Published in final edited form as:
Expert Rev Anti Infect Ther. 2012 November ; 10(11): 1343–1356. doi:10.1586/eri.12.118.

Immunomodulation in *Plasmodium falciparum* malaria: experiments in nature and their conflicting implications for potential therapeutic agents

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Abstract

Effective *Plasmodium falciparum* immunity requires a precisely timed and balanced response of inflammatory and anti-inflammatory immune regulators. These responses begin with innate immune effectors and are modulated over the course of an infection and between episodes to limit inflammation. To date, there are no effective immunomodulatory therapies for severe malaria. Some of the most potent immunomodulators are naturally occurring infections, including helminthic and chronic viral infections. This review examines malaria coinfection with these organisms, and their impact on malaria morbidity and immune responses. Overall, there is compelling evidence to suggest that chronic coinfections can modulate deleterious malaria-specific immune responses, suggesting that therapeutic agents may be effective if utilized early in infection. Examination of the mechanisms of these effects may serve as a platform to identify more targeted and effective malaria immunomodulatory therapeutics.

Keywords

coinfection; helminth; hepatitis; HIV; immunomodulation; malaria; *Plasmodium falciparum*

The pathophysiology of malaria has been difficult to characterize over the past century, in part because of the wide spectrum in the severity and presentation of disease. In some cases, it presents as a profoundly life-threatening disease, and in other cases as a benign infection without any symptoms. With increased focus on the immunology of *Plasmodium* infection in pursuit of a vaccine, it has become clear that this spectrum of disease can be explained in part by the complex relationship between the parasite and the host immune response. Like many other infections, the pathology experienced by the host is often a consequence of the immune system response to the parasite. There is now evidence that the host immune response is modulated over the course of infection and in subsequent infections to limit malaria morbidity. Because of this, there has been significant investigation into immunomodulators as adjunctive therapy for the treatment of severe malaria. However, despite promising results in mouse studies, little to no benefit has been demonstrated in human studies.

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Financial & competing interests disclosure
The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.
No writing assistance was utilized in the production of this manuscript.

How can we determine which immune responses influence human malaria protection and pathology, so that we can utilize immunmoodulation as a therapeutic modality? We have relied heavily on mouse models, which can give us valuable clues through direct augmentation of the immune responses with cell line depletions and knockout studies. However, the conclusions that can be drawn from these studies are limited by the fundamental differences in immune responses between mice and humans, and the differences in murine and human *Plasmodium* species. The same immunomodulatory experiments cannot be carried out in humans, and therefore, we are often limited to observational studies. One way to examine the relative importance of these immune responses in humans is to observe the effect of naturally occurring immunomodulators on the vulnerability to malaria infection and disease. In this review, understanding about effective natural immune responses to malaria and the data on how common malaria coinfections affect this response, specifically helminthic infections and chronic viral infections, are examined. This area of research holds tremendous opportunity for the discovery of therapeutic modalities, as it increases understanding of the role that parasites and viral infections play in modulating immune responses. Indeed as a result of these kinds of studies, whole organisms such as *Trichuris suis* for allergic diseases are already being pursued as immunomodulatory therapies [1].

Since *Plasmodium falciparum* is the most common cause of severe disease by far, this review focuses almost exclusively on immunomodulation in *P. falciparum* malaria in humans and in animal models designed to mimic *P. falciparum* infection in humans. It should also be noted that in many areas of this review, severe malaria is described as a single clinical entity; but in fact, this description includes multiple syndromes with varying pathophysiology. For this review, the general category of ‘severe malaria’ is used because many of the clinical studies cited did not explicitly differentiate between these syndromes when published. The lack of clarity on the precise pathology being studied may explain some of the discrepant results described in malaria coinfection literature. As we move forward in our understanding of severe malaria pathophysiology, it will be important for future studies to differentiate between these syndromes, particularly when examining immunopathology and modulation.

Host immune responses in malaria infection & disease

Evidence in both humans and mice suggests that effective natural immunity is characterized by a rapid initial inflammatory response, quickly followed by regulation of this response [2,3]. The critical role of this initial inflammatory response has been shown in a variety of ways, including multiple studies that have demonstrated that peripheral blood mononuclear cell IFN-γ production in response to *ex vivo* malaria antigen stimulation is associated with malaria infection protection [4]. However, a number of proinflammatory cytokines have also been implicated in the development of severe disease, including IFN-γ, TNF, IL-1α, IL-1β, IL-6 and IL-2 receptor [4,5]. Furthermore, relatively low levels of the anti-inflammatory cytokines, such as IL-10 and IL-6, have been found in individuals suffering from severe malaria [5–7]. Overall, this suggests that an unregulated inflammatory response is deleterious to the host. The importance of a balanced response was demonstrated in a Ghanian study where a higher ratio of proinflammatory (IFN-γ, TNF-α and IL-12) to anti-inflammatory (IL-10 and TGF-β) cytokines was associated with lower rates of infection, but a greater likelihood of having clinical disease when infected [8]. Overall, it seems that a lack of the initial inflammatory stage will lead to uncontrolled proliferation of the parasite. However, an uncontrolled inflammatory immune response will lead to immune-mediated pathology **(Figure 1)**. Further investigations are focusing on what factors tip the balance in one direction or the other, ultimately leading to disease.

Early inflammatory response

The role of innate immunity in the initial inflammatory response to malaria is under investigation. The strongest evidence for the interaction between malaria and the innate immune system comes from mouse models, specifically *Plasmodium berghei, Plasmodium yoelii* and *Plasmodium chabaudi*. Toll-like receptor (TLR) and other pattern recognition receptor signaling play a critical role in the activation of early immune system responders including macrophages, dendritic cells and natural killer cells. *In vitro* studies have demonstrated that the activation of TLR2 and TLR9 by various parasite targets leads to proinflammatory cytokine responses [9–12]. However, as these responses are examined over the course of infection, the picture becomes more complex. Activation of dendritic cell (DC) TLRs taken from mice early in *Plasmodium* infection appears to cause inflammatory cytokine release from T cells through the production of IL-12 [13]. However, DCs isolated from later stages in infection, when activated through TLRs, produce and stimulate T cells to produce IL-10 [14]. The molecular signals that modulate this change have yet to be understood.

Whether these early innate immune responses contribute to disease pathology is under investigation. There is evidence that cerebral malaria pathophysiology is immune mediated, with a priming of αβ T cells on first exposure to the parasite leading to the exaggerated inflammatory response in subsequent infections [15]. This was supported by the observations that children afflicted with cerebral malaria already have high levels of antimalaria antibodies, suggesting earlier infection without severe disease. As mentioned, the inflammatory cytokine IFN-γ plays a critical role in controlling parasitemia and in disease protection, and also in cerebral malaria pathophysiology [16–20]. Mice that do not express IFN-γ receptor do not develop cerebral malaria [21]. Similarly, early innate responses seem necessary for the development of cerebral disease. In mouse models, cerebral malaria-type syndromes are seen in *P. berghei* ANKA-infected wild-type mice. Mice that are deficient in TLR2, TLR9 or MyD88 (the downstream mediator of most TLR activation pathways) do not experience CNS symptoms when infected with *P. berghei* ANKA, which normally causes murine cerebral malaria syndromes [22]. The suggestion that TLRs play a role in disease severity has spurred multiple studies on TLR polymorphisms. Polymorphisms in human TLR4, TLR9 and Toll-IL-1 receptor domain-containing adaptor proteins have all been associated with increased susceptibility to severe malaria disease [23–26]. These findings support the role of early innate immune responses in the development of malaria-related pathology and present potential targets for immunomodulating therapy to augment disease therapy or vaccine efficacy.

Immune tolerance & Tregs

Early effective immune responses are characterized by inflammatory responses set in motion by the innate responders detailed earlier, but how are these responses later replaced by anti-inflammatory cytokine signaling? It may be reasonable to surmise that this change takes place as the erythrocytic parasite load is controlled. However, murine studies suggest that inflammatory cytokines peak early but decline prior to the decline of peripheral parasitemia [27–29]. Tregs, characterized as $CD4^+CD25^+FoxP3^+$, are now being investigated as the mediator of this switch from a predominantly inflammatory to a predominately anti-inflammatory response. Data in murine and human studies suggest that these cells are associated with a muted inflammatory response to malaria and increased parasite burden [30]. In human observational studies, it appears that malaria induces FoxP3 expression and increased number of Treg cells [31,32]. Furthermore, longitudinal evaluation of $CD4^+FoxP3^+$ Treg cell levels in a human sporozoite challenge study documented that an increased number of these cells was associated with higher parasite loads and a decline in proinflammatory cytokines [31]. Recent studies have examined the development of Treg-

mediated immune tolerance to malaria *in utero*. There is evidence that newborns of women with placental malaria are at an increased risk of malaria infection and disease, a vulnerability that persists for years [33–35]. Data suggest that this effect may be mediated by induction of tolerance to blood-stage malaria antigens *in utero*. These findings include an increased frequency of Treg ($CD4^+CD25^{hi}$ $FoxP3^+$) in the cord blood of newborns born in malaria endemic areas and *in vitro* data that Th1-responses to *P. falciparum* blood-stage antigens are suppressed in these study subjects [36,37].

In vitro culture experiments suggest that IL-2, IL-10 and TGF-β may drive the induction and expansion of these Tregs [38]. IL-10 plays an important role in balancing inflammatory responses to infections versus immune-related pathology [39,40]. This has been demonstrated both in viruses [41] and in parasites, where increased IL-10 is associated with a chronic infection, while low levels lead to acute infectious disease patterns. Increased IL-10 levels in asymptomatic malaria have been found in pregnant women [42] and children [6]. It has been demonstrated that despite the seemingly short life of inflammatory immune responses to malaria (as characterized by the presence of IFN-γ-producing T cells), IL-10-producing T cells persist for many years after infection in a low transmission setting [43]. This is consistent with the observation that those with historical immunity of malaria but a recent lack of exposure will still maintain protection from severe disease.

Nitric oxide/CD23 pathway

Modulation of signaling in the CD23/nitric oxide (NO) pathway has also been the proposed mechanism by which malaria immune responses are modulated, an area in which coinfection research has contributed considerably. NO production via inducible nitric oxide synthetase (iNOS) upregulation has been intensely investigated as a mediator, either as the cause or alleviator of human malaria disease. On the whole, NO production has been associated with protection from cerebral malaria in both murine and human studies [44–47]. Reactive nitrogen intermediates (RNIs), which are present when NO is produced, have been negatively correlated with the peripheral schizont to trophozoite ratio, which is used as a surrogate for the quantity of sequestered parasites [48]. It is postulated that NO decreases endothelial activation and parasite sequestration by downregulating the production of receptors such as ICAM-1 by way of the angiopoietin/TIE2 system and systemic anti-inflammatory effects [46]. NO can be produced as the downstream effect of an inflammatory cytokine response, and can also result from the activation of CD23 through low-affinity binding with IgE complexes. CD23 activation will induce iNOS transcription and also can lead to the release of cytokines that both potentiate an inflammatory reaction, such as TNF, as well as regulate it, such as IL-10, when activated chronically [48]. CD23 when not bound is cleaved and becomes a soluble signaling molecule, increasing IgE expression and activating multiple immune effector cells [49]. With chronic activation of this pathway, it is thought that less soluble CD23 is produced, leading to a more restrained NO and inflammatory cytokine reaction during an acute malaria infection [50].

Antibody-dependent cellular cytotoxicity

Finally, as mentioned earlier, control of parasitemia by antibody-dependent mechanisms may not, on its own, explain the transition from an inflammatory to anti-inflammatory profile. However, antibody-dependent cellular cytotoxicity has been a focus of investigation as a possible mechanism behind develop ment of immunity to symptomatic parasitemia. Certain *P. falciparum* antibodies are recognized as being associated with protection from malaria disease, and particular subclasses have been more strongly associated with these protective effects, specifically the cytophilic antibody subclasses IgG1 and IgG3 [51,52]. It has been theorized that early, ineffective antibody responses are later replaced by effective responses through subclass switching, and with this subclass switching comes a more

effective immune response [53]. The growing data on the protection from disease provided by blood-stage antigen antibodies support the role of this mechanism as a part of the immunomodulatory response to malaria infection, and the differentiation of B cells to produce a particular type of IgG subclass is probably in part dictated by the presence and timing of specific cytokine signals, set in motion by the mechanisms described earlier.

Immunoregulation in the setting of coinfections

Microorganisms can have powerful effects on immune responses of their host. This is particularly the case in chronic infections that modulate immune responses in order to escape detection or in diseases that attack or exhaust the host immune response. A significant amount of research has been devoted to examining the impact of malaria coinfection with other microorganisms. The findings from this research provide insight into the effects of known immunomodulators on malaria morbidity.

Helminth infections

As with malaria infection, helminthic infection is often asymptomatic and when there is pathology, it is often derived from the host immune response. These organisms, which include both soil-transmitted helminths, schistosomes and filarial infections, have well-described potent immunomodulatory effects on their hosts, a trait that allows them to live in humans for long periods of time undetected. This has been attributed to their anti-inflammatory effect, which dampens IFN-γ responses and biases immune responses towards a Th2-type response through Tregs, Th2-inducing DCs and alternatively activated macrophages [54,55]. Early on, it was demonstrated that helminthic infection decreases host responses to various vaccines, including tetanus [56] and cholera [57]. Given the anti-inflammatory effects of helminths, it is expected that, based on what is known of malaria immunology and pathophysiology, a helminth infection may lead to increased rates of infection and parasitemia by dampening inflammatory signaling and decreased rates of immune-mediated disease. Overall, this hypothesis is born out in some studies but contradicted in others. This suggests that our understanding of malaria immunity and the homogeneity of helminthic immunomodulation may be overly simplistic [58]. Most important, however, is the overwhelming evidence that these infections can influence malaria-specific immune responses, and helminth infection must, therefore, be assessed in studies aimed at characterizing malaria immunity.

Soil-transmitted helminths

The impact of soil-transmitted helminths on malaria infection and severity is the best characterized among all the helmithic infections **(Table 1)**. Several studies on *Ascaris lumbricoides* have been performed out of concern that intestinal worm treatment campaigns may have the unintended effect of increasing the rate and severity of malaria infection. In studies that screen purely for infection, both symptomatic and asymptomatic, some have found increased malaria infection rates among individuals with *Ascaris* infection [59], while others have shown decreased rates [60] or no effect [61]. Randomized controlled trials of the antihelminthic levamisole in the 1990s showed that after children were treated for *Ascaris* infection, they had increased parasitemia rates with malaria infections [62,63]. Studies that went further, examining the malaria morbidity, have found that *Ascaris* infection is associated with decreased drops in hemoglobin from malaria [64], and decreased odds of cerebral malaria and renal complications of severe malaria [65], all complications that may occur in part owing to immune-mediated mechanisms [66]. By contrast, a study suggested that *Ascaris* infection was associated with increased rates of severe malaria among children, although the majority of subjects in this study met criteria for severe malaria through vomiting, which can be symptoms of *Ascaris* infection itself [67]. On the whole, there are

insufficient data on whether *Ascaris* infection increases or decreases vulnerability to infection; but the preponderance of data suggests that *Ascaris* infection may decrease the morbidity associated with malaria infection.

There are less data on other soil-transmitted helminths including hookworm and *Trichuris trichiura*, and many studies do not differentiate by the type of soil-transmitted helminth. The studies that have examined the effect of hookworm alone found more consistently that malaria infection is greater among hookworm-infected individuals, including increased infection rates and increased *Plasmodium* parasitemia [60,68–74]. Clinical studies examining any type of soil-transmitted helminth have yielded mixed results [60,61,68,75–77]; however, an interesting study demonstrated that stool soil-transmitted helminth infection was associated with protection from cerebral malaria in Thai adults, and that individuals with helminth infections had increased concentrations of RNIs than those without helminth infection [50]. This was attributed to the chronic activation of the CD23 receptor and decreased circulating levels of soluble CD23. This group has postulated that acute CD23 activation, in the setting of high levels of sCD23, produces pathologic NO and TNF production. In the setting of low circulated soluble CD23, CD23 activation will lead to a restrained NO release, limiting parasite sequestration.

With the inconsistent data, research addressing the immunologic mechanisms at play is useful. Cytokine profiling in *Plasmodium* and helminth coinfected individuals reveals increased expression of IL-10 when compared with those with *Plasmodium* infection alone [78]. Another study has also demonstrated increased expression of the Treg marker FoxP3 among those with helminthic infection [78]. There were similar findings in an Asian retrospective case–control study examining the influence of worm infection on malaria vulnerability [79]. In *Ascaris*, in particular, it seems that soil-transmitted helminths may be able to decrease the severity of infection by biasing a Th2-mediated response. However, the effect of helminthic infection of controlling parasitemia is less clear. If the initial inflammatory response is critical for controlling parasitemia, then one might expect higher parasitemia in the setting of helminthic infection. This has been seen in hookworm infections, but the opposite has been demonstrated with *Ascaris*. Overall, there is evidence that soil-transmitted helminths immunomodulate responses to malaria infection, but there are inconsistencies between helminth type and other factors including host age.

Schistosomes

The same issue of conflicting findings exists in the research on malaria-schistosome coinfection **(Table 2)**. Limited studies in humans suggest that *Schistosoma mansoni* is associated with increased risk of malaria infection [80]. In murine models, *S. mansoni* coinfection results in higher parasitemia and lethal disease from *P. yoelii*, an infection that is normally not fatal in mouse models [81]. In an examination of possible immune-mediated pathology, a *P. berghei* murine model found that coexisting *S. mansoni* infection appears to decrease rates of cerebral malaria [82]. Even more confusingly, researchers examining *P. falciparum* and *Schistosoma haematobium* have found decreased rates of uncomplicated malaria among individuals with *S. haematobium* [83,84]. In this population, *S. haematobium* is associated with elevated IL-6, IL-4 and IFN-γ levels. When infected with malaria, IL-6 and IL-10 levels rose in all children, but were blunted in Schistsoma-infected individuals [85]. Further studies showed that *S. haematobium* infection was associated with decreased Tregs during malaria infection, decreased *Plasmodium* parasitemia and increased IFN-γ and IL-2 [74,86]. The parasitemia and cytokine profile is similar to what would be expected based on the results of the sporozoite challenge studies examining Treg cells, but why does *S. haematobium* infection, which in isolation causes a potent Th2 response, lead to an increased inflammatory response to malaria infection? It is likely that we are

oversimplifying the immunomodulatory of *S. haematobium* and other helminths. Interestingly, one factor addressed by the schistosomiasis coin-fection literature is the role of the intensity of helminth infection in malaria outcomes [83]. In the same study cited above, where *S. mansoni* infection was associated with increased malaria risk, a subanalysis demonstrated that those with only moderate intensity infection, malaria attack rates were lower, although this analysis did not reach statistical significance [80].

Filaria

Chronic filarial infections (Wuchereria bancrofti, Mansonella perstans, Brugia malayi and *Brugia timori*), like other helminth infections have been associated with an anti-inflammatory immune response (decreased IL-2 and IFN-γ), thought to be mediated through increased circulating IL-10-producing $CD4^+$ lymphocytes [87–89]. In fact, the filarial glycoprotein ES-62 is an excellent example of a potential immunomodulatory therapy to arise from coinfection research. This protein has been shown to inhibit mast cell activity in hypersentivity reactions and may hold therapeutic promise in allergy-based pathophysiology [90]. In malaria coinfection research, filarial infection has been demonstrated to modulate malaria-specific IL-12p70, IP-10 (CXCL-10), IFN-γ, TNF-α and IL-17A levels [91,92]. This is thought to take place through interferon regulatory factor-1 signaling by CD4 cells, suppressing IL-12 secretion from antigen presenting cells [93]. In the murine cerebral malaria model using *P. berghei* ANKA, filiarial infection with *Litomosoide sigmodontis* was protective against the development of cerebral malaria, an effect that was lost when the same experiment was carried out in *IL-10*-knockout mice [94]. Although robust research has been carried out on the immunologic mechanisms of filarial and *Plasmodium* coinfection, there are limited clinical data on these coinfections. Therefore, it is not known whether the bias towards a regulatory immune environment actually leads to more infection and less immune-mediated disease.

The variability in responses, both clinical and immunologic, reinforces that helminthic infection should not be considered a uniform immunologic entity. Investigation into the precise mechanisms by which helminthic infection augments the immune response is currently underway. Multiple TLR targets have been identified so perhaps distinct immunologic signaling with the innate immune system is one explanation for the observed variability. Lipids derived from *Schistosoma* and *Ascaris* activate TLR2 [95], schistosomal egg dsRNA activates TLR3 [96] and a schistosomal mansoni egg polylactosamine sugar (lacto-*N*-fucopentaose III) and filarial glycoprotein ES-62 activate TLR4. Each of these is thought to do so through unknown coreceptors that push DC response in an anti-inflammatory direction [89], and also possibly interfere with TLR signaling of other microorganisms.

HIV infection

There is great interest in understanding the interaction of HIV and malaria, given the magnitude and geographic distribution of the two epidemics. However, there are major obstacles in studying these two infections. Diagnostically, the high prevalence of multiple opportunistic infections in persons with HIV infection and immunodeficiency makes accurate assessments of malaria burden difficult. Furthermore, the widespread use of cotrimoxazole prophylaxis, which has antimalarial activity, may obscure the immunomodulatory effects of HIV on malaria infection. Finally, from a mechanistic standpoint, immunomodulation in HIV is difficult to characterize because it has such profound effects on multiple aspects of the immune system, causing dysfunction in innate, cell-mediated and humoral aspects of immunity. Despite this, there is considerable clinical evidence that HIV increases susceptibility to malaria **(Table 3)** [97–101], and this increased vulnerability has been reported both in infection rates and disease severity [102].

Interestingly, observations that HIV-positive persons are at increased risk for severe disease have come primarily from studies carried out in areas of unstable transmission and in pregnant women. This suggests that the impact of HIV infection seems to be greatest in those who have a greater likelihood of being without pre-existing immunity to malaria at the time of HIV infection. Perhaps, the immunomodulatory effects of HIV most profoundly impact one's ability to effectively acquire immunity to malaria and to a lesser degree impairs already acquired immune responses.

In terms of the mechanisms behind this immunomodulation, it was found early in the HIV epidemic that progression of the disease is characterized by the gradual transition to lower levels of inflammatory cytokines and higher levels of anti-inflammatory cytokines [103]. In viremic HIV-infected persons, IL-10 mRNA is found to be unregulated in several peripheral blood mononuclear cell lines [104]. Cytokine profiles in response to another chronic parasitic infection, leishmaniasis, have found that there are decreased IFN-γ:IL-10 ratios in individuals with HIV and leishmaniasis as compared with HIV-negative individuals with the infection. There is very little direct research examining the immunologic mechanisms by which HIV affects malaria vulnerability. A study on pregnant women found that HIV infection significantly alters the cytokine milieu of the placenta. IL-10 mRNA levels in the placenta decrease while TNF-α mRNA levels increase. However, the presence of peripheral or placental *P. falciparum* parasitemia did not differ according to HIV infection status, although HIV infection was associated with higher level of placental parasitemia [105]. Further research is required in the area of HIV and malaria coinfection.

Hepatitis B infection

Viral hepatitis coinfection in malaria has been largely overlooked, with only a handful of clinical studies available in the literature **(Table 3)**. These infections, like the other chronic infections discussed in this article, have been described as having anti-inflammatory effects on their hosts. A study conducted in the Amazon included immunologic outcomes and found that individuals with active hepatitis B infection were more likely to be asymptomatic in response to *Plasmodium* infection, tended to present with lower levels of parasitemia and had decreased proinflammatory cytokine levels [106]. However, another study in the same region found that there were no clinical differences in those individuals with coinfection versus malaria infection alone [107]. Parasitemia rates were lower and antibody levels were higher in hepatitis B-infected subjects; but these findings did not reach statistical significance. In Thailand, one group found that the prevalence of hepatitis B among severe malaria patients was much higher than in the general population (24 vs 10%) [108]. This phenomenon has also been documented in Africa among children with uncomplicated malaria infection [109]. Few conclusions can be drawn, given that there are only a handful of studies addressing this coinfection, and it would also be valuable to understand the impact of hepatitis C coinfection.

Other viral infections

Other immunomodulatory viruses include Epstein–Barr virus (EBV) and cytomegalovirus (CMV). While numerous articles have been written about the effects of malaria on EBV infection [110–112], little is known about how EBV infection affects the risk of malaria or immune response to *P. falciparum*. Very little is known about CMV infection in malaria endemic areas, and the few studies published on CMV and malaria have noted the effect of malaria on CMV infection (e.g., placental malaria is associated with increased congenital CMV infection) [113], but not the effect of CMV infection on risk of malaria or on immune response to malaria. Further research in this area is also strongly indicated.

Conclusion

Implications of immunomodulatory therapeutics

In this review, we have examined the effect on malaria infection of common infections with known immunomodulatory effects, but can we apply what we learn from these ‘experiments in nature’ on malaria coinfections to help develop immunomodulatory therapeutics? Based on the data on helminthic or viral coinfections with malaria reviewed here, immunomodulatory therapies in malaria will need to ride the fine balance between control of parasitemia and control of the immune response to *P. falciparum*. Excessive attenuation of the proinflammatory response could lead to an increased level of parasitemia, while therapies that do not adequately control the proinflammatory response may be ineffective at decreasing severe disease. Therapies that have been investigated include broadly anti-inflammatory agents such as glucocorticoids and TNF-α-targeting agents whether through antibodies, pentoxifylline or thalidomide [114,115]. Smaller studies have also examined targeting other cytokines, and there are also agents that target downstream immune effects including drugs targeting the expression of adherence molecules and drugs aimed at limiting oxidative damage.

At present, none of the major immunomodulatory therapies have been shown to improve clinical outcomes in humans, although attenuation of the inflammatory response might have an effect on long-term effects such as cognitive impairment in survivors of severe malaria [116]. In terms of improving short-term outcomes including mortality, the timing of immunomodulatory therapy remains an issue. It has been postulated that these agents have not had great success because they are administered too late in the pathogenesis of the disease; at the time a child presents with severe disease, an already strong proinflammatory response is underway. The demonstrated ability of coinfections, which are present at the onset of infection, to modulate disease outcomes supports this hypothesis. It is possible that concomitant highly effective antiparasitic therapy such as artemisinin combination therapy with immunomodulatory agents may decrease the risk of uncontrolled parasitemia with these agents; but there are no well-designed trials to provide a strong basis for such a prediction. Murine models may provide some insight into timing of immunomodulatory therapy, but the differences in *Plasmodium* species between human infection and murine models and in the human and murine immune response may limit the extrapolation of these data [117]. Two recent reviews highlighted some promising new therapies, including arginine and inhaled NO to increase NO concentrations, and PPAR-γ agonists such as rosiglitazone, which can modify CD36 transcription and TLR2-dependent innate inflammatory immune responses, as potential agents to favorably modulate human immune response to malaria [114,115]. These studies reflect insights learned from murine models and also from the studies of helminth coinfection with malaria, ‘experiments in nature’ that provided support for helminth-induced increase in RNIs as a factor in protection against cerebral malaria [50], and helminth-associated alterations in TLR responses [118,119].

In the case of limiting or preventing severe disease, the only way to administer pharmacologic immunomodulators sooner may be to treat all infected individuals with risk factors of severe malaria, but before they develop severe disease in order to prevent rather than treat severe malaria [89]. There are investigations underway to identify biomarkers for severe disease [120]; but in reality we are still a long way from useful clinical tools to identify high-risk individuals. With this in mind, when considering prevention strategies for those with severe disease, we should consider what has been seen with HIV infection. It is possible that immunomodulation may prevent the acquisition of immunity. Any immunomodulatory therapy that is studied should be evaluated longitudinally to assure that it does not impair the acquisition of effective natural immunity in a way that leads to greater risk later in life.

Finally, the diversity of immunomodulatory responses seen among the helminth infections suggests that certain anti-inflammatory pathways decrease malaria severity while others do not. This may point towards choosing specific immunomodulatory targets rather than using broadly anti-inflammatory agents. This variability also brings up the possibility that confounding factors may be at play here, and that more data are needed to understand the true impact of coinfection on malaria morbidity and mortality. Epidemiological studies may not be able to completely control the increased risk of both infections among certain sub populations. Age and socioeconomic factors have been linked with the risk of coinfection [73]. Studies examining only clinical outcomes do not allow us to understand the mechanism for increased or decreased malaria vulnerability. It is possible that in the setting of a pre-existing condition such as HIV or helminthic infection, an individual has less physiologic reserve to deal with malaria infection as opposed to a truly immunomodulatory mechanism. Because of these issues with clinical and epidemiologic studies, it is valuable to have studies that examine immunologic mechanisms behind interactions including cytokine profiling and T-cell phenotyping.

In conclusion, we have evaluated some of the critical naturally acquired immune responses to malaria infection and examined how some of these responses are altered by naturally occurring immunomodulators. Overall, this line of research may lend insight into effective ways to alter immune response to decrease malaria morbidity, but sometimes the contradictory evidence from different studies points to the complexity of the interplay of pathogens and host immune response. The relative paucity of data on coinfections, notably but not exclusively for viral coinfections, points to a need for continued research in these areas including both clinical assessments and studies on immunologic mechanisms of disease or protection.

Expert commentary

At present, there are still major research gaps in the field of immunomodulation in malaria. Limited data on coinfection with known immunomodulatory infections suggest that immunomodulation can ameliorate severe disease outcomes. However, the precise targets that can simultaneously control deleterious immune responses without leading to uncontrolled parasitemia remain elusive. More research is required to provide insights that could lead to new immunomodulatory therapies in malaria. The research presented here on experiments in nature suggests that coinfection research can lend insights into the development of these therapies. Data from animal models cannot be the sole basis for human immunomodulatory targets, because the immunology of severe malaria in animal models differs in important ways from that of human malaria. Data from studies of coinfections in humans may inform us about important factors in the human immune response that modulate parasitemia and severity of disease.

Five-year view

The field of malaria immunology is rapidly expanding. Over the next several years, the ‘optimal’ immune response to *P. falciparum* may be understood more fully through high-throughput assays and systems biology approaches. Diagnostic tools that allow for more accurate field-based diagnostics may also be developed, including tools that identify children who are at greatest risk for severe disease early in their course. These studies and tools could allow for the development of efficacious immunomodulatory therapies by providing insights on how to more precisely modulate the host immune response, and by allowing introduction of this therapy in a timely manner. The development of these therapeutics would be greatly facilitated by continued research on coinfections in malaria.

Acknowledgments

This work was supported in part by a grant awarded to CC John from the National Institute of Neurological Disorders and Stroke and the Fogarty International Center (R01 NS055349).

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Key issues

- Effective *Plasmodium falciparum* immunity requires a precisely timed and balanced response of inflammatory and anti-inflammatory immune regulators.
- Limited data from parasitic and viral coinfections in individuals with *P. falciparum* infection suggest that immunomodulatory therapies that alter host Toll-like receptor and Treg responses to *P. falciparum* or augment host nitric oxide levels are most likely to result in reduction in disease severity.
- Data on helminthic coinfection in malaria suggest that specific anti-inflammatory profiles are associated with protection, while others are not.
- HIV infection increases the incidence and severity of malaria infection. This phenomenon is particularly notable in areas of low transmission.
- There is a paucity of data on coinfection in malaria, particularly viral coinfection, and further studies in this area are needed.
- To date, no immunomodulatory therapies have reproducibly demonstrated improvement in clinical outcomes in human studies, but some promising adjunctive therapies are currently in clinical trials.

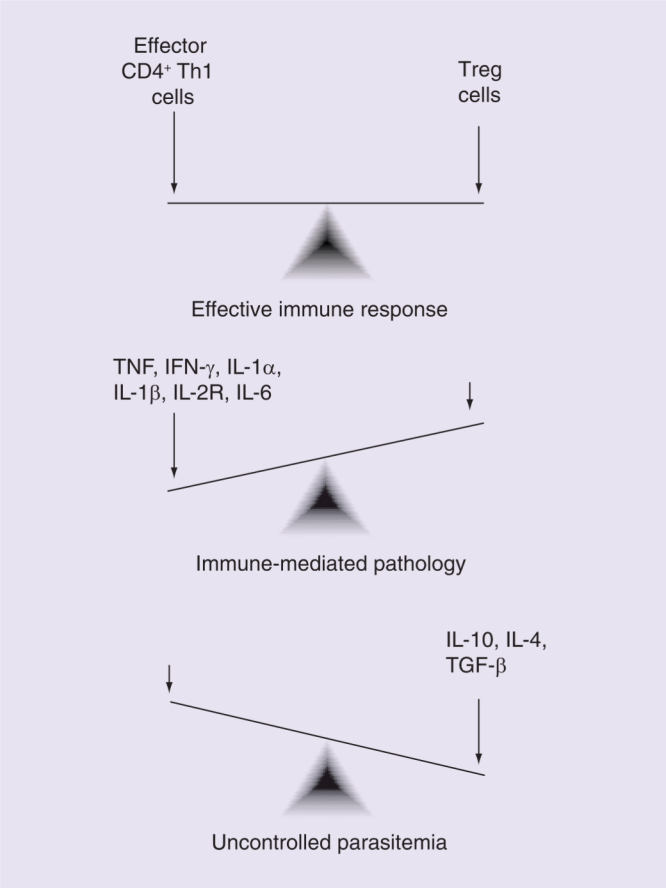

Figure 1. Balancing inflammatory and anti-inflammatory immune responses to malaria

This figure represents cellular immune responses theorized to mediate disease outcomes in the setting of *Plasmodium falciparum* coinfection, notably intestinal helminth infections. The figure is a simplified representation of these responses, which highlights mechanisms that may be amenable to therapeutic targets, but does not fully represent the multiple and complex immune mechanisms that occur in humans in response to *P. falciparum* infection.

Table 1

Key clinical publications on soil-transmitted helminth and malaria coinfection.

Organism	Country	*Ascaris* spp.	Hookworm	*Trichuris* spp.	*Strongyloides* spp.	All soil-transmitted helminths	Findings	Ref.
Hookworm	Malawi		↑ IR				Hookworm infection associated with malaria infection in a cross-sectional study	[74]
Ascaris lumbricoides	Gabon	↑ IR					*A. lumbricoides* associated with *Plasmodium* infection during pregnancy. Sensitivity of hookworm assay not considered sufficient for analysis. Study was a cross-sectional analysis within a clinical trial	[59]
Soil-transmitted helminths	Thai–Burmese border	↓ IR	↑ IR ↑ DS				*A. lumbricoides* infection was associated with a decreased risk of malaria in a cross-sectional analysis. Hookworm infection was associated with an increased risk of malaria and anemia. Any spp. of malaria was evaluated	[60]
A. lumbricoides, hookworm, *Trichuris* spp.	Brazil	↓ DS	↓ DS	↓ DS			Decreased drop in hemoglobin during acute *Plasmodium vivax* infection for helminth-infected children	[64]
A. lumbricoides, hookworm, *Trichuris* spp.	Uganda		↑ DS				Hookworm infection associated with lower hemoglobin level during malaria infection in school-aged children. Spatial and household clustering of coinfection noted	[69]
A. lumbricoides, hookworm, *Trichuris* spp.	Ethiopia		↑ DS			↓ DS	Higher malaria parasite density among those with hookworm	[68]

Organism	Country	*Ascaris* spp.	Hookworm	*Trichuris* spp.	*Strongyloides* spp.	All soil-transmitted helminths	Findings	Ref.
							infection. Soil-transmitted helminth infection negatively correlated with the severe malaria symptoms	
A. lumbricoides, hookworm, *Trichuris* spp.	Ghana						Whole blood samples from helminth-infected children were exposed to malaria infected RBCs and expressed increased levels of IL-10, SOCS-3, FoxP3 and PD-1	[78]
A. lumbricoides, hookworm, *Trichuris* spp., *Strongyloides* spp.	Ghana	↑ IR	↑ IR	No Assoc.	No Assoc.		Pregnant women with hookworm and *Ascaris* infection had increased rates of malaria infection. No association was seen with *Strongyloides* or *Trichura* spp.	[73]
All gastrointestinal helminth, *Ascaris*	Kenya	No Assoc.					Gastrointestinal helminth infection and eosinophilia were statistically not associated with malaria susceptiblity	[76]
Hookworm, *Trichuris* spp., *Strongyloides* spp.	Uganda		↑ IR	No Assoc.	No Assoc.		Cross-sectional study in which hookworm infection is associated with malaria infection. No association noted with *Trichuris* or *Strongyloides* spp. infection	[71]
Hookworm, *Trichuris, Ascaris*	Zimbabwe		↑ IR				Hookworm infection is associated in a cross-sectional analysis with *P. falciparum* malaria infection	[70]
A. lumbricoides	Madagascar	↓ DS					*Ascaris* treatment with levamisole associated with increase in *P.*	[62,63]

Organism	Country	*Ascaris* spp.	Hookworm	*Trichuris* spp.	*Strongyloides* spp.	All soil-transmitted helminths	Findings	Ref.
							falciparum densities among 4–15-year-olds	
A. lumbricoides, hookworm, *Trichuris* spp.	Uganda					No Assoc.	No association observed between intestinal helminth infection and malaria risk in a cross-sectional household-based sample	[61]
A. lumbricoides	Senegal	↑ DS					Higher prevalence in a prospective case–control study of *Ascaris* infection in individuals with severe malaria	[67]
Soil-transmitted helminths	Thailand					↑ DS	Helminth-infected subjects were more likely to develop falciparum clinically symptomatic malaria in a prospective trial	[75]
A. lumbricoides, hookworm	Thailand	↓ DS	↓ DS				*A. lumbricoides* and *Necator americanus* (hookworm) was associated with protection from cerebral malaria in a case–control trial. Helminth infection was associated with lower rates of renal failure, jaundice and peripheral mature schizonts. Helminth-infected individuals had higher reactive nitrogen intermediates in blood samples	[65,75,79]

DS measures include parasite density, severity of anemia and prevalence/incidence of severe malaria (or one of the severe malaria criteria including cerebral malaria). IR refers to any measure of infection, including incidence or prevalence.

Assoc.: Association; DS: Disease severity; IR: Infection rate; PD: Programmed death; RBC: Red blood cell.

Table 2

Key clinical publications on schistosomiasis and malaria coinfection.

Organisms	Country	Findings	Ref.
Schistosoma haematobium	Malawi	*S. haematobium* associated with lower malaria parasite densities	[74]
S. haematobium	Gabon	*S. hematobium* was not found to be associated with malaria infection	[59]
S. haematobium	Kenya	Schistosomiasis was not associated with malaria susceptiblity	[76]
Schistosoma mansoni	Uganda	No association between *S. mansoni* and malaria infection found	[71]
S. mansoni, S. haematobium	Zimbabwe	*S. mansoni* infection associated with *Plasmodium falciparum* malaria infection	[70]
S. haematobium	Mali	Levels of Tregs were lower in malaria–*S. haematobium* coinfected children versus children with malaria alone. Increased Tregs were associated with decreased serum Th1 cytokine levels and elevated parasitemia	[86]
S. haematobium	Mali	IL-6 and IL-10 elevations in acute malaria were blunted in schistosomiasis-infected children from 4 to 8 years old	[85]
S. haematobium	Senegal	Subjects with low-density *S. haematobium* had lower *P. falciparum* parasitemia	[83]
S. haematobium	Mali	Prospective study demonstrating delay in time to clinical malaria infection, decreased number of malaria episodes and decreased *Plasmodium* parasitemia among schistosomiasis-infected children	[86]
S. mansoni	Senegal	*S. mansoni*-infected individuals had increased incidence of clinical malaria. Effect most apparent in those with high schistosomiasis organism burden. Nonsignificant observation that malaria attack rates were lower in ‘medium-grade’ *S. mansoni* infections	[80]

Table 3

Key clinical publications on chronic viral and malaria coinfection.

Infection	Country	Findings	Ref.
HIV	Malawi	HIV infection was associated with malaria infection in a cross-sectional analysis within a clinical trial	[74]
	Uganda	HIV-1 infection is associated with cerebral malaria in children in a cross-sectional analysis	[102]
	Cameroon	HIV-1 infection was not associated with placental and peripheral *Plasmodium falciparum* infection. HIV was associated with increased placental *P. falciparum* parasitemia	[105]
	Zambia	Case–control trial in which HIV-1 infection was a risk factor for adults with severe malaria compared with controls with uncomplicated malaria asymptomatic controls. Disease severity was associated with low CD4	[99]
	Malawi	Prospective study evaluating malaria treatment failure with SP in the setting of HIV infection and considerable levels of SP resistance. Treatment failure was not associated with CD4 cell count	[100]
	Malawi	A longitudinal observational trial demonstrating an increased incidence of clinical malaria episodes in subjects with CD4 cell counts <200 cells/mm^3	[101]
	Malawi	Prospective trial showing association of HIV-1 infection with parasitemia. Treatment failure was not associated with HIV infection	[98]
	Uganda	Prospective observational trial in which HIV infection, low CD4 cell count and advanced HIV clinical stage was associated with increased incidence of clinical malaria. Among HIV-infected subjects, low CD4-cell counts were associated with increased parasitemia	[97]
Hepatitis B	Brazil	An observational study with active and passive *Plasmodium* case finding. Subjects with active HBV were more likely to be asymptomatic when infected with malaria and have decreased parasitemia. HBV infection was also associated with a decreased inflammatory cytokine profile	[106]
	Brazil	Among subjects with acute *P. falciparum* and *Plasmodium vivax* malaria, clinical characteristics were similar between those with and without serologic markers for HBV infection	[107]
	Vietnam	Among patients hospitalized for severe *P. falciparum* malaria, the prevalence of hepatitis B surface antigen positivity was higher than that in the reported population	[108]
	Gambia	In a case–control trial of severe malaria and match controls, severe cases were more likely to carry hepatitis B	[109]

HBV: Hepatitis B virus; SP: Sulfadoxine–pyrimethamine.