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Dihydroartemisinin regulates the immune system by promotion of CD8$^+$ T lymphocytes and suppression of B cell responses

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Artemisia annua is an anti-fever herbal medicine first described in traditional Chinese medicine 1,000 years ago. Artemisinin, the extract of *A. annua*, and its derivatives (dihydroartemisinin (DHA), artemether, and artesunate) have been used for the treatment of malaria with substantial efficacy. Recently, DHA has also been tested for the treatment of lupus erythematosus, indicating that it may function to balance the immune response in immunocompromised individuals. In the present study, the regulatory effect of artemisinin on the murine immune system was systematically investigated in mice infected with two different protozoan parasites (*Toxoplasma gondii* and *Plasmodium berghei*). Our results revealed that the mouse spleen index significantly increased (spleen enlargement) in the healthy mice after DHA administration primarily due to the generation of an extra number of lymphocytes and CD8$^+$ T lymphocytes in both the spleen and circulation. DHA could increase the proportion of T helper cells and CD8$^+$ T cells, as well as decrease the number of splenic and circulatory B cells. Further, DHA could reduce the production of proinflammatory cytokines. Our study revealed that apart from their anti-parasitic activity, artemisinin and its derivatives can also actively modulate the immune system to directly benefit the host.

artemisinin, immune cells, cytokine, immunomodulation, *Toxoplasma gondii*, *Plasmodium berghei*

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INTRODUCTION

Artemisinin was first extracted from *Artemisia annua* in 1972 by Chinese scientists who were searching for anti-malarial drugs (Li, 2012). Artemisinin and its derivatives (e.g., dihydroartemisinin (DHA), artemether, and artesunate) quickly clear circulatory malaria parasites in mammalian hosts by inhibiting the uptake of hemoglobin (Klonis et al., 2011), and the key structure for antimalarial activity is the endoperoxide bridge (Meshnick, 2002). Although the drugs have an effect on all stages of the malaria parasites and achieve 100% growth inhibition within 2–4 h, the early trophozoite stage (also called the ring stage) of the plasmodial parasites is more vulnerable to artemisinin (Skinner et al., 1996; Chimanuka et al., 2001). Currently, artemisinin and its derivatives are used in combination with classical anti-malarial drugs (e.g., lumefantrine, mefloquine, amodiaquine, sulfadoxine/pyrimethamine, piperaquine, and chlorproguanil/dapsone) to provide an adequate cure rate and delay the development of drug resistance (http://www.who.int/malaria/areas/treatment/overview/en/).

Apart from its anti-malarial effects, artemisinin and its derivatives have been shown to inhibit the early stages of schistosomal parasites (Xiao et al., 2000; Utzinger et al.,

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2001) by reducing the activity of the glutathione reductase and cytochrome c peroxidase of the schistosomal parasites (Wu et al., 1996; Guo et al., 1997; Abdin et al., 2013). Furthermore, studies have shown that artemisone and artemiside have an inhibitory effect on *Toxoplasma gondii* both *in vitro* and *in vivo* (Dunay et al., 2009). Additionally, artemisinin may disrupt the calcium homeostasis of *T. gondii*, supporting the hypothesis that Ca^{2+}-ATPases are potential drug targets in these parasites (Nagamune et al., 2007).

Additionally, the immune regulatory function of artemisinin has recently been recognized. One study demonstrated that the artemisinin analog, artesunate, remarkably ameliorated the arthritis in K/BxN mouse, and artesunate treatment prevented the development of arthritis in young K/BxN mice, a classic murine model of human rheumatoid arthritis. Artesunate treatment was found to rapidly reduce germinal center (GC) B cells in the K/BxN mice likely by inhibiting GC formation and the production of autoantibodies (Hou et al., 2014). In addition to these findings in animal models, artesunate derivatives also display promising effects for human rheumatoid arthritis. Artesunate has also been found to decrease the secretion of vascular endothelial growth factor (VEGF) and IL-8 from TNF-α or hypoxia-stimulated rheumatoid arthritis fibroblast-like synoviocytes (RA FLS) (He et al., 2011). Furthermore, artemisinin and its derivatives are also able to regulate macrophage activation, T cell activation and proliferation, T cell subsets differentiation (Th1, Th17, Treg) (Li et al., 2012; Li et al., 2013a; Li et al., 2013b). The topical administration of artemisinin effectively induced the production of regulatory T cells (Tregs) possibly via the blockage of AKT signaling (Li et al., 2012). Moreover, it was reported that p38 MAPK could be modulated by Treg peptide tolerance (Lourenco et al., 2009). Further studies showed that artemisinin could effectively increase the activity of phospho-p38 MAPK, likely due to the impairment of AKT phosphorylation, which was associated with the upregulation of p38 MAPK activation (Li et al., 2012). Artemisinin was also found to significantly inhibit the production of IL-17 in mice (Xu et al., 2007; Cheng et al., 2011). Another recent study also demonstrated that DHA treatment could effectively inhibit Th cell differentiation *in vitro* and effectively reduce the onset of autoimmune encephalomyelitis (EAE). It further improved sustained EAE in mice and weakened mTOR signaling in T cells, indicating that DHA alleviated the clinical symptoms by modulating the mTOR pathway (Zhao et al., 2012).

Systemic lupus erythematosus (SLE) is a chronic, systemic autoimmune disease that affects many different organs, including the heart, spleen, and kidneys. In addition, studies have found that DHA was able to ameliorate the symptoms of lupus in BXSB mice (Li et al., 2006). While B cells, autoantibodies, and cytokines have been implicated in the intricate pathogenesis of SLE (Gordon et al., 2009), T cells enhance the production of autoantibodies by offering substantial help to B cells via stimulating their differentiation, proliferation, and maturation in SLE (Shlomchik et al., 2001; Lewis et al., 2013). Furthermore, it was found that thymic B cells, as well as both $CD8^-CD4^+$ and $CD4^-CD8^+$ T cells were increased, whereas the proportion of $CD4^+CD8^+$ T cells was reduced during SLE (Xing et al., 2017). This suggests that the impaired regulatory function of NK cells together with the increased number of DC-like NK cells may play an important role in the development of SLE (Cruz-González et al., 2018).

In the present study, we investigated the potential immune regulatory role of dihydroartemisinin (DHA), a derivative of artemisinin by systematically analyzing the immune response in mice infected with either *T. gondii* or *P. berghei* before and after treatment with DHA.

RESULTS

DHA promoted the spleen index and enhanced the production of splenic $CD8^+$ T cells and circulating Th cells, but downregulated circulating B cells in healthy mice

The number of splenic $CD8^+$ T cells in the DHA group were significantly increased compared to that of the control group on days 6 ($P<0.05$), 8 ($P<0.05$), and 15 ($P<0.001$) (Figure 1A and B). The spleen indexes were significantly increased after DHA administration for 15 ($P<0.01$) and 25 days ($P<0.001$) (Figure 1C). Similarly, the circulating Th cells in the DHA group were increased compared to that of the control group on days 2 ($P<0.001$), 4 ($P<0.001$), 6 ($P>0.05$), and 8 ($P<0.01$) (Figure 2A and B). However, the circulating B cells in the DHA group were decreased compared to that of the control group on days 2 ($P>0.05$), 4 ($P<0.05$), 6 ($P>0.05$), and 8 ($P>0.05$) (Figure 2C and D).

DHA reduced the levels of serum TNF-α, IL-2, and IL-13, whereas the level of IL-5 was elevated in healthy mice

To investigate the influence of DHA on lymphocyte activity, we quantitatively analyzed the serum levels of IL-2, IFN-γ, TNF-α, IL-4, IL-5, IL-17A, and IL-22 on days 0, 4, 8, 12, 16, 20, 24, 28, and 32 post-DHA administration. The mouse sera from day 0 were used as the controls. The expression of TNF-α (Figure 3A and C), IL-2 (Figure S1A and C in Supporting Information), and IL-13 (Figure S1B and C in Supporting Information) was significantly reduced in the DHA group, whereas Th2 cytokines (e.g., IL-5) were increased in the DHA group compared to that of the control group (Figure 3B and C).

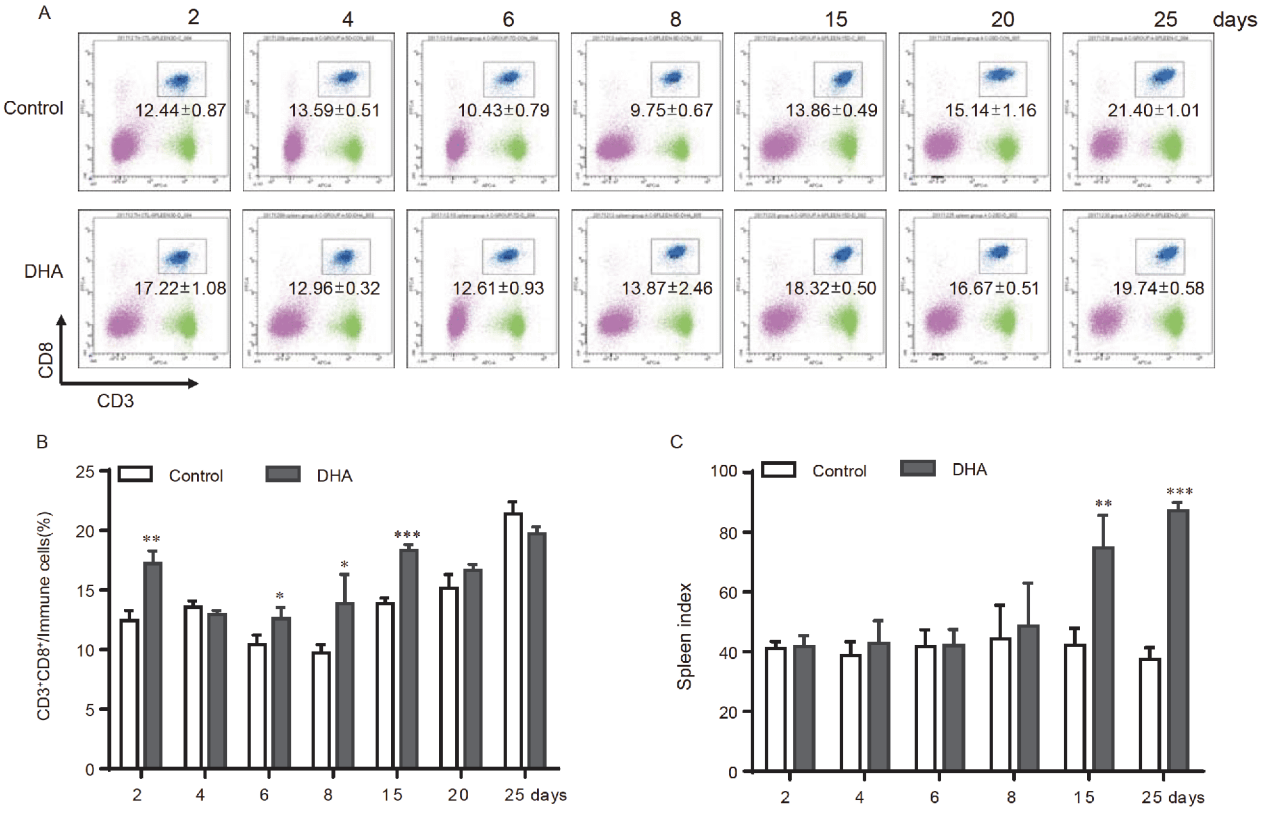

Figure 1 The number of splenic CD8$^+$ T cells in the DHA group. The population distribution of splenic CD8$^+$ T cells (A and B) was detected by flow cytometry on days 2, 4, 6, 8, 15, 20, and 25 following treatment with DHA. The spleen was observed after DHA treatment, and the spleen indexes (spleen weight (mg)×10/body weight (g) × 100%) were calculated. C, The spleen index of the control and DHA groups. The results are representative of three independent experiments with three to five mice per group per experiment. Data are presented as the mean±SD. *, P<0.05; **, P<0.01; ***, P<0.001.

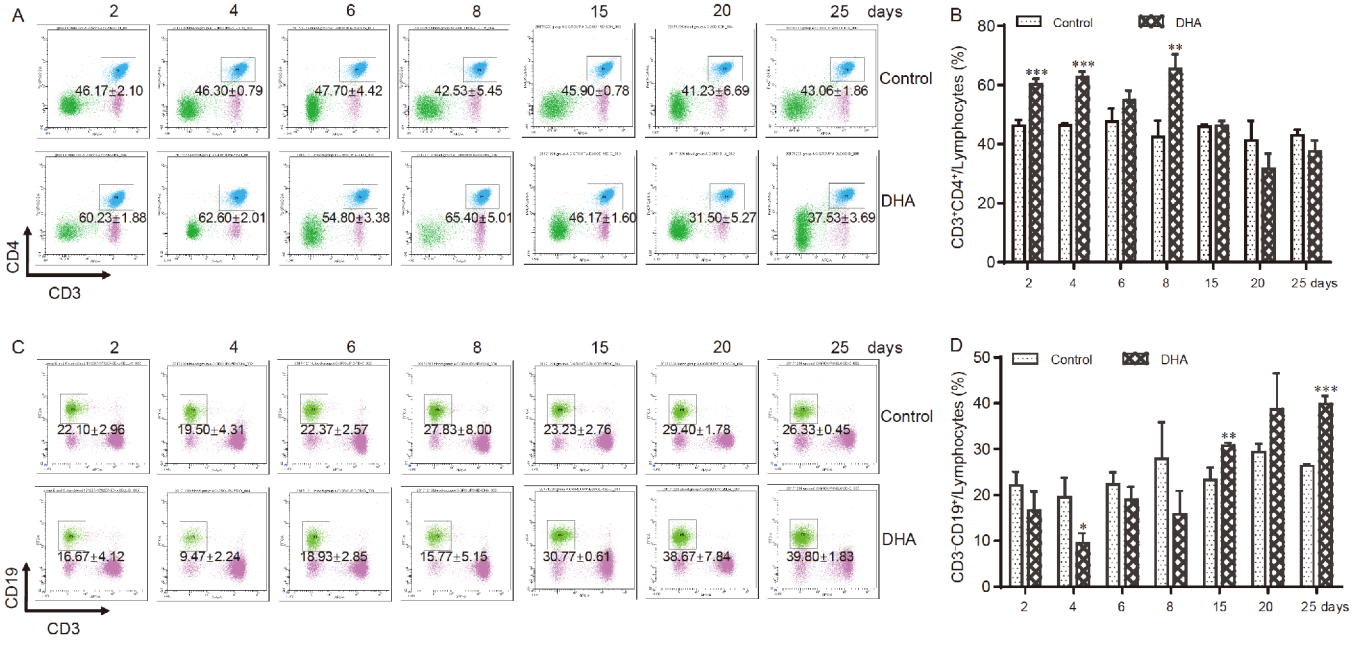

Figure 2 DHA stimulated the proliferation of circulatory Th cells, but down-regulated circulatory B cells in healthy mice over a certain period of time. The number and distribution of circulatory Th (A and B) and circulatory B cells (C and D) were detected by flow cytometry on days 2, 4, 6, 8, 15, 20, and 25 after treatment with DHA. The results are representative of three independent experiments with three to five mice per group per experiment. Data are presented as the mean±SD. *, P<0.05; **, P<0.01; ***, P<0.001.

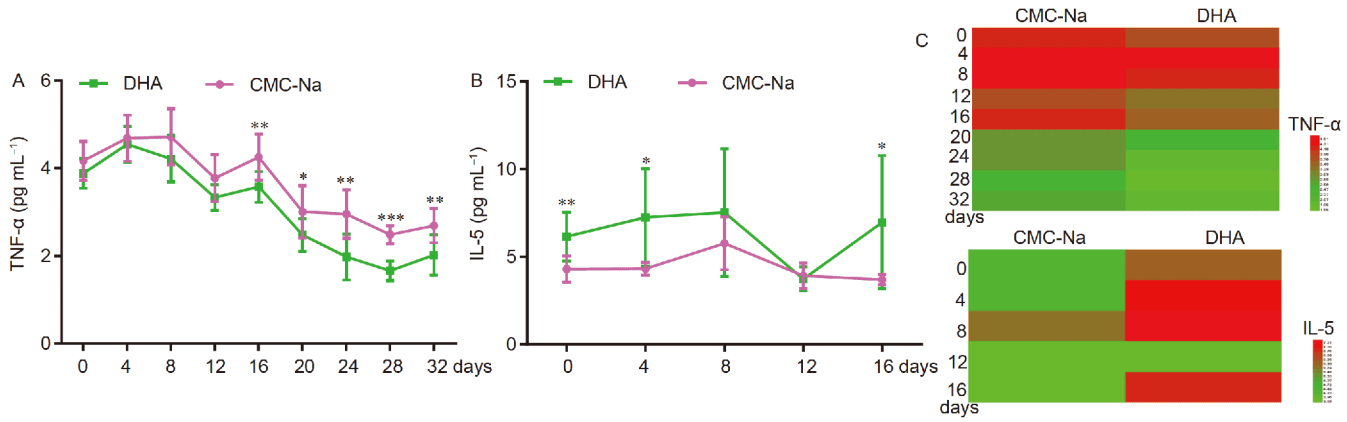

Figure 3 Detection of cytokine production during gavage by flow cytometry. A, Comparison of the Th1 cytokine levels (TNF-α) in the sera from the CMC-Na group and CMC-Na+DHA group. B, Comparison of the Th2 cytokine levels (IL-5) in the sera from the CMC-Na group and CMC-Na+DHA group. C, Heatmaps directly showing the difference in the detected cytokines. The results are representative of three independent experiments with seven to ten mice per group per experiment. Data are expressed as the mean±SD. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

DHA suppressed splenomegaly in *T. gondii*-infected mice, downregulated splenic B cells, and up-regulated splenic Th cells and CD8$^+$ T cells

The spleen indexes of *T. gondii* ME49 strain-infected mice increased on days 7, 9, and 11 post-infection; however, the spleen indexes of the DHA-treated group decreased on days 7 and 11 (Figure S2 in Supporting Information).

DHA treatment significantly deceased the number of splenic B cells in the *T. gondii*-infected group compared to that of the untreated group on days 3, 5, 7, and 9 ($P<0.05$) (Figure S3A and B in Supporting Information). However, the number of splenic Th cells in the DHA group was significantly increased compared to that of the untreated *T. gondii*-infected group on days 5 ($P<0.05$), 7 ($P<0.05$), and 9 (Figure S3C and D in Supporting Information). Furthermore, the number of splenic CD8$^+$ T cells in the DHA-treated group was significantly increased compared to that of the untreated group on days 5 ($P<0.05$), 7 ($P<0.001$), 9 ($P<0.05$), and 11 ($P>0.05$) (Figure S3E and F in Supporting Information).

DHA promoted the generation of circulating Th cells and CD8$^+$ T cells but suppressed the circulating B cells in *T. gondii*-infected mice

DHA treatment significantly decreased the number of circulating B cells in the mice of the *T. gondii*-infected group compared to that of the untreated group on days 7 ($P<0.01$), 9 ($P<0.001$), and 11 ($P<0.01$) (Figure 4A and B). However, the number of circulating Th cells in the DHA-treated group was significantly increased compared to that of the untreated group on days 7, 9, and 11 ($P<0.05$) (Figure 4C and D). Similarly, the number of circulating CD8$^+$ T cells in the DHA-treated group was significantly increased compared to that of the untreated *T. gondii*-infected group on days 5 ($P>0.05$), 7 ($P<0.01$), 9 ($P<0.05$), and 11 ($P>0.05$) (Figure 4E and F).

DHA suppresses the proinflammatory cytokine responses in *T. gondii*-infected mice

The expression profiles of IL-2, TNF-α, IFN-γ, IL-4, IL-5, IL-17A, and IL-22 in the sera of DHA-treated *T. gondii*-infected and untreated mice were monitored on days 3, 5, 7, 9, and 11. The expression of TNF-α, IL-5, IL-17A, and IL-22 was increased after *T. gondii*-infection (Figure 5A–E); however, cytokine expression was significantly decreased on days 7, 9, and 11 after DHA treatment (Figure 5A–E).

DHA suppressed splenomegaly in *P. berghei*-infected mice and up-regulated the number of splenic CD8$^+$ T, NK, and NKT cells

The spleen indexes of the *P. berghei*-infected group increased on days 4, 7, 10, 12, and 14 post-infection. However, the spleen indexes of the DHA-treated group decreased significantly on days 4 ($P<0.01$), 10 ($P<0.01$), 12 ($P<0.001$), and 14 ($P<0.001$) (Figure S4A in Supporting Information). Furthermore, the number of splenic CD8$^+$ T cells in the *P. berghei*-infected group significantly increased following DHA treatment compared to that of the untreated group on days 8, 10, 12, and 14 ($P<0.001$) (Figure 6A and B). The number of splenic NK and NKT cells in the DHA-treated group was also significantly increased compared to that of the untreated *P. berghei*-infected group on days 7, 8, 10, 12, and 14 ($P<0.001$) (Figure 7A–C). Similarly, DHA treatment significantly increased the number of circulating Th, NK, and NKT cells from days 4 to 14 ($P<0.01$) (Figures 8A and B, 9A–C).

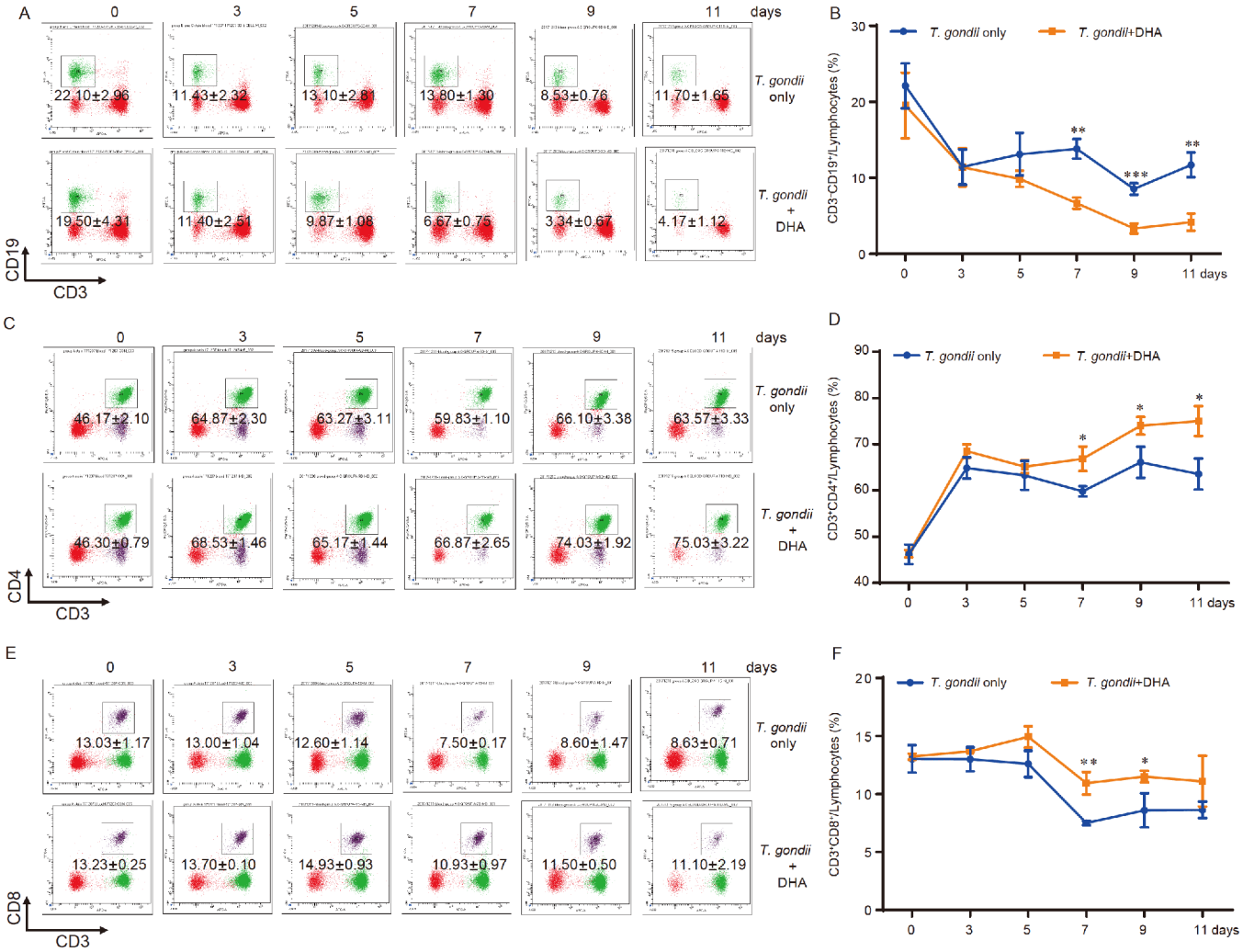

Figure 4 The ratio of circulating Th, $CD8^+$ T, and B cells in the *T. gondii* (ME49 strain) and the treatment groups. B cells (A and B), Th cells (C and D), and $CD8^+$ T cells (E and F) were detected by flow cytometry on days 0, 3, 5, 7, 9, and 11 after DHA treatment. A, Representative dot plots of $CD3^-CD19^+$ B cells among the circulating lymphocytes. B, Comparison of the number of B cells in the circulation between the ME49 and treatment groups. C, Representative dot plots of $CD3^+CD4^+$ Th cells among the circulating lymphocytes. D, Comparison of the number of Th cells in the circulation between the ME49 and treatment groups. E, Representative dot plots of $CD8^+$ T cells among the circulating lymphocytes. F, Comparison of the number of $CD8^+$ T cells in the circulation between the ME49 and treatment groups. The results are representative of three independent experiments with three to five mice per group per experiment. Data are expressed as the mean±SD. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

DHA suppresses proinflammatory cytokine responses in *P. berghei*-infected mice

The expression profiles of TNF-α, IFN-γ, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-17A, and IL-22 in the serum of DHA-treated *P. berghei*-infected and untreated mice were monitored on days 0, 4, 7, 10, 12, and 14. The expression of IL-2, TNF-α, IFN-γ, IL-4, IL-6, and IL-10 increased after infection (Figure 10A–D, F, G, and K). However, the expression of these cytokines significantly decreased on days 4, 7, 10, 12, and 14 following DHA treatment (Figure 10A–D, F, G, and K). The expression of IL-5 and IL-22 also significantly increased on days 7, 10, 12, and 14 after DHA treatment (Figure 10E, I–K).

DISCUSSION

Artemisinin was first extracted from *Artemisia annua* in 1972 by Chinese researchers, and was subsequently characterized as an effective antimalarial treatment (Listed, 1979). Since then, artemisinin and its derivatives have been extensively used as a first line treatment for malaria (Li et al., 1994). Moreover, recent reports indicated that artemisinin also has an inhibitory effect on other pathogens (e.g., *Schistosoma japonicum* and *S. mansoni*), primarily at the cercarial stage (Xiao et al., 2000; Utzinger et al., 2001); however, the molecular mechanisms remain poorly understood. Apart from its anti-malarial effects, the immune regulatory function of artemisinin has been recognized (Li et al.,

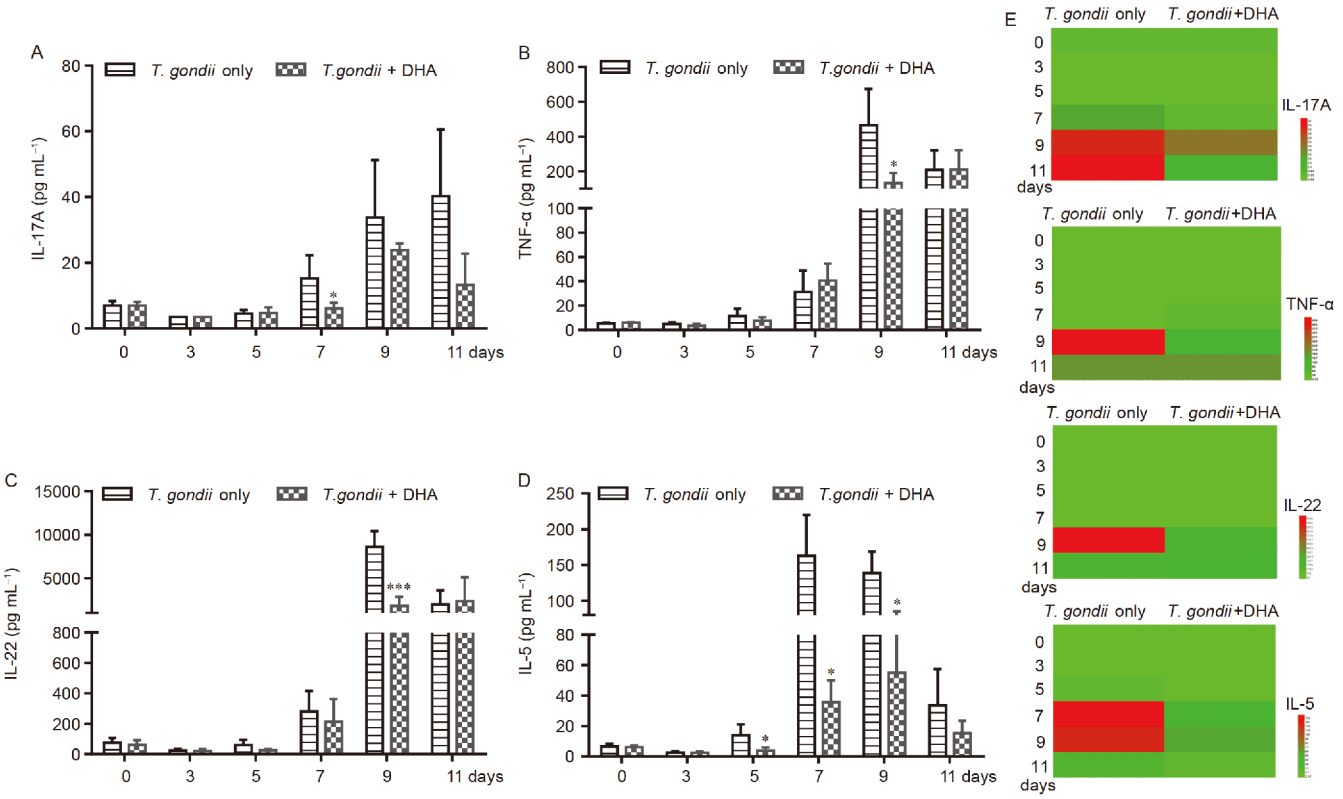

Figure 5 Detection of cytokine production during infection with *T. gondii* and after DHA treatment via flow cytometry. A and C, Comparison of type 17 cytokine levels (IL-17A and IL-22) in the sera collected from the ME49 group and the treatment group. B, Comparison of Th1 cytokine levels (TNF-α) in the sera collected from the ME49 group, and the treatment group. D, Comparisons of Th2 cytokine levels (IL-5) in the sera collected from the ME49 group and the treatment group. E, Heatmaps directly showing the difference in the level of detected cytokine. The results are representative of three independent experiments with three to five mice per group per experiment. Data are expressed as the mean±SD. *, P<0.05; **, P<0.01; ***, P<0.001.

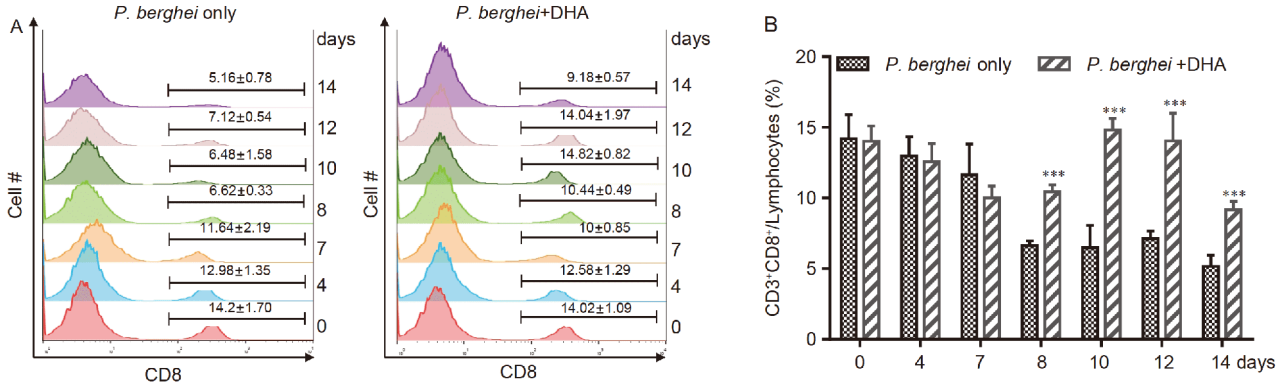

Figure 6 The number of splenic CD8$^+$ T cells in the *P. berghei* (ANKA strain)-infected and the DHA-treated group. CD8$^+$ T cells (A and B) were detected by flow cytometry on days 0, 4, 7, 8, 10, 12, and 14 post-infection. A, Representative histograms of CD8$^+$ T cells among the circulating lymphocytes. B, Comparison of the number of CD8$^+$ T cells in the circulation between the ANKA and DHA-treatment groups. The results are representative of three independent experiments with five to seven mice per group per experiment. Data are expressed as the mean±SD. *, P<0.05; **, P<0.01; ***, P<0.001.

2012; Hou et al., 2014). DHA treatment has been shown to effectively reduce the onset of autoimmune encephalomyelitis (EAE) through modulating mTOR pathway (Zhao et al., 2012). Artemisinin could suppress both T cell mediated delayed-type hypersensitivity (DTH) and B cell-mediated quantitative hemolysis of SRBC (QHS) reactions. Further, the ability of mouse spleen cells to produce IL-2 and the proliferative response to T cell mitogen Con A and B cell mitogen LPS were inhibited, suggesting a potential role for artemisinin as immune modulating agents (Zhou et al., 2010). Moreover, previous studies have shown that DHA has certain therapeutic effects on mice with systemic lupus erythematosus (SLE) by decreasing proinflammatory cytokine TNF-α production from macrophage (Dong et al., 2003; Li et

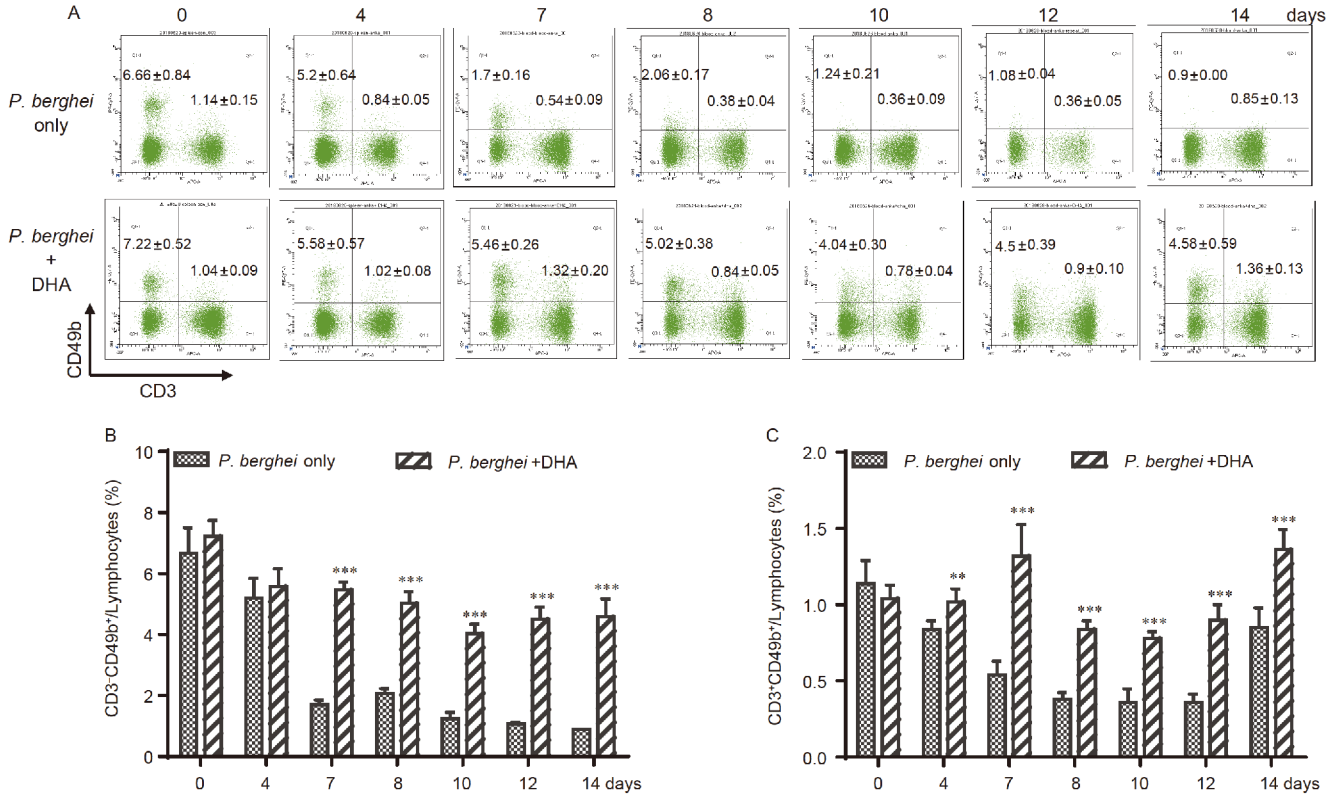

Figure 7 The number of splenic NK and NKT cells of the *P. berghei* (ANKA strain)-infected and DHA-treated group. Splenic NK cells (A and B) and NKT cells (A and C) were detected by flow cytometry on days 0, 4, 7, 8, 10, 12, and 14 post-infection. A, Representative dot plots of $CD3^-CD49b^+$ NK cells and $CD3^+CD49b^+$ NKT cells among the splenic lymphocytes. B, Comparison of the number of NK cells among the splenic lymphocytes between the ANKA and DHA-treated groups. C, Comparison of the number of NKT cells among the splenic lymphocytes between the ANKA and DHA-treated groups. The results are representative of three independent experiments with five to seven mice per group per experiment. Data are expressed as the mean±SD. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

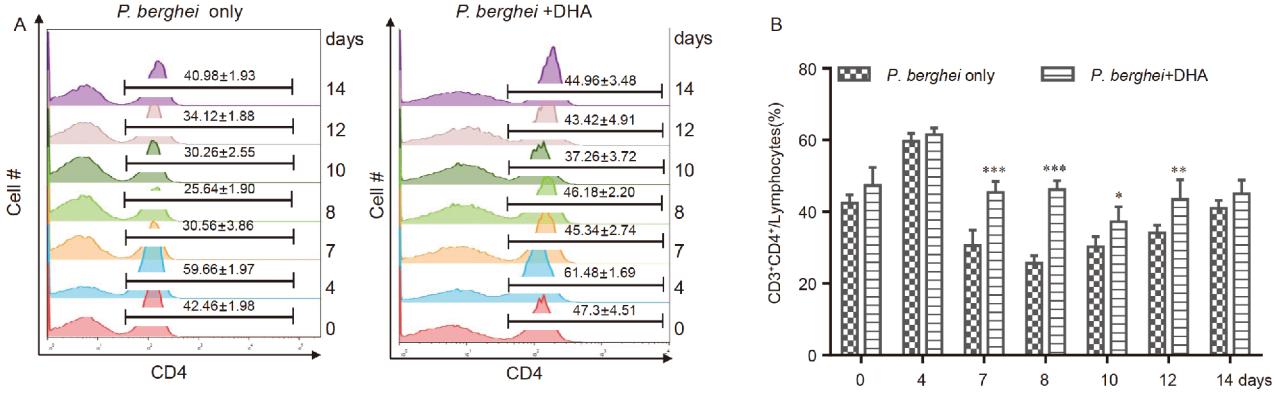

Figure 8 The number of circulating Th cells in the *P. berghei* (ANKA strain)-infected and DHA-treated group. Th cells (A and B) were detected by flow cytometry on days 0, 4, 7, 8, 10, 12, and 14 post-infection. A, Representative histogram of $CD3^+CD4^+$ Th cells among the circulating lymphocytes. B, Comparison of the number of Th cells among the circulating lymphocytes between the ANKA and DHA-treated groups. The results are representative of three independent experiments with five to seven mice per group per experiment. Data representative of the mean±SD. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

al., 2006). Thus, DHA may function to improve the symptoms of SLE by modulating B cells, T cells, pathogenic anti-dsDNA IgG autoantibodies and cytokines (Li et al., 2006; Tsokos, 2011). It has been hypothesized that DHA may be capable of suppressing the activation of the TLR4-IRF signaling pathway triggered by lipopolysaccharide (LPS) in the splenocytes of MRL/lpr mice (Huang et al., 2014). SM934, a derivative of artemisinin, limited the glomerulonephritis in the MRL/lpr mice by inhibiting Th1 and Th17 responses and prolong the mouse lifespan (Hou et al., 2011). Furthermore, DHA was also found to inhibit the growth of cancer cells (Du et al., 2013; Zhao et al., 2015; Efferth, 2017; Shi et al., 2017;

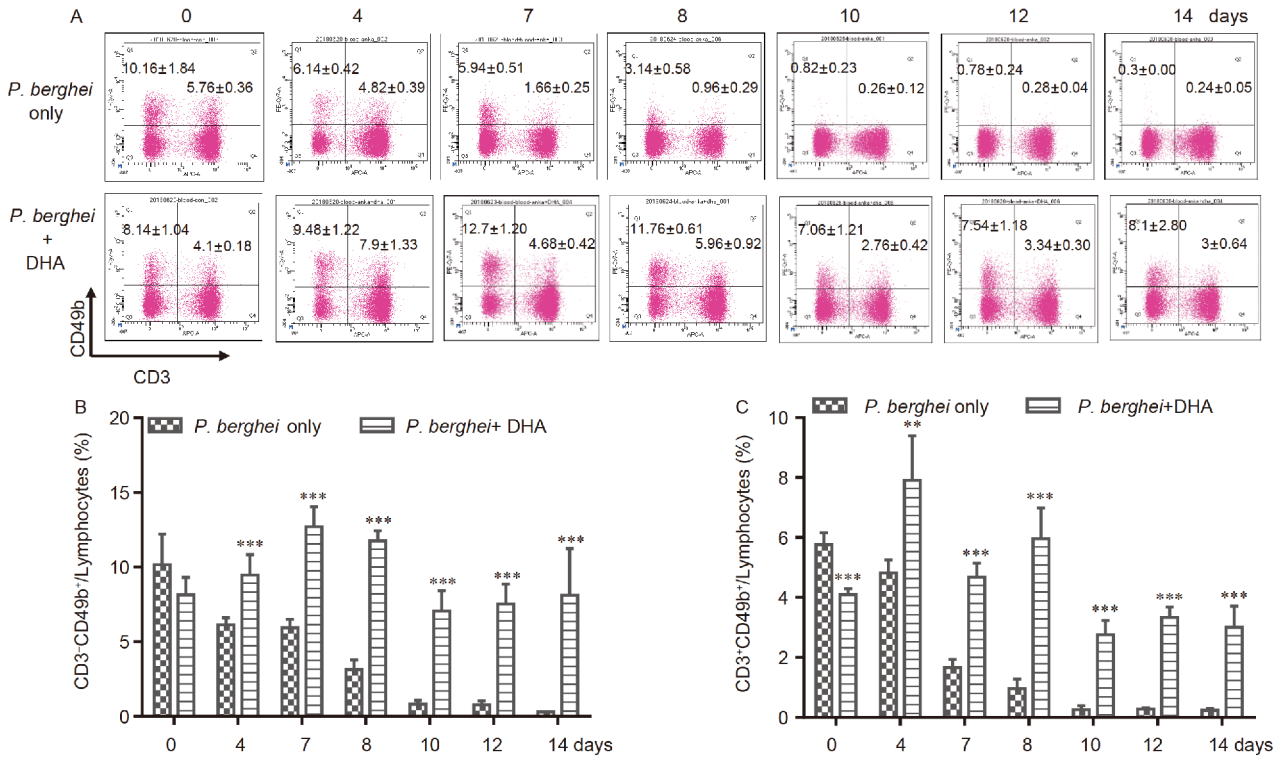

Figure 9 The number of circulating NK and NKT cells in the *P. berghei* (ANKA strain)-infected and DHA-treated groups. Circulating NK cells (A and B) and NKT cells (A and C) were detected by flow cytometry on days 0, 4, 7, 8, 10, 12, and 14 post-infection. A, Representative dot plots of $CD3^-CD49b^+$ NK cells and $CD3^+CD49b^+$ NKT cells among the circulating lymphocytes. B, Comparison of the number of NK cells among the circulating lymphocytes between the ANKA and DHA-treated groups. C, Comparison of the number of NKT cells among the circulating lymphocytes between the ANKA and DHA-treated groups. The results are representative of three independent experiments with five to seven mice per group per experiment. Data are expressed as the mean ±SD. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

Xu et al., 2017). Following treatment with DHA, both C6 glioma (Xu et al., 2017) and acute myeloid leukemia (AML) cells (Zhao et al., 2015) were found to exhibit progressive apoptosis and proliferation inhibition. Furthermore, in both esophageal and human tongue squamous cell carcinomas, treatment with DHA was found to reduce the viability of cancer cells in a dose-dependent manner (Du et al., 2013; Shi et al., 2017). Studies have also demonstrated that DHA can induce the apoptosis of human gastric cancer cells via the c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (p38 MAPK) signaling pathways (Sun et al., 2013; Zhang et al., 2017).

To further understand the potential function of DHA on the modulation of the immune system, we investigated the changes in the host immune response following DHA treatment in the context of two protozoan parasite infections: *T. gondii* and *P. bergheri*. While *T. gondii* is not highly sensitive to DHA, *P. bergheri* is extremely sensitive. In general, we found that DHA could beneficially modulate the host immune response. First, DHA increased the spleen index by promoting splenic $CD8^+$ T and Th cells, but suppressed the proliferation of circulating B cells. This finding indicates that DHA can specifically inhibit the generation of B cells, as well as promote the proliferation of $CD8^+$ T cells and Th cells (Figures 1 and 2). These findings are in line with a previous report, indicating that treatment with artesunate abolished GC B cells (Hou et al., 2014), as well as the findings of the study by You-You Tu et al. that DHA treatment inhibited the proliferation of B cells and autoantibody production (Xu and Chen, 2002; Li et al., 2006). However, in mice infected with either *T. gondii* or *P. berghei*, the spleen indexes in the DHA-treated groups were significantly reduced compared to that of the untreated groups (Figures S2 and S4 in Supporting Information). It has been established that the immune system becomes activated in response to infection. In the context of a malaria infection, the spleen plays a critical role in the clearance of infected erythrocytes, thus macrophages and other immune cells (i.e., $CD8^+$ T cells) are extensively recruited to this organ. Furthermore, parasitic factors will induce an extensive inflammatory reaction in the spleen. Thus, the reduced spleen indexes observed in the DHA-treated group in the present study suggest that DHA may beneficially suppress the over-reaction of the immune response.

Several concordant studies have documented the presence of high levels of TNF-α in SLE patients (Studnicka-benke et al., 1996; Aderka et al., 2010) and TNF-inhibitory agents have been used for the treatment of SLE (Shakoor et al.,

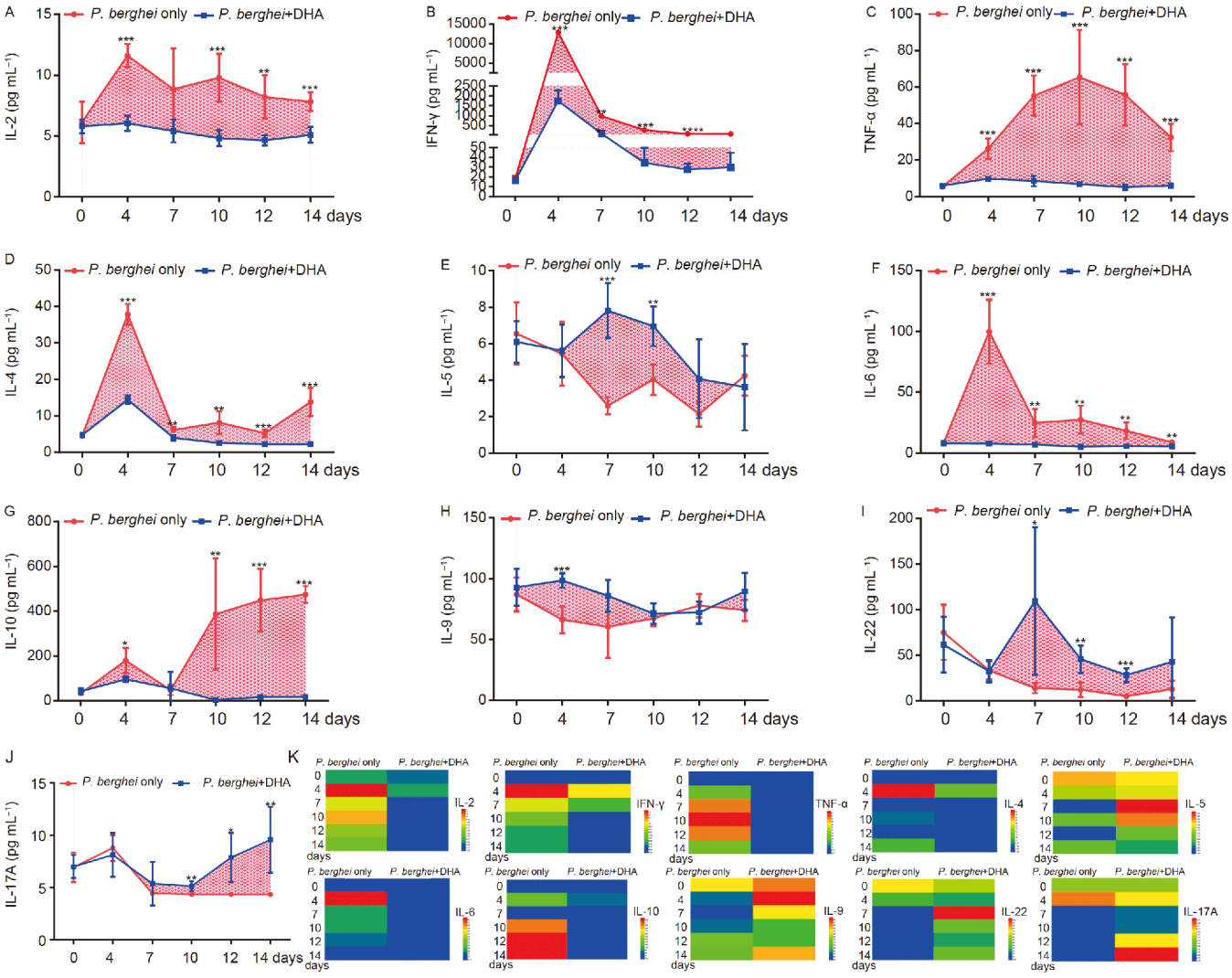

Figure 10 Detection of cytokine production during infection with *P. berghei* (ANKA strain) and after DHA treatment by flow cytometry. A-C, Comparison of Th1 cytokine levels (IL-2, IFN-γ, and TNF-α) in the sera from the ANKA group and DHA-treated group. D-G, Comparison of Th2 cytokine levels (IL-4, IL-5, IL-6, and IL-10) in the sera collected from the ANKA group and DHA-treated group. H, Comparison of the Th9 cytokine levels (IL-9) in the sera from the ANKA group and DHA-treated group. I and J, Comparison of the type 17 cytokine levels (IL-22 and IL-17A) in the sera from the ANKA group and DHA-treated group. K, Heatmaps directly showing the difference in the cytokines detected. The results are representative of three independent experiments with five to seven mice per group per experiment. Data are expressed as the mean±SD. *, P<0.05; **, P<0.01; ***, P<0.001.

2002). Studies have also indicated that the dysregulated, continuously elevated expression of IL-5 in SLE-prone mice may directly or indirectly mediate the skewed signaling of the proliferation and differentiation of self-antigen-activated B1 cells, thereby suppressing the development of autoimmune disease (Wen et al., 2004). Our present study found that DHA could reduce the production of the Th1 cytokine, TNF-α, and elevate the Th2 cytokine, IL-5, in healthy mice (Figure 3), which supports the application of DHA for the treatment of SLE.

In *T. gondii* infection, the generation of parasite-specific $CD4^+$ and $CD8^+$ cells was found to contribute to the host defence against *T. gondii* through the production of IFN-γ and other pro-inflammatory cytokines (e.g., TNF-α, IL-6, and IL-1) (Sibley et al., 1991; Langermans et al., 1992). $CD8^+$ T cells have been reported to be essential for controlling chronic infections (Bhadra et al., 2011a) and played a key role in suppressing *T. gondii* in chronically infected hosts and preventing parasite reactivation (Bhadra et al., 2011b). Moreover, $CD4^+$ T cells released IL-2, which promoted $CD8^+$ T cell proliferation (Munoz et al., 2015). Furthermore, previous studies have found that incubating tachyzoites with purified secretory immunoglobulin A (IgA) from women with acute toxoplasmosis is associated with a 50%-75% reduction in infected enterocytes *in vitro* (Mack and Mcleod, 1992). More recently, *T. gondii*-specific IgM was shown to prevent cellular invasion and limit the systemic dissemination of tachyzoites during the early phase of acute *T. gondii* infection (Couper et al., 2005). Thus, B cells and different antibody classes appear to contribute to protection against *T.*

gondii infection. In our experiments, the number of splenic and circulating $CD8^+$ T cells and Th cells in *T. gondii*-infected mice were significantly increased following DHA treatment (Figure 4). This finding strongly suggests that DHA enhances the host's cellular immune response, which is also in line with the findings of previous studies (Feng et al., 2012). However, DHA decreased the number of B cells (Figure 4) in *T. gondii* infected mice, which indicated the primary effect of DHA was to promote cellular immune responses.

In patients with toxoplasmosis, the level of serum IL-5, IL-6, and TNF-α were found to be significantly elevated, which was indicative of an inflammatory state (Matowicka-Karna et al., 2009; Meira et al., 2014). It has also been shown that the level of TNF-α mRNA expression, a contributing factor associated with neuropathic pain, was significantly increased in infected mice in comparison with uninfected BALB/c mice (Mahmoudvand et al., 2016). Further, IL-17A is a proinflammatory cytokine produced by Th17 cells that contributes to various inflammatory responses (Stumhofer et al., 2006; Tajima et al., 2008). IL-22 has also been reported to be a potential factor contributing to pathogenic inflammation in the intestine following oral *T. gondii* infection (Wilson et al., 2010). In the present study, the level of serum IL-17A, TNF-α, IL-22, and IL-5 significantly increased in mice following *T. gondii* infection. However, these cytokines were suppressed following treatment with DHA for a certain period of time (Figure 5). Therefore, DHA can beneficially regulate the immune response to prevent an overt inflammatory reaction.

Following *P. berghei* infection, Th1 cytokines and $CD8^+$ T cells are critical for protection against malaria (Schofield et al., 1987). Moreover, we found that the number of splenic $CD8^+$ T cells decreased following *P. berghei* infection, whereas there was an increase in this cell type following DHA treatment (Figure 6). Previous studies have shown that IFN-γ could limit the number of splenic T cells during a *P. berghei* ANKA infection by promoting cellular apoptosis (Villegas-Mendez et al., 2011). In the present study, there was an initial increase in the level of IFN-γ following infection with *P. berghei*, which decreased following treatment with DHA (Figure 10). This finding suggests that DHA can promote the generation of splenic $CD8^+$ T cells during malarial parasite infection. Similarly, the responses of both splenic and circulatory NK and NKT cells, as well as increased inflammatory cytokine production benefited the host following DHA treatment (Figures 7-10).

In summary, the present findings demonstrate that DHA modulated the host immune response by promoting cellular immunity and suppressing B cell responses in both healthy and infected mice, irrespective of the pathogens. Collectively, these data support the application of artemisinin and its derivatives as immune modulating agents in addition to their anti-malarial function.

MATERIALS AND METHODS

Animals

BALB/C female mice, 4–6 weeks of age, were purchased from the Liaoning Changsheng Biological Technology Company in China. Mice were housed under pathogen-free conditions and acclimatized for one week before experimentation. All experimental procedures were conducted in accordance with the animal husbandry guidelines of Shenyang Agricultural University. The Ethical Committee of Shenyang Agricultural University approved the laboratory animal experiments (Permit No. SYXK<Liao>2017-0001).

DHA preparation and drug administration

A dose of 0.1 g sodium carboxymethyl cellulose (CMC-Na) was dissolved in 20 mL warm distilled water, stirred with a magnetic stirrer for 1 h. When the solution was restored to room temperature, 0.2 g DHA was added and continually stirred for 1 h in the dark. To determine the immune regulatory function of DHA, the mice were divided into seven groups: (1) control group; (2) CMC-Na group; (3) CMC-Na +DHA group; (4) *T. gondii* group; (5) *T. gondii*+DHA group; (6) *P. berghei* group; and (7) *P. berghei*+DHA group. All of the mice in the control group were healthy. The CMC-Na group was perfused into the stomach of mice with 200 μL CMC-Na suspension daily. The CMC-Na+DHA, *T. gondii* +DHA, and *P. berghei*+DHA groups were perfused into the stomach of mice daily with 200 μL DHA (2 mg) in a CMC-Na solution.

Parasites and infection

For the experiments involving *T. gondii*, the ME49 strain was used. The peritoneal fluid of the infected mice was repeatedly squeezed with a 5 mL curved syringe, and the parasites were filtered through a 5.0 μm filter and checked under the microscope to ensure that they were all a single parasite. For the infection assay, mice (*T. gondii* and *T. gondii* +DHA groups) were both infected with 100 *T. gondii* bradyzoites in 0.2 mL 1× phosphate-buffered saline (PBS, pH 7.4). Mice in the *T. gondii*+DHA group were orally administered daily with 200 μL DHA (2 mg) in a CMC-Na solution three days post-infection, and the uninfected group was given only CMC-Na in the same manner as the infected group.

In the malaria parasite group, the *P. berghei* ANKA strain was used. Mice were intraperitoneally infected with 1×10^5 parasitized RBCs. DHA was administered daily as 200 μL DHA (2 mg) in CMC-Na for three days post-parasite infec-

tion. The uninfected group was administered only CMC-Na in the same manner as the infected group.

Preparation of splenic and circulatory immune cells

All mice were instantly sacrificed before the spleens were harvested and blood was collected. The spleens were cut into pieces and minced. Red blood cells (RBCs) were depleted using a RBC lysis solution (155 mmol L^{-1} NH_4Cl, 10 mmol L^{-1} $KHCO_3$, 0.11 mmol L^{-1} $EDTA.Na_2$, pH 7.2). Anticoagulated blood was obtained and the circulating immune cells were isolated by depleting the RBCs as described above.

To determine the possible intrinsic immune regulation of DHA in healthy mice, both splenic and circulating immune cells were collected at 2, 4, 6, 8, 15, 20, and 25 days (Figure S5A in Supporting Information) after treatment with DHA. Serum was collected on days 4, 8, 12, 16, 20, 24, 28, and 32 (Figure S5B in Supporting Information) after treatment. Further splenic and circulating immune cells, as well as the serum of the mice infected with either *T. gondii* or *P. berghei* were obtained on days 3, 4, 5, 7, 8, 9, 10, 11, 12, and 14 post-infection (Figure S5C and D in Supporting Information).

Flow cytometry

The immune cells were adjusted to 10^7 cells mL^{-1} and stained for 15 min in the dark with Zombie NIR™ Fixable Viability dye (Biolegend#423106), which distinguishes live and dead cells since it is non-permeable to live cells, but permeable to the cells with compromised membranes. The cells were then pre-incubated with an anti-mouse CD16/32 antibody (Biolegend#101310) for 5–10 min on ice to block the non-specific binding of immunoglobulins to Fc receptors. A total of 10^6 cells in 100 μL were incubated with the following specific antibodies or isotype-matched controls at the manufacturers' recommended concentrations at 4°C for 30 min. The labelled cells were then washed twice with PBS and reserved for flow cytometric analysis. The following antibodies were all purchased from Biolegend: Pacific Blue™: anti-mouse CD45 (Biolegend#103126); APC anti-mouse CD3 (Biolegend#100236); FITC anti-mouse CD8a (Biolegend#100706); PerCP anti-mouse CD4 (Biolegend#100432); FITC anti-mouse CD19 (Biolegend#115506); PE/Cy7 anti-mouse CD49b (Biolegend#103518); Pacific Blue™ Rat IgG2b, κ Isotype Ctrl (Biolegend#400627); APC Rat IgG2b; κ Isotype Ctrl (Biolegend#400611); FITC Rat IgG2a; κ Isotype Ctrl (Biolegend#400505); PerCP Rat IgG2b; κ Isotype Ctrl (Biolegend#400629); PE/Cy7 Armenian Hamster IgG Isotype Ctrl (Biolegend#400922); LEAF™ Purified anti-mouse CD16/32 antibody (Biolegend#101310); and Zombie NIR™ Fixable Viability dye (Biolegend#423106).

Cytokine detection

The cytokine profiles in the mice of each group were analyzed using a LEGENDplex Mouse Th Cytokine Panel (BioLegend#740005) in accordance with the manufacturer's instructions. Bead ID was used to associate a bead population with a particular analyte using the LEGENDplex™ Data Analysis software. Samples were treated according to the BioLegend standard protocol and examined using FACSAria III (BD Biosciences) driven by FACSDiva software (BD Biosciences).

Statistical analysis

All data were analyzed using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA) and PASW Statistics 18 (IBM Co., Armonk, NY, USA). The results were analyzed using a two-tailed paired *t*-test. The mean and standard deviation (SD) were determined using three to five biological replicates. A threshold of $P<0.05$ was considered significant. Cytokine calculations were performed using the LEGENDplex 8.0 application (VigeneTech Inc., Carlisle, MA, USA). A heatmap was drawn using Heml 1.0.3.7 software (Huazhong University of Science and Technology, Hubei, Wuhan, China).

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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References

Abdin, A.A., Ashour, D.S., and Shoheib, Z.S. (2013). Artesunate effect on schistosome thioredoxin glutathione reductase and cytochrome c peroxidase as new molecular targets in *Schistosoma mansoni*-infected mice. Biomed Environ Sci, 26, 953–961.

Aderka, D., Wysenbeek, A., Engelmann, H., Cope, A.P., Brennan, F., Molad, Y., Hornik, V., Levo, Y., Maini, R.N., Feldmann, M., et al. (2010). Correlation between serum levels of soluble tumor necrosis factor receptor and disease activity in systemic lupus erythematosus. Arthrit Rheumat 36, 1111–1120.

Bhadra, R., Gigley, J.P., and Khan, I.A. (2011a). The CD8 T-cell road to immunotherapy of toxoplasmosis. Immunotherapy 3, 789–801.

Bhadra, R., Gigley, J.P., Weiss, L.M., and Khan, I.A. (2011b). Control of Toxoplasma reactivation by rescue of dysfunctional $CD8^+$ T-cell response via PD-1-PDL-1 blockade. Proc Natl Acad Sci USA 108, 9196–9201.

Cheng, C., Ho, W.E., Goh, F.Y., Guan, S.P., Kong, L.R., Lai, W.Q., Leung, B.P., and Wong, W.S.F. (2011). Anti-malarial drug artesunate attenuates experimental allergic asthma via inhibition of the phosphoinositide 3-kinase/Akt pathway. PLoS ONE 6, e20932.

Chimanuka, B., Francois, G., Timperman, G., Heyden, Y.V., Holenz, J., Plaizier-Vercammen, J., and Bringmann, G. (2001). A comparison of the stage-specific efficacy of chloroquine, artemether and dioncophylline B against the rodent malaria parasite *Plasmodium*

chabaudi chabaudi in vivo. Parasitol Res 87, 795–803.
Couper, K.N., Roberts, C.W., Brombacher, F., Alexander, J., and Johnson, L.L. (2005). *Toxoplasma gondii*-specific immunoglobulin M limits parasite dissemination by preventing host cell invasion. Infect Immun 73, 8060–8068.
Cruz-González, D.J., Gómez-Martin, D., Layseca-Espinosa, E., Baranda, L., Abud-Mendoza, C., Alcocer-Varela, J., González-Amaro, R., and Monsiváis-Urenda, A.E. (2018). Analysis of the regulatory function of natural killer cells from patients with systemic lupus erythematosus. Clin Exp Immunol 191, 288–300.
Dong, Y.J., Li, W.D., and Tu, Y.Y. (2003) Effect of dihydro-qinghaosu on auto-antibody production, TNF alpha secretion and pathologic change of lupus nephritis in BXSB mice (in Chinese). Zhongguo Zhong Xi Yi Jie He Za Zhi 23, 676–679.
Du, X.X., Li, Y.J., Wu, C.L., Zhou, J.H., Han, Y., Sui, H., Wei, X.L., Liu, L., Huang, P., Yuan, H.H., et al. (2013). Initiation of apoptosis, cell cycle arrest and autophagy of esophageal cancer cells by dihydroartemisinin. Biomed Pharmacother 67, 417–424.
Dunay, I.R., Chan, W.C., Haynes, R.K., and Sibley, L.D. (2009). Artemisone and artemiside control acute and reactivated toxoplasmosis in a murine model. Antimicrobial Agents Chemother 53, 4450–4456.
Efferth, T. (2017). From ancient herb to modern drug: *Artemisia annua* and artemisinin for cancer therapy. Seminars Cancer Biol 46, 65–83.
Feng, Y., Zhu, X., Wang, Q., Jiang, Y., Shang, H., Cui, L., and Cao, Y. (2012). Allicin enhances host pro-inflammatory immune responses and protects against acute murine malaria infection. Malar J 11, 268.
Gordon, C., Li, C.K., and Isenberg, D.A. (2009). Systemic lupus erythematosus. N Engl J Med 38, 73–80.
Guo, Y., Xu, P., Xuan, Y., Wu, L., and Li, S. (1997) Effect of artesunate on vltrastructure of schistosomula *Schistosoma japonicum* (in Chinese). Chin J Schistosom Contr 9, 34–36.
He, Y., Fan, J., Lin, H., Yang, X., Ye, Y., Liang, L., Zhan, Z., Dong, X., Sun, L., and Xu, H. (2011). The anti-malaria agent artesunate inhibits expression of vascular endothelial growth factor and hypoxia-inducible factor-1α in human rheumatoid arthritis fibroblast-like synoviocyte. Rheumatol Int 31, 53–60.
Hou, L.F., He, S.J., Li, X., Yang, Y., He, P.L., Zhou, Y., Zhu, F.H., Yang, Y. F., Li, Y., Tang, W., et al. (2011). Oral administration of artemisinin analog SM934 ameliorates lupus syndromes in MRL/lpr mice by inhibiting Th1 and Th17 cell responses. Arthrit Rheumat 63, 2445–2455.
Hou, L., Block, K.E., and Huang, H. (2014). Artesunate abolishes germinal center B cells and inhibits autoimmune arthritis. PLoS ONE 9, e104762.
Huang, X., Xie, Z., Liu, F., Han, C., Zhang, D., Wang, D., Bao, X., Sun, J., Wen, C., and Fan, Y. (2014). Dihydroartemisinin inhibits activation of the Toll-like receptor 4 signaling pathway and production of type I interferon in spleen cells from lupus-prone MRL/lpr mice. Int Immunopharmacol 22, 266–272.
Klonis, N., Crespo-Ortiz, M.P., Bottova, I., Abu-Bakar, N., Kenny, S., Rosenthal, P.J., and Tilley, L. (2011). Artemisinin activity against *Plasmodium falciparum* requires hemoglobin uptake and digestion. Proc Natl Acad Sci USA 108, 11405–11410.
Langermans, J.A., Van der Hulst, M.E., Nibbering, P.H., Hiemstra, P.S., Fransen, L., and Van Furth, R. (1992). IFN-gamma-induced L-arginine-dependent toxoplasmastatic activity in murine peritoneal macrophages is mediated by endogenous tumor necrosis factor-alpha. J Immunol 148, 568–574.
Lewis, J.E., Fu, S.M., and Gaskin, F. (2013). Autoimmunity, end organ damage, and the origin of autoantibodies and autoreactive T cells in systemic lupus erythematosus. Discov Med 15, 85–92.
Li, G.Q., Guo, X.B., Fu, L.C., Jian, H.X., and Wang, X.H. (1994). Clinical trials of artemisinin and its derivatives in the treatment of malaria in China. Trans R Soc Trop Med Hygiene 88, 5–6.
Li, T., Chen, H., Wei, N., Mei, X., Zhang, S., Liu, D., Gao, Y., Bai, S., Liu, X., and Zhou, Y. (2012). Anti-inflammatory and immunomodulatory mechanisms of artemisinin on contact hypersensitivity. Int Immunopharmacol 12, 144–150.
Li, T., Chen, H., Yang, Z., Liu, X.G., Zhang, L.M., and Wang, H. (2013a). Evaluation of the immunosuppressive activity of artesunate *in vitro* and *in vivo*. Int Immunopharmacol 16, 306–312.
Li, W., Dong, Y., Tu, Y., and Lin, Z. (2006). Dihydroarteannuin ameliorates lupus symptom of BXSB mice by inhibiting production of TNF-alpha and blocking the signaling pathway NF-kappa B translocation. Int Immunopharmacol 6, 1243–1250.
Li, X., Li, T.T., Zhang, X.H., Hou, L.F., Yang, X.Q., Zhu, F.H., Tang, W., and Zuo, J.P. (2013b). Artemisinin analogue SM934 ameliorates murine experimental autoimmune encephalomyelitis through enhancing the expansion and functions of regulatory T cell. PLoS ONE 8, e74108.
Li, Y. (2012). Qinghaosu (artemisinin): Chemistry and pharmacology. Acta Pharmacol Sin 33, 1141–1146.
Listed, N. (1979). Antimalaria studies on Qinghaosu. Chin Med J (Engl) 92, 811–816.
Lourenco, E.V., Procaccini, C., Ferrera, F., Iikuni, N., Singh, R.P., Filaci, G., Matarese, G., Shi, F.D., Brahn, E., Hahn, B.H., et al. (2009). Modulation of p38 MAPK activity in regulatory T cells after tolerance with anti-DNA Ig peptide in (NZB × NZW)F1 lupus mice. J Immunol 182, 7415–7421.
Mack, D.G., and Mcleod, R. (1992). Human *Toxoplasma gondii*-specific secretory immunoglobulin A reduces *T. gondii* infection of enterocytes *in vitro*. J Clin Invest 90, 2585–2592.
Mahmoudvand, H., Ziaali, N., Ghazvini, H., Shojaee, S., Keshavarz, H., Esmaeilpour, K., and Sheibani, V. (2016). *Toxoplasma gondii* infection promotes neuroinflammation through cytokine networks and induced hyperalgesia in BALB/c mice. Inflammation 39, 405–412.
Matowicka-Karna, J., Dymicka-Piekarska, V., and Kemona, H. (2009). Does *Toxoplasma gondii* Infection Affect the Levels of IgE and Cytokines (IL-5, IL-6, IL-10, IL-12, and TNF-alpha)? Clin Dev Immunol 2009(1), 1–4.
Meira, C.S., Pereira-Chioccola, V.L., Vidal, J.E., de Mattos, C.C.B., Motoie, G., Costa-Silva, T.A., Gava, R., Frederico, F.B., and de Mattos, L.C. (2014). Cerebral and ocular toxoplasmosis related with IFN-γ, TNF-α, and IL-10 levels. Front Microbiol 5, 492.
Meshnick, S.R. (2002). Artemisinin: mechanisms of action, resistance and toxicity. Int J Parasitol 32, 1655–1660.
Munoz, M., Liesenfeld, O., and Heimesaat, M.M. (2015). Immunology of *Toxoplasma gondii*. Immunol Rev 240, 269–285.
Nagamune, K., Beatty, W.L., and Sibley, L.D. (2007). Artemisinin induces calcium-dependent protein secretion in the protozoan parasite *Toxoplasma gondii*. Eukaryot Cell 6, 2147–2156.
Qinghaosu Research Group, Institute of Biophysics Academia Sinica. (1980). Crystal structure and absolute configuration of Qinghaosu. Sci China Ser A, 380–396.
Shakoor, N., Michalska, M., Harris, C.A., and Block, J.A. (2002). Drug-induced systemic lupus erythematosus associated with etanercept therapy. Lancet 359, 579–580.
Schofield, L., Villaquiran, J., Ferreira, A., Schellekens, H., Nussenzweig, R., and Nussenzweig, V. (1987). γ Interferon, $CD8^+$ T cells and antibodies required for immunity to malaria sporozoites. Nature 330, 664–666.
Shi, X., Wang, L., Li, X., Bai, J., Li, J., Li, S., Wang, Z., and Zhou, M. (2017). Dihydroartemisinin induces autophagy-dependent death in human tongue squamous cell carcinoma cells through DNA double-strand break-mediated oxidative stress. Oncotarget 8, 45981–45993.
Shlomchik, M.J., Craft, J.E., and Mamula, M.J. (2001). From T to B and back again: positive feedback in systemic autoimmune disease. Nat Rev Immunol 1, 147–153.
Sibley, L.D., Adams, L.B., Fukutomi, Y., and Krahenbuhl, J.L. (1991). Tumor necrosis factor-alpha triggers antitoxoplasmal activity of IFN-gamma primed macrophages. J Immunol 147, 2340–2345.
Skinner, T.S., Manning, L.S., Johnston, W.A., and Davis, T.M.E. (1996). *In vitro* stage-specific sensitivity of *Plasmodium falciparum* to quinine and artemisinin drugs. Int J Parasitol 26, 519–525.
Studnicka-Benke, A., Steiner, G., Petera, P., and Smolen, J.S. (1996).

Tumour necrosis factor alpha and its soluble receptors parallel clinical disease and autoimmune activity in systemic lupus erythematosus. Rheumatology 35, 1067–1074.
Stumhofer, J.S., Laurence, A., Wilson, E.H., Huang, E., Tato, C.M., Johnson, L.M., Villarino, A.V., Huang, Q., Yoshimura, A., Sehy, D., et al. (2006). Interleukin 27 negatively regulates the development of interleukin 17–producing T helper cells during chronic inflammation of the central nervous system. Nat Immunol 7, 937–945.
Sun, H., Meng, X., Han, J., Zhang, Z., Wang, B., Bai, X., and Zhang, X. (2013). Anti-cancer activity of DHA on gastric cancer—an *in vitro* and *in vivo* study. Tumor Biol 34, 3791–3800.
Tajima, M., Wakita, D., Noguchi, D., Chamoto, K., Yue, Z., Fugo, K., Ishigame, H., Iwakura, Y., Kitamura, H., and Nishimura, T. (2008). IL-6–dependent spontaneous proliferation is required for the induction of colitogenic IL-17–producing $CD8^+$ T cells. J Exp Med 205, 1019–1027.
Tsokos, G.C. (2011). Systemic lupus erythematosus. N Engl J Med 365, 2110–2121.
Utzinger, J., Chollet, J., You, J., Mei, J., Tanner, M., and Xiao, S. (2001). Effect of combined treatment with praziquantel and artemether on *Schistosoma japonicum* and *Schistosoma mansoni* in experimentally infected animals. Acta Trop 80, 9–18.
Villegas-Mendez, A., de Souza, J.B., Murungi, L., Hafalla, J.C.R., Shaw, T. N., Greig, R., Riley, E.M., and Couper, K.N. (2011). Heterogeneous and tissue-specific regulation of effector T cell responses by IFN-gamma during *Plasmodium berghei* ANKA infection. J Immunol 187, 2885–2897.
Wilson, M.S., Feng, C.G., Barber, D.L., Yarovinsky, F., Cheever, A.W., Sher, A., Grigg, M., Collins, M., Fouser, L., and Wynn, T.A. (2010). Redundant and pathogenic roles for IL-22 in mycobacterial, protozoan, and helminth infections. J Immunol 184, 4378–4390.
Wu, L., Xu, Y., Guo, Y., Xu, P., and Li, S. (1996). Studies on the effect of artesunate to the energy-metabolic enzymes (in Chinese). Chin J Schistosom Contr 5, 267–269.
Wen, X., Zhang, D., Kikuchi, Y., Jiang, Y., Nakamura, K., Xiu, Y., Tsurui, H., Takahashi, K., Abe, M., Ohtsuji, M., et al. (2004). Transgene-mediated hyper-expression of IL-5 inhibits autoimmune disease but increases the risk of B cell chronic lymphocytic leukemia in a model of murine lupus. Eur J Immunol 34, 2740–2749.
Xiao, S.H., Booth, M., and Tanner, M. (2000). The prophylactic effects of artemether against *Schistosoma japonicum* infections. Parasitol Today 16, 122–126.
Xing, C., Zhu, G., Xiao, H., Fang, Y., Liu, X., Han, G., Chen, G., Hou, C., Shen, B., Li, Y., et al. (2017). B cells regulate thymic $CD8^+$T cell differentiation in lupus-prone mice. Oncotarget 8, 89486–89499.
Xu, C.H., Liu, Y., Xiao, L.M., Guo, C.G., Zheng, S.Y., Zeng, E.M., and Li, D.H. (2017). Dihydroartemisinin treatment exhibits antitumor effects in glioma cells through induction of apoptosis. Mol Med Rep 16, 9528–9532.
Xu, L.M., Chen, X.R., and Tu, Y.Y. (2002). Effect of hydroartemisinin on lupus BXSB mice (in Chinese). Chin J Dermatovenerol Integr Trad West Med 1, 19–20.
Xu, H., He, Y., Yang, X., Liang, L., Zhan, Z., Ye, Y., Yang, X., Lian, F., and Sun, L. (2007). Anti-malarial agent artesunate inhibits TNF-alpha-induced production of proinflammatory cytokines via inhibition of NF-kappaB and PI3 kinase/Akt signal pathway in human rheumatoid arthritis fibroblast-like synoviocytes. Rheumatology 46, 920–926.
Zhang, S., Shi, L., Ma, H., Li, H., Li, Y., Lu, Y., Wang, Q., and Li, W. (2017). Dihydroartemisinin induces apoptosis in human gastric cancer cell line BGC-823 through activation of JNK1/2 and p38 MAPK signaling pathways. J Recept Signal Transduct 37, 174–180.
Zhao, X., Zhong, H., Wang, R., Liu, D., Waxman, S., Zhao, L., and Jing, Y. (2015) Dihydroartemisinin and its derivative induce apoptosis in acute myeloid leukemia through Noxa-mediated pathway requiring iron and endoperoxide moiety. Oncotarget 6, 5582–5596.
Zhao, Y.G., Wang, Y., Guo, Z., Gu, A., Dan, H.C., Baldwin, A.S., Hao, W., and Wan, Y.Y. (2012). Dihydroartemisinin ameliorates inflammatory disease by its reciprocal effects on Th and regulatory T cell function via modulating the mammalian target of rapamycin pathway. J Immunol 189, 4417–4425.
Zhou, W., Wu, J., Wu, Q., Wang, J., Zhou, Y., Zhou, R., He, P., Li, X., Yang, Y., Zhang, Y., et al. (2010). A novel artemisinin derivative, 3-(12-beta-artemisininoxy) phenoxyl succinic acid (SM735), mediates immunosuppressive effects *in vitro* and *in vivo*. Acta Pharmacol Sin 26, 1352–1358.

SUPPORTING INFORMATION

Figure S1 Detection of cytokine production after treatment with DHA via flow cytometry.

Figure S2 Spleen indexes.

Figure S3 The number of splenic Th cells, $CD8^+$ T, and B cells from the *T. gondii* ME49 strain-infected and the DHA treatment group.

Figure S4 Spleen indexes. The spleen was observed 14 days after treatment with DHA and the spleen indexes (spleen weight (mg)×10/body weight (g) × 100%) were calculated. The number of splenic Th cells and circulating $CD8^+$ T cells in the *P. berghei* (ANKA strain) infected and the DHA-treated groups.

Figure S5 Splenic immune cells, circulating immune cells, and serum collection.

The supporting information is available online at http://life.scichina.com and https://link.springer.com. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.