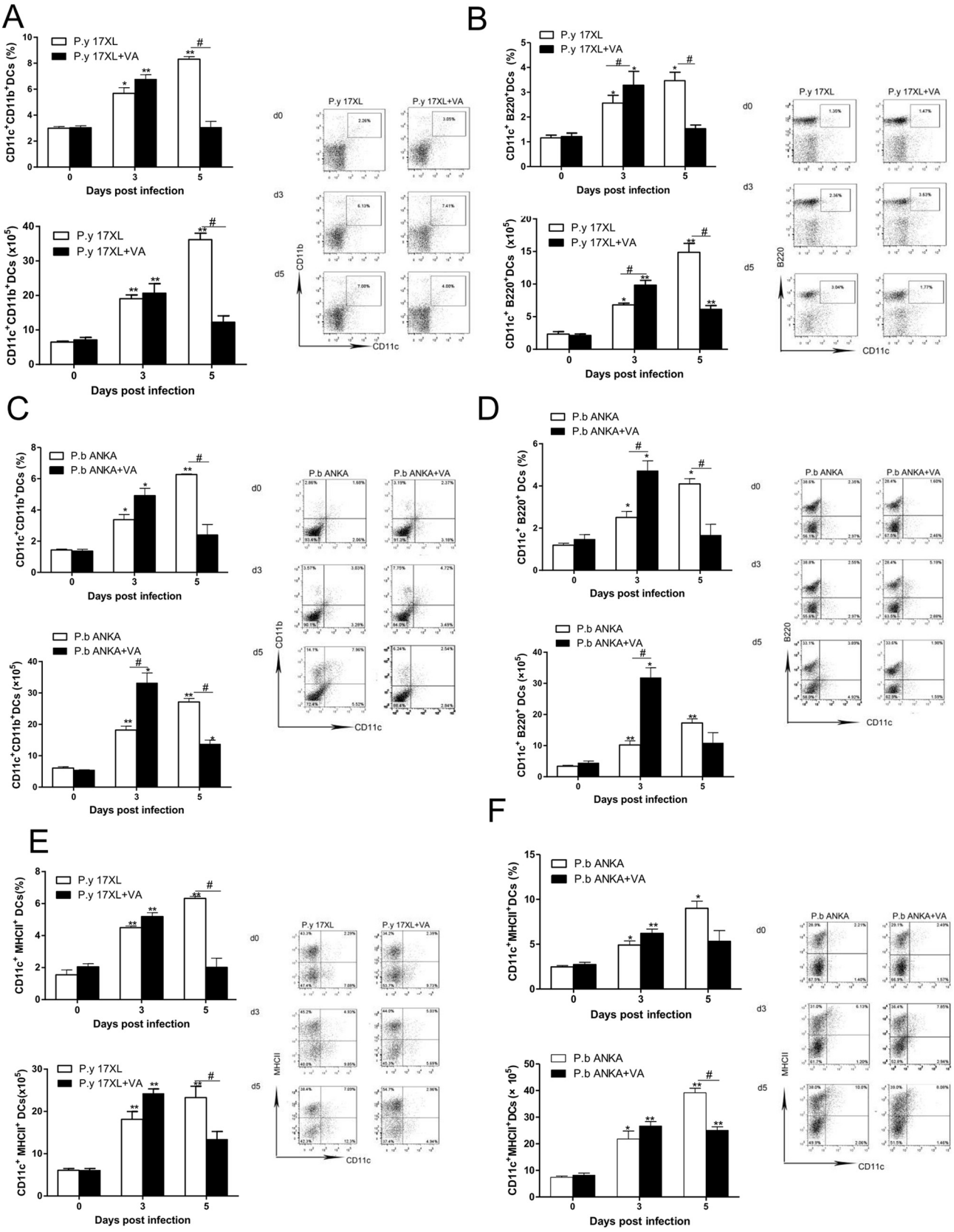

Fig. 2. Effects of VA pretreatment on DCs during *P.y*17XL and *P.b*ANKA infection. Proportion, absolute cell numbers (Column diagram, left) and representative dot plots (right) of mDCs, pDCs and the expression of MHC II on DCs in *P.y*17XL(A, B, E) and *P.b*ANKA (C, D, F) infection. Flow cytometry was performed on spleen cells from C57BL/6J mice by double staining with FITC-anti-CD11c, PE-anti-CD11b, PerCP-anti-CD45R/B220 and APC-anti-MHCII. Results were presented as the arithmetic mean of four mice per group ± SE. $^{*}P < 0.05$ and $^{*}P < 0.01$ vs. control mice (non-infected mice; day 0) using a students *t*-test. $^{\#}P < 0.05$ and $^{\#}P < 0.01$ between infected and Tregs depletion groups mice using one way ANOVA. *P*-values were calibrated using Bonferroni correction. The data are representative of three separate experiments. VA, Vitamin A; DC, dendritic cells; *P.y*17XL, *Plasmodium yoelii* 17XL; *P.b*ANKA, *Plasmodium berghei* ANKA; m, myeloid; p, plasmacytoid; MHC II, major histocompatibility complex class II.

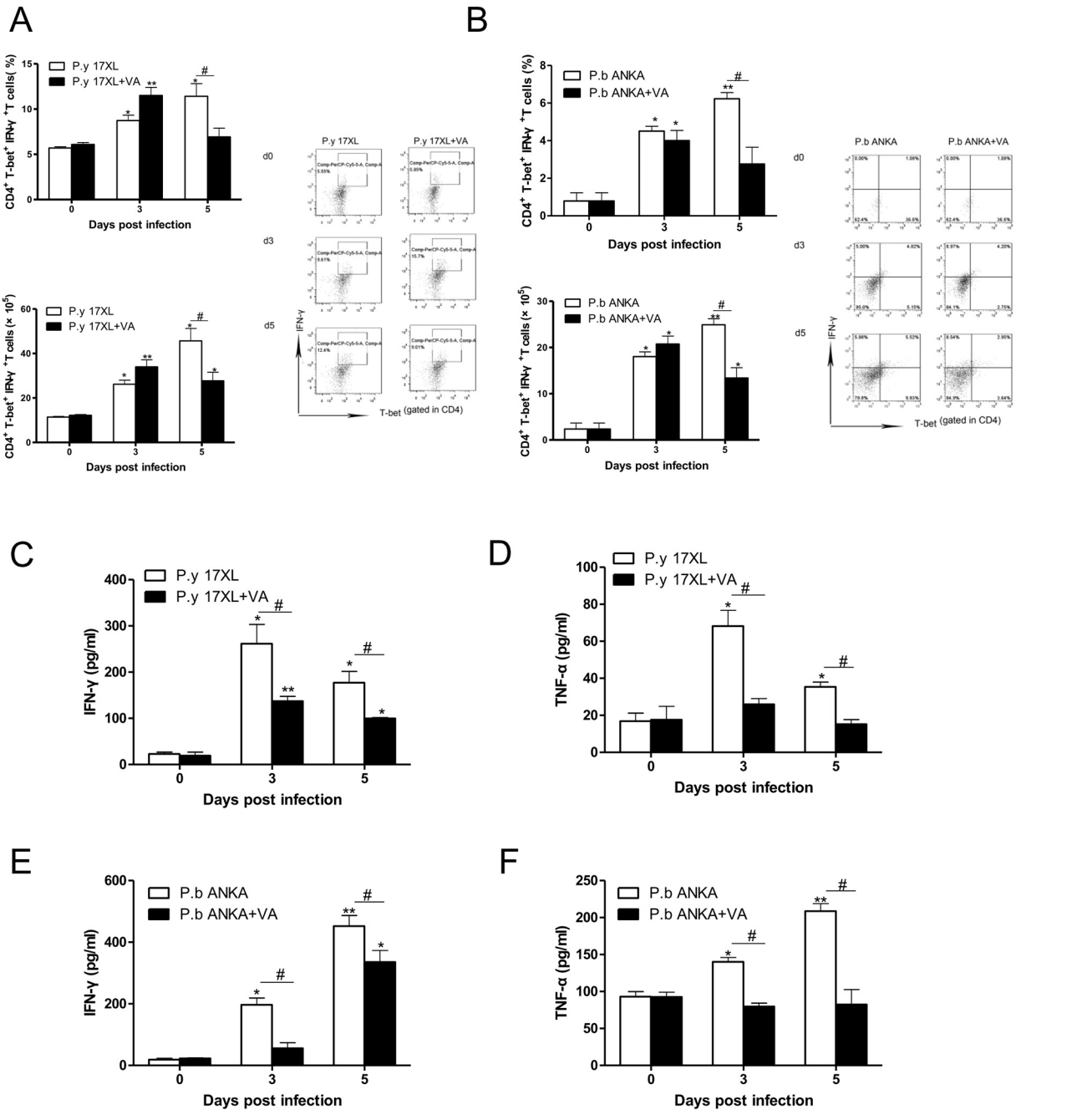

Fig. 3. Effects of VA pretreatment on Th1 during *P.y*17XL and *P.b*ANKA infection. Proportion, absolute cell numbers (Column diagram, left) and representative dot plots (right) of Th1 cells in *P.y*17XL (A) and *P.b*ANKA (B) infected C57BL/6J mice were displayed. The levels of IFN-γ and TNF-α (right) in *P.y*17XL (C, D) and *P.b*ANKA (E, F) infected C57BL/6J mice. Flow cytometric analysis was performed on spleen cells from C57BL/6J mice by triple-staining with FITC-anti-CD4, PE-anti-T-bet and APC-anti-IFN-γ. Results were presented as the arithmetic mean of four mice per group ± SE. $^{*}P < 0.05$ and $^{*}P < 0.01$ vs. control mice (non-infected mice; day 0) using a students *t*-test. $^{\#}P < 0.05$ and $^{\#}P < 0.01$ between infected and Tregs depletion groups mice using one way ANOVA. P-values were calibrated using Bonferroni correction. The data are representative of three separate experiments. VA, Vitamin A; *P.y*17XL, *Plasmodium yoelii* 17XL; *P.b*ANKA, *Plasmodium berghei* ANKA.

expression on days 3 and 5 p.i. in *P.y*17XL/*P.b*ANKA infected C57BL/6J mice compared with the normal infection group (Fig. 3C and D; $P < 0.01$; $P < 0.05$). These results demonstrated that VA pretreatment preferentially suppressed the production of pro-inflammatory mediators during acute malaria infection in *P.y*17XL/*P.b*ANKA infected C57BL/6J mice.

3.4. VA supplementation downregulated the percentage of Tregs in *P.y17XL/P.bANKA infected C57BL/6J mice*

Retinoic acid (RA) can regulate T cell differentiation and function. Under homeostatic conditions, VA promotes the transformation of naive $CD4^{+}$T cells to regulatory T cells and inhibits the development of Th17 cells, which is associated with transforming growth factor β [29]. The proportion and absolute cell numbers of Tregs was also detected, which were revealed to exhibit an inhibitory effect on Th1 immune responses. Compared with the normal infection group, in *P.y*17XL

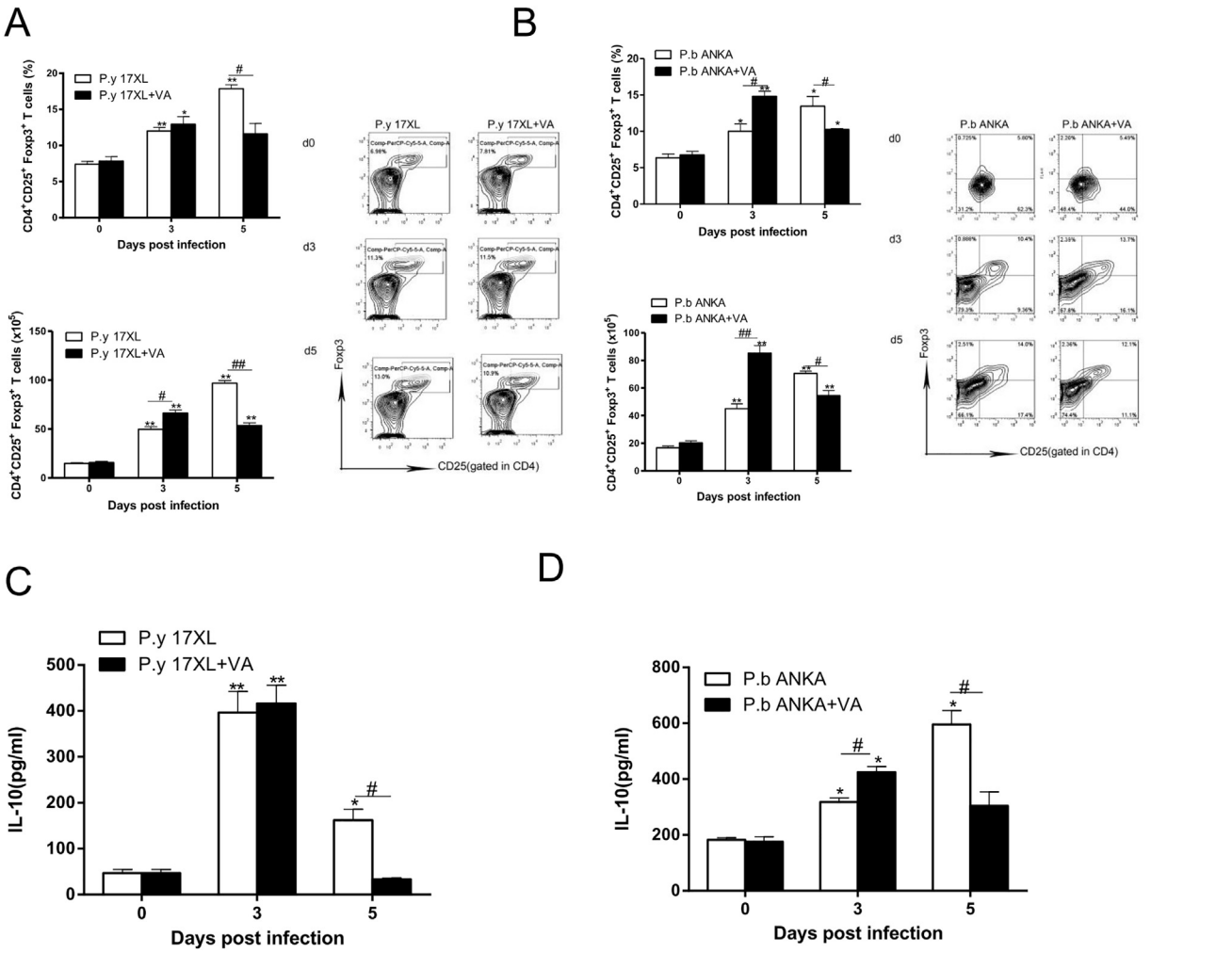

Fig. 4. Effects of VA pretreatment on Tregs during *P.y*17XL and *P.b*ANKA infection. Proportion, absolute cell numbers (Column diagram, left) and representative contour (right) of Tregs in (A) *P.y*17XL and (B) *P.b*ANKA infected C57BL/6J mice were displayed. The level of IL-10 production in *P.y*17XL (C) and *P.b*ANKA (D) infected C57BL/6J mice. Flow cytometric analysis was performed on spleen cells from C57BL/6J mice by triple-staining with FITC-anti-CD4, PE-anti-CD25 and APC-anti-Foxp3. ELISA was performed on supernatants of cultured spleen cells from C57BL/6J mice. Results were presented as the arithmetic mean of four mice per group ± SE. $^{*}P < 0.05$ and $^{*}P < 0.01$ vs. control mice (non-infected mice; day 0) using a students *t*-test. $^{\#}P < 0.05$ and $^{\#}P < 0.01$ between infected and Tregs depletion groups mice using one way ANOVA. P-values were calibrated using Bonferroni correction. The data are representative of three separate experiments. VA, Vitamin A; *P.y*17XL, *Plasmodium yoelii* 17XL; *P.b*ANKA, *Plasmodium berghei* ANKA; IL, interleukin.

infected C57BL/6J mice, following VAS treatment, the number of Tregs revealed a weaker increase on day 3 p.i., however, no statistically significant difference was observed (Fig. 4A). In contrast, VAS increased the number of Tregs on day 3 p.i., then decreased and was lower compared with the normal infection group on day 5 p.i. in *P.b*ANKA infected C57BL/6J mice ($P < 0.05$; $P < 0.01$; Fig. 4B).

Additionally, to investigate the possible modulating mechanisms of Tregs, the level of IL-10 production was analyzed in *P.y*17XL/*P.b*ANKA infected C57BL/6J mice. Consistently, VAS decreased the level of IL-10 on day 5 p.i. ($P < 0.05$) in C57BL/6J mice (Fig. 4C and D).

4. Discussion

The current study demonstrated that VAS delayed the onset of CM and can improve the outcome of malaria infection. These data indicated that VA exhibits varying effects during the early stages of *P.y*17XL and *P.b*ANKA infection. VA was also indicated to be associated with the regulation of immune-mediated protection and pathology in malaria. Pretreatment with VA did not significantly influence parasitaemia and survival rate during *P.y*17XL infection. However, in *P.b*ANKA infected C57BL/6 mice, VA pretreatment increased parasitaemia ($P > 0.05$). The results revealed that the onset of cerebral malaria exhibited a lag in the infectious early stage. VAS protected the onset of CM by reducing inflammation, was correlated with the downregulation of Th1 by modifying the function of DCs and Tregs, but did not exhibit these effects during *P.y*17XL infection. The results of the current study indicated that VAS can decrease neuroinflammation to regulate immune-mediated pathology in CM, but didn't protect *P.y*17XLinfectious mice.

CM is the most severe complication of infection of *Plasmodium falciparum* parasite infection It has complex immunopathology including prominent neuroinflammation [30]. While $CD4^+$ T cells mediate the early induction phase of CM, $CD8^+$ T cells are required for advanced immunopathology which directly contribute to the damage of blood brain barrier (BBB) [31–33]. In addition, in our previous studies, we assessed the relationship between the brain pathology in ECM and the expression levels of adhesion of leukocytes in the brain microvasculature, microhemorrhages, disruption of BBB integrity and immune response [34,35]. The results indicated that neuropathology is associated with an overwhelming inflammatory response and T cell response. Thus, adjunctive therapies that target the underlying pathophysiology of severe malaria as additional approaches induced an interest to reduce malaria-associated morbidity and mortality. As a

serious public health problem, malnutrition affects millions of people worldwide. Studies have indicated that malnutrition is a risk factor that can increase the incidence and/or severity of infection. Nutrient-deficient hosts can acquire infections more easily than healthy hosts, and this may be associated with their impaired immune function [36]. VAD is a serious global health problem that affects 100–140 million children all over the world [37]. Recent studies have indicated that malaria and malnutrition, in children, are major causes of morbidity and mortality in low- and middle-income countries [38,39]. Multiple clinical trials in infants and young children have also identified that VAS can exhibit protective effects against malaria, including a decrease in the number and time to first clinical episode, risk of febrile illness, spleen enlargement and mean parasite density [40].

VAD mice possess impaired innate and adaptive immunity. Epidemiological studies, clinical trials and experimental studies have clearly demonstrated that VA can regulate immunity, and that VAD is the cause of broad immune alterations including decreased humoral and cellular responses, inadequate immune regulation, weak response to vaccines and poor lymphoid organ development [41]. Additionally, studies have also indicated that VA, as a regulator, can induce phagocytosis, which contributes to the clearance of *Plasmodium falciparum*-infected erythrocytes via RA [42].

After phagocytizing iRBCs or free merozoites, DCs lose the ability of parasite clearance [43]. Furthermore, studies have also demonstrated that VA level can influence the development and function of conventional DCs in mucosal and lymphoid tissues [44]. The maturation of DCs is impaired in malaria, and results in the loss of antigen presentation function. Immature DCs also lose the ability to phagocytize and migrate towards T cell-rich areas to initiate the adaptive immune response [45]. Presently, why the maturation of DCs is damaged during infection with malaria, and whether VA can regulate the impaired function of DCs, is undetermined. Therefore, in the current study, assessments of the frequency, phenotype and function of circulating DCs were performed in *P.y*17XL/*P.b*ANKA-infected C57BL/6J mice. The results indicated that VA pretreatment improved the impaired function on DC maturation (the expression of MHC II on DCs) during *P.b*ANKA infection, but not during *P.y*17XL infection (Fig. 2C and D).

VA can activate the adaptive immunity and the development of T-helper (Th) cells and B-cells, and RA can inhibit the Th1 response [15]. Furthermore, DCs are particularly important in the activation of $CD4^+$T cells, which attack the parasite. The mechanisms have been indicated to be correlated with the production of inflammatory cytokines, which activate other immune cells. Therefore, the current study aimed to investigate whether VAS serves a role in the regulation of the Th1 immune response via modifying the maturation and function of DCs in malaria. As aforementioned, the results demonstrated that VA treatment decreased the percentage of Th1 cells compared with the normal infection group at day 5 p.i. in *P.y*17XL/*P.b*ANKA infected C57BL/6J mice.

VA is referred to as “the anti-infective vitamin” and exhibits an anti-inflammatory agent role [15,46]. In the present study, VA treatment did not affect parasitaemia compared with the control group, and did not extend the survival rate in *P.y*17XL/*P.b*ANKA infected C57BL/6J mice. However, VA treatment decreased pro-inflammatory cytokines (TNF-α and IFN-γ) production and the percentage of Th1 cells compared with normal infection group at day 5 p.i. in *P.y*17XL/*P.b*ANKA infected C57BL/6J mice. This may be the main reason that the onset of CM exhibited a lag during early stage *P.b*ANKA infection, as VAS decreased the pro-inflammatory response. Additionally, RA has been demonstrated to regulate neural differentiation, neuronal patterning and axon outgrowth. Due to this, studies have focused on the therapeutic potential of RA [47,48]. The brain is an immunologically privileged site, and under normal condition, the central nervous system (CNS) cannot be infected. However, in pathologic conditions, an organized immunologic response can develop within the CNS to eliminate inflammation without tissue damage [49]. Previous research has indicated that RA may be an agent for the treatment of neuroinflammation [50]. This may explain why VAD is associated with the reduced effector T cell responses, suboptimal immune responses to some vaccines and an increased risk for certain infections. These conclusions demonstrated that increased parasite clearance and moderate reduced intensity of pro-inflammatory responses to infection may explain the beneficial effects of supplementation with VA in malaria, especially in CM.

Studies have also considered that during infection, RA may promote the differentiation of $Foxp3^+$ inducible regulatory T cells by inducing DCs to express CD103. VA can maintain homeostasis at the intestinal barrier and equilibrate immunity and tolerance, including in gut dysbiosis. Retinoids perform a wide variety of functions in many settings, especially the central nervous system [50]. RA can regulate the differentiation and function of T cells, and during homeostatic conditions, RA induces naive $CD4^+$T cells to transform into regulatory T cells and inhibits the development of Th17 cells with TGF-β help. Tregs may modulate effector $CD4^+$ T cell responses in malaria, and exhibit a protective immune response that results in the prevention of immune-mediated pathology [51]. The results of the current study demonstrated that, in *P.y*17XL infected C57BL/6J mice after VAS treatment, the number of Tregs increased on day 5 p.i., but no statistically significant difference was observed. In contrast, VAS increased the number of Tregs day 3 p.i., This was followed by a decreased number which was lower compared with the normal infection group on day 5 p.i. in *P.b*ANKA infected C57BL/6J mice. These results indicated that the effect of VAS on these associations depended on the role of pro-inflammatory or Th1 immune responses via modifying the function of DCs and Tregs in malaria. Pretreatment with VA was not indicated to serve a role in the immune-mediated protection of *P.y*17XL infected mice. However, VAS delayed the onset of immune-mediated pathology in *P.b*ANKA infected mice. The dose of VA that was used in the current study (50 mg/kg) is lower compared with the clinical dose used presently [52].

Recently, many similar studies have indicated that VAS decreases the severity of malaria infection in children aged 6 months to 5 years old [53]. However, some studies have indicated that VA cannot prevent or treat malaria infection in pregnancy or childhood, based on RCT evidence [54]. However, combined with the results of the current study, due to the extensive role of VA in immune cells and the immune response, it is possible that VAS reduces mortality in children [55]. Additionally, VA exhibits extensive regulatory activity and can be used to control inflammatory diseases not only in the intestine [56,57] but also in other tissues, including in the central nervous system [58–60] and pulmonary mucosa [61,62].

5. Conclusions

In summary, VA is an essential nutrient required for maintaining immune function, and has been demonstrated to serve an important role in the regulation of Th1 response. The results of the current study support the hypothesis that a lower level of VA may be associated with relative type 1 cytokine dominance. VAS at least partly protects against the onset of immune-mediated pathology in CM, and is associated with the downregulation of Th1 via modifying the function of DCs and Tregs. However, this is not exhibited during infection with *P.y*17XL. These results have led to an increased interest in adjunctive therapies that target the underlying pathophysiology of severe malaria as additional approaches that may further reduce malaria-associated morbidity and mortality [63].

Current evidence suggests that VAS is a beneficial treatment that can be used to enhance immune function. However, the results of the current study revealed that VA was not improved in the plasmodium infectious VAD group. VAS reduces the incidence of uncomplicated malaria by ~33%, but does not appear to reduce malaria-associated mortality [64]. Therefore, it is necessary that VAS is supplemented in

the food supply in malaria endemic regions.

In patients infected with malaria, the problem of VAD should be treated first, but treatment with VAS must be performed with caution, as VA has been indicated to regulate the pro-inflammatory response. If this response is inadequate or inappropriate, it can accelerate the pathological damage exhibited by the host.

Abbreviations

*P.y*17XL	*Plasmodium yoelii* 17XL
*P.b*ANKA	*Plasmodium berghei* ANKA
VA	Vitamin A
ELISA	Enzyme-linked Immunosorbent Assay
VAD	Vitamin A deficiency
VAS	Vitamin A supplements
RA	Retinoic acid
CNS	Central nervous system
CM	Cerebral malaria

Ethics approval and consent to participate

Ethical approval was obtained from the Ethical Review Committee (ERC) at China Medical University, approved all procedures in the present study involving animals, which were in accordance with ethical standards.

Financial support

This work was supported by grants from the National Natural Science Foundation of China (81101278); Key project of the Natural Science Foundation of Heilongjiang Province (ZD2017020).

Declaration of competing interest

The authors declare no competing financial interest.

References

[1] World Health Organization, World Malaria Report, http://www.who.int/malaria/news/2018/launch-world-malaria-report-app/en/, (2018).
[2] J. Achan, J. Mwesigwa, C.P. Edwin, U. D'alessandro, Malaria medicines to address drug resistance and support malaria elimination efforts, Expert. Rev. Clin. Pharmacol. 11 (2018) 61–70.
[3] Q. Bassat, M. Tanner, P.J. Guerin, K. Stricker, K. Hamed, Combating poor-quality anti-malarial medicines: a call to action, Malar. J. 15 (2016) 302.
[4] C.C. John, E. Kutamba, K. Mugarura, R.O. Opoka, Adjunctive therapy for cerebral malaria and other severe forms of Plasmodium falciparum malaria, Expert Rev. Anti-Infect. Ther. 8 (2011) 997–1008.
[5] A. Sengupta, T. Keswani, S. Sarkar, S. Ghosh, S. Mukherjee, A. Bhattacharyya, Autophagic induction modulates splenic plasmacytoid dendritic cell mediated immune responsein cerebral malarial infection model, Microbes. Infect. (2019), https://doi.org/10.1016/j.micinf.2019.05.004 (pii: S1286-4579(19)30060-7). [Epub ahead of print] PMID:31185303.
[6] J. Dunst, F. Kamena, K. Matuschewski, Cytokines and chemokines in cerebral malaria pathogenesis, Front. Cell. Infect. Microbiol. 7 (2017) 324 Jul 20.
[7] J. Langhorne, P.E. Duffy, Expanding the antimalarial toolkit: targeting host-parasite interactions, J. Exp. Med. 213 (2016) 143–153.
[8] N. Van Braeckel-Budimir, S.P. Kurup, J.T. Harty, Regulatory issues in immunity to liver and blood-stage malaria, Curr. Opin. Immunol. 42 (2016) 91–97.
[9] R. Reifen, E. Levy, Z. Berkovich, O. Tirosh, Vitamin A exerts its anti-inflammatory activities in colitis through preservation of mitochondrialactivity, Nutrition 31 (2015) 1402–1407.
[10] R. Reifen, Vitamin A as an anti-inflammatory agent, Proc. Nutr. Soc. 61 (2002) 397–400.
[11] S. Bar-El Dadon, R. Reifen, Vitamin A and the epigenome, Crit. Rev. Food Sci. Nutr. 57 (2017) 2404–2411.
[12] J.A. Silverman, Y. Chimalizeni, S.E. Hawes, E.R. Wolf, M. Batra, H. Khofi, E.M. Molyneux, The effects of malnutrition on cardiac function in African children, Arch. Dis. Child. 101 (2) (2016) 166–171, https://doi.org/10.1136/archdischild-2015-309188 (Epub 2015 Nov 9. PMID:26553908).
[13] Z.A.S.R. Bhutta, Global nutrition epidemiology and trends, Ann. Nutr. Metab. 61 (2012) 8.
[14] A. Shankar, B. Genton, R. Semba, M. Baisor, J. Paino, S. Tamja, et al., Effect of vitamin A supplementation on morbidity due to Plasmodium falciparum in young children in Papua New Guinea: a randomised trial, Lancet 354 (1999) 203–209.
[15] C.B. Stephensen, Vitamin A, infection, and immune function, Annu. Rev. Nutr. 21 (2001) 167–192.
[16] N. Solomons, Pathways to the impairment of human nutritional status by gastrointestinal pathogens, Parasitology 107 (1993) S19–S35.
[17] L. Mata, Diarrheal disease as a cause of malnutrition, Am. J. Trop. Med. Hyg. 47 (1992) 16–27.
[18] V. Herbert, The five possible causes of all nutrient deficiency: illustrated by deficiencies of vitamin B 12, Am. J. Clin. Nutr. 26 (1973) 77–86.
[19] E.C. dos Santos-Valente, R. da Silva, M.I. de Moraes-Pinto, R.O. Sarni, B.T. Costa-Carvalho, Assessment of nutritional status: vitamin A and zinc in patients with common variable immunodeficiency, J Investig Allergol Clin Immunol 22 (2012) 427–431.
[20] M.A. Alexandre, S.G. Benzecry, A.M. Siqueira, S. Vitor-Silva, G.C. Melo, W.M. Monteiro, et al., The association between nutritional status and malaria in children from a rural community in the Amazonian Region: a longitudinal study, PLoS Negl. Trop. Dis. 9 (2015) e0003743.
[21] A.E. Frosch, B.N. Ondigo, G.A. Ayodo, J.M. Vulule, C.C. John, S.E. Cusick, Decline in childhood iron deficiency after interruption of malaria transmission in highland Kenya, Am. J. Clin. Nutr. 100 (2014) 968–973.
[22] M. Chopra, I. Darnton-Hill, Responding to the crisis in sub-Saharan Africa: the role of nutrition, Public Health Nutr. 9 (2006) 544–550.
[23] S. Kawazu, T. Nozaki, T. Tsuboi, Y. Nakano, K. Komaki-Yasuda, N. Ikenoue, et al., Expression profiles of peroxiredoxin proteins of the rodent malaria parasite Plasmodium yoelii, Int. J. Parasitol. 33 (2003) 1455–1461.
[24] A.H. Shankar, Nutritional modulation of malaria morbidity and mortality, J. Infect. Dis. 182 (2000) S37–S53.
[25] L.E. Caulfield, S.A. Richard, R.E. Black, Undernutrition as an underlying cause of malaria morbidity and mortality in children less than five years old, Am. J. Trop. Med. Hyg. 71 (2004) 55–63.
[26] X. Qin, J. Liu, Y. Du, Y. Li, L. Zheng, G. Chen, et al., Different doses of vitamin C supplementation enhances the Th1 immune response to early Plasmodium yoelii 17XL infection in BALB/c mice, Int. Immunopharmacol. 70 (2019) 387–395.
[27] B. Wu, Y. Du, Y. Feng, Q. Wang, W. Pang, Z. Qi, et al., Oral administration of vitamin D and importance in prevention of cerebral malaria, Int. Immunopharmacol. 64 (2018) 356–363.
[28] A.W. Taylor-Robinson, R.S. Phillips, B cells are required for the switch from Th1 to Th2 regulated immune responses to *Plasmodium chabaudi* infection, Infect. Immun. 62 (1994) 490–498.
[29] B. Cassani, E.J. Villablanca, J. De Calisto, S. Wang, J.R. Mora, Vitamin A and immune regulation: role of retinoic acid in gut-associated dendritic cell education, immune protection and tolerance, Mol. Asp. Med. 33 (1) (2012) 63–76 Feb.
[30] S. Torre, D. Langlais, P. Gros, Genetic analysis of cerebral malaria in the mouse model infected with Plasmodium berghei, Mamm. Genome 29 (7–8) (2018) 488–506. Aug.
[31] E. Belnoue, M. Kayibanda, A.M. Vigario, J.C. Deschemin, N. van Rooijen, M. Viguier, G. Snounou, L. Rénia, On the pathogenic role of brain-sequestered alphabeta CD8+ T cells in experimental cerebral malaria, J. Immunol. 169 (11) (2002 Dec 1) 6369–6375.
[32] Haque A1, S.E. Best, F.H. Amante, S. Mustafah, L. Desbarrieres, F. de Labastida, T. Sparwasser, G.R. Hill, C.R. Engwerda, CD4+ natural regulatory T cells prevent experimental cerebral malaria via CTLA-4 when expanded in vivo, PLoS Pathog. 6 (12) (2010) e1001221 Dec 9.
[33] N. Ghazanfari, S.N. Mueller, W.R. Heath, Cerebral malaria in mouse and man, Front. Immunol. 9 (2018) 2016 Sep 10.
[34] X. He, J. Yan, X. Zhu, Q. Wang, W. Pang, Z. Qi, M. Wang, E. Luo, D.M. Parker, M.T. Cantorna, L. Cui, Y. Cao, Vitamin D inhibits the occurrence of experimental cerebral malaria in mice by suppressing the host inflammatory response, J. Immunol. 193 (3) (2014) 1314–1323. Aug 1.
[35] Y. Du, G. Chen, X. Zhang, C. Yu, Y. Cao, L. Cui, Artesunate and erythropoietin synergistically improve the outcome of experimental cerebral malaria, Int. Immunopharmacol. 48 (2017) 219–230 Jul.
[36] M.K. Ibrahim, M. Zambruni, C.L. Melby, P.C. Melby, Impact of childhood malnutrition on host defense and infection, Clin. Microbiol. Rev. 30 (2017) 919–971.
[37] L.L. Iannotti, I. Trehan, M.J. Manary, Review of the safety and efficacy of vitamin A supplementation in the treatment of children with severe acute malnutrition, Nutr. J. 12 (2013) 125.
[38] A.H. Shankar, Nutritional modulation of malaria morbidity and mortality, J. Infect. Dis. 182 (Suppl. 1) (Sep 2000) S37-53 Review. PMID:10944483.
[39] D. Das, R.F. Grais, E.A. Okiro, K. Stepniewska, R. Mansoor, S. van der Kam, et al., Complex interactions between malaria and malnutrition: a systematic literature review, BMC Med. 16 (2018) 186.
[40] M.G. Hollm-Delgado, F.B. Piel, D.J. Weiss, R.E. Howes, E.A. Stuart, S.I. Hay, et al., Vitamin A supplements, routine immunization, and the subsequent risk of Plasmodium infection among children under 5 years in sub-Saharan Africa, Elife 3 (2015) e03925.
[41] M.R. Bono, G. Tejon, F. Flores-Santibañez, D. Fernandez, M. Rosemblatt, D. Sauma, Retinoic acid as a modulator of T cell immunity, Nutrients 8 (2016) (pii: E349).
[42] S.G. Benzecry, M.A. Alexandre, S. Vítor-Silva, J.L. Salinas, G.C. de Melo, H.A. Marinho, et al., Micronutrient deficiencies and plasmodium vivax malaria among children in the Brazilian Amazon, PLoS One 11 (2016) e0151019.
[43] M.F. Good, C. Engwerda, Defying malaria: arming T cells to halt malaria, Nat. Med. 17 (2011) 49–51.
[44] M.R. Beijer, G. Kraal, J.M. den Haan, Vitamin A and dendritic cell differentiation, Immunology 142 (2014) 39–45.
[45] Y. Wu, Q.H. Wang, L. Zheng, H. Feng, J. Liu, S.H. Ma, et al., Plasmodium yoelii:

distinct CD4 + CD25 + regulatory T cell responses during the early stages of infection in susceptible and resistant mice, Exp. Parasitol. 115 (2007) 301–304.
[46] H. Dommisch, D. Kuzmanova, D. Jönsson, M. Grant, I. Chapple, Effect of micronutrient malnutrition on periodontal disease and periodontal therapy, Periodontol 2000 78 (1) (Oct 2018) 129–153, https://doi.org/10.1111/prd.12233 (Review. PMID: 30198127).
[47] M. Maden, Retinoic acid in the development, regeneration and maintenance of the nervous system, Nat. Rev. Neurosci. 8 (2007) 755–765.
[48] M. Machado-Pereira, T. Santos, L. Ferreira, L. Bernardino, R. Ferreira, Anti-inflammatory strategy for M2 microglial polarization using retinoic acid-loaded nanoparticles, Mediat. Inflamm. 2017 (2017) 6742427.
[49] L.L. Muldoon, J.I. Alvarez, D.J. Begley, R.J. Boado, G.J. Del Zoppo, N.D. Doolittle, et al., Immunologic privilege in the central nervous system and the blood-brain barrier, J. Cereb. Blood Flow Metab. 33 (2013) 13–21.
[50] L.M. Oliveira, F.M.E. Teixeira, M.N. Sato, Impact of retinoic acid on immune cells and inflammatory diseases, Mediat. Inflamm. 2018 (2018) 3067126.
[51] M.M. Stevenson, R. Ing, F. Berretta, J. Miu, Regulating the adaptive immune response to blood-stage malaria: role of dendritic cells and $CD4^{+}Foxp3^{+}$ regulatory T cells, Int. J. Biol. Sci. 7 (2011) 1311–1322.
[52] S. Owusu-Agyei, S. Newton, E. Mahama, L.G. Febir, M. Ali, K. Adjei, et al., Impact of vitamin A with zinc supplementation on malaria morbidity in Ghana, Nutr. J. 12 (2013) 131.
[53] M.Y. Yakoob, M. Qadir, O.E. Hany, Vitamin A Supplementation for Prevention and Treatment of Malaria during Pregnancy and Childhood: A Systematic Review and Meta-analysis, J. Epidemiol. Glob. Health. 8 (1-2) (2018) 20–28, https://doi.org/10.2991/j.jegh.2018.04.104 PMID:30859783.
[54] M.Y. Yakoob, M. Qadir, O.E. Hany, Vitamin A supplementation for prevention and treatment of malaria during pregnancy and childhood: a systematic review and meta-analysis, J. Epidemiol. Glob. Health 8 (2018) 20–28.
[55] Y. Sakai, C. Meno, H. Fujii, J. Nishino, H. Shiratori, Y. Saijoh, et al., The retinoic acid inactivating enzyme CYP26 is essential for establishing an uneven distribution of retinoic acid along the anterioposterior axis within the mouse embryo, Genes Dev. 15 (2011) 213–225.
[56] G. Tejón, V. Manríquez, J. De Calisto, F. Flores-Santibáñez, Y. Hidalgo, N. Crisóstomo, et al., Vitamin A impairs the reprogramming of Tregs into IL-17-producing cells during intestinal inflammation, Biomed. Res. Int. 2015 (2015) 137893.
[57] H.L. Penny, T.R. Prestwood, N. Bhattacharya, F. Sun, J.A. Kenkel, M.G. Davidson, et al., Restoring retinoic acid attenuates intestinal inflammation and tumorigenesis in APCM in/+ mice, Cancer Immunol. Res. 4 (2016) 917–926.
[58] A.A. Saboor-Yaraghi, M.H. Harirchian, N. Mohammadzadeh Honarvar, S. Bitarafan, M. Abdolahi, F. Siassi, et al., The effect of vitamin A supplementation on FoxP3 and TGF-β gene expression in Avonex-treated multiple sclerosis patients, J. Mol. Neurosci. 56 (2015) 608–661.
[59] N. Mohammadzadeh Honarvar, M.H. Harirchian, M. Abdolahi, E. Abedi, S. Bitarafan, et al., Retinyl palmitate supplementation modulates T-bet and interferon gamma gene expression in multiple sclerosis patients, J. Mol. Neurosci. 59 (2016) 360–365.
[60] M. Raverdeau, C.J. Breen, A. Misiak, K.H. Mills, Retinoic acid suppresses IL-17 production and pathogenic activity of γδ T cells in CNS autoimmunity, Immunol. Cell Biol. 94 (2016) 763–773.
[61] S. Goswami, P. Angkasekwinai, M. Shan, K.J. Greenlee, W.T. Barranco, S. Polikepahad, et al., Divergent functions for airway epithelial matrix metalloproteinase 7 and retinoic acid in experimental asthma, Nat. Immunol. 10 (2009) 496–503.
[62] C. Niu, N. Liu, J. Liu, M. Zhang, L. Ying, L. Wang, et al., Vitamin A maintains the airway epithelium in a murine model of asthma by suppressing glucocorticoid-induced leucine zipper, Clin Exp Allergy 46 (2016) 848–860.
[63] J. Achan, A. Mpimbaza, Vitamin A as adjunct therapy for cerebral malaria: what is the evidence? Afr. Health Sci. 12 (2012) 87–88.
[64] B.H. Kim, K.S. Kang, Y.S. Lee, Effect of retinoids on LPS-induced COX-2 expression and COX-2 associated PGE (2) release from mouse peritoneal macrophages and TNF-alpha release from rat peripheral blood mononuclear cells, Toxicol. Lett. 150 (2004) 191–201.