Parasite virulence, co-infections and cytokine balance in malaria

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Strong early inflammatory responses followed by a timely production of regulatory cytokines are required to control malaria parasite multiplication without inducing major host pathology. Here, we briefly examine the homeostasis of inflammatory responses to malaria parasite species with varying virulence levels and discuss how co-infections with bacteria, viruses, and helminths can modulate inflammation, either aggravating or alleviating malaria-related morbidity.

Keywords: Malaria, Immunity, Cytokines, Inflammation

Introduction

A delicate balance between inflammatory and regulatory cytokine responses is crucial to determine the outcome of infections with intracellular pathogens, such as viruses, bacteria, and protozoa. A strong, T_H1 -driven pro-inflammatory response is typically elicited during acute infections and exerts a major microbicidal effect, but the failure to produce sufficient regulatory cytokines, such as interleukin (IL)-10 and transforming growth factor (TGF)- β , can lead to excessive inflammation and significant tissue damage. Nevertheless, high levels of IL-10 suppress phagocytosis and the release of inflammatory mediators from monocytes and reduce the ability of dendritic cells to present antigens and activate T cells,¹ overriding effector responses mediated by interferon (IFN)- γ and other inflammatory cytokines and promoting pathogen persistence.^{2,3}

Malaria parasite products such as DNA bound to hemozoin⁴ and glycosylphosphatidylinositol anchors⁵ trigger the production of pro-inflammatory cytokines via activation of toll-like receptors (TLRs).^{6–8} An early production of tumor necrosis factor (TNF)- α , IFN- γ , IL-1, IL-6, and IL-12, among other inflammatory cytokines and chemokines,⁹ allows for a fast parasite clearance in human *Plasmodium falciparum* malaria,^{10–12} but may cause significant endothelial dysfunction and aggravate the sequestration of parasitized red blood cells in small blood vessels.¹³ Once parasite multiplication has been controlled, regulatory cytokines are required to reduce the risk of disproportionate inflammation and severe disease.^{14–16}

However, an early release of high levels of IL-10 may lead to a less effective parasite clearance.¹⁷

Similar cytokine balance patterns are seen in experimental murine malaria: while the timely production of IFN- γ by $CD4^+$ T cells protects mice from overwhelming acute-phase parasitemias, down-regulating the inflammatory response is required to reduce the risk of cerebral malaria, severe malaria-related anemia, hypothermia, and hepatic pathology over the next few days.^{18–21} The extent to which the pathophysiological bases of cerebral malaria in humans and experimental murine models are comparable remains a matter of intense debate, but at least a central role for inflammation in both processes has been largely recognized.²² The dendritic cell response to repeated TLR stimulation becomes anti-inflammatory in the later stages of experimental murine malaria, with decreased production of TNF- α and IL-12 and increased release of IL-10.^{23,24} Interestingly, prolonged IL-10 responses have been associated with hyperparasitemia and parasite persistence in chronic *Plasmodium chabaudi* infections in B-cell-deficient $JH^{-/-}$ mice.²⁵

The iron regulatory hormone hepcidin is a key player in the association between inflammation and anemia, one of the major complications of human and experimental malaria. Pro-inflammatory cytokines up-regulate the secretion of hepcidin from macrophages and hepatocytes,²⁶ which in turn inhibits iron absorption and its release from macrophages by down-regulating the concentration of ferroportin. In fact, high levels of circulating hepcidin have been found in acute *P. falciparum* infections^{27–30} as well as in experimental *Plasmodium berghei* infections in mice.³¹ Since a timely IL-10 response during acute *P. falciparum* infections can prevent

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severe anemia in humans,^{32,33} one could speculate that IL-10 prevents anemia by down-regulating hepcidin. Nevertheless, to the contrary, there is recent evidence that IL-10 actually induces hepcidin secretion in primary macrophages, in a dose-dependent manner.³⁴ These findings suggest that the protective effect of IL-10 against severe anemia in *P. falciparum* malaria is not mediated by hepcidin.

We hypothesize that the optimal immunoregulatory strategies might vary according to the virulence of malaria parasites, as suggested by comparisons between experimental murine infections with lethal and non-lethal *Plasmodium yoelii* strains.^{35,36} Significantly, high levels of IL-10 induced by nonlethal rodent parasites, *P. berghei* XAT and *P. yoelii* 17X, can protect mice against experimental cerebral malaria caused by co-infection with a more virulent strain, *P. berghei* ANKA.³⁷ Furthermore, co-infections with a wide range of unrelated pathogens, such as Gram-negative bacteria, HIV, and helminths, might also modulate immune responses elicited by malaria parasites. Here, we briefly discuss how infections with relatively less virulent plasmodia and co-infections with a second pathogen can affect the finely tuned inflammatory cytokine balance that characterizes uncomplicated malaria.

Maintaining the Balance: Competing Priorities

Classical studies of the highly virulent parasite *P. falciparum* have contributed to our current understanding of the immunopathology of human malaria, but the regulation of inflammatory responses elicited by the more benign species *Plasmodium vivax* remains understudied. However, *P. vivax* is a major public health challenge in Central and South America, the Middle East, Central, South, and Southeast Asia, Oceania, and East Africa, where 2.85 billion people are currently at risk of infection³⁸ and 70–80 million clinical cases are reported each year.³⁹

In contrast with *P. falciparum*, *P. vivax* elicits an early regulatory cytokine response, which counteracts the effects of TNF- α , IFN- γ , and IL-12 and prevents major inflammation-related pathology. High levels of inflammatory cytokines are typically found in the plasma of acute *P. vivax* malaria patients,^{40–43} with a sharp increase in TNF- α levels preceding febrile paroxysms.⁴⁴ *Plasmodium vivax* has recently been suggested to elicit a greater inflammatory response per parasite than does *P. falciparum*,⁴⁵ but subsequent studies using real-time polymerase chain reaction (PCR) to quantify parasite densities failed to confirm this finding.⁴³ Moreover, a prominent regulatory response can be simultaneously found in acute vivax malaria, with median plasma concentrations of IL-10 up to eight-fold greater than those measured in falciparum or mixed-species malaria patients living in the same area of endemicity.^{43,46} Accordingly, higher

IL-10/TNF- α , IL-10/IFN- γ , and IL-10/IL-6 ratios are found in uncomplicated vivax malaria, compared to uncomplicated falciparum or mixed-species malaria,⁴³ consistent with a bias toward regulatory cytokines in human immune responses to *P. vivax*.

An IL-10-dominated environment prevents excessive inflammation but may theoretically favor parasite multiplication. In fact, plasma concentrations of IL-10/TNF- α and IL-10/IFN- γ ratios correlate positively with *P. vivax* parasitemias^{41,43,47,48} and return to their normal range after antimalarial treatment.^{43,46,49} Interestingly, however, high IL-10 levels do not eventually translate into overwhelming parasitemias, since *P. vivax* blood stages can only infect reticulocytes, which comprise less than 1% of the circulating pool of red blood cells.

In conclusion, the mechanisms maintaining the homeostasis of inflammatory responses in human and experimental malaria remain unclear,⁵⁰ but available data indicate that hosts have developed different strategies to deal with different species of parasites. The fast-multiplying species *P. falciparum*, similarly to the most virulent rodent malaria species, requires a double-edged strategy in which predominantly inflammatory responses can prevent hyperparasitemia but may lead to severe disease, such as cerebral malaria and severe anemia, while *P. vivax* elicits IL-10-dominated cytokine responses without the risk of allowing for overwhelming parasite multiplication.

Breaking the Balance: Role of Co-infections

Any factor affecting the delicate balance between inflammatory and regulatory responses may theoretically affect the outcome of experimental and human malaria.⁵¹ We next examine the effect of co-infections with either different species of plasmodia or unrelated pathogens on the immune homeostasis in malaria patients.

P. falciparum and *P. vivax* co-infections

Outside of Africa, *P. falciparum* invariably co-exists with other human malaria parasite species – the most important of which is *P. vivax*. Most available data show a reduced *P. falciparum*-associated morbidity in co-infections with *P. vivax*, consistent with intra-host competition between species being beneficial to the host.⁵² In a large prospective study, the risk of severe disease decreased four-fold in Karen subjects co-infected with *P. vivax*, compared with those with *P. falciparum*-only infections, suggesting that *P. vivax* might attenuate the severity of concomitant *P. falciparum* infections.⁵³ Mixed-species infections were also associated with lower risk of anemia, lower recrudescence rate after antimalarial treatment,^{54,55} and lower average parasite density.^{43,52} In addition, mixed-species infections may be associated with milder symptoms, compared to single-species infections with *P. vivax* or *P. falciparum*.⁴³ However, there

is so far no evidence that a *P. vivax*-induced shift in the inflammatory balance contributes to reduced morbidity in mixed-species malarial infections.⁴³ In addition, there is a single report suggesting that co-infections with *P. falciparum* and *P. vivax* in Western Thailand are associated with higher fever than single infections with either species.⁵⁶

Co-infections with Gram-negative bacteria

Available evidence suggests that bacterial co-infections can worsen malaria outcomes by exacerbating the inflammatory responses that characterizes acute-phase malaria. Accordingly, bacteremia has been diagnosed in 24% critically ill adult malaria patients in France;⁵⁷ moreover, the risk of developing severe malaria is elevated 8.5-fold in children with bacteremia.^{58,59}

Several lines of evidence point to co-infections with nontyphoid *Salmonella* species (NTS) and other Gram-negative bacteria, which are common in developing countries, as major contributors to malaria-related morbidity in African children.^{60,61} Human malaria can increase the susceptibility to concurrent bacteremia by inducing neutrophil dysfunction, with reduced chemotaxis and oxidative burst.^{49,62} Induction of heme oxygenase-1 (HO-1) production following parasite-induced hemolysis is one of the putative mechanisms that could impair neutrophil function and resistance to NTS during acute malaria. Heme oxygenase-1 induction is thought to be essential to reduce heme-mediated tissue damage in hemolytic diseases such as malaria and sickle-cell anemia, but inhibits oxidative burst in granulocytes and can favor bacterial survival within these cells.⁶³ Moreover, malaria can increase the iron content in macrophages, as a result of enhanced erythrophagocytosis, thus favoring the survival of iron-dependent NTS within these cells.^{64,65}

Interestingly, malaria not only predisposes to concurrent infections but can also increase the responsiveness to inflammatory stimuli of bacterial origin, contributing to overall disease severity. A classical experiment has shown that *Plasmodium vinckei*- and *P. berghei*-infected mice are 100-fold more susceptible than naïve mice to the lethal effects of a TLR4 ligand, lipopolysaccharide (LPS) from *Escherichia coli*.⁶⁶ This hyper-responsiveness is nowadays known to result from the pro-inflammatory priming of innate immune responses during acute malaria⁶⁻⁸ and can be reversed by TLR antagonists.⁶⁷ A series of elegant experiments have recently shown that hypersensitivity to septic shock, in experimental *P. chabaudi* infections in mice, results from MyD88-mediated caspase-1 activation, which depends on IFN-gamma-priming and expression of the inflammasome components ASC, P2X7R, NLRP3, and/or NLRP12. As a result, infected mice produce extremely high levels

of the pro-inflammatory cytokine IL-1beta upon a second microbial stimulus.⁵⁹ Interestingly, administration of an IL-1 receptor antagonist prevents bacterial-induced lethality in *P. chabaudi* infections. Altogether, these data suggest that the inflammasome and IL-1beta are major factors in the pro-inflammatory priming leading to hypersensitivity to bacterial products in experimental malaria.⁵⁹

The pathophysiology of severe disease caused by the relatively benign human malaria parasite *P. vivax*, which is increasingly common in South and Southeast Asia, Oceania, and South America,^{68,69} remains poorly understood.⁷⁰ We hypothesize that undiagnosed bacterial or viral co-infections may contribute to the striking inflammatory imbalance seen in severe vivax malaria.⁴² As in experimental murine infections, peripheral blood mononuclear cells from *P. vivax* malaria patients produce extremely high levels of IL-1beta when exposed *ex vivo* to bacterial components such as LPS.^{8,49,59} In fact, infectious comorbidities were common in two recent case series of severe vivax malaria^{71,72} and may have further contributed to potentially inflammation-related complications of these infections, such as endothelial dysfunction leading to respiratory distress and circulatory shock, as well as severe anemia.

Co-infections with viruses

Co-infection with malaria and influenza in young African children is associated with longer hospitalization than single infections in the same population,⁷³ while more complications are seen in co-infections with *P. vivax* and dengue virus in French Guyana than in single-pathogen infections.⁷⁴ However, most studies suggesting an adverse effect of viral infection on malaria-related outcomes refer to co-infections with *P. falciparum* and HIV in sub-Saharan Africa.

Acute HIV infection elicits a strong inflammatory response that may contribute to intracellular virus replication. Accordingly, high levels of TNF-alpha, TNF-beta, IL-1, IL-2, IL-3, IL-12, granulocyte macrophage colony-stimulating factor (GM-CSF), and IL-6 appear to enhance HIV replication and disease progression;⁷⁵ IFN-alpha and IFN-beta appear to inhibit HIV replication, whereas TGF-beta, IL-4, IL-10, IL-13, and IFN-gamma may either stimulate or inhibit HIV replication under different experimental conditions.^{76,77} IL-4 has been shown to up-regulate (and IL-10 to down-regulate) the expression of the HIV co-receptor CXCR4 on the surface of CD4⁺ T lymphocytes.⁷⁸

HIV-related disease progression is characterized by a marked change in cytokine balance, with decreased production of IL-12 and IFN-gamma and increase levels of IL-10 in AIDS patients.⁷⁹ Increased IL-10 affects both innate and adaptive immune responses to

HIV infection, leading to impaired T_H1 responses and dendritic cell dysfunction.⁷⁹ HIV-infected individuals with low $CD4^+$ cell counts are at increased risk of acquiring malaria,⁸⁰ developing severe malarial disease,^{81,82} and experiencing parasite recrudescences.⁸³ Furthermore, acute malaria is associated with increased HIV loads⁸⁴ and a steeper decline in $CD4^+$ cell counts⁸⁵ in AIDS, most likely as a consequence of malaria-induced inflammation and enhanced HIV cell invasion. However, the effects of further co-infections with opportunistic bacterial, viral, and protozoan pathogens on clinical outcomes remain to be determined in HIV-malaria co-infections.

Co-infections with helminths

Chronic infections with tissue-invasive helminths provide another example of an immunoregulatory environment dominated by IL-10 and TGF- β that mediates suppression of T-cell proliferation and decreased production of IL-2 and IFN- γ in response to both related and unrelated antigens.^{86–88} The impact of helminth infections on antimalarial immunity has been characterized in more detail for filarial nematodes, with an IL-10-dependent decrease in IL-12p70, IFN- γ , and IP-10 responses upon T-cell stimulation with malarial antigens.⁸⁹ Malaria-specific T_H1 and T_H17 responses are suppressed during chronic filarial infections in Africa, with increased frequencies of natural $CD4^+$ T regulatory cells (Treg) and IL-10-producing Treg/Tr1 cells.⁹⁰ Interestingly, pre-existent filarial infection protected against malaria-related anemia, without necessarily reducing the overall severity of *P. falciparum* infection, in a cohort of Malian children.⁹¹

Most experimental models of co-infection with filarial worms and plasmodia in rodents have shown a protective effect against severe disease. In CBA/J mice, for example, co-inoculation with irradiated infective larva (L_3) of *Brugia pahangi* and *P. berghei*-infected erythrocytes induced high levels of T_H2 cytokines and protected mice from cerebral malaria.⁹² Similarly, C57BL/6 mice co-infected with *P. berghei* and *Litomosoides sigmodontis* were protected against cerebral malaria in an IL-10-dependent manner,⁹³ while 30% of BALB/c infected with *L. sigmodontis* failed to develop parasitemia after *P. berghei* sporozoite infection.⁹⁴ The same protective effect, however, is not necessarily seen in infections with intestinal helminths.⁹⁵

Concluding Remarks

The outcome of human and experimental malaria is largely defined by how much inflammation is elicited by infection. Varying levels of parasite virulence and co-infections with unrelated pathogens are key factors to determine the balance between inflammatory and regulatory immune responses, leading to

either increased malaria-related morbidity or some degree of protection against infection. Of particular interest is the putative role of co-infections in altering the clinical course of infections with the relatively benign malaria parasite *P. vivax*, aggravating inflammation and causing severe and potentially lethal disease.

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