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Review

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Immunomodulation of artemisinin and its derivatives

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Abstract In the 1970s, artemisinin ("qinghaosu" in Chinese), a sesquiterpene lactone with an unusual peroxide bridge, was isolated from *Artemisia annua* L. It showed promising antimalarial activity, particularly by eliminating parasites resistant to chloroquine. For more than 30 years, artemisinin has contributed to worldwide health as a new type of antimalarial drug. Artemisinin and its analogs, such as dihydroartemisinin, artemether, artesunate, artemiside, artemisone, and arteether, possess not only potent antimalarial activity but also anti-viral, antifungal, anticancer, and anti-inflammatory properties. In this review, we discuss the current understanding of how artemisinin and its analogs affect the immune system and immune-related diseases.

Keywords Artemisinin · Immune system · Immune-related diseases · Macrophage · T-cell · B-cell

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1 Introduction

Because of their strong antimalarial activity even against chloroquine-resistant malarial organisms, artemisinins (artemisinin and its derivatives) are regarded as the "best hope for the treatment of malaria" by the World Health Organization [1]. The antimalarial action of artemisinin requires the presence of its peroxide bridge structure. The widely accepted mechanism for artemisinin is that it exhibits antimalarial effects due to breaking of the peroxide bridge by heme (present in hemoglobin), leading to degradation of the molecular structure of artemisinin and formation of heme–artemisinin adducts. These adducts produce free radicals that cause death of the malaria parasites. In animals, parasite-infected red blood cells are susceptible to artemisinin due to the elevated level of intracellular free radicals and associated lipid peroxidation [2–5]. In addition to their antimalarial activity, artemisinins, in different dose ranges, have been tested in experimental immune-related disease models, including those for rheumatoid arthritis (RA), multiple sclerosis (MS), experimental allergic encephalomyelitis (EAE), systemic lupus erythematosus (SLE), collagen-induced arthritis (CIA), inflammatory bowel disease (IBD), lupus nephritis, sepsis, uveitis, Alzheimer's disease, endometriosis, dermatitis, asthma, anaphylaxis, and delayed-type hypersensitivity [6–27].

More recently, it has been reported that artemisinins possess immunoregulatory properties and modulate components of the immune system. As a requirement for an investigational new drug application to the FDA, all new drugs under development must be evaluated for their potential effects on immune function, such as immunosuppression, immunomodulation, autoimmunity, hypersensitivity, and immunotoxicity. In this review, we

describe the effects of artemisinins on immune cells, including neutrophils, macrophages, T-cells, B-cells, mast cells, γδT cells, eosinophils, and basophils under physiological and pathological conditions.

2 Artemisinins and innate immunity

2.1 Artemisinins and neutrophils

At sites of acute tissue damage and infection, neutrophils are the first defenders. Artemisinins affect neutrophil counts. For patients in sub-Saharan Africa with uncomplicated *Plasmodium falciparum* malaria, treatment with artemisinin-based combination therapy caused decreased neutrophil numbers [2]. Another clinical trial in sub-Saharan Africa showed that artesunate–amodiaquine therapy caused neutropenia in patients with uncomplicated *P. falciparum* infections [28]. Prior studies had similar findings. Following treatment of malaria with artesunate–amodiaquine, HIV-infected children had higher incidence of neutropenia compared with uninfected ones [29]. Artesunate–fosmidomycin, used to treat 50 children with *P. falciparum* malaria, caused transient neutropenia in eight of them [30]. Consistent results were obtained from experimental models. Artesunate decreased neutrophil counts and other related symptoms in joint of monoarthritis rat model [31]. In rat model of lipopolysaccharide (LPS)-activated neutrophils, extracts of *A. annua* reduced production of TNF-α and PGE2 dose-dependently [32]. In a mouse model of septic lung injury, attenuate decreased sepsis-induced lung damage and mortality, relieved lung pathological syndromes and infiltration of neutrophil, and reduced TNF-α and interleukin (IL)-6 secretion through upregulated heme oxygenase-1 [33]. Artesunate displayed antioxidant effects in ovalbumin-challenged allergic asthma mice; it decreased the number of total cell, eosinophil, and neutrophil after ovalbumin challenge and reduced several oxidative injury markers in bronchoalveolar lavage fluid [10].

Another investigation revealed artemisinin, dihydroartemisinin, and artesunate impaired phagocytic capacity of neutrophils after *Escherichia coli* exposure, but with the ROS increased [34]. Since neutrophils are thought to be involved in early innate immunity against malaria [35], further studies should be conducted for assessment of artemisinins-related neutropenia along with changes in neutrophil function regarding malaria immunity to have an explicit picture about immunomodulating properties of neutrophils.

2.2 Artemisinins and macrophages

Macrophages are pivotal components of innate immunity regulating immune homeostasis. During inflammation, they produce various cytokines mediated by an NF-κB signaling pathway [36]. Artemisinins could disrupt the macrophage-related homeostatic functions via interfering transcriptional signaling pathways in macrophages, resulting in the reduction in proinflammatory cytokines secretion. NF-κB is the most important transcription factor that regulates the expression of genes associated with immune response [37]. In human monocytes, artemisinin suppresses expression of MMP-9 and production of TNFα and IL-1β via regulating NF-κB signaling as well [38, 39].

In a murine macrophage cell line RAW264.7, dihydroartemisinin, a semisynthetic analog of artemisinin, inhibits secretion of TNF-α, IL-6, and NO from LPS-stimulated RAW264.7 cells [40]. Artemisinin also exhibits anti-inflammatory potential on PMA-treated human THP-1 cells [41]. Artesunate inhibits TNF-α secretion dose-dependently from heat-killed *Staphylococcus aureus* or peptidoglycan-generated mice peritoneal macrophages through reducing mRNA expression of TLR2, Nod2, and translocation of NF-κB [14]. In a prior investigation by the same group, treatment of murine peritoneal macrophages with artesunate inhibited production of TNF-α, IL-6 dose-dependently stimulated by CpG ODN, LPS, or heat-killed *E. coli*, resulting in reduced release of proinflammatory cytokines and lower endotoxin levels via NF-κB translocation and decreases in mRNA expression of TLR4 and TLR9 [42]. Similarly, in experimental murine colitis, artesunate alleviated colitis developed by DSS or TNBS, but not that induced by oxazolone; it ameliorated weight loss, disease activity, and colonic injury. In TNBS or DSS colitis, the levels of IFN-β, IL-17, and TNF-α were downregulated due to artesunate suppression of NF-κBp65 and p-IkBa. Artesunate also restrained TNF-α production by murine primary peritoneal macrophages activated by LPS [26]. SM934, a water-soluble artemisinin derivative, enhanced IL-10 secretion by macrophages in SLE mice, ovalbumin-induced mice, and IFN-γ-challenged mice. In primary peritoneal macrophages, SM934 boosted IL-10 secretion by IFN-γ stimulation [43]. In a mouse model of myocardial infarction, artemisinin reduced macrophage infiltration and inhibited inflammation through downregulation of IL-1β and TNF-α protein expression [44]. In a mouse orthotopic (HO8910PM) model of ovarian cancer, dihydroartemisinin inhibited ovarian cancer metastasis to intestine, liver, and peritoneum; reduced macrophage infiltration; and suppressed phosphorylated focal adhesion kinase, MMP-2, and Von Willebrand factor [45]. In a mouse model of cecal ligation/puncture sepsis, artesunate protected mice from sepsis through reduced serum cytokines production and LPS levels [46]. Additionally, it improved hepatic function through promotion of scavenger receptors at transcriptional level and enhancing both peritoneal macrophages and liver Kupffer cells internalization of LPS.

In human THP-1 cells, artemisone, artesunate, and dihydroartemisinin, but not artemisinin itself, enhanced haemozoin or TNFα-induced MMP-9 secretion by THP-1 cells to different degrees, a result consistent with mRNA levels. These derivatives exhibited immune suppression properties via suppressing haemozoin or TNFα-induced NF-κB transcription [47]. In RAW 264.7 cells, dihydroartemisinin exhibited anti-inflammatory activity [48]. It decreased COX-2 expression through downregulating the AKT and MAPK pathways. It also reduced luciferase activities of NF-κB, AP-1, C/EBP, and CREB, which were closely related to COX-2. Dihydroartemisinin hindered nuclear translocation of PMA-triggered transcription factors as well. These results partially revealed underlying mechanisms of the anti-inflammatory properties of dihydroartemisinin. Artemisinin reduced LPS-stimulated secretion and mRNA expression of IFN-β and production of nitric oxide (NO) in RAW264.7. In same cells, artemisinin suppressed STAT-1 signaling, which is involved in IFN-β-induced responses [49]. Also, in LPS-challenged RAW 264.7, artemisinin promoted IL-12p40 release by inhibiting JNK activation, a result potentially of benefit for treatment of cancer and infectious diseases [50]. In these cells, SM905, another water-soluble artemisinin analog, inhibited LPS-triggered NO production and secretion of TNFα, IL-1β, and IL-6, simultaneously suppressed mRNA and protein levels of iNOS and COX-2 through downregulating the MAPK and NF-κB pathways [51]. Artemisinin and five of its derivatives inhibited NO production and mRNA expression of iNOS; artesunate was most active in RAW 264.7 [52]. After artesunate exposure of cells, microarray analysis indicated that several NO metabolism pathways-related genes were altered. Additionally, Wnt and cAMP signaling pathways regulated relevant genes at RNA level. There is a dispute related to artemisinin in regard to NO generation and *iNOS* mRNA level. Evidently, artemisinin suppressed NO synthesis and transcriptional level of iNOS of LPS-stimulated RAW 264.7 cells, an effect dependent on suppression of IFN-β secretion and inhibition of STAT-1 pathway, not NF-κB repression [49]. But another study of mice infected with *Leishmania donovani* revealed that artemisinin maintained host homeostasis in macrophages by attenuating NO production and mRNA expression of *iNOS*; it also ameliorated spleen weight and parasite burden, these effects accompanied by restoration of T helper 1 cytokines [53].

Thus, to modulate immune surveillance, multiple pathways have been demonstrated to participate in artemisinins-elicited genes expression and proinflammatory cytokines secreted by monocytes/macrophages. Nevertheless, the complete anti-inflammatory effects of artemisinins remain to be elucidated.

3 Artemisinins and adaptive immunity

3.1 Artemisinins and T-cells

T-cells, as fundamental immune effectors, play a crucial role in the cell-mediated adaptive immune response. By producing various cytokines, they also affect B-cell-mediated humoral immunity.

Cross-linking of T-cell receptors (TCRs) activates T-cells from a quiescent condition and leads to expression of the IL-2, and IL-2Rα (CD25). IL-2, derived from autocrine/paracrine signaling, enhances proliferation and sustains vitality of activated T-cells. Once pathogens are cleared, the production of proinflammatory cytokines stops, activated T-cells apoptosis occurs, and the host returns to immune homeostasis. IL-2 is involved in T lymphocyte expansion, differentiation, and maintenance. Artemisinin or dihydroartemisinin showed suppression of IL-2 production in mice [19], indicating that artemisinins suppressed T-cell proliferation and T-cell-related immune response by governing release of the IL-2 and other relevant cytokines. Dihydroartemisinin inhibited enhancement of LPS-challenged splenic cells dose-dependently due to the suppression of TLR4 signaling cascade in SLE mice [54]. As determined with mouse models of delayed-type hypersensitivity or ovalbumin immunization, artemether arrested T-cells in the G0/G1 phase, inhibited T-cell expansion, and reduced IL-2 and IFN-γ generation via blockage of the Ras-ERK1/2 signaling activation [55]. A clinical trial, malarial patients coinfected with HIV and with CD4 counts ≤200 cells/μL showed a decrease in their CD4 counts after treatment with dihydroartemisinin [56]. In cultured human lymphocytes, artesunate caused genotoxic and cytotoxic effects through increased apoptosis and necrosis [57].

Relative to artemisinin, artesunate, and artemether, a series of new dihydroartemisinin derivatives caused stronger suppression of T-cell and B-cell expansion challenged by ConA and LPS, respectively [58]. In phytohemagglutinin-stimulated peripheral blood mononuclear cells, artesunate and dihydroartemisinin decreased proportions of activated CD4 and CD8 T-cells [59]. Artesunate depressed CD4 T lymphocytes expansion and IL-2 secretion. Further, in CD4 T-cells, it decreased the expression of activation-associated receptors, CD25 and CD69; however, it enhanced the function of effector T-cells by inducing production of IFN-γ in Th1 cells and IL-4 in Th2 cells [60]. In mice, dihydroartemisinin ameliorated experimental autoimmune encephalomyelitis (EAE) via decreased T helper cells, but increased Tregs cells. The effects on T-cells were related to the mTOR pathway but were diminished by enhancement of Akt

activity. Further, dihydroartemisinin suppressed T helper cell differentiation in vitro [27].

SM933, an artemisinin derivative, exhibited anti-inflammatory properties to ameliorate EAE through regulating the Rig-G/JAB1 pathway-mediated cell cycle arrest of encephalitogenic T-cells. Its effect was selective for activated T lymphocytes; remaining T lymphocytes were not changed [25]. An artemisinin derivative, SM735, inhibited production of proinflammatory cytokines (IL-12 etc.) stimulated by LPS or PMA in a dose-dependent manner but left IL-2 untouched. Furthermore, SM735 repressed both delayed-type hypersensitivity and quantitative hemolysis mediated by T-cell and B-cell, respectively, in mice [61]. SM905 showed immunosuppressive properties and inhibited IL-2 and IFN-γ produced by T-cells dose-dependently and inhibited CD3/CD28-stimulated T lymphocytes activating and proliferate via suppressing MAP kinases and Ras signaling pathway [62]. Administered to mice, SM905 inhibited type II bovine collagen-challenged T-cells proliferation and IL-17A and IL-6 secretion [21]. Another artemisinin derivative, SM934, exhibited similar immunosuppressive properties but by a different mechanism [12]. SM934 suppressed the proliferation of splenocytes and CD4 T-cells accompanied by apoptosis of the CD69 population. It also inhibited IFN-γ production stimulated by the mixed lymphocyte reaction or by anti-CD3/28. In contrast, SM934 restricted IL-2-induced proliferation and maintenance of T lymphocytes via suppressing AKT phosphorylation. SM934 moved activated T-cells into apoptosis to a greater extent than resting T-cells. Furthermore, in ovalbumin-challenged mice, SM934 inhibited ovalbumin-stimulated T-cell expansion and cytokines secretion [12]. Additionally, SM934 repressed Th1 cell and Th17 cell responses via suppressing IFN-γ and IL-17 secreted from activated CD4 T-cells. In female MRL/lpr mouse model, it also caused differentiation of naïve CD4 T-cells into Th1 and Th17 cells [11]. SM934 attenuates murine EAE through increased numbers of Tregs. As determined in ex vivo experiments, SM934 suppressed Th17 and Th1; blocked production of IL-2, IFN-γ, IL-17, and IL-6; and increased generation of IL-10 and TGF-β. Furthermore, SM934 reduced the infiltration of CD4 T-cells and elevated percentage of Treg cells through mediating Treg differentiation and expansion [63]. In a rat model of experimental membranous nephropathy, SM934 attenuated pathogenetic progress of glomerulonephritis and renal fibrosis via suppressing TGF-β1/Smad signaling pathway [64]. In female NZB/W F1 mice, SM934 suppressed enhancement of T effector cells and T memory cells, promoted CD4 T-cells apoptosis, and induced the differentiation of Treg cells as well [43].

In contrast, artemisinins, acting through immune enhancement or reconstitution, enhanced the functions of T-cells. Artesunate promoted immune restoration in a long-term T-cell deficiency mice model triggered by bone marrow transplantation [65]. A toxicological study revealed dihydroartemisinin increased the total white cell counts and the percentage of lymphocytes [66]. Another study of SLE mice indicated dihydroartemisinin enhanced expansion of CD4 and CD8 T lymphocytes due to inhibition of B-cells [67].

Tregs, an immunosuppressive population of T lymphocytes, suppress innate and adaptive immunity. In a murine model of breast cancer, artemisinin decreased Treg counts and elevated the splenocyte IFNγ/IL-4 ratio, reflecting its immunoenhancing properties [68]. In *Schistosoma mansoni*-infected mice, a combination of artemether and praziquantel shifted the ratio of Th/cytotoxic cells to Th differentiation and improved liver functions [69]. In an orthotopic mouse model of cervical cancer, artesunate decreased the percentages of Treg cells and expression of Foxp3 in T-cells dose-dependently [70]. For Balb/c mice bearing 4T1 breast cancer cells, arteether reduced the tumor volume and the numbers of splenic Treg cells, but the reduction was not significant [71]. For Balb/c mice, dosing with dihydroartemisinin reduced the level of IL-4 and counts of Treg cells in the spleen [72]. In the ret-transgenic mouse model of melanoma, administration of artesunate did not alter splenic T lymphocyte subsets, but the numbers of Treg cells in lymph nodes were decreased [73]. Artemisinin indirectly augmented generation of Th1 responses through mediating IL-12p40 secretion by LPS-stimulated macrophages [50].

Although most evidence suggests the T-cell immunosuppressive properties of artemisinins, it also caused immune enhancement [74]. Yet the mechanisms are unclear, and the interactions between artemisinins and T-cells remain a matter of debate.

3.2 Artemisinins and B-cells

In all vertebrates, B-cells, as a fundamental component of the adaptive immunity, are involved in the humoral immunity by producing antibodies. In mice, artemisinin, dihydroartemisinin, and arteether suppressed the humoral response based on the hemolytic plaque assay [75]. In BXSB mouse model of SLE, You–You Tu et al. found dihydroartemisinin treatment suppressed the proliferation of B-cells and autoantibody production [67]. In K/BxN mouse model of autoimmune arthritis, artesunate prevented germinal center formation and autoantibody generation during development of arthritis, and in established arthritis, it reduced germinal center B-cells. In contrast, artesunate exerted limited effects on K/BxN serum-induced arthritis, which indicated it had little influence on antibody production during inflammation. Consequently, artesunate

attenuated the humoral immune response via targeting the proliferative germinal center B-cells [76]. Artesunate decreased autoantibody in serum through inhibiting B-cell-activating factor in MRL/lpr SLE mice [13].

Some artemisinin derivatives showed suppressive activity on LPS-induced B-cell proliferation and ameliorated B-cell-mediated hemolysis of sheep red blood cells [58, 61, 77, 78]. In SLE mice, SM934 relieved glomerulonephritis and reduced the production and accumulation of IgG2a and IgG3autoantibodies in serum and renal tissue, suppressed enhancement of effector/memory T-cells, and increased Treg cells counts [43]. More recently, SM934 was found ameliorated the progression of SLE through suppressing the B cells expansion and activation and also blocking plasma cells generation in mice [79].

4 Artemisinins and other immune cells

The interactions of artemisinins and immune components under various physiological and pathological conditions are being elucidated. In a mast cell-mediated anaphylactic responses mouse model, artesunate ameliorated IgE-induced cutaneous vascular permeability, temperature altered, and histamine level increased in mice dose-dependently and pulmonary mast cells degranulation [8]. Similarly, artesunate inhibited IgE-induced mast cells degranulation in rat basophil leukemic cell line and primary human mast cells [8]. Furthermore, in mast cells, artesunate inhibited IgE-induced phosphorylation of Syk and PLCγ1, lowered production of IP3, and elevated cytosolic Ca^{2+} levels [8]. In the ret-transgenic mouse model of melanoma, however, artesunate exerted only minor effects on CD4 and CD8 T lymphocytes, Tregs, and natural killer cells [73].

Dihydroartemisinin enhanced γδT cells expansion, a subset of T-cells possess a distinct TCR on their surface and elevated γδT cell-mediated killing of cultured pancreatic cancer cells. γδT cells treated with dihydroartemisinin apparently exerted more effective anti-pancreatic cancer activity through upregulation of intracellular perforin, granzyme B expression, and IFN-γ production [80].

In mice with allergic asthma, artesunate exerted protective activity by decreasing ovalbumin-increased eosinophil counts [10]. Artesunate, administered intraperitoneally to BALB/c mice, inhibited ovalbumin- and house dust mite-induced eosinophilia in bronchoalveolar lavage fluid [7]. In pregnant rats, there were dose-dependent increases in basophils at 9 d after artesunate administration [81].

5 Summary and perspectives

Artemisinins have an immunomodulatory effect on diverse components of the immune system through affecting various immune cells responses. They suppress the secretion of cytokines and related signaling pathway, induce decrease in neutrophils, reduce macrophage functional responses, and inhibit lymphocyte proliferation and maintenance. Also, artemisinins affect signaling pathway cascades, including those for TLR, PLCγ, PKC, Akt, MAPK, Wnt, STATs, NF-κB, and Nrf2/ARE [7, 8, 10, 12, 14, 16, 17, 42, 74, 82, 83].

Artemisinin research should now focus on clarifying artemisinins–host relationships; more evidence is needed to elucidate the mechanisms. Further, the new analogs discovery and therapeutic approaches to targeting immune-related diseases, including viral and non-viral infections, inflammation, autoimmune disorders, and cancers, are now appropriate. New artemisinin derivatives are promising candidates to treat inflammation-associated diseases. Their development will allow scientists to continue the search for natural products for immunotherapy-based approaches to treating diverse immune-related diseases [84]. Continued identification of natural products with immunomodulatory activity can lead to a new age of drug discovery.

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