

Review Article

Role of Interleukin-10 in Malaria: Focusing on Coinfection with Lethal and Nonlethal Murine Malaria Parasites

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Interleukin- (IL-) 10, anti-inflammatory cytokine, is known to inhibit the protective immune responses against malaria parasites and to be involved in exacerbating parasitemia during *Plasmodium* infection. In contrast, IL-10 is regarded as necessary for suppressing severe pathology during *Plasmodium* infection. Here, we summarize the role of IL-10 during murine malaria infection, focusing especially on coinfection with lethal and nonlethal strains of malaria parasites. Recent studies have demonstrated that the major sources of IL-10 are subpopulations of CD4⁺ T cells in humans and mice infected with *Plasmodium*. We also discuss the influence of innate immunity on the induction of CD4⁺ T cells during murine malaria coinfection.

1. Introduction

Malaria, caused by protozoan parasites of the genus *Plasmodium*, is the major parasitic disease in tropical and subtropical regions, including parts of the Americas, Asia, and Africa. With more than 200–300 million clinical cases globally and approximately 1 million deaths per year, malaria represents the most important infectious disease worldwide. Four species of *Plasmodium* infect humans: *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*.

Human malarial parasites develop through two stages in humans: a liver stage and a blood stage. The asexual blood stage of the parasite is the cause of malarial pathologies. Therefore, it is important to prevent the replication of this stage of parasite. Particularly, *P. falciparum* causes severe pathologies such as cerebral malaria, severe anemia, and respiratory injury in the blood stage. It is necessary to understand the mechanism of protective immunity against the blood stage of the parasite during malaria infection. Nevertheless, it is difficult to investigate the human *in vivo* immune response against malaria parasite for many reasons. Consequently, murine malaria models with *P. berghei*, *P. yoelii*, and *P. chabaudi* have been used to elucidate the immune interaction in hosts and to demonstrate many factors associated with malarial defense mechanisms [1].

2. Parasite Killing: The Role of Proinflammatory Cytokines during Human and Murine Malaria Infection

Interferon- (IFN-) γ and Interleukin- (IL-) 12 play a crucial role in the clearance of intracellular pathogens [2–5]. Low levels of IFN- γ and IL-12 production have been observed in young African children with severe anemia during infection with *P. falciparum* [6]. The IFN- γ -mediated responses have been shown to be involved in protection against infection with *P. falciparum* [7]. In murine malaria, IFN- γ produced by CD4⁺ T cells has been shown to play a pivotal role in protective immunity against *P. chabaudi* (Pc) AS [8], nonlethal *P. berghei* (Pb) XAT [9], and *P. yoelii* (Py) 17XNL [10] infection. Actually, IFN- γ -depleted mice infected with murine malaria parasites show high levels of parasitemia and eventually die. IL-12 is a necessary factor for clearance of nonlethal Pc AS [11], Pb XAT [12], and Py 17XNL [13], suggesting that IL-12 plays an important role in protective immunity via IFN- γ production in murine malaria. Production of IFN- γ and IL-12 is suppressed by anti-inflammatory cytokines such as IL-10. It is possible that enhancement of IL-10 production contributes to suppression of parasite killing, considering that IL-10 plays a detrimental role during *P. falciparum* infection.

3. Source and Biological Effect of IL-10

IL-10, an anti-inflammatory cytokine, plays an important role in regulating immune responses in hosts, as does TGF- β . The major source of IL-10 is known to be T cell subsets including Th1 cells, Th2 cells, Tr1 cells (CD25⁺Foxp3⁻), and regulatory T (Treg) cells (CD25⁺Foxp3⁺). In antigen-primed T cells, Th2 cells were originally believed to be the major source of IL-10. Stimulation of Th1 cells with IL-27 upregulates IL-10 production and enhances IFN- γ expression slightly [14, 15]. Tr1 were identified as a subset of CD4⁺ cells that produce high levels of IL-10, low levels of IL-2, but not IL-4. They develop from naïve T cells under the influence of IL-27 [15–17]. IL-10 is also produced by naturally occurring Treg cells [18]. TGF- β induces the expression of IL-10 [19]. Moreover, IL-2, an important activator of suppressive activity by Treg cells, enhances IL-10 production [20, 21]. Today, it is known that the source of IL-10 is not only T cell subsets but also almost all leukocytes [22–25].

Apparently, monocytes/macrophages are the main target cells of inhibitory IL-10 effects [26]: IL-10 inhibits the release of proinflammatory mediators from monocytes/macrophages, and thereby inhibits the LPS- and IFN- γ -induced secretion of TNF- α , IL-1 β , IL-6, IL-8, G-CSF, and GM-CSF [27, 28]. Furthermore, IL-10 inhibits the antigen presentation of monocytes/macrophages. Moreover, the IL-10-induced inhibition of IL-12 synthesis in antigen-presenting cells results in reduced IFN- γ production in T cells [29]. Actually, IL-10 inhibits both the proliferation and the cytokine synthesis of CD4⁺ T cells, including the production of IL-2 and IFN- γ by Th1 and of IL-4 and IL-5 by Th2 [30, 31].

4. Detrimental Effect of IL-10 on the Outcome of Human and Murine Malaria Infection

High levels of IL-10 and TNF in plasma have been characteristic of young African children with malarial anemia and high levels of parasitemia [32–39]. In common IL-10 promoter variants, the -1082A/-819T/-592A (ATA) haplotype has been associated with increased susceptibility to severe anemia [39]. Their IL-10:IL-12 ratio was higher than that in the non-ATA haplotype. On the other hand, the -1082G/-819C/-592C (GCC) haplotype has been associated with protection against severe anemia [39]. The IL-10:IL-12 ratio in the GCC haplotype was lower than that in the ATA haplotype. These findings suggest that a high IL-10:IL-12 ratio is associated with the downregulation of IFN- γ production and that it causes development of severe anemia during *P. falciparum* infection.

Lethal *Py* 17XL-infected mice show higher levels of IL-10 and TGF- β production than nonlethal *Py* 17XNL-infected mice early in infection [40, 41]. High levels of IL-10 and TGF- β are associated with inhibition of proinflammatory response, resulting in high levels of parasitemia, severe anemia by which RBCs ruptured, causing parasite replication and the death of infected mice. Depletion or deficiency of IL-10 [40, 42], or the blockade of IL-10 receptor [41] regulates parasitemia during lethal *Py* 17XL infection and prolongs

survival of infected mice. Couper et al. [42] reported that the major source of IL-10 in lethal *Py* 17XL-infected mice is CD4⁺ Tr1 cells, just as it is in toxoplasmosis [43] and cutaneous leishmaniasis [44] (Figure 1).

In nonlethal *Py* 17XNL-infected mice, the production of IL-10 and TGF- β is induced in the late phase of infection [41]. The population of CD4⁺ Tr1 cells has been shown to be the major source of IL-10 in nonlethal *Py* 17XNL as well as lethal *Py* 17XL infection. Moreover, IL-10-deficient mice show marked suppression of the replication of parasites compared with that in wild-type mice [42] (Figure 1). These findings suggest that enhanced-IL-10 production suppresses inflammatory response against malaria parasites, resulting in high levels of parasitemia and anemia by replication of parasites in infected mice. Results show that IL-10 plays a detrimental role during human and murine malaria infection.

5. Role of Anti-Inflammatory Cytokines during Murine Malaria Infection

Reportedly, a low IL-10/TNF ratio is associated with severe malarial anemia [36–38]. These results suggest that low levels of IL-10 production are associated with enhancement of TNF production, followed by increased IFN- γ production. The enhancement of TNF production might be associated with the aggravation of disease severity, such as severe anemia, by which phagocytosis of uninfected RBC occurs [45], or dyserythropoiesis [46]. Moreover, results obtained using mouse models have suggested that IL-10 plays a protective role in the host during murine malaria infection. Although IL-10-deficient mice show lower levels of parasitemia than wild-type mice do during murine malaria infection, they indicate severe diseases such as hepatic pathology [42, 47, 48] and cerebral pathology [49, 50]. Actually, inflammation, which is involved in parasite killing, is upregulated in IL-10-deficient mice, but excessive inflammation, such as the increase of IFN- γ production, also presents the risk of developing hepatic pathology and/or cerebral pathology. Therefore, it seems that IL-10 might be necessary for suppression of hepatic pathology and cerebral pathology in the host during infection.

B6 mice infected with *Pb* NK65 display hepatic pathology and die within 2 weeks. The development of severe hepatic pathology is involved in IL-12 [11], IFN- γ , and CD8⁺ T cells [9]. The IL-12 production is induced through a MyD88-dependent pathway in DCs or macrophage and engenders hepatic pathology in a perforin/granzyme-dependent manner during *Pb* NK65 infection [51]. Coinfection with nonlethal *Pb* XAT or *Py* 17XNL prevents the development of hepatic pathology caused by *Pb* NK65 infection and prolongs survival of mice [47]. In fact, IL-10 KO mice coinfecting with nonlethal *Pb* XAT or *Py* 17XNL showed severe hepatic pathology, suggesting that IL-10 is involved in suppression of disease severity during coinfection [47] (Figure 2(b)). During lethal *Py* 17XL or nonlethal *Py* 17XNL infection, IL-10, which is derived from CD4⁺ Tr1 cells, is also necessary for the prevention of hepatic pathology [42]. Nevertheless, it remains unclear whether IFN- γ and CD8⁺ T cells are

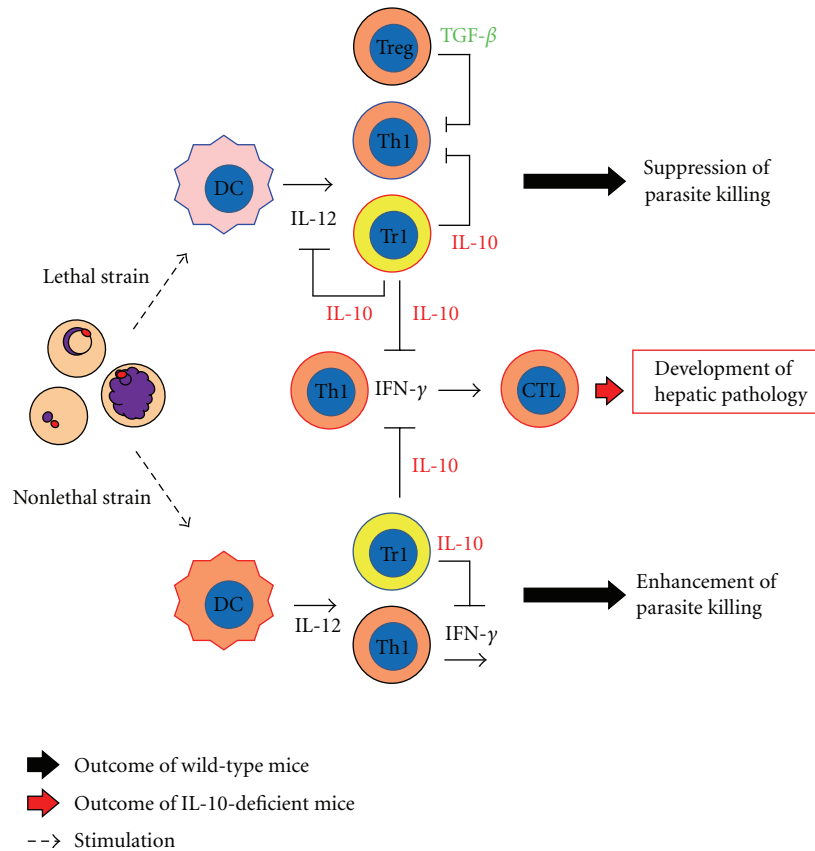


FIGURE 1: Scheme of immune responses during lethal *P. yoelii* 17XL and nonlethal *P. yoelii* 17XL infection: DC: dendritic cell; Th: helper T cell ($CD4^+$ T cells); Tr1, IL-10-producing $CD4^+$ T cells; Treg, regulatory T cells; CTL, cytotoxic T cells ($CD8^+$ T cells). In a lethal *P. yoelii* 17XL infection, Tr1 are induced. IL-10 inhibits proinflammatory cytokine production for parasite killing, producing high levels of parasitemia and the death of mice. Tr1 are also induced in nonlethal *Py* 17XNL as well as lethal *Py* 17XL infection. However, parasite killing occurs during nonlethal *Py* 17XNL infection. When IL-10 is ineffective (depletion or deficiency of IL-10 or the blockade of IL-10 receptor) in mice infected with nonlethal *Py* 17XNL or lethal *Py* 17XL, excessive inflammation is induced in association with the development of hepatic pathology.

associated with development of hepatic pathology in mice infected with *Py* 17XL or *Py* 17XNL (Figure 1).

Mice infected with *Pb* ANKA show similar features to human cerebral malaria (CM) regarding neurologic signs and histopathological findings, considering that *Pb* ANKA infection in mice might be an experimental model of CM (ECM) [52, 53]. Proinflammatory cytokines, such as IFN- γ and lymphotoxin- α , are known to accelerate ECM development [54] (Figure 2(c)). In contrast, anti-inflammatory cytokines, such as IL-10, prevent ECM development [55, 56]. However, it remains unclear how IL-10 suppresses ECM development, because high levels of IL-10 production were observed in spleen [57] and plasma [58] of mice singly infected with *Pb* ANKA.

The ECM development is suppressed by the simultaneous presence of murine AIDS during *Pb* ANKA infection [55]. Results demonstrated that murine AIDS-mediated protection of ECM is dependent on IL-10, which is produced by splenic $CD4^+$ T cells, with the use of anti-IL-10 mAb. It is particularly interesting that coinfection with parasites such as nonlethal *Pb* XAT [50] or *Filaria* [59] has also been shown to prevent ECM development. The suppressive effect of

coinfection with nonlethal *Pb* XAT or *Filaria* on ECM during *Pb* ANKA infection was abrogated in IL-10 KO mice [50, 59], suggesting that IL-10 plays a crucial role in the suppression of ECM during coinfection with other parasites (Figure 2(b)).

In contrast to coinfection with nonlethal *Pb* XAT, the suppressive effect of coinfection with nonlethal *Py* 17XNL on ECM during *Pb* ANKA infection is independent of IL-10 [50, 60]. A recent study demonstrated that Treg cells, which are expanded by IL-2/anti-IL-2 complexes, suppress the recruitment of pathogenic $CD4^+$ and $CD8^+$ T cells to brains and protect mice from developing ECM during *Pb* ANKA [61]. The IL-2/anti-IL-2 complexes enhanced the levels of Foxp3 and CTLA-4 expression and increased the levels of IL-10 production from Treg cells during *Pb* ANKA infection. However, the suppression of ECM by Treg cells was dependent on CTLA-4 but not on IL-10 [61] (Figure 2(c)). The suppressive effect of coinfection with nonlethal *Py* 17XNL on ECM during *Pb* ANKA infection was not reversible by depleting antibodies against $CD25^+$ -bearing $CD4^+$ T cells or CTLA4 $^+$ -bearing $CD4^+$ T cells [60]. A key factor that has a suppressive effect on ECM by coinfection with nonlethal *Py* 17XNL has not yet been discovered.

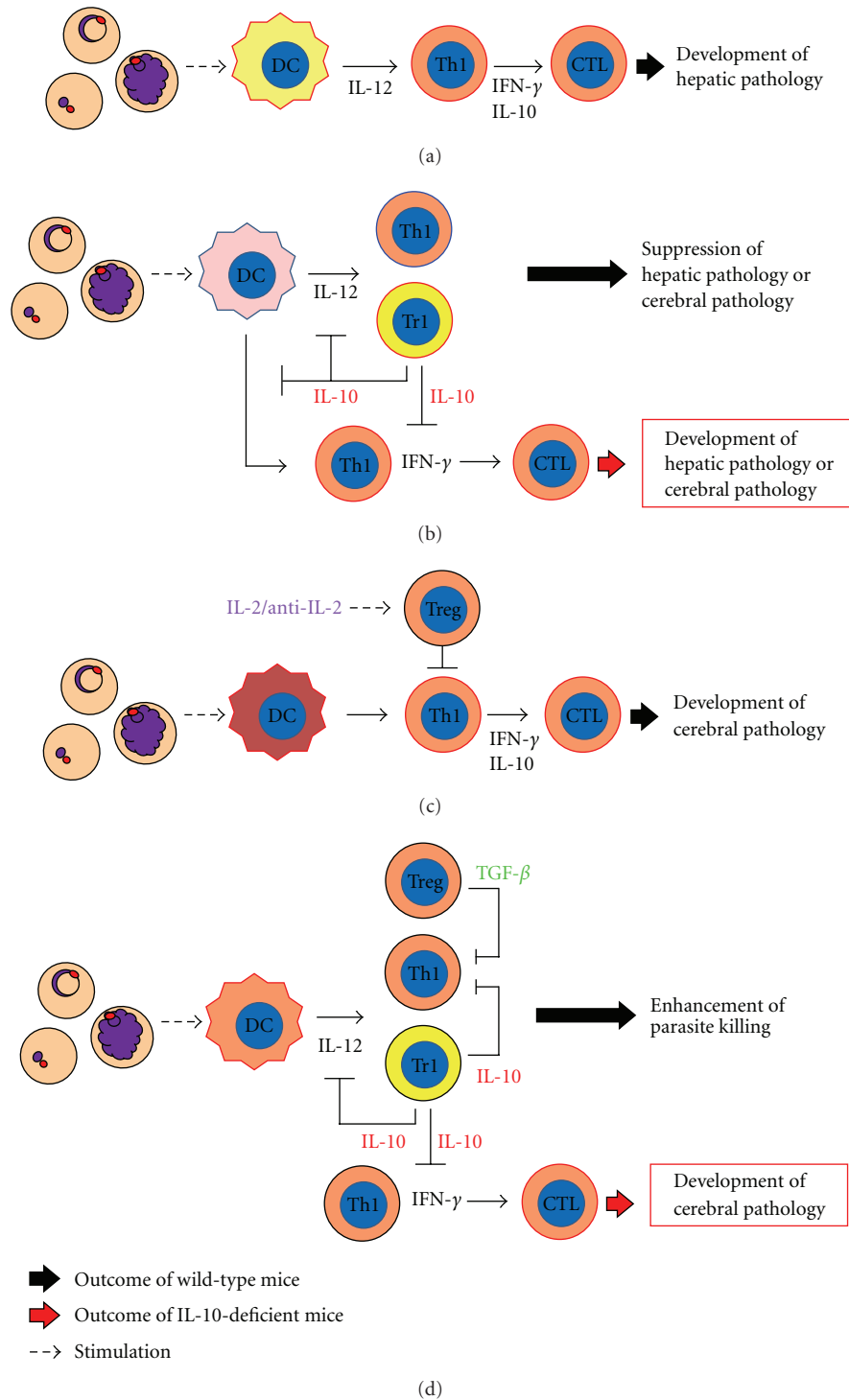


FIGURE 2: Role of anti-inflammatory responses during murine malaria infection. (a) Immune responses during lethal *P. berghei* NK65 infection. (b) Possible mechanism by which development of disease severity such as hepatic or cerebral pathology is suppressed by coinfection with nonlethal murine malaria parasites. (c) Immune responses during lethal *P. berghei* ANKA infection. (d) Immune responses during *P. chabaudi* AS infection. (a) and (c) A type of DC activated by lethal *P. berghei* NK65 or *P. berghei* ANKA might induce pathological Th1 and CTL. The pathological Th1 and CTL are involved in excessive inflammation and the development of severe pathology, such as hepatic pathology (*P. berghei* NK65) or experimental cerebral malaria (ECM) (*P. berghei* ANKA). (b) A type of DC activated by nonlethal malaria parasites before activation by lethal parasites might expand Tr1, but not pathological Th1, and might subsequently suppress severe disease. IL-10, which might be produced by Tr1, inhibits proinflammatory cytokine production and expansion of pathological Th1 during coinfection. Therefore, coinfecting IL-10 deficient mice develop severe pathology, such as hepatic pathology or ECM. (d) In *P. chabaudi* AS infection, IL-10 from Tr1 and Treg are associated with suppression of proinflammatory cytokine production and expansion of pathological Th1. A deficiency of IL-10 or TGF- β contributes to development of ECM.

Development of severe disease such as hepatic pathology and cerebral pathology generally involves excessive inflammation in murine malaria parasites. Little is known about the differences between the developmental mechanisms of hepatic pathology and cerebral pathology during *Pb* NK65 and *Pb* ANKA infection, respectively. However, IL-10 can downregulate excessive inflammation during *Pb* NK65 or *Pb* ANKA infection. It is associated with the suppression of hepatic pathology and cerebral pathology. Results show that IL-10 plays a protective role in the host during *P. falciparum* infection.

6. A Different Type of DC Induced by Lethal and Nonlethal Murine Malaria Infection

How are different subsets of CD4⁺ T cells, such as pathological CD4⁺ T cells [62, 63], IL-10-producing CD4⁺ T cells [42], and Treg cells [64], induced between lethal and nonlethal murine malaria infection? The development of CM is inhibited completely by the simultaneous presence of nonlethal *Py* 17XNL [60], lethal *Pb* K173 [57], and nonlethal *Pb* XAT [50]. However, protection from CM was not induced in mice when they were infected with *Py* 17XNL on day 4 after *Pb* ANKA infection [60]. Similarly, coinfection with *Pb* XAT on day 1 or day 3 after infection with *Pb* ANKA failed to protect mice from cerebral malaria (Niikura et al. unpublished data). In simultaneous infection with *Pb* ANKA and *Pb* K173, suppression of ECM was associated with the induction of cytokines such as IFN- γ , IL-10, and IL-12 on day 1 after infection [57]. These findings suggest that the presence of other parasites might modulate some key factors/cells that are involved in innate immunity in early infection with *Pb* ANKA. Actually, DCs are important for initiating immune responses against malaria parasites. It is possible that immune responses induced by DCs produce protective and pathological effects, respectively, when mice are infected with nonlethal and lethal parasites. Therefore, DCs might contribute to the determination of the virulence of malaria parasites. In coinfection, a type of DC activated by nonlethal malaria parasites before activation by lethal parasites might fail to expand pathological CD4⁺ T cells and subsequently fail to suppress severe disease.

Wykes et al. [13] showed that although DCs from mice infected with nonlethal *Py* 17XNL were fully functional, DCs from mice infected with lethal *Py* YM were unable to produce IL-12 or present antigens to T cells. Apparently, lethal malaria causes a failure of DC function, resulting in the suppression of Th1 immune responses (Figure 1). Similar to lethal *Py* 17XL infected mice, it is possible that mice infected with lethal *Py* YM induce IL-10-producing CD4⁺ T cells. Although IL-10 might inhibit the DC function, such as antigen presentation and release of proinflammatory cytokines, little is known about whether IL-10 associates with a different type of DC induced between lethal and nonlethal murine malaria infection. Toll-like receptors (TLRs) play an important role in the innate immune system against pathogens [65]. Therefore, TLRs might be associated with disease severity during malaria infection. During lethal *Py*

17XL infection, TLR9 signaling in DCs is known to be crucial for the activation of Treg cells that suppress Th1 immune responses, causing high levels of parasitemia [64]. In contrast, MyD88, but not TLR signaling, has been shown to be necessary for elimination of parasites in mice infected with nonlethal *Py* 17XNL [66]. Accordingly, a different type of DC induced between lethal and nonlethal murine malaria infection might induce different subsets of CD4⁺ T cells, such as IL-10-producing CD4⁺ T cells or Treg cells.

7. Is IL-10 Necessary for Host Protection against Murine Malaria Parasites?

Although IL-10-deficient mice suppressed an increase of parasitemia during coinfection with lethal and nonlethal parasites, mice were unable to eliminate parasites completely and eventually died [47, 50]. These results suggest that the lethal strains of malaria parasites may modulate the induction of adaptive immunity independent of IL-10. Millington et al. [67] demonstrated that *Plasmodium* infection inhibits the induction of adaptive immunity to heterologous antigens by modulating DC function. According to their paper, hemozoin (HZ), rather than infected RBC membranes, was a key factor involved in the suppression of murine DC function. On the other hand, it has been shown that repeated stimulation through TLR9, which is the receptor for HZ, engenders tolerance to signaling through TLR4 [68].

In fact, HZ activates DCs through the TLR9-MyD88 pathway [69]. A recent study has demonstrated that parasite protein-DNA complex, but not HZ, plays a crucial role in TLR9-mediated activation of DCs during infection [70]. Stimulation through TLR9 might be associated with development of severe hepatic pathology, because MyD88 pathway, which is activated by TLR9 stimulation, is known to be involved in severe hepatic pathology caused by *Pb* NK65 [51]. Coban et al. [71] and Griffith et al. [72] reported that the TLRs-MyD88 signaling pathway might play a critical role in ECM during lethal *Pb* ANKA infection. It has been shown that ECM is prevented by oral treatment with E6446, which is a synthetic antagonist of nucleic acid-sensing TLRs [73]. In contrast, it is demonstrated that murine cerebral malaria development is independent of Toll-like receptor signaling [74, 75]. It remains controversial whether TLRs-MyD88 signaling pathway is associated with ECM development.

In summary, IL-10 is necessary for suppression of hepatic pathology or ECM in the host although IL-10 entails a risk of downregulation of protective immunity against malaria parasites. CD4⁺ T cells of different kinds, such as pathological CD4⁺ T cells, IL-10-producing CD4⁺ T cells, or Treg cells, are induced during different kinds of *Plasmodium* spp infection. To induce a more effective immune response in host defense against *Plasmodium* spp, it is necessary to elucidate the interaction of innate and acquired immune cells such as DCs, $\alpha\beta$ T cells, and $\gamma\delta$ T cells.

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