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The lower levels of IL-27 and severity of *Plasmodium falciparum* malaria: role of IL-27 in malaria disease control

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Abstract: The blood stages of the malaria parasite, *Plasmodium falciparum*, induce a proinflammatory response in the host, which although important for the clearance of parasite, can lead to severe immune-mediated pathology. In humans and in mice, high levels of the proinflammatory cytokines as interferon (IFN)-γ, interleukin (IL) IL-1, IL-2, IL-6, IL-18, IL-31, IL-33 and chemokines CCL4, CCL20, CXCL8, CXCL9, CXCL10 and CXCL16 have been shown to correlate with complications during malaria, such as severe anemia, hypoglycemia, and cerebral malaria. IL-27 seems to play either a positive or negative role in protecting the host from infectious disease or the associated immune-driven pathology. However, the low levels of IL-27 observed in children were gradually associated with severe malaria and the absence of IL-27 receptor (IL-27R) in mice ($WSX\text{-}1^{-/-}$) have greater amounts of plasma IFN-γ, IFN-α, and IL-12, higher mortality, and more pronounced pathology than wild-type (WT) ones. A successful response must strike a balance between protection from the parasite and immunopathology, and IL-27 appears to be one of the means by which this balance can be established. The early induction of regulatory cytokine IL-27 may be a protection of malaria patients from severe clinical complications; this cytokine may also be use as a monitoring parameter in biological control of severe malaria treatment to prevent cases of recurrence or drug resistance. The purpose of this review is to describe the state of knowledge regarding the predominant role of IL-27 during malaria so appropriate strategies can be found to limit the pathology.
Key words: Interleukin (IL)-27, cytokines, chemokines, *Plasmodium falciparum* malaria.

Introduction

Cytokines and chemokines are essential mediators during *Plasmodium falciparum* infection and the balance between pro and anti-inflammatory cytokines may be important for the clinical outcome of malaria [1, 2, 3, 4]. As a major cause of morbidity and mortality in many tropical regions of the world, malaria still remains an important global public health concern. For example, according to WHO, an estimated 3.4 billion people were at risk of malaria in 2012. Of this total, 2.2 billion were at low risk (<1 reported case per 1000 population),

of whom 94% were living in geographic regions other than the African Region. The 1.2 billion at high risk (>1 case per 1000 population) were living mostly in the African Region (47%) and the South-East Asia Region (37%) [5]. Growing problem of antimalarial drug resistance and lack of an effective vaccine makes the insight into the complex pathogenesis of malaria vital for the development of new therapeutic tools and control of the disease [6, 7].

Cytokines seem to be involved both in protection and pathology in malaria infection. Early and effective inflammatory response, mediated by IFN-γ in IL-12 and IL-18 dependent-manner, seems to be crucial for the control of parasitaemia and resolution of malaria infection through the mechanisms of the tumor necrosis factor-α (TNF-α) induction and enhanced release of the antiparasitic reactive nitrogen and oxygen radicals [4, 8]. On the other hand, severe malaria has long been associated with high circulating levels of type 1 immunity (Th1) proinflammatory cytokines such as TNF-α, IFN-γ, IL-1 and IL-6 [9]. Their excessive production may affect the disease outcome through their direct systemic effect and by increasing cytoadherence of parasitized erythrocytes to the endothelium via upregulation of adhesion molecules in *Plasmodium falciparum* infections [10]. Cytokine responses reflect different host strategies for controlling malaria infection. Controlling virulent, fast-multiplying parasites such as *Plasmodium falciparum* blood stages may require a double-edged strategy: a strong inflammatory response that can prevent fulminant infections but which may lead to severe disease [11].

Interleukin-27 is a novel cytokine of the IL-6/IL-12 family that has been reported to be involved in the pathogenesis of diseases such as malaria and has a pivotal role as both a pro- and anti-inflammatory cytokine [11, 12]. A recent report [13] showed that IL-27 transgenic mice exhibited a systemic inflammatory condition accompanied by an increased percentage of activated T cells and an elevated IFN-γ level. In that study, IL-27 transgenic mice lacked regulatory T (Treg) cells in lymphoid organs, suggesting that the inappropriate inflammation was caused by a Treg deficiency. Regarding the T helper type 1 (Th1) cells; IL-27 is thought to mediate the proinflammatory response by modulating the early stage of Th1 cell differentiation via induction of the IL-12 receptor b2 expression [14]. On the contrary, IL-27R$^{-/-}$ CD4^{+} T cells produce more IL-2 than wild-type cells during Th1 differentiation, suggesting that IL-27 has anti-inflammatory properties [15]. Taken together, the in vivo and in vitro consequences of the response of immune cells to IL-27 appear to be a complicated and a complex problem.

Several studies [16, 17, 18, 19] shown that IL-10 is required to limit morbidity and mortality during malaria infection. Surprisingly, other studies proved that the Th1 response was quantitatively and qualitatively similar in IL-10 receptor deficient mice (IL-10R1$^{-/-}$) and WT mice during malaria infection. These data strongly suggest that IL-27 receptor (WSX-1) does not regulate Th1 responses in vivo during infection specifically through IL-10-dependent mechanisms. Consistent with this, IL-27 has previously been shown to mediate IL-10-independent mechanisms. Thus, under physiological conditions, IL-27 and IL-10 appear to have discrete immunoregulatory functions in vivo during malaria infection [20]. In our previous study in children it was clear that the levels of IL-27 decreased continuously with the severity of malaria at the same time the rate of IL-10 remained growing [21]. This result also shows that IL-27 decreasing leads to severe malaria and this cytokine do not regulate Th1 responses in vivo during malaria infection specifically through IL-10-dependent mechanisms. Thus, it is necessary to define the appropriate pathways that IL-27 regulates proinflammatory responses during malaria infection so that new therapeutic strategies can be developed.

1- Manifestations of malaria and cytokines expression

Pathogenesis of malaria

All the manifestations of malarial illness are caused by the infection of the red blood cells (RBCs) by the asexual forms of the malaria parasite and the involvement of the red cells makes malaria a potentially multisystem disease, as every organ of the body is reached by the blood [22, 23]. All types of malaria manifest with common symptoms such as fever; some patients may progress into severe malaria. Severe malaria is more often seen in cases of *P. falciparum* infection, with complications and even deaths especially in children. At the completion of the schizogony within the red cells, each cycle lasting 48 hours of the infecting parasite, newly developed merozoites are released by the lysis of infected erythrocytes and along with them, numerous known and unknown waste substances, such as red cell membrane products, hemozoin pigment, and other toxic factors such as glycosylphosphatidylinositol (GPI) are also released into the blood. These products, particularly the GPI, activate macrophages and endothelial cells to secrete cytokines and inflammatory mediators such as TNF-α, IFN-γ, IL-1, IL-6, IL-8, macrophage colony-stimulating factor, and lymphotoxin, as well as superoxide and nitric oxide (NO). Many studies have implicated the GPI tail, common to several merozoite surface proteins

such as MSP-1, MSP-2, and MSP-4, as a key parasite toxin [24, 25]. The systemic manifestations of malaria such as headache, fever and rigors, nausea and vomiting, diarrhea, anorexia, tiredness, aching joints and muscles, thrombocytopenia, immunosuppression, coagulopathy and central nervous system manifestations have been largely attributed to the various cytokines released in response to these parasite and red cell membrane products [2, 26]. In addition to these factors, the plasmodial DNA is also highly proinflammatory and can induce cytokinemia and fever. The plasmodial DNA is presented by hemozoin (produced during the parasite development within the red cell) to interact intracellularly with the Toll-like receptor-9, leading to the release of proinflammatory cytokines that in turn induce COX-2-upregulating prostaglandins leading to the induction of fever [27]. Hemozoin has also been linked to the induction of apoptosis in developing erythroid cells in the bone marrow, thereby causing anemia [28, 29].

The excessive inflammation in *Plasmodium falciparum* malaria

The infection of the red cells by malaria parasites, particularly *Plasmodium falciparum*, results in progressive and dramatic structural, biochemical, and mechanical modifications of the red cells that can worsen into life-threatening complications of malaria. Several pathophysiological factors such as the parasite biomass; 'malaria toxin(s)' and inflammatory response; cytoadherence, resetting and sequestration; altered deformability and fragility of parasitized erythrocytes; endothelial activation, dysfunction and injury; and altered thrombostasis have been found to be involved in the development of severe malaria. All these phenomena are more profound and wide spread in *Plasmodium falciparum* infection compared to non-falciparum infections. As a result, except for severe anemia, complications such as cerebral malaria, hypoglycemia, metabolic acidosis, renal failure, and respiratory distress are more commonly seen in *P. falciparum* infections [19, 22, 2316, 19, 20].

With its wide array of receptor families and highly redundant, alternate invasion pathways [24], *P. falciparum* has the ability to invade RBCs of all ages, and with repeated cycles of development within the red cells, the parasite numbers exponentially grow into very high parasite burdens if the infection is uninhibited by treatment or host immunity. On the contrary, *Plasmodium vivax* preferentially infects only young RBCs, thus limiting its reproductive capacity and resultant parasite loads. Thus, the parasite load in *Plasmodium falciparum* infections can be very high, even exceeding 20-30%, whereas in vivax malaria it rarely exceeds 2%, even in case of severe disease [19, 22].

Cytoadherence, sequestration and rosetting

Structural changes in the infected red cells and the resulting increase in their rigidity and adhesiveness are major contributors to the virulence for *P. falciparum* malaria. Owing to the increased adhesiveness, the red cells infected with late stages of *Plasmodium falciparum* (during the second half of the 48 hour life cycle) adhere to the capillary and postcapillary venular endothelium in the deep microvasculature (cytoadherence) [25]. The infected red cells also adhere to the uninfected red cells, resulting in the formation of red cell rosettes (rosetting). Cytoadherence leads to sequestration of the parasites in various organs such as the heart, lung, brain, liver, kidney, intestines, adipose tissue, subcutaneous tissues, and placenta. Sequestration of the growing *Plasmodium falciparum* parasites in these deeper tissues provides them the microaerophilic venous environment that is better suited for their maturation and the adhesion to endothelium allows them to escape clearance by the spleen and to hide from the immune system. These factors help the falciparum parasites to undergo unbridled multiplication, thereby increasing the parasite load to very high numbers. Due to the sequestration of the growing parasites in the deeper vasculature, only the ring-stage trophozoites of *Plasmodium falciparum* are seen circulating in the peripheral blood, while the more mature trophozoites and schizonts are bound in the deep microvasculature, hence seldom seen on peripheral blood examination. If the cytoadherence-rosetting-sequestration of infected and uninfected erythrocytes in the vital organs goes on uninhibited, it ultimately blocks blood flow, limits the local oxygen supply, hampers mitochondrial ATP synthesis, and stimulates cytokine production; all these factors contributing to the development of severe disease [26, 30, 31, 33, 34, 35].

Certain proteins expressed on the surface of the infected red cells mediate the adhesion of parasitized RBCs to the endothelium and to uninfected red cells. The most important of such proteins is the *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1), an antigenically diverse protein family that is expressed on the thousands of knob-like excrescences on the surface of red cells infected with *Plasmodium falciparum* trophozoites and schizonts [31, 36]. Rosetting is mediated by binding of PfEMP1-DBLα on the surface of infected red cells to complement receptor 1, CD31, and heparan sulfate-like glycosaminoglycans of

uninfected RBCs [31, 33, 35]. Rosetting is found to be lesser in blood group O erythrocytes compared with groups A, B, and AB, and thus patients with blood group O may be protected from severe malaria [37].

Altered red cell membrane rigidity and deformability also contribute to the pathogenesis of severe malaria. In patients with severe falciparum malaria, the entire red cell mass, comprising mostly of unparasitized red cells and also parasitized red cells, becomes rigid [38]. Several mechanisms such as hemin-induced oxidative damage of the red cell membrane, alterations in the phospholipid bilayer and attached spectrin network by the proteins transported to the red cell membrane, thermally driven membrane fluctuations due to fever, and inhibition of the Na^+/K^+ pump on the red cell membrane, possibly by nitric oxide (NO) may be responsible for the increase in rigidity and reduction in deformability of the red cells in falciparum malaria [26, 38, 39]. Reduced red cell deformability leads to increased splenic clearance and loss of red cells, causing anemia.

Role of Cytokines in Severe Malaria

The pathogenesis of severe malaria involves a cascading interaction between parasite and red cell membrane products, cytokines and endothelial receptors, leading to inflammation, activation of platelets, hemostasis, a procoagulant state, microcirculatory dysfunction and tissue hypoxia, resulting in various organ dysfunctions manifesting in severe malaria [40]. The cytokines of the proinflammatory cascade like tumor necrosis factor (TNF), interleukins, interferon-γ, and nitric oxide act as double-edged swords in the pathogenesis of malaria [1, 41]. Cytokines act as homeostatic agents and an early proinflammatory cytokine response helps in limiting the infection, with the cytokines inhibiting the growth of malarial parasites in lower concentrations. On the other, failure to down-regulate this inflammatory response results in progressive immune pathology, leading to complications. Excessive levels of cytokines can lead to decreased mitochondrial oxygen use and enhanced lactate production; increased cytoadherence that in turn causes microvascular obstruction and more hypoxia; disturbed auto-regulation of local blood flow leading to poor circulation and further tissue hypoxia; dyserythropoiesis, poor red cell deformability and multifactorial anemia; reduced gluconeogenesis and hypoglycemia; myocardial depression and cardiac insufficiency; loss of endothelial integrity and vascular damage in the lungs and brain; selective upregulation of vascular and intercellular adhesion molecules (ICAMs), particularly in the brain and placenta leading to cerebral malaria and placental dysfunction; and activation of leukocytes and platelets, promoting procoagulant activity [23, 26, 32, 42, 43]. It can therefore be said that the outcome of malaria infection is determined by the balance between the pro- and anti-inflammatory cytokines [23, 26, 32]. Hemolysis, suppression of erythropoeisis by cytokines, and hemozoin-induced apoptosis in developing erythroid cells also contribute to the development of anemia in severe malaria [26, 28, 38].

Cytokines and chemokines involved in the malaria severity changes

Several cytokines and chemokines are discussed in malaria. The most commonly cited are shown in Figure 1 [1, 3, 4, 10, 11, 21] below with the changes in their levels of expression depending on the clinical status of the disease. Cytokines seem to be involved both in protection and pathology in malaria infection. Early and effective inflammatory response, mediated by IFN-γ in IL-12 and IL-18 dependent -manner, seems to be crucial for the control of parasitaemia and resolution of malaria infection through the mechanisms of TNF-α induction and enhanced release of the antiparasitic reactive nitrogen and oxygen radicals [1, 9, 44]. On the other hand, severe malaria has long been associated with high circulating levels of proinflammatory cytokines such as TNF-α, IFN-γ, IL-1 and IL-6, IL-18, IL-31, IL-33 (Figure 1). Their excessive production may affect the disease outcome through their direct systemic effect and by increasing cytoadherence of parasitized erthrocytes to the endothelium via upregulation of adhesion molecules in *Plasmodium falciparum* infections [10].

The expression of cytokines in general as well as the balance of pro and anti-inflammatory response are supposed to be involved in malaria pathogenesis, but their relationship with the pattern and extent of vital organ dysfunction in malaria infection has not been well defined yet. Severe malarial anemia has been associated with low serum levels of IL-12 and IL-10 to TNF-α serum concentrations ratio in a few studies of childhood malaria in holoendemic areas [45, 46]. However, the manifestations of severe malaria vary with geographic location and malaria transmission intensity as well as with the age of the patient [5, 47]. In non-immune adults severe malaria often presents as a multiorgan disorder with renal failure, hepatic dysfunction with jaundice and shock while in African children cerebral malaria and severe anemia predominate [48]. Recent *in vitro* experiments have shown that peripheral blood mononuclear cells from clinically immune individuals from areas of high endemicity produce lower amounts of IFN-γ in response to *P. falciparum* schizont antigens than those from previously unexposed donors, indicating that the control of clinical symptoms may depend on the host ability to

regulate strictly the inflammatory response [49]. Studies in mice undergoing primary malaria infection have suggested that the profile of cytokines, including IFN-γ, released early in the course of the infection, may predict the final outcome of the disease [44].

Cytokines and chemokines are secreted proteins with growth, differentiation, and activation functions that regulate the nature of immune responses. Cytokines are involved in nearly every facet of immunity and inflammation, from induction of the innate immune response to the generation of cytotoxic T cells and the development of antibodies by the humoral immune system. The combination of cytokines that are produced in response to an immune insult determines which arm of the immune system will be activated [50]. For this update, recent advances in our understanding of immune regulatory cytokines will be discussed, which includes the IL-10 and IL-27 families.

Pro-inflammatory Cytokines and Chemokines	**Negative Control**	**Mild Malaria**	**Severe Malaria**
IFN	+	++	+++
IL-1		++	
IL-2	+	++	+++
IL-6	+	++	+++
IL-12	+	+++	++
IL-17	++	+++	+++
IL-18	+	++	+++
IL-31	+	++	+++
IL-33	+	++	+++
CCL4	+	++	+++
CCL20	+	++	+++
CXCL8	+	++	+++
CXCL9	+	++	+++
CXCL10	+	++	+++
CXCL16	+	++	+++
Anti-inflammatory Cytokines			
TGF	+	-	- -
IL-4	+	-	- -
IL-13	+	++	+++
Regulatory Cytokines			
IL-10	+	++	+++
IL-27	+	-	- -

Figure 1: Biological effects of cytokines and chemokines commonly involved in malaria disease [1, 3, 4, 10, 11, 21]

+ : Normal expression in unaffected individual with malaria (Negative control)
++ : Increased expression
+++ : strongly increased expression
- : decreased expression
- - : strongly decreased expression

2- The important regulatory role of IL-27

The IL-27 deficient expression, source of malaria severity

The anti-inflammatory cytokines recognized in malaria disease are TGF-β, IL-4, IL-10, IL-13 and IL-27 recently whose role seems to be decisive. IL-10 has been widely reported to play an anti-inflammatory role as IL-13. However, his high levels in children with severe malaria do not systematically prevent clinical complications leading to death. On the contrary, the rates of anti-inflammatory cytokines IL-4 and TGF-β also decrease during severe malaria [3, 51] as IL-27; but IL-27 appears to play a pivotal role in the clinical outcome of malaria. In addition, IL-27 and IL-10 are known as the key regulatory cytokines. Interestingly, while IL-10

expression stilled high in children with severe malaria, IL-27 levels lessened. In our previous study [21], severe malaria was consequential to the lack of expression of the main regulatory cytokine IL-27 (Figure 2).

It is well established that much of the pathology associated with blood-stage malaria infections is a result of excessive production of proinflammatory cytokines, including TNFα, lymphotoxin, and IFN-γ or insufficient production of anti-inflammatory cytokines, including IL-4, IL-13, IL-10 and TGF-β [3, 11, 16, 51]. The comparison of the outcome between virulent *Plasmodium berghei* NK65 infection in WSX-$1^{-/-}$ mice and the WT control mice have shown that, the WT mice developed unremitting parasitemia and succumbed to infection after over 30 days. Interestingly, the IL-27 receptor deficient mice (WSX-$1^{-/-}$) succumbed to infection much more rapidly than the WT ones despite very low parasite burdens. The death of *Plasmodium berghei* NK65-infected WSX-1-/- mice was due to liver necrosis secondary to exacerbated Th-1 responses [16]. It also emerges that the loss of IL-27 immunoregulation specifically leads to Th1 cell terminal differentiation during malaria infection and very few non-Th1 cells express marker of cell terminal differentiation (KLRG1) [20].

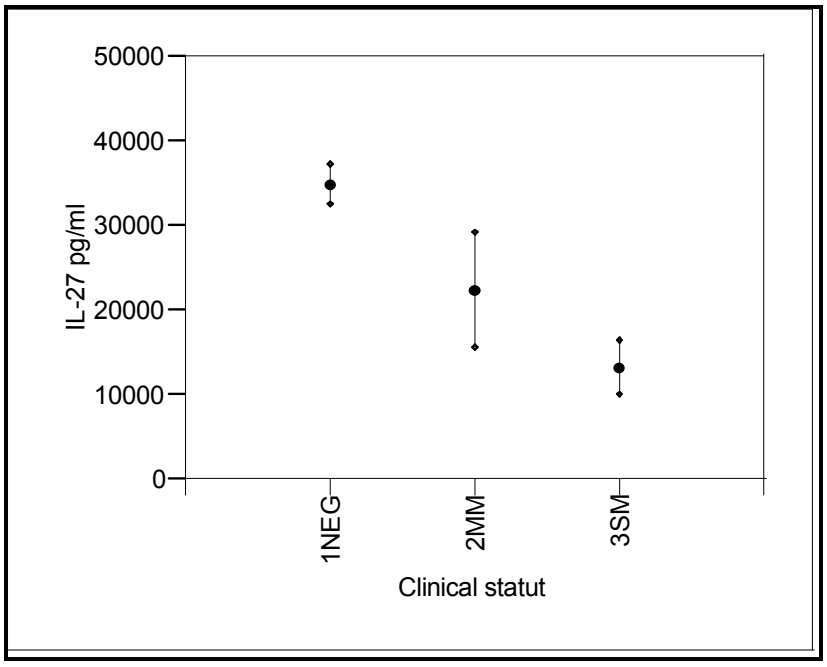

Figure 2: The defect in the levels of IL-27 with malaria severity according to WHO criteria, taken from Ayimba et al., *Clinical and experimental Immunology* 2011 [21].

NEG: Control infants (n=81); MM: Mild Malaria (n=184); SM: Severe Malaria (n=127)

IL-27 as pro- and anti-inflammatory cytokine

As a cytokine with dual regulatory capacity, IL-27 will first initiate Th1-type IFN-γ responses and promote IL-10 synthesis by regulatory T cells, then attenuate inflammatory Th2 and Th17 cells [52] and depress pro-inflammatory cytokines and chemokines [53]. IL-27 is a member of a heterodimeric cytokine produced by antigen-presenting cells (APCs), including monocytes and dendritic cells. It belongs to the IL-12 cytokine family, which also includes IL-23 and IL-35. IL-27 is composed of the Epstein–Barr virus-induced gene 3 (EBI3) and p28 subunits, and has been demonstrated to have a pivotal role as both a pro- and anti-inflammatory cytokine [12, 54]. The IL-27R complex consists of the specific IL-27Ra subunit, WSX-1, a type I cytokine receptor, and gp130, the IL-6R subunit, and is expressed by numerous cells of the immune system, including $CD4^+$ T cells and $CD8^+$ T cells as well as monocytes, Langerhans cells, DCs, and NK cells. IL-27R activates STAT1, STAT3, STAT4, and STAT5, with disparate downstream effector functions depending on the precise signaling pathway used. IL-27 exerts both proinflammatory and suppressive effects on T cells, augmenting Th1 polarization by the induction of T-bet and increasing expression of ICAM-1 and responsiveness to IL-12, and suppressing $CD4^+$ T cell proliferation and effector function, for example, via suppressor of cytokine signaling 3-dependent downregulation of CD28-mediated IL-2 production or downregulation of the RORc/IL-17 pathway [16, 55]. IL-12 has a profound effect on committed Th17 cells by readily converting them into Th1 cells, even in the presence of IL-23. These findings demonstrate that Th17 cells have “unstable phenotype” and can transition into Th1 cells [56].

IL-27 has also been shown to inhibit development of $Foxp3^+$-inducible regulatory T cells [57, 58]. These early reports have emphasized the proinflammatory functions of IL-27. However, subsequent studies showed a more complex role for IL-27, because it also exerts anti-inflammatory functions. Two such reports have shown increased central nervous system (CNS) inflammation in IL-27R-deficient ($WSX\text{-}1^{-/-}$) mice either with experimental autoimmune encephalomyelitis (EAE) or infected with *Toxoplasma gondii*. This enhanced inflammation was associated with increased numbers of Th17 cells in the CNS [53, 59]. In addition, delivery of exogenous IL-27, during the priming phase of anti-myelin response, ameliorates EAE, with evidence of suppression of both Th1 and Th17 responses [60]. In vitro, IL-27 efficiently counters the effect of TGF-β and IL-6 on naïve $CD4^+$ T cells, resulting in near complete inhibition of de novo Th17 development in a STAT1-dependent manner [56, 59]. Further study of the mechanism of action of IL-27 on Th17 development has revealed that this cytokine inhibits the expression of RORγt. More recent findings showing the ability of IL-27 to induce IL-10 secretion from both $CD4^+$ and $CD8^+$ T cells provide a new mechanism that may explain the anti-inflammatory effects of IL-27. Accordingly, T cells from $WSX\text{-}1^{-/-}$ mice infected with *Toxoplasma gondii* displayed a reduced capacity to produce IL-10 and to dampen excessive immune response. Similarly, IL-27-mediated inhibition of EAE was IL-10 dependent [56, 61].

The addition of recombinant IL-27 to naive T cells in culture under Th2-polarizing conditions results in decreased expression of GATA-3, a transcription factor important for Th2 development. Concurrent with the decrease in GATA-3 was a decrease in IL-4 production. The decrease in Th2 cytokines caused by IL-27 is a result of inhibition of Th2 cell development. These results suggest that IL-27 might serve a dual role in T-cell development and the immune response by stimulating production of Th1 responses while inhibiting production of Th2 inflammatory responses [50]. IL-27 also promotes the production of IL-10 by various effector $CD4^+$ T cell populations, including Th1 and Th2 cells and $CD4^+$ T cells polarized under Th17-inducing conditions. Although the role of pro-inflammatory and anti-inflammatory cytokine is attributed to IL-27, its anti-inflammatory properties are essentially known in malaria [16].

Mechanisms of IL-27 regulatory role and malaria disease resolution

The ability of IL-27 to both promote and inhibit inflammation suggested that it may play a role during malaria infection, where the outcome of infection is determined by the balance of pro- and anti-inflammatory responses. The recently identified regulatory cytokine, IL-27, has been shown to play an important role during a variety of infections, but its role during malaria infection has not yet been examined [62].

Overall, the findings presented above highlight the complex and pleiotropic role of IL-27 in immune responses. Although IL-27 is one of the most potent inhibitors of Th17 differentiation, little is known about how IL-27 regulates committed Th17 cells. This aspect of effector/memory Th17 cell biology is crucial to understanding the mechanisms that regulate inflammation in peripheral tissues during the effector phase of an immune response. This view is supported by the finding that IL-27 augmented IFN-γ production by naive T cells stimulated in non-polarizing conditions, while it suppressed IFN-γ secretion by activated $CD4^+$ T cells [63]. In addition, differentiated Th17 cells seem to acquire resistance to suppression by IL-4 and IFN-γ, two cytokines that, similarly to IL-27, have inhibitory effects on the initial development of Th17 cells. Thus, to assess the therapeutic potential of exogenous IL-27, it is essential to know whether IL-27 negatively regulates committed Th17 cells, given that in a clinical setting pathogenic Th17 cells have already developed before initiation of treatment.

In the study using in vitro-differentiated Th17 cells, it was found that IL-27 does not affect an established Th17 phenotype. Even though committed Th17 cells retain expression of IL-27R and respond to IL-27 by phosphorylating both STAT1 and STAT3, IL-27 failed to suppress expression of RORγt, RORα, and IL-23R or to modify responsiveness of these cells to IL-23. Unlike in the case of developing Th17 cells, IL-27 did not up-regulate expression of T-bet in committed Th17 cells or converted their phenotype to Th1 lineage as IL-12 does [56]. In addition, IL-27 did not suppress encephalitogenicity of Th17 cells in an adoptive EAE model. Taken together, these data clearly demonstrate that Th17 cells, depending on the stage of their development, exhibit a sharp difference in their susceptibility to IL-27, with differentiating Th17 cells being susceptible and committed Th17 cell being resistant to suppression by IL-27 [56, 60]. On other hand, IL-17 was not found associated with development of cerebral malaria in *Plasmodium berghei* infected mice [64]. In Ghanaian children, cerebral malaria mortality did not associate with IL-17 [65] and in our study [21] IL-17F levels were similarly high in negative, Mild Malaria and Severe Malaria infants. This also suggests that, IL-27 does not regulate the inflammatory response via Th17 in malaria disease.

In line with these disparate roles, IL-27 signaling is essential for the generation of early protective T cell responses during Leishmania major and bacillus Calmette-Gue´rin infections but is required for the suppression of Th1- and Th17-mediated inflammation during *Toxoplasma gondii*, *Trypanosoma cruzi*, *Mycobacterium tuberculosis*, *Leishmania donovani*, and nonhealing *Leishmania major* infections [16, 66, 67]. As with $CD4^+$ T cells, IL-27 also positively and negatively regulates APCs. IL-27R–deficient DCs are hyperresponsive to LPS, with increased expression of CD80 and CD86 compared with wild-type (WT) DCs [68]. In addition, administration of rIL-27 suppresses production of reactive oxygen intermediates, TNF-α and IL-12, by activated macrophages in vitro [69]. Although not responsible for the differentiation of alternatively activated macrophages, IL-27 may play an important role in the modulation of alternatively activated macrophage function [70]. In contrast, IL-27 augments production of TNF-α, IL-12, IL-6, and IFN-γ by human monocytes/macrophages [71].

As IL-27 can induce IL-10 production by effector $CD4^+$ T cell populations, including during malaria infection, the hyperactive Th1 phenotype observed in WSX-$1^{-/-}$ mice would be recapitulated in IL-$10^{-/-}$ or IL-$10R1^{-/-}$ mice. Indeed, IL-10 is required to limit morbidity and mortality during malaria infection [16, 17]. But the fact that the Th1 response was quantitatively and qualitatively similar in IL-$10R1^{-/-}$ mice and WT ones during malaria infection suggest that WSX-1 does not regulate Th1 responses in vivo during infection specifically through IL-10 dependent mechanisms. Consistent with this, IL-27 has previously been shown to mediate IL-10-independent mechanisms [61]. Thus, under physiological conditions, IL-27 and IL-10 appear to have discrete immunoregulatory functions in vivo during malaria infection. The $Foxp3^+$ regulatory T cell population has also been shown largely unaltered in WSX-$1^{-/-}$ mice during malaria infection; the frequency, absolute number and phenotype (T-bet, CXCR3 and IFN-c) of $Foxp3^+$ Tregs were essentially the same in infected WT and WSX-$1^{-/-}$ mice. Thus, although it is not entirely sure that the Foxp3 Tregs maintain their regulatory function during malaria infection in WSX-$1^{-/-}$ mice, there is no evidence that WSX-1 regulates the collapse of the $Foxp3^+$ T cell population during malaria infection. Moreover, it does not appear that WSX-1 controls the functional adaptation of $Foxp3^+$ Tregs to become Th1-$Foxp3^+$ Treg ($CXCR3^+$ $Foxp3^+$) during malaria infection, as is observed during *Toxoplasma gondii* infection [72]. Irrespective of the role of IL-27 in modifying the nature of the $Foxp3^+$ regulatory cell compartment, depletion of $Foxp3^+$ regulatory T cells throughout the course of malaria infection does not lead to the expansion or terminal differentiation of Th1 cells. Thus, IL-27 controls Th1 responses during malaria infection through $Foxp3^+$ regulatory T cell independent mechanisms. In summary, IL-27/WSX-1 signalling regulates Th1 responses in vivo during infection. It was shown that WSX-1 signalling regulates the molecular programming of Th1 cells, inhibiting the formation of terminally differentiated KLRG-1^+ Th1 cells, and thereby establishes an upper threshold limit of T-bet expression within the $CD4^+$ effector T cell population. Importantly, IL-27 mediates its effects independently of IL-10 and $Foxp3^+$ Tregs [20, 21]. Thus, this data highlight a critical role for IL-27/WSX-1 signalling in regulating the size and quality of the Th1 response during infection. Manipulation of the IL-27 pathway may therefore represent a therapeutic approach to limit T cell dependent immunopathology and/or enhance pathogen control during chronic inflammatory disorders.

Conclusion

Cytokines may be key determinants of malaria severity and outcome and are thus potential targets for therapeutic interventions if their effects can be better understood. Our study support the hypothesis that effective *Plasmodium falciparum* infection resolution along with uncomplicated clinical manifestation of the disease. So, the favorable outcome depend on strict regulation of pro and anti-inflammatory responses mediated by relevant cytokines and IL-27 seems to be a candidate target for therapeutic intervention and biological parameter for malaria severity monitoring as the decrease in its levels is synonym of the malaria pathology increase. Through this review, we have demonstrated that IL-27 regulates Th1 response during malaria disease independently from IL-10 effects. In addition, the severity of malaria is due to the inadequacy of the regulatory effect of IL-27. However, the results of these investigations should be considered as preliminary. Therefore, further practical researches are needed to determine the IL-27 involvement in particular clinical manifestations of the disease by stimulation IL-27 expression during malaria or by administering this cytokine to patients.

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