

Immunomodulatory Therapy Associated to Anti-Parasite Drugs as a Way to Prevent Severe Forms of Malaria

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Abstract: Malaria is an important problem of public health. It is estimated that 350 to 500 million clinical cases occur annually, which cause 1.1 and 1.3 million deaths every year. The excessive activation of the immune system plays an important role in the pathogenesis of the disease. The cells of the immune system of *Plasmodium*-infected individuals not only produce large amounts of cytokines, which have anti-parasite effects, but also participate in the pathogenesis of the severe complications of malaria. A central feature of *P. falciparum* infection is the sequestration of parasitized erythrocytes within the small vessels of major organs. This involves molecular interactions between antigens of parasitized erythrocytes and host receptors, expressed on the surface of endothelial cells. The increased production of pro-inflammatory cytokines and nitric oxide, followed by the up regulation of endothelial cell adhesion molecules, influences the progression of cerebral lesions. The association of drugs capable of modulating the immune response to anti plasmodial drugs has been evaluated. Antibodies to tumor necrosis factor, pentoxifylline, and thalidomide have been tried for this purpose with variable success. This review submitted this subject to a critical assessment and suggests ways to take advantage of immunomodulatory drugs, associated to anti parasite therapy, to reduce the morbimortality of malaria.

Key Words: Malaria, *Plasmodium falciparum*, cytokines, tumor necrosis factor, TNF, cerebral malaria, immunopathogenesis, immunomodulation, pentoxifylline, thalidomide, sequestration, adherence molecules, nitric oxide, reactive oxygen species.

A. INTRODUCTION

Malaria is a major cause of suffering and death, and one of the most important problems of public health in vast areas of the world, mainly due to the severe forms of *Plasmodium falciparum* infection in children, previously unexposed adults and pregnant women [1]. It is currently estimated that a total of 350 to 500 million clinical cases occur annually, which cause approximately 1.1 and 1.3 million deaths every year [2-4].

It is estimated that in African areas of high transmission, a total of 400 infectious mosquito bites would originate 200 plasmodial infections, which would give rise to 100 clinical cases of malaria. From those, approximately 2% would present severe malaria, and half of them would die [5]. Naïve individuals of any age acutely infected with malaria parasite always present disease. However, in highly endemic areas of the disease, children bear the brunt of the morbidity and mortality of malaria. Although these children in their first months of life may become parasitized, they are usually resistant to serious disease, possibly due to the passive transfer of maternal protective antibodies against the parasite and the impair of parasite development by fetal hemoglobin. From six months to two-three years of life, in parallel with a decrease in maternal antibodies, children become increasingly susceptible to malaria, mainly to severe anemia, and from around two-three years to six-seven years of age, they become susceptible to severe cerebral malaria. From five years old, the frequency of the disease begins to decrease and, from the adolescence onwards, severe disease exceptionally occurs [6].

Severe manifestations and complications due to *P. falciparum* life-threatening disease in children include cerebral malaria, severe anemia, metabolic acidosis, hypoglycemia and respiratory distress. In areas of lower transmission, severe disease may also occur in adults, in whom severe disease involves additional disturbances, such as renal failure, pulmonary edema, shock and jaundice. Overall, cerebral malaria and severe anemia are the most common causes of hospitalization and death, especially in naïve individuals [1, 7-14].

The reasons that only a small percentage of individuals infected with malaria parasite develop severe life-threatening disease are still unclear. Probably different factors, including those dependent on the host, the parasite, the vector and the environment, are important [5,6,15], as shown in Table 1.

B. IMMUNOPATHOGENESIS OF CEREBRAL MALARIA

It has been considered that the excessive activation of the immune system by some parasite components is critical for pathogenesis of severe malaria [13,16,17]. Glycosylphosphatidylinositol, the anchor molecule of *Plasmodium* merozoite surface protein, induces expression of many genes that are implicated in malaria pathogenesis, as tumor necrosis factor (TNF), interleukin-1 (IL-1), IL-12, inducible nitric oxide synthase, and several adhesion molecules that are expressed on the surface of the vascular endothelium and are recognized by *P. falciparum* EMP1 molecule [14,18]. The production of pro-inflammatory cytokines (TNF and IL-1) is further stimulated upon activation of macrophages by interferon- γ (IFN- γ), produced mainly by CD4+ T lymphocytes [19-21].

Both TNF and IFN- γ have anti-plasmodial effects and are associated with the benign forms of malaria [22-27]. How-

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Table 1. Putative Factors Associated with Severe Malaria

<p>(a) Dependent on the host</p> <p>low or absent immunity (including infants and older people) pregnancy (specially the first) delayed, incorrect or no diagnosis delayed, incorrect or no treatment high degree of activation of the immune system high expression of adherence molecules by endothelial cells co-morbidities (anemia, immunodeficiency, other infections)</p>
<p>(b) Dependent on the parasite</p> <p>species (<i>P. falciparum</i> more frequently associated with severe malaria) high parasitemia resistance to antimalarial drugs high virulence of the clone high antigenic polymorphism high antigenic variation capacity of cytoadherence to endothelial cells and non-parasitized erythrocytes capacity to evade host immune defense capacity to induce high levels of pro-inflammatory cytokines</p>
<p>(c) Dependent on the vector</p> <p>high vectorial capacity high anthropophily (preference for biting humans) high density resistance to insecticides seasonality of transmission</p>
<p>(d) Dependent on the environment</p> <p>high frequency of mosquito breeding sites</p>

ever, if these cytokines are overproduced, or if they act on hyperreactive endothelial cells, they play a role in the pathogenesis of severe complications of malaria, as anemia and cerebral involvement [17,21,28].

Anemia is a frequent manifestation of malaria and a major cause of death among African children, and cerebral involvement is the second cause of death in malaria. Cerebral malaria is a consequence of the local patchy involvement of cerebral capillaries and postcapillary venules. It starts with the adherence of *P. falciparum*-parasitized erythrocytes to endothelial cells and to other erythrocytes. This sequestration may interfere with the local blood flow and, therefore, facilitate the accumulation of leucocytes, which are activated by parasite products released during local multiplication of plasmodium [29]. The local production of pro-inflammatory cytokines by activated monocytes and macrophages, particularly TNF, and of nitrogen and oxygen species, leads to the activation of the endothelial cells and, eventually, to their own damage and damage of cells in close contact [14]. Following activation, endothelial cells increase the production of nitric oxide and the expression of adherence molecules, which aggravate sequestration of erythrocytes. The consequences of endothelial alterations are perivascular edema, hypoxia of the surrounding brain parenchyma and petechial

hemorrhages [30,31]. Depending on the extent of the structural damage of the brain and the degree of metabolic disturbance, cerebral involvement can progress to coma and multiple convulsions (Fig. 1).

C. IMMUNOMODULATION IN MALARIA

Since the hallmark of the pathogenesis of severe malaria is the hyperactivation of the immune system with excessive production of pro-inflammatory cytokines, several attempts have been made to reduce the severity of the disease through the modulation of the immune response.

1. Immunomodulatory Activity of Antimalarial Drugs

All antimalarial drugs indirectly influence the immune response by means of their ability to destroy malaria parasites and, therefore, reduce the amount of antigens capable of activating the immune system. Besides this effect, some antiplasmodial drugs also exert a direct effect on the immune system, similar to that occurring for quinine, chloroquine and mefloquine. These drugs may improve the evolution of the disease by decreasing the production of pro-inflammatory cytokines such as tumor necrosis factor [32,33] and IL-2 [34], which are involved in the initial steps of pathogenesis of severe malaria.

Furthermore, quinine, chloroquine, primaquine, pyrimethamine, artemisinin, mefloquine and proguanil may decrease the adherence of parasitized erythrocytes, probably by down regulating the expression of adhesion receptors [35,36]. This effect may reduce the sequestration of parasitized erythrocytes in the small vessels and, therefore, increase their destruction during their passage through the spleen.

On one hand, chloroquine, quinine, mefloquine, amodiaquine, artemether [37] and artemisinin [38] may decrease the defense against parasite by inhibiting phagocytosis of malaria parasites by monocytes or neutrophils. Chloroquine may also reduce chemotaxis of human monocytes [39]. On the other hand, artemisinin and artemether are able to enhance the generation of reactive oxygen intermediates by phagocytes [38], and chloroquine and artemether are able to increase the production of reactive nitrogen intermediates [40], which may improve the defense against malaria parasites.

The factors involved in the interplay between the anti parasitic effects of antimalarial drugs and their ability to interfere with the immune response are not as yet determined. A better understanding of these factors is necessary to allow taking advantage of the immunomodulatory actions of antimalarial drugs.

2. Immunomodulatory Therapy

Different approaches have been adopted to downmodulate the hyperactive immune system in malaria, including the inhibition of cytokine production or action, the reduction of the excessive synthesis of nitric oxide, or the expression of adherence molecules by endothelial cells (Fig. 1). Most studies involved experimental models, and some of them provided quite promising results, as shown in Tables 2,3, and 4.

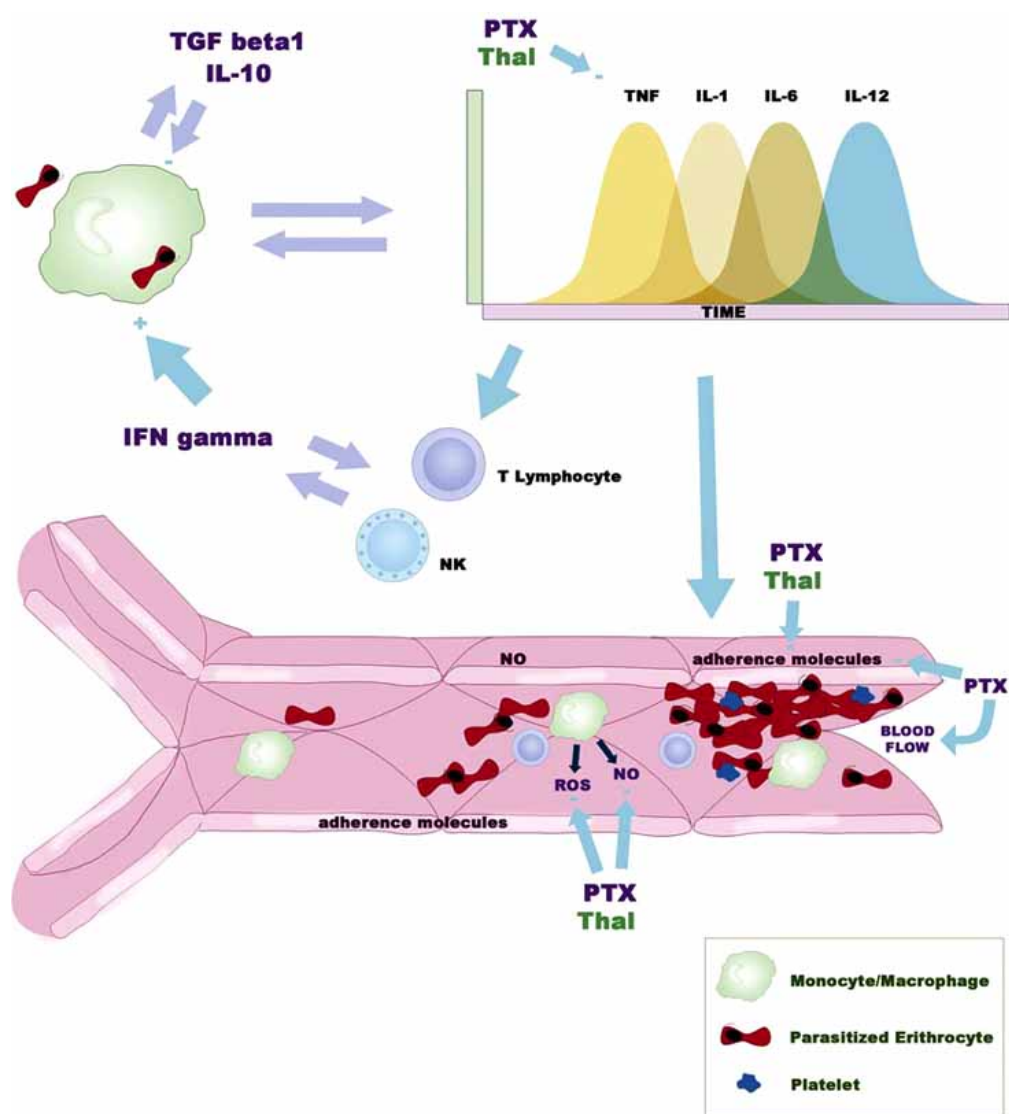


Fig. (1). Antigens of *Plasmodium falciparum* stimulate the local production of inflammatory cytokines, mainly TNF and IL-12, by monocytes/macrophages, which stimulate the production of interferon- γ by NK cells and T lymphocytes. Depending on the host and the parasite relationship, it local overproduction of these cytokines may occur and, consequently, local patchy involvement of capillaries and postcapillary venules. The enhanced levels of these cytokines may increase the production of nitrogen and oxygen species. These molecules lead to endothelial cells damage and damage of all cells in close contact. In addition, NO may impair neuronal transmission. The increased levels of inflammatory cytokines also stimulate the expression of adherence molecules on endothelial cells increasing the sequestration of parasitized erythrocytes and consequently, it may impair blood flow. The consequences of endothelial alterations are perivascular edema, hypoxia of the surrounding brain parenchyma and petechial hemorrhages. Depending on the extent of the structural damage of the brain and the degree of metabolic disturbance, cerebral involvement can progress to coma and multiple convulsions. Pentoxifylline (PTX) inhibits TNF, IL-1, and IL-8 production, platelet aggregation, and free oxygen radicals formation. It also has endothelium-protective properties and improves blood flow. Thalidomide (Thal) inhibits TNF production and the expression of adherence molecules. It modulates free radicals production, and improves the immune defense against plasmodium.

2.a. Cytokines

1. Anti-Inflammatory Drugs

Dexamethasone was the first drug used in an attempt to revert the inflammatory manifestations of cerebral malaria. In fact, it is recognized that glucocorticoids inhibit the ex-

pression of multiple inflammatory molecules, such as cytokines (TNF, IL-1, IL-6 and GM-CS), chemokines (IL-8, RANTES, and eotaxin), and enzymes (nitric oxide synthase). They also regulate the expression of adherence molecules, as ICAM-1 and VCAM-1 [41]. However, the association of dexamethasone with antimalarial drugs in patients with cere-

bral malaria caused no modification of mortality rates or the duration of coma, and increased the frequency of infectious complications [42] (Table 2).

Iloprost, a synthetic prostacyclin analog, successfully prevented the development of cerebral malaria in mice and inhibited malaria antigen-induced TNF production by macrophages. Furthermore, this drug increased the survival of *Plasmodium* infected-mice [43] (Table 2).

A new anti-inflammatory compound, LMP-420 or 2 NH₂-6CI-9-[(5-dihydroxyboryl)-pentyl] purine, which acts as a transcriptional inhibitor of TNF, was recently suggested as a potential drug for cerebral malaria. The compound was able to abolish cytoadherence mediated by ICAM-1 and VCAM-1 on brain-derived endothelial cells and reduced the activation of endothelial cells induced by TNF or lymphotoxin [44].

2. Inhibition of TNF Production or Action

TNF is the cytokine best evaluated in severe malaria and its inhibition thought to improve the morbimortality of the

disease was the most assessed aspect. Both the inhibition of its action by anti-TNF antibodies and of its production by drugs, such as pentoxifylline and thalidomide, have been assessed (Table 3).

Since the suggestion by Clark in 1978 [45] that increased levels of TNF could play a role in the pathogenesis of malaria, several publications have confirmed this association [46-51]. This cytokine modulates the production of other cytokines involved both in defense and pathology of the disease. Furthermore, TNF modulates the expression of adherent molecules on endothelial cells and, therefore, increases the sequestration of parasitized erythrocytes in the small vessels. Thus, TNF- α/β -deficient mice were completely resistant to *P. berghei* ANKA-induced cerebral malaria, and no up regulation of ICAM-1 and nitric oxide was detected. The resistance to cerebral malaria in these mice was associated with reduced IFN- γ and IL-12 expression in the brain [52]. It seems that the TNF-receptor 2 is the receptor involved in cerebral disease, because mice genetically deficient in this receptor were protected from cerebral malaria, whereas TNF-receptor 1 deficient mice were as susceptible to it as wild-

Table 2. Responses of Malarial Individuals or Animals to Cytokine Inhibition or Administration

Target	Treatment	Response	Model	Reference
Several	Dexamethasone	Worsened Prolonged coma	Human	Warrell <i>et al.</i> , 1982 [42]
Several	Iloprost (prostacyclin analog)	Improved Prolonged survival	Mice	Sliwa <i>et al.</i> , 1991 [43]
IFN- γ	Anti IFN- γ mAb	Improved Decreased incidence CM, prolonged survival	Mice	Grau <i>et al.</i> , 1989 [91]
IL-6	Anti-IL6mAb	No effect	Mice	Grau <i>et al.</i> , 1990 [94]
IL-3 plus GM-CSF	Anti-IL-3 IgG plus Anti-GM-CSF IgG	Improved Prolonged survival	Mice	Grau <i>et al.</i> , 1988 [93]
TGF- β	TGF- β	Worsened Unable to control infection	Mice	Tsutsui and Kamiyama, 1999 [92]
	Anti TGF- β mAb	Improved Production of IFN- γ and NO and acquisition of resistance to infection	Mice	Tsutsui and Kamiyama, 1999 [92]
IL-2	IL-2	Worsened	Mice	Haque <i>et al.</i> , 2001 [98]
IL-12	IL-12	Improved a) Protected against challenge b) Increased survival	Mice Mice	a) Sedegah <i>et al.</i> , 1994 [96] b) Stevenson <i>et al.</i> , 1995 [95]
	IL-12	Improved Protected against challenge	Monkey	Hoffman <i>et al.</i> , 1997 [97]

Table 3. Responses of Malarial Individuals or Animals to TNF Inhibition or Administration

Target	Treatment	Response	Model	Reference
TNF	Anti-TNF Ab	No effect on evolution Decreased fever	Children	Kwiatkowski <i>et al.</i> , 1993 [55]
	Anti-TNF Ab	No effect on evolution Increased neurological sequelae	Children	van Hensbroeck <i>et al.</i> , 1996 [56]
TNF (Several)	Pentoxifylline	Improved evolution	Human	Graninger <i>et al.</i> , 1991 [72]
	Pentoxifylline	Improved Treated mice did develop CM	Mice	Kremsner <i>et al.</i> , 1991 [69]
	Pentoxifylline	Improved Increased survival	Mice	Santos-Neto <i>et al.</i> , 1992 [70]
	Pentoxifylline	Improved Shorter coma time and lower mortality	Children	Di Perri <i>et al.</i> , 1995 [71]
	Pentoxifylline	Improved Prevented selective damage of hippocampal neurons	Mice	Stoltenburg-Didinger <i>et al.</i> , 1993 [75]
	Pentoxifylline	No clinical effect	Adult with various clinical forms of severe malaria	Looreesuwan <i>et al.</i> , 1998 [74]
	Pentoxifylline	No effect on clinical and laboratory outcome Several side effect of the pentoxifylline	Severe and non-severe adult patients with malaria	Hemmer <i>et al.</i> , 1997 [73]
	Pentoxifylline	Inconclusive no clinical effect	Severe and non-severe adult patients with malaria	Wenisch <i>et al.</i> , 1998 [76]
TNF (Several)	Thalidomide	Improved hypoglycemia was less severe	Mice	Ramirez-Villafuerte <i>et al.</i> , 1998 [86]
	Thalidomide	Improved Increased survival	Mice	Muniz-Junqueira <i>et al.</i> , 2005 [87]
TNF	TNF	Improved	Mice	Postma <i>et al.</i> , 1999 [88-90]

type mice. The mechanism by which deficient TNF-receptor 2 mice were protected from cerebral malaria seems to be linked to the role of this receptor in ICAM-1 upregulation in brain microvessels. It was suggested that the membrane form of TNF, rather than the soluble TNF, might have a critical role in cerebral malaria [53]. In fact, it was shown that membrane-bound TNF preferentially interacts with TNF-receptor 2 [54]. It was suggested that the membrane-bound TNF present on sequestered leukocytes might be able to induce ICAM-1 expression on cerebral microvessels by preferentially triggering TNF-receptor 2 [53].

Since the role played by TNF on the pathophysiology of malaria was disclosed, novel possibilities of therapeutic intervention have been tried aiming at reducing the morbidity

and lethality of the disease. Several approaches, as anti-TNF Ab, pentoxifylline, and thalidomide, have been used to reduce the lesions resulting from the overproduction of TNF and the subsequent cascade of products that are induced, such as other cytokines, oxygen and nitrogen intermediates, and the over expression of adhesion molecules. Fig. 1 shows the major strategies used for modulating the immune system in malaria.

2.1. Anti-TNF Antibodies

Administration of anti-TNF monoclonal antibody to children with cerebral malaria caused inhibition of fever, but no modification of the severity of the disease or in the fatality rate was observed [55]. A major drawback of this observa-

Table 4. Responses of Malarial Individuals or Animals to Adherence Molecules, Platelets and ROS Inhibition

Target	Treatment	Response	Model	Reference
ICAM-1	Anti-LAF-1	Improved Increased survival	Mice	Grau <i>et al.</i> , 1991 [122] Falanga and Butcher, 1991 [123]
P-selectin	Anti-P-selectin Ab	No influence	Mice	Combes <i>et al.</i> , 2004 [124]
Platelet	Anti-LAF-1	Improved Abrogated cerebral sequestration of platelets, protected against cerebral malaria	Mice	Grau <i>et al.</i> , 1993 [135]
N-acetylcysteine	Scavenger ROI	Improved	Humans	Watt <i>et al.</i> , 2002 [177]
Desferrioxamine	Scavenger ROI	Worsened Increased mortality	Humans	Thuma <i>et al.</i> , 1998 [176]
Butylated hydroxyanisole	Scavenger ROI	Improved Increased survival Decrease hemorrhages	Mice	Thumwood <i>et al.</i> , 1989 [165]

tion was the fact that the patients had developed cerebral malaria before receiving anti-TNF antibody. Since TNF acts at the initial stages of the process of cerebral lesion, it is not expected that its late inhibition would affect the prognosis. Surprisingly, the administration of anti-TNF antibody can negatively affect the course of malaria, as shown by van Hensbrock *et al.* [56]. The authors treated children presenting cerebral malaria with anti-TNF monoclonal antibody and obtained no improvement in their survival. On the contrary, the patients presented a significant increase in neurological sequelae. A possible explanation is that the antibody might have retained TNF within the circulation prolonging its effects on vascular endothelium.

2.2. Pentoxifylline

Attempts to modify the outcome of malaria by inhibiting the synthesis of TNF with pentoxifylline have led to controversial results. Pentoxifylline is a methylxanthine derivative that inhibits endotoxin-induced TNF production, both *in vitro* and *in vivo*, and exerts this control by inhibiting endotoxin-induced transcription of the TNF gene [57,58] and reducing TNF bioactivity in a dose-dependent manner [59]. It may also inhibit TNF synthesis *via* inhibition of phosphodiesterase and the increase of intracellular cyclic adenosine monophosphate [1]. This drug is a hemorrheological agent for the treatment of conditions involving a defective regional microcirculation. It acts primarily by increasing red blood deformability, reducing blood viscosity, and decreasing the potential for platelet aggregation and thrombus formation [60]. Both *in vitro* and *in vivo* studies showed that pentoxifylline significantly decreases the adhesion of blood platelets to the vascular wall. It may also intervene with the process of blood coagulation by decreasing the pathologically raised level of fibrinogen and influencing the process of thrombin formation. The process of fibrinolysis is partly influenced by the decrease of antiplasmin activity and also by the increase of the level of plasminogen activator. In addition, pentoxifylline stimulates the synthesis and release of PGI₂, inhibits the formation of thromboxane A₂, and has an

inhibitory effect on the formation of free oxygen radicals [61-64].

Pentoxifylline is a potent inhibitor of inflammatory damage due to two major mechanisms: (a) it reduces the production of inflammatory cytokines TNF [57], IL-1 β , IL-6 and IL-8 [65] by phagocytes, and (b) reverses the effects of these cytokines on phagocytes. However, production of IL-6 by mononuclear cells stimulated by plasmodium antigen may be enhanced by pentoxifylline [66]. Pentoxifylline may counteract different effects of inflammatory cytokines on phagocytes, such as the increased adherence, shape change resulting in larger size and rigidity of phagocytes, increased oxidative burst, increased degranulation, and decreased chemotactic movement [67].

Based on the recognized mechanisms of action of pentoxifylline, this drug could exert different beneficial effects in cases of cerebral malaria, including: (1) improvement of cerebral blood flow; (2) improvement of microcirculation through its hemorrheological effect due to increased deformability of erythrocytes and reduction of blood viscosity; (3) decrease of platelet aggregability; (4) anti-inflammatory effect due to the inhibition of pro-inflammatory cytokines and free radicals; (5) endothelium-protective properties; and (6) inhibition of erythrocyte rosette formation and disruption of formed rosettes [68].

Treatment of severe malaria with pentoxifylline has shown controversial results. While some authors found improvement of the clinical course of the disease [see 69-72], others showed no effect [see 73,74] (Table 3). The use of this immunomodulatory drug in plasmodium-infected mice usually showed better results than its use in humans, probably due to a better controlled experimental design, and the precocious onset of treatment in the experimental models.

It was found that pentoxifylline decreased serum levels of TNF, prevented cerebral malaria [69], and increased the survival of CBA mice infected with *Plasmodium berghei* Anka [70]. In another model of the disease, C57/B16 mice

infected with *Plasmodium berghei* K173, pentoxifylline prevented selective damage of hippocampal neurons. However, there was no significant difference in the course of parasitemia, in the survival time, and in the occurrence of cerebral malaria [75] (Table 3).

The results of pentoxifylline treatment in human malaria are difficult to interpret. Some authors observed a better evolution after treatment with the drug. Di Perri *et al.* [71] found that the association of pentoxifylline to the standard quinine regimen given to comatose malaria-infected children caused an inhibitory effect on TNF synthesis, significantly shortened coma duration, and caused a trend toward lower mortality. Other observations in humans did not show these beneficial effects of pentoxifylline on severe malaria. Looareesuwan *et al.* [74] assessed the influence of the drug associated to artesunate and found no difference in TNF serum levels or in the clinical course between groups. However, the heterogeneity of the clinical presentation of the patients and the small size of the groups do not allow for definite conclusions. Wenisch *et al.* [76] showed that high doses of pentoxifylline markedly reduced serum levels of TNF, IL-6, and TNF soluble receptor when compared to those patients who received low doses of the drug or a placebo regimen. However, it was not possible to draw any conclusion about the effect of the drug on the clinical course of malaria (Table 3).

Hemmer *et al.* [73] evaluated a group of patients with heterogeneous clinical manifestations. Most of them did not have severe disease, but they had similar TNF serum levels. They were submitted to different treatment schedules with antimalarial drug. The authors found no difference in the length of hospital stay, the time until deffervescence, total acetaminophen consumption and in clinical laboratory parameters between the groups treated or not with pentoxifylline. Significantly more patients using pentoxifylline had side effects, such as nausea and abdominal discomfort compared to the placebo group. The study was terminated earlier than planned because there was not improvement of the course of the disease (Table 3).

Why was no concordance found between the expected effects of pentoxifylline, theoretically capable of acting on the key points of the pathogenesis of cerebral malaria, and the reported observations? In this context, some aspects deserve attention. It appears that pentoxifylline is more efficient in children with cerebral malaria because it may cause a reduction in coma duration.

The published trials with pentoxifylline raise some concerns: the patients presented different clinical pictures of malaria and constituted very heterogeneous groups; the criteria of severity of the disease were not always met or established, the drug schedules were extremely variable, and the size of the samples was not always sufficient to allow for definite conclusions. Therefore, it is still not possible to draw any conclusion about the possible benefits of pentoxifylline to prevent or treat severe malaria.

An important drawback of most studies using immunomodulatory drugs in malaria is when they are administered. According to our understanding of the immunopathogenesis of severe malaria, immunomodulatory drugs should be pre-

viously initiated in order to prevent severe disease by down modulating the immune response before the lesions are established. Furthermore, since TNF participates in the defense against plasmodium, pentoxifylline should not be used very early in the infection to avoid inhibiting its protective effect against the parasite [26]. Therefore, it can be postulated that the better time to use this drug is when individuals show risk of severity of the disease, such as high parasitemia in children or in previously unexposed individuals. However, this drug should be used before the establishment of clinical parameters of severity, such as coma, pulmonary or renal involvement and severe anemia.

2.3. Thalidomide

Thalidomide is a drug of controlled use due to its teratogenicity. In July 1998, this drug was approved by the FDA (USA) for treating erythema nodosum of leprosy [77]. This drug is another inhibitor of TNF production, which has also been evaluated in malaria. The immunomodulatory effects of thalidomide are evident in several aspects of the immune function. This drug inhibits the production of TNF by human monocytes stimulated with lipopolysaccharide (LPS), both *in vitro* [78] and *in vivo* [79]. This effect is thought to be due to the increase in the rate of TNF mRNA degradation [80]. The drug may act as a co-stimulatory signal for T cell activation *in vitro* resulting in increased production of interleukin-2 and interferon- γ [81]. Thalidomide causes two opposite effects on interleukin-12 production: one is the inhibition of the production of this cytokine by peripheral mononuclear blood cells stimulated with LPS, and the other is an increased production of IL-12 when cells are co-stimulated *via* T cell receptor [81,82], suggesting that the drug had an inhibitory effect on monocytes, but exerted a co-stimulatory activity on T cell responses. It has been suggested that this combination of effects may contribute to the immunomodulatory properties of the thalidomide [81]. Although thalidomide did not by itself alter the expression of E-selectin, ICAM-1, or VCAM on resting umbilical vein endothelial cells, the up regulation of these molecules following activation by LPS or recombinant TNF was inhibited by thalidomide. Furthermore, reduction of the percentage of endothelial cells expressing ICAM-1, after incubation with TNF and thalidomide, was also observed [83-85]. These observations show that thalidomide may interfere with key points of the immunopathogenesis of severe malaria, which indicates that this drug might be useful to prevent the complications of the disease by modulating the over-production of TNF and the subsequent inflammatory cascade that follows to it.

Only two reports assessed the influence of thalidomide in malaria. Ramirez-Villafuerte *et al.* [86] showed that hypoglycemia was less severe in *Plasmodium chabaudi chabaudi*-infected mice treated with thalidomide. In the other report [87], thalidomide treatment of *P. berghei* ANKA-infected CBA mice was started on the second day after infection in order to minimize the interference with the antiparasite effect of TNF, since this cytokine also participates in the defense against malaria parasites [26]. It was shown that thalidomide delayed the onset of death and caused a significant increment of two days in the duration of life of infected mice. Moreover, this drug also increased the phagocytic ca-

capacity of macrophages, and the production of hydrogen peroxide and nitric oxide by macrophages. Nonetheless, no effect on parasitemia was detected. This latter observation suggests that the drug affected mainly the function of the immune system and/or modulated the pathophysiology of the disease [87].

Some aspects on the potential use of thalidomide and its derivatives in malaria patients deserve consideration. Based on experimental models of malaria, it was concluded that this drug should not be used very early in the infection to avoid interference with the protective effect of TNF against the malaria parasite [26]. However, the beneficial effect of thalidomide may be reduced if it is administered too late in the course of the infection because local production of TNF would be already elevated and the expression of adherence molecules established. Therefore, it can be postulated that thalidomide is potentially beneficial for individuals who are at risk for severe malaria, provided it is used before the establishment of clinical parameters of severity, such as coma, pulmonary or renal involvement and severe anemia [87]. Although fully contraindicated for pregnant women, the available data point to the possibility to test thalidomide as a novel alternative therapy in human malaria, in association with antimalarial drugs, in order to prevent severe forms of the disease, and, therefore, decrease the morbidity and mortality of malaria [87].

3. Administration of Recombinant TNF

Adding complexity to our understanding and concerns about the possibility to modulate the immune response in malaria, it was observed that recombinant TNF, liposome-bound or thiolated recombinant TNF injected on the fifth day of infection were able to decrease parasitemia and protect against *P. berghei* K173 experimental cerebral malaria [88-90]. Although the increased production of TNF has been associated with severe disease, this cytokine is also important in the defense against malaria parasite [26], which may justify this apparently contradictory result.

4. Other Cytokines

Several cytokines are important in the pathogenesis of malaria, so the effect of cytokines and the inhibition of their effect with anti cytokine antibodies have been assessed. All reports were in experimental model, and most of them provided quite successful results.

The administration of antibodies against interferon- γ [91], TGF- β [92], and the association of IL-3 and GM-CSF [93] improved the evolution of the disease, whereas anti-IL-6 [94] had no effect. The administration of IL-12 improved the evolution of malaria [95] and protected against challenge [96,97], whereas TGF- β [92] and IL-2 [98] worsened it (Table 2).

The role played by IFN- γ in immunity and pathogenesis of malaria has been demonstrated. Increased IFN- γ mRNA levels were detected in cerebral malaria-susceptible mice but not in the resistant ones during the neurological syndrome. IFN- γ increases TNF mRNA levels and upregulates TNF receptors on the target cell surface [21,91]. Administration of anti-IFN- γ antibody to *P. berghei*-infected CBA mice im-

proved the evolution of malaria by decreasing serum TNF levels and preventing the development of cerebral pathology [91] (Table 3).

The increased production of IFN- γ in malaria depends on the unbalanced response of Th1 and Th2 lymphocytes, and is involved in the pathogenesis of the disease and in the complex cytokine network triggered by the parasite, which involves the production of IFN- γ , TNF, IL-6, IL-12, IL-18, IL-10, and TGF- β , among others. The balance between Th1 and Th2 immune response and between pro-inflammatory and anti-inflammatory cytokines is important in determining the level of parasitemia, disease outcome and rates of recovery [99-105]. The overproduction of both pro- and anti-inflammatory cytokines may be responsible for disease severity and mortality in malaria, and although highly assessed, the role played by this cytokine network in malaria is still unclear. Plasma IL-6, IL-10 and TNF concentrations were significantly higher in adult patients who died from severe falciparum malaria than in survivors. Hyperparasitemia, jaundice and shock were associated with raised levels of IL-6, IL-10 and IFN- γ . However, those who exclusively had cerebral malaria had significantly lower levels of these cytokines, suggesting a more localized pathology for cerebral disease. The imbalance between pro- and anti-inflammatory responses is an important determinant of mortality [101].

The level of IL-10 and its relationship with TNF appears to be an important determinant of severity of disease. It seems that higher levels of IL-10 over TNF may prevent development of anemia by controlling the excessive inflammatory activities of TNF [106]. IL-10 deficient mice infected with *P. chabaudi* (AS) suffer a more severe form of the disease and show a higher rate of mortality than control C57BL mice [107].

TGF- β was evaluated both by its inhibition with antibody as well as by administration of the cytokine itself. Administering anti-TGF- β Ab increased serum IFN- γ and NO and the mice resisted to infection, whereas administering the recombinant TGF- β to infected mice decreased their resistance by suppressing IFN- γ and NO, causing the animals to succumb to infection [92] (Table 2).

The effects of cytokines in malaria are complex and still unclear and controversial. The levels of the cytokines IL-12 and TGF- β 1 regulate the balance between pro- and anti-inflammatory cytokines. TGF- β is an important regulator of inflammation at low concentrations and an anti-inflammatory agent at high concentrations and may modify the severity of malaria. Serum levels of IL-12, IL-18 and TGF- β are higher in patients with malaria. However, they are imbalanced in severe disease. IL-18 cooperates with IL-12 to stimulate IFN- γ production by T and NK cells. They are produced by mononuclear phagocytes and have immunoregulatory functions with effects on the immune response to the blood stage of disease. The levels of IL-12 and IL-18 were lower in patients who suffered a more severe form of disease, whereas the level of TGF- β was higher in severe disease, mainly anemia [107-112]. In contrast, Perkins and Weinberg observed that IL-12 and TGF- β 1 were significantly lower, whereas TNF and IL-10 were higher in chil-

dren with severe anemia and parasitemia [108]. Luty *et al.* [113] observed that pretreatment plasma IL-12 and IFN- α levels were lower in severe anemia and hyperparasitemia, and that IL-12 shows a strong inverse correlation with parasitemia, while TNF and IL-10 were higher in those with severe malaria.

The sequence of production of cytokines was also assessed and found relevant. A relationship between virulence of infection and the time of production of TGF- β was shown. C57BL6 mice infected with a nonlethal Py17X strain of *P. yoelii* produced TGF- β 5 days post-infection, which correlated with the resolution of parasitemia, down regulation of TNF and recovery. On the other hand, infection with lethal strain Py17L induced high levels of circulating TGF- β within 24 h and was associated with delayed and blunted IFN- γ and TNF responses, failure to clear parasites and mortality [114]. TGF- β seems to induce protective immune responses, leading to slower parasite growth early in the infection and, subsequently, to downregulate pathogenic response late in the infection [115].

Recombinant IL-12 was useful in the treatment of blood-stage malaria infection. Treatment during the first 5-6 days of infection delayed the onset of parasitemia, significantly reduced the peak of parasitemia, and prevented lethal infection in the *P. chabaudi*-susceptible mice. IL-12 was also effective in correcting malarial anemia [95,116] (Table 2). Administration of recombinant IL-12 protected Balb/c mice against challenge with *P. yoelii* sporozoites [96] (Table 2), and protected rhesus monkeys against challenge with *P. cynomolgi* through IFN- γ and NO-dependent elimination of the parasite [97] (Table 2).

In conclusion, it appears that severe malaria is characterized by the suppression of the protective effect of TGF- β 1 and IL-12 and overproduction of TNF [108,117], and that different patterns of immunological deregulation in cerebral malaria and severe anemia occur. This indicates that an appropriate immune response to malarial infection requires a delicate balance between the beneficial and detrimental effects of inflammation. The loss of this balance in either direction can have dire consequences [118].

Inhibition of IFN- γ [91], TGF- β [92] and IL-3 plus CSF-GM [93] with monoclonal antibodies and administration of IL-12 [95-97] were very efficient in improving the evolution of the disease and decreasing the pathological alterations of the animals. However, no attempt to evaluate the effects of the inhibition or administration of these cytokines in human malaria was made. The modulation of immune response in patients with malaria seems highly complex and still unclear. Apparently, the inhibition or stimulation of single cytokine in human malaria may have unexpected effects due to the complex interaction and kinetics of cytokines and their pleiotropic action on immune system. Furthermore, any approach to modulate cytokines needs to begin before the establishment of lesions in order to prevent the disease.

2.b. Sequestration, Adherence Molecules and Platelets

A central feature of *P. falciparum* infection is the sequestration of parasitized erythrocytes within the small vessels of major organs, mainly brain and lungs [119-121], and its ex-

acerbation is a direct consequence of the imbalance of cytokines, mainly overproduction of TNF. Only three reports assessed the influence of antibodies against molecules of adherence [see 122-124] (Table 4). Their exacerbated expression in endothelial vessel wall might increase the sequestration process and, consequently, enhance the severity of the disease. The reports showed opposite results. In two of them, an anti-LAF1 Ab, the ligand of ICAM-1 [122,123], was used, which improved the evolution of the treated mice. The other study used anti-P-selectin Ab, which showed no effect [124] (Table 4).

The precise mechanisms involved in the onset of neuropathology remain unknown, but parasite sequestration in the brain, metabolic disturbances and host immune responses all play a role [125]. The process of sequestration occurs in every *P. falciparum* infection, including immune individuals with asymptomatic parasitemia and nonimmune patients with life-threatening illness [126]. Erythrocytes containing young parasites circulate in the peripheral blood for the first 18-24 h of the 48 h life cycle of *P. falciparum*, and red blood cells containing the more mature trophozoites and schizonts are rarely seen in peripheral blood because they are sequestered in the microvessels of various organs [127,128].

Sequestration is a complex phenomenon involving molecular interactions between antigens located on erythrocyte, such as EMP1, and host receptors expressed on the surface of endothelial cells, platelets or dendritic cells. EMP1 is a name collectively given to members of a family of variant cell-surface proteins encoded by *P. falciparum*. They are expressed or not as knob-like protrusions on the surface of parasitized erythrocytes and are able to engage multiple host receptors, including CD36, thrombospondin, ICAM-1/CD54, PECAM/CD31, E-selectin, P-selectin, VCAM-1, chondroitin sulfate A, and hyaluronic acid [14,129-131].

Overproduction of TNF induces an upregulation of adhesion molecules on endothelia, notably ICAM-1 and VCAM-1 [52, 132], which may increase the adhesion of leukocytes and other cells and thereby disturb the microcirculation in the brain and other organs [133]. It has been observed that mice ICAM-1 deficient [134] or treated with anti-LAF-1 (CD11a) monoclonal antibody, the ligand of ICAM-1 [122, 123], show delayed mortality (Table 4).

Several endothelial receptors have been identified for *P. falciparum* parasitized red blood cells adhesion. However, it is still unclear which receptors are involved in cerebral malaria. CD36 is the most common receptor found, however, this receptor is constitutively present in the microvasculature and its expression is not affected by inflammatory cytokines, such as TNF. The distribution of this receptor on endothelial surfaces in the brain is marginal and its contribution to cerebral malaria is unclear. It has been suggested that platelets could function as a bridge between pRBC and endothelial receptors, and that pRBCs could interact with CD36 on platelets [13,14,129].

In a murine cerebral malaria model, platelets accumulate in brain microvasculature, adhere to and probably damage brain endothelial cells. *In vivo* treatment with a mAb to LAF-1, which is expressed on platelets, selectively abro-

gated the cerebral sequestration of platelets and correlated with the prevention of cerebral malaria (Table 4). Furthermore, malaria-infected animals rendered thrombocytopenic were significantly protected against cerebral disease. The CD11a-dependent interaction between platelets and endothelial cells appears pivotal to microvascular damage [135]. *In vitro*, platelets acted as effectors of vascular damage when endothelial cells were previously stimulated by TNF [21]. By immunostaining brain samples from children whose fatal illness was cerebral malaria, platelets between malaria pigment and leukocytes, associated with malaria pigment and alone, were observed. The surface area of platelet staining and the proportion of vessels showing platelet accumulation were significantly higher in patients with cerebral malaria [136]. Platelets might provide an adhesion receptor to microvascular beds, reorienting the sequestration of different parasites and playing a role in the pathogenesis of severe disease [137].

The selectin family mediates initial tethering to the endothelial cells and rolling of leukocytes [138]. Plasma concentration of soluble E-selectin is increased in human severe malaria [139-142]. Infection of mice with *P. berghei* ANKA leads to P-selectin up regulation in the brain vessels of cerebral malaria-susceptible mice, but not of cerebral malaria-resistant ones. Despite the protection from mortality of P-selectin deficient mice, treatment with anti-P-selectin mAb failed to prevent the development of the neurological syndrome [124] (Table 4).

The other important molecule that binds parasitized erythrocyte is ICAM-1, a membrane glycoprotein expressed on lymphocytes, macrophages and vascular endothelium. It is present in most microvasculature surfaces and is upregulated by TNF and IFN- γ . Its primary role is to mediate cellular adhesion within the immune system via the leukocyte integrins LFA-1 and MAC-1 [13,143]. Inhibition of the interaction between ICAM-1 and infected erythrocytes by using recombinant soluble ICAM-1 as competitor was unable to reduce adhesion to ICAM-1 *in vitro* [144].

ICAM-1 also exists in a soluble form released from the surface of endothelial cells by proteolytic cleavage. This plasma soluble form is elevated in malaria patients [139, 145]. In addition, immunohistochemical studies on post-mortem tissue have demonstrated its widespread expression at microvascular endothelium [127,129], concomitant with increased parasite sequestration. Co-localization of parasitized erythrocytes with ICAM-1 expressing vascular endothelial cells was observed in cerebral malaria brain tissue [129,145]. Furthermore, monocytes, which express ICAM-1 and can bind to ICAM-1, were often co-localized with parasites and microhemorrhages within the cerebral vessels in cerebral malaria [146]. *In vitro* studies have demonstrated that the affinity of most pRBC to ICAM-1 is weak and synergic cooperation with other receptors might be necessary for a stable adhesion [13, 144,147]. There was an increased survival of > 15 days of ICAM-1 deficient mice infected with *P. berghei* ANKA, whereas the wild-type mice died 6-8 days after infection. Moreover, sequestration of macrophages and parasitized erythrocytes was less evident in deficient mice [134]. Treatment of mice with anti-LAF-1, the ligand of

ICAM-1, reduced the incidence of cerebral malaria and increased their survival [122,123] (Table 4).

Platelet-endothelial cell adhesion molecule 1 (PECAM/CD31), located in the junction of endothelial cells [148], is also involved as a receptor to *P. falciparum* parasitized erythrocyte adhesion [149,150]. Increased expression and redistribution of this molecule have been observed after IFN- γ stimulation [151]. In addition, its expression in brain tissue of malaria patients has been noted [152].

Post-mortem brain tissue from patients who died from cerebral malaria expressed multiple cell adhesion molecules, including VCAM -1, E-selectin (ELAM-1), CD36, ICAM-1, and TSP, on cerebral microvascular endothelium not expressed in the brains of individuals who died from other causes [127,129]. The multi-functional binding sites on the parasitized red cell, which recognized these structurally unrelated molecules, demonstrate the versatile adhesive properties of malaria-infected erythrocytes [150,153,154]. Studies on clinical isolates directly from malaria patients show that parasites from those suffering severe disease were more adhesive and could bind to several human receptors [154]. Multi-adhesion phenotype could be an important feature of virulent parasites. To be able to sequester in human hosts of different genetic background, the parasites might need to possess affinity to several receptors simultaneously. To be able to adhere simultaneously to several receptors is advantageous to parasites, assuring their survival in the hosts and giving them a possibility to hide in the postcapillary regions where they can escape from clearance by the spleen before initiating the next erythrocytic cycle [12].

The complexity and variety of molecules involved in the process causing sequestration of parasitized erythrocytes in microvessels suggest that no single approach will be effective. Any approach to inhibit adhesion molecules must consider acting on several adhesion molecules simultaneously. Despite its complexity, the main effect of targeting these molecules should be the inhibition of interactions between parasitized erythrocytes and endothelial cells, which, if exacerbated, lead to increased sequestration and severe malaria.

Some drugs with pleiotropic action, such as statins [155-157] and thalidomide [83-85], can also inhibit the expression of adherence molecules and need to be considered for evaluation in human malaria.

Several anti adhesion therapies have already been investigated for the treatment of inflammation, ischemia/reperfusion injury, autoimmune diseases, allergic diseases and cancer. In the field of clinical development, some new anti-adhesion drugs have been approved, including some monoclonal antibodies and small molecules [158-162]. These novel approaches also need to be considered for the prevention and treatment of severe malaria.

2.c. Reactive Species of Oxygen and Nitrogen

Reactive oxygen [163-166] and nitrogen intermediates [95,167-169], produced by phagocytes, particularly TNF-producing activated macrophages [169, 170], are also involved both in the antiparasitic defense as well as in the pathogenesis of the severe forms of malaria. Interactions

between NO and oxygen intermediates can produce lipid peroxidation products, which are increased in malaria and are also involved in the pathogenesis and protection against malaria parasite [171, 172].

1. Reactive Oxygen Species

Reactive oxygen species are among the main microbicidal mechanisms of macrophages. Several observations point to a role of oxygen radicals against malaria parasites. Both non-lethal *Plasmodium yoelii* and lethal *Plasmodium berghei* were killed *in vitro* by hydrogen peroxide [163]. *In vitro* studies showed the appearance of crisis forms of *Plasmodium falciparum* inside erythrocytes incubated with hydrogen peroxide, suggesting that oxygen-dependent microbicidal system of phagocytes has a killing effect on the parasite [173]. *Plasmodium yoelii* incubated in micro-chambers separated from macrophages by a 0.45 μ m filter were susceptible to hydrogen peroxide released by cytokine-activated macrophages, which were abrogated by pre-incubating macrophages with catalase [164]. Moreover, macrophages from *Plasmodium chabaudi* AS-infected mice exhibit defects in oxygen metabolism that may affect their susceptibility to the parasite [166]. Human malaria infection is associated with an increased production of reactive oxygen species by phagocytic cells, suggesting that these molecules participate in the host defense mechanisms against the parasite [174]. However, patients with severe malaria show increased generation of oxygen species, supporting the hypothesis of Clark *et al.* [175] that oxygen species could be responsible for the tissue damage observed in severe forms of malaria [174]. The effects of reactive oxygen species can be both beneficial and pathological, depending on the amount and the place of its production [170].

Some antioxidant adjuvant therapies for malaria have been evaluated in humans (pentoxifylline, desferrioxamine and N-acetylcysteine) and mice (butylated hydroxyanisole) (Table 4). Desferrioxamine is an iron chelator, which also inhibits free radical activity. This drug used in cerebral malaria children did not affect the coma recovery and was associated with increased mortality [1,176]. N-acetylcysteine inhibits TNF release, impedes cytoadherence and is a potent scavenger of free oxygen radicals, which are produced in response to TNF and mediate some of its toxic effects. This drug was tested as an adjunctive therapy for severe malaria and normalized serum lactate levels twice quickly [177]. Mice fed with a diet containing 0.75% w/w butylated hydroxyanisole, a potent radical scavenging, lived 2 weeks longer and had less hemorrhage [165] (Table 4).

2. Nitric Oxide

It has been proposed that NO may be involved in defense against the parasite. However, high levels of this molecule could impair neuronal signaling and cause oxidant damage of endothelial, both of which are involved in the pathogenesis of cerebral malaria. In addition, NO may damage red blood cells and increase anemia [178]. Inhibitors of NO excessive production or NO scavengers might be an effective adjunct therapy. An opposite alternative hypothesis, which claims that NO production is limited during malaria mainly by scavenging molecules, such as cell-free plasma hemoglo-

bin and superoxide, has been suggested. Therefore, restoring NO bioavailability might represent an effective anti disease therapy [178].

The possible effects of nitric oxide on malaria parasite are still controversial. A direct killing effect of NO on *P. falciparum* was not observed, but oxidation products of nitric oxide were toxic to the parasite [171,172,179]. Several works showed the important role played by reactive species of nitrogen on defense against plasmodium. It was shown that human monocytes stimulated with interferon- γ inhibit *Plasmodium falciparum in vitro* growth via the secretion of reactive nitrogen intermediates [167]. Moreover, IL-12-induced protection against blood stage *Plasmodium chabaudi* AS requires IFN- γ and TNF, and occurs via an NO-dependent mechanism [95]. The finding of a correlation between high plasma levels of NO and a rapid parasitological and clinical cure of *Plasmodium falciparum* infection suggests a role of this radical in defense against parasite. However, increased levels of NO are also associated with severe disease [168, 180]. The concentration of NO in cerebrospinal fluid was significantly higher in children who died than in survivors of falciparum malaria [181], which could indicate the participation of this product in the pathogenesis of severe malaria. Furthermore, increased expression of inducible nitric oxide synthase was shown in the brain of fatal cerebral malaria [182].

A better understanding of the role played by reactive oxygen and nitrogen species in defense or pathogenesis in the microvessels of patched lesions of severe malaria is important in order to choose whether to enhance or inhibit these molecules to treat or prevent severe disease.

CONCLUSION

The key points of immunopathogenesis of malaria in which it is possible to interfere in order to decrease the severity of malaria are: 1) to modulate the overproduction of inflammatory cytokines, including TNF, 2) to prevent the sequestration process, modulating the expression of adherence molecules and the activation of platelets to improve the blood flow, and 3) to prevent the lesions or metabolic dysfunction due to oxygen and nitrogen intermediate reactive overproduction.

Several approaches assessed the modulation of immune system in order to treat severe malaria. Most of them were done in experimental model and were quite promising. However, the majority of reports in human malaria were inconclusive. TNF was the most evaluated key point of pathogenesis of malaria and several reports inhibiting its overproduction showed controversial results. Antibodies against TNF were not efficient. In addition, the results found with pentoxifylline are still inconclusive and thalidomide has not yet been tested in human malaria. Anti adhesion therapies to prevent the sequestration process were not assessed in humans, and a report about antioxidant adjuvant therapies tested in human malaria worsened the evolution of the disease.

Early, appropriate and efficient antimalarial chemotherapy is still the easiest and the most important known approach to prevent severe forms of malaria [183]. The pathogenesis of the severe disease is complex and involves multi-

ple mechanisms. It is possible that no one drug acting on a single point can treat severe disease. Some drugs already tested in malaria, such as pentoxifylline and thalidomide, have pleiotropic actions and act on several points of the pathogenesis of the disease. The possible therapeutic benefits of these drugs should still be taken into consideration. Other potentially useful but not yet assessed ones are statins and anti adhesion therapies, which should also be considered.

It is possible that the beneficial effect of any one drug may be reduced if its administration occurs too late during the course of the infection because, local production of cytokines would already be elevated, the expression of adherence molecules established, and reactive oxygen and nitrogen species would already have caused lesion on tissue. Therefore, it can be postulated that these drugs would be potentially beneficial for individuals who have risk factors of severe disease, provided that they are used before the establishment of clinical parameters of severity. This indicates that these drugs need to be used to prevent, not to treat severe malaria. A better understanding of these immunomodulatory therapies in human malaria may decrease the unacceptably high morbidity and mortality of this disease in the world.

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REFERENCES

- [1] World Health Organization Division of Control of Tropical Diseases. Severe *P. falciparum* malaria. *Trans R Soc Trop Med Hyg* 2000; 94 Suppl 1: 1-90.
- [2] Sachs J, Malaney P. The economic and social burden of malaria. *Nature* 2002; 415: 680-5.
- [3] World Health Organization. *World Malaria Report 2005*
- [4] Snow RW, Guerra CA, Noor AM, Myint HI, Hay SI. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature* 2005; 434:214-217.
- [5] Greenwood B, Marsh K, Snow R. Why do some African children develop severe malaria? *Parasitol Today* 1991; 7: 277-81.
- [6] Marsh K. Malaria – a neglected disease? *Parasitology* 1992; 104: S53-69.
- [7] Sitprija V. Nephropathy in falciparum malaria. *Kidney Int* 1988; 34: 867-77.
- [8] Warrel DA. Pathophysiology of severe falciparum malaria in man. *Parasitology* 1987; 94: S53-76.
- [9] Boulos M, Costa JM, Tosta CE. Comprometimento pulmonar na malária. *Rev Inst Med Trop São Paulo* 1993; 35: 93-102.
- [10] Barsoum RS. Malarial nephropathies. *Nephrol Dial Transplant* 1998; 13: 1588- 97.
- [11] Taylor WRJ, White NJ. Malaria and the lung. *Clin Chest Med* 2002; 23: 457-68.
- [12] Naqvi R, Ahmad E, Akhtar F, Naqvi A, Rizvi A. Outcome in severe acute renal failure associated with malaria. *Nephrol Dial Transplant* 2003; 18: 1820-3.
- [13] Rasti N, Wahlgren M, Chen Q. Molecular aspects of malaria pathogenesis. *FEMS Immunol Med Microbiol* 2004; 41: 9-26.
- [14] Schofield L, Grau GE. Immunological processes in malaria pathogenesis. *Nat Rev Immunol* 2005; 5: 722-35.
- [15] Miller LH, Baruch DI, Marsh K, Doumbo OK. The pathogenic basis of malaria. *Nature* 2002, 415; 673-9.
- [16] Clark IA, Cowden WB. The pathophysiology of falciparum malaria. *Pharmacol Ther* 2003; 99: 221-260.
- [17] Hunt NH, Grau GE. Cytokines: accelerators and brakes in the pathogenesis of cerebral malaria. *Trends Immunol* 2003; 9: 491-9.
- [18] Schofield L, Hackett F. Signal transduction in host cell by a glycosylphosphatidylinositol toxin of malaria parasite. *J Exp Med* 1993; 177: 145-53.
- [19] Grau GE, Piguet PF, Engers HD, Louis P, Vassali P, Lambert PH. L3T4+ T lymphocytes play a major role in the pathogenesis of murine cerebral malaria. *J Immunol* 1986; 137:2348-54.
- [20] Scragg IG, Hensmann M, Bate CA, Kwiatkowski D. Early cytokine induction by *Plasmodium falciparum* is not a classical endotoxin-like process. *Eur J Immunol* 1999; 29: 2636-44.
- [21] Lou J, Lucas R, Grau GE. Pathogenesis of cerebral malaria: recent experimental data and possible applications for humans. *Clin Microbiol Rev* 14: 810-820; 2001.
- [22] Urquhart AD. Putative pathophysiological interactions of cytokines and phagocytic cells in severe human falciparum malaria. *Clin Infect Dis* 1994; 19: 117-31.
- [23] Eling WMC, Kremsner PG. Cytokines in malaria, pathology and protection. *Biotherapy* 1994; 7: 211-21.
- [24] Richards AL. Tumour necrosis factor and associated cytokines in the host's response to malaria. *Int J Parasitol* 1997; 27:1251-63.
- [25] Hensmann M, Kwiatkowski D. Cellular basis of early cytokine response to *Plasmodium falciparum*. *Infect Immun* 2001; 69: 2364-71.
- [26] Muniz-Junqueira MI, Santos-Neto LL, Tosta CE. Influence of tumor necrosis factor- α on the ability of monocytes and lymphocytes to destroy intraerythrocytic *Plasmodium falciparum* *in vitro*. *Cell Immunol* 2001; 208, 73-79.
- [27] Ângulo I, Fresno M. Cytokines in the pathogenesis of and protection against malaria. *Clin Diag Lab Immunol* 2002; 9: 1145-52.
- [28] Souza JB, Riley EM. Cerebral malaria: the contribution of studies in animal models to our understanding of immunopathogenesis. *Microbes Infect* 2002; 4: 291-300.
- [29] Bate CAW, Taverne J, Playfair JHL. Malarial parasites induce tumour necrosis factor production by macrophages. *Immunology* 1988; 64, 227-31.
- [30] Turner G. Cerebral malaria. *Brain Pathol* 7: 569-582; 1997.
- [31] Pongponratn E, Turner G, Day NPJ, *et al.* An ultrastructural study of the brain in fatal *Plasmodium falciparum* malaria. *Am J Trop Med Hyg* 2003; 69: 345-9.
- [32] Picot S, Peyron F, Vuillez JP, Polack B, Ambriose-Thomas P. Chloroquine inhibits tumor necrosis factor production by human macrophages *in vitro*. *J Infect Dis* 1991; 164: 830.
- [33] Kwiatkowski D, Bate C. Inhibition of tumor necrosis factor (TNF) production by antimalarial drugs used in cerebral malaria. *Trans R Soc Trop Med Hyg* 1995; 89: 215-6.
- [34] Landewe RBM, Miltenburg AMM, Verndonk MJA, *et al.* Chloroquine inhibits T- cell proliferation by interfering with IL-2 production and responsiveness. *Clin Exp Immunol* 1995; 102: 144-51.
- [35] Udomsangpetch R, Pipitaporn B, Krishna S, *et al.* Antimalarial drugs reduce cytoadherence and resetting of *Plasmodium falciparum*. *J Infect Dis* 1996; 173: 691-8.
- [36] Goldring JPD, Nemaorani S. Antimalarial drugs modulate the expression of monocyte receptors. *Int J Immunopharmacol* 1999 21: 599-607.
- [37] Shalmiev G, Krugliak M, Turrini F, Ginsburg H. Antimalarial drugs inhibit the phagocytosis of erythrocytes infected with *Plasmodium falciparum*. *Trans R Soc Trop Med Hyg* 1996; 90: 558-62.
- [38] Wenisch C, Parschall B, Zedwitz-Liebenstein K, Wernsdorfer W, Graninger W. The effect of artemisinin on granulocyte function assessed by flow cytometry. *J Antimicrob Chemother* 1997; 39: 99-101.
- [39] Osorio LM, Fonte L, Finlay CM. Inhibition of human monocyte function by prophylactic doses of chloroquine. *Am J Trop Med Hyg* 1992; 46: 165-8.
- [40] Prada J, Muller S, Bienzle U, Kremsner PG. Upregulation of reactive oxygen and nitrogen intermediates in *Plasmodium berghei* infected mice after rescue therapy with chloroquine or artemether. *J Antimicrob Chemother* 1996; 38: 95-102.
- [41] Bloom J. New insights into the molecular basis of glucocorticoid action. *Immunol Allergy Clin North Am* 1999; 19: 653-70.
- [42] Warrel DA, Looareesuwan S, Warrel MJ, *et al.* Dexamethasone proves deleterious in cerebral malaria. *N Engl J Med* 1982; 306: 313-9.
- [43] Sliwa K, Grundmann HJ, Neifer S, *et al.* Prevention of murine cerebral malaria by a stable prostacyclin analog. *Infect Imm* 1991; 59: 3846-8.

- [44] Wassmer SC, Cianciolo GJ, Combes V, Grau GE. Inhibition of endothelial activation: a new way to treat cerebral malaria? *PLOS Med* 2005; 2: 885-90.
- [45] Clark IA. Does endotoxin cause both the disease and parasite death in acute malaria and babesiosis? *Lancet* 1978; ii: 75-7.
- [46] Clark IA, Chaudhri G, Cowden WB. Roles of tumour necrosis factor in the illness and pathology of malaria. *Trans R Soc Trop Med Hyg* 1989; 83: 436-40.
- [47] Clark IA, Yaman FMA, Jacobson IS. The biological basis of malarial disease. *Int J Parasitol* 1997; 27: 1237-49.
- [48] Clark IA, Alleva LM, Mills AC, Cowden WB. Pathogenesis of malaria and clinically similar conditions. *Clin Microbiol Rev* 2004; 17: 509-39.
- [49] Grau GE, Fajardo LF, Piquet PF, Lambert PH, Vassali P. 1987. Tumor necrosis factor (cachectin) as an essential mediator in murine cerebral malaria. *Science* 1987; 237: 1210-2.
- [50] Grau GE, Taylor TE, Molyneux ME, Wirima JJ, Vassalli P, Hommel M, Lambert PH. Tumor necrosis factor and disease severity in children with falciparum malaria. *N Engl J Med* 1989; 320: 1586-91.
- [51] Santos-Neto L, Muniz-Junqueira MI, Tosta CE. Severe cases of *Plasmodium falciparum* malaria show increased level of both E-selectin and tumor necrosis factor- α . *Clin Res* 1994; 42: 283A.
- [52] Rudin W, Eugster H-P, Bordmann G, et al. Resistance to cerebral malaria in tumor necrosis factor- α / β -deficient mice is associated with a reduction of intercellular adhesion molecule-1 up-regulation and T helper type 1 response. *Am J Pathol* 1997; 150: 257-66.
- [53] Lucas R, Lou J-N, Juillard P, Moore M, Bluethmann H, Grau GE. Respective role of TNF receptors in the development of experimental cerebral malaria. *J Neuroimmunol* 1997; 72: 143-8.
- [54] Grell M, Douni E, Wajant H, et al. The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor. *Cell* 1995; 83: 793-802.
- [55] Kwiatkowski D, Molyneux ME, Stephens S, et al. Anti-TNF therapy inhibits fever in cerebral malaria. *QJ Med* 1993; 86: 91-8.
- [56] van Hensbroek MB, Palmer A, Onyiah E, et al. The effect of a monoclonal antibody to tumor necrosis factor on survival from childhood cerebral malaria. *J Infect Dis* 1996; 174: 1091-7.
- [57] Doherty GM, Jensen JC, Alexander HR, Buresh CM, Norton JA. Pentoxifylline suppression of tumor necrosis factor gene transcription. *Surgery* 1991; 110: 192-8.
- [58] Zabel P, Schade FU, Schlaak M. Inhibition of endogenous TNF formation by pentoxifylline. *Immunobiology* 1993; 187: 447-63.
- [59] Strieter RM, Remick DG, Ward PAI. Cellular and molecular regulation of tumor necrosis factor release from macrophages. *Biochem Biophys Res Commun* 1988; 155: 1230-6.
- [60] Ward A, Clissold SP. Pentoxifylline. A review of its pharmacodynamic and pharmacokinetic properties, and its therapeutic efficacy. *Drugs* 1987; 34: 50-97.
- [61] Hand WL, Butera ML, King-Thompson NL, Hand DL. Pentoxifylline modulation of plasma membrane functions in human polymorphonuclear leukocytes. *Infect Immun* 1989; 57: 3520-6.
- [62] Crouch SPM, Fletcher J. Effect of ingested pentoxifylline on neutrophil superoxide anion production. *Infect Immun* 1992; 60: 4504-09.
- [63] Kozaki K, Egawa H, Bermudes L, Feduska NJ, So S, Esquivel CO. Pentoxifylline inhibits production of superoxide anion and tumor necrosis factor by Kupffer cells in rat liver preservation. *Transplant Proc* 1993; 25: 3025-26.
- [64] Kriska M, Kristová V, Turčáni P. Pentoxifylline - a pharmacodynamically versatile agent. *Slovakofarma Revue VIII* 1998; 2: 41-7.
- [65] Neuner P, Klosner G, Schauer M, et al. Pentoxifylline *in vivo* down-regulates the release of IL-1 β , IL-6, IL-8 and tumor necrosis factor- α by human peripheral blood mononuclear cells. *Immunology* 1994; 83: 262-7.
- [66] Prada J, Prager C, Neifer S, Bienzle U, Kremsner PG. Production of interleukin-6 by human and murine mononuclear leukocytes stimulated with *Plasmodium* antigens enhanced by pentoxifylline, and tumor necrosis factor secretion is reduced. *Infect Immun* 1993; 61: 2737-40.
- [67] Mandell GL. Cytokines, phagocytes, and pentoxifylline. *J Cardiovasc Pharmacol* 1995; 25 (Suppl): 20-22.
- [68] Lehman LG, Vu-Quoc B, Carlson J, Kremsner PG. *Plasmodium falciparum*: inhibition of erythrocyte rosette formation and detachment of rosettes by pentoxifylline. *Trans R Soc Trop Med Hyg* 1997; 91: 74-5.
- [69] Kremsner PG, Grundmann H, Neifer S, et al. Pentoxifylline prevents murine cerebral malaria. *J Infect Dis* 1991; 164: 605-8.
- [70] Santos-Neto L, Muniz-Junqueira MI, Brandt MC, Tosta CE. Has pentoxifylline a place in the therapy of malaria? *Rev Soc Brasil Med Trop* 1992; 25 (Suppl II): 66-7.
- [71] Di Perri G, Di Perri IG, Badona G, et al. Pentoxifylline as a supportive agent in the treatment of cerebral malaria in children. *J Infect Dis* 1995; 171: 1317-22.
- [72] Graninger W, Thalhammer F, Locker G. Pentoxifylline in cerebral malaria. *J Infect Dis* 1991; 164: 829.
- [73] Hemmer CJ, Hort G, Chiwakata CB, et al. Supportive pentoxifylline in falciparum malaria: no effect on tumor necrosis factor alpha levels or clinical outcome: a prospective, randomized, placebo-controlled study. *Am J Trop Med Hyg* 1997; 56: 397-403.
- [74] Looareesuwan S, Wilairatana P, Vanaratana V, et al. Pentoxifylline as an ancillary treatment for severe falciparum malaria in Thailand. *Am J Trop Med Hyg* 1998; 58: 348-53.
- [75] Stoltenberg-Didinger G, Neifer S, Bienzle U, Eling WMC, Kremsner PG. Selective damage of hippocampal neurons in murine cerebral malaria prevented by pentoxifylline. *J Neurol Sci* 1993; 114: 20-4.
- [76] Wenisch C, Looareesuwan S, Wilairatana P, et al. Effect of pentoxifylline on cytokine patterns in the therapy of complicated *Plasmodium falciparum* malaria. *Am J Trop Med Hyg* 1998; 58: 343-7.
- [77] Calabrese L, Fleischer AB. Thalidomide: current and potential clinical applications. *Am J Med* 2000; 108: 487-95.
- [78] Sampaio EP, Sarno EN, Galilly R, Cohn ZA, Kaplan G. Thalidomide selectively inhibits tumor necrosis factor- α production by stimulated human monocytes. *J Exp Med* 1991; 173: 699-703.
- [79] Sampaio EP, Kaplan G, Miranda A, Nery JAC, Miguel CP, Viana SM, Sarno EM. The influence of thalidomide on the clinical and immunologic manifestation of erythema nodosum leprosum. *J Infect Dis* 1993; 168: 408-14.
- [80] Moreira AL, Sampaio EP, Zmuidzinas A, Frindt P, Smith KA, Kaplan G. Thalidomide exerts its inhibitory action on tumor necrosis factor- α by enhancing mRNA degradation. *J Exp Med* 1993; 177: 1675-80.
- [81] Corral L, Kaplan G. Immunomodulation by thalidomide and thalidomide analogues. *Ann Rheum Dis* 1999; 58 (Suppl 1), 1107-13.
- [82] Moller DR, Wysocka M, Greenlee BM, Ma X, Wahl L, Flockhart DA, Trinchieri G, Karp CL. Inhibition of IL-12 production by thalidomide. *J Immunol* 1997; 159: 5157-61.
- [83] Geitz H, Handt S, Zwingenberger K. Thalidomide selectively modulates the density of cell surface molecules involved in the adhesion cascade. *Immunopharmacology* 1996; 31: 213-21.
- [84] Zwingenberger K, Wnendt S. Immunomodulation by thalidomide: systematic review of the literature and of unpublished observations. *J Inflamm* 1996; 46: 177-211.
- [85] Teo SK, Stirling DI, Zeldis JB. Thalidomide as a novel therapeutic agent: new uses for an old product. *Drug Discov Today* 2005; 10: 107-14.
- [86] Ramirez-Villafuertes JM, Oltra-Ramirez A, Favila-Castillo L. The effect of thalidomide on parasitemia, antibody response, anemia and blood glucose in CB6F1 mice infected with *Plasmodium chabaudi chabaudi* AS. *Parasitol Int* 1998; 47 (Suppl): 346.
- [87] Muniz-Junqueira MI, Silva FO, Paula-Júnior MR, Tosta CE. Thalidomide influences the function of macrophages and increases the survival of *Plasmodium berghei*-infected CBA mice. *Acta Trop* 2005; 94: 128-38.
- [88] Postma NS, Hermsen CC, Crommelin DJ, Zuidema J, Eling WM. Treatment with recombinant human tumor necrosis factor-alpha reduces parasitaemia and prevents *Plasmodium berghei* K173-induced experimental cerebral malaria in mice. *Parasitology* 1999 118; 7-15.
- [89] Postma NS, Hermsen CC, Crommelin DJ, Eling WM, Zuidema J. Thiolated recombinant human tumor necrosis factor-alpha protects against *Plasmodium berghei* K173-induced experimental cerebral malaria in mice. *Antimicrob Agents Chemother* 1999 43; 1027-33.
- [90] Postma NS, Hermsen CC, Crommelin DJ, Eling WM, Zuidema J. Treatment with liposome-bound recombinant human tumor necrosis factor- α suppresses parasitemia and protects against *Plasmodium berghei* K173-induced experimental cerebral malaria in mice. *J Pharmacol Exp Ther* 1999; 288: 114-20.
- [91] Grau GE, Heremans H, Piquet PF, et al. Monoclonal antibody against interferon-gamma can prevent experimental cerebral malaria and its associated overproduction of tumor necrosis factor. *Proc Natl Acad Sci USA* 1989; 86: 5572-4.

- [92] Tsutsui N, Kamiyama T. Transforming growth factor β -induced failure of resistance to infection with blood-stage *Plasmodium chabaudi* in mice. *Infect Immun* 1999; 67: 2306-11.
- [93] Grau GE, Kindler V, Piguet P-F, Lambert P-H, Vassalli P. Prevention of experimental cerebral malaria by anticytokine antibodies. *J Exp Med* 1988; 168: 1499-504.
- [94] Grau GE, Frei K, Piguet P-F, Fontana A, Heremans H, Billiau A, Vassalli P, Lambert P-H. Interleukin 6 production in experimental cerebral malaria: modulation of anticytokine antibodies and possible role in hypergammaglobulinemia. *J Exp Med* 1990; 172: 1505-8.
- [95] Stevenson MM, Tam MF, Wolf SF, Sher A. IL-12 induced protection against blood-stage *Plasmodium chabaudi* AS requires IFN- γ and TNF- α and occurs via a nitric oxide dependent mechanism. *J Immunol* 1995; 155: 2545-56.
- [96] Sedegah M, Finkelman F, Hoffman SL. Interleukin 12 induction of interferon γ -dependent protection against malaria. *Proc Natl Acad Sci USA* 1994; 91: 10700-02.
- [97] Hoffman SL, Crutcher JM, Puri SK *et al*. Sterile protection of monkeys against malaria after administration of interleukin-12. *Nat Med* 1997; 3: 80-3.
- [98] Haque A, Echchannaoui H, Seguin R, Schwartzman J, Kasper LH, Haque S. Cerebral malaria in mice. Interleukin-2 treatment induces accumulation of $\gamma\delta$ T cells in the brain and alters resistant mice to susceptible-like phenotype. *Am J Pathol* 2001; 158: 163-72.
- [99] Kossodo S, Grau GE. Profiles of cytokine production in relation with susceptibility to cerebral malaria. *J Immunol* 1993; 151: 4811-20.
- [100] Winkler S, Wilhelm M, Baier K *et al*. Reciprocal regulation of Th1 and Th2 cytokine-producing T cells during clearance of parasitemia in *Plasmodium falciparum* malaria. *Infect Immun* 1998; 66: 6040-4.
- [101] Day NPJ, Hien TT, Schollaardt T, Loc PP, Chuong LV, Chau TTH, Mai TH, Phu NH, Sinh DX, White NJ, Ho M. The prognostic and pathophysiologic role of pro- and anti-inflammatory cytokines in severe malaria. *J Infect Dis* 1999; 180: 1288-97.
- [102] Riley EM. Is T cell priming required for initiation of pathology in malaria infection? *Immunol Today* 1999; 20: 228-33.
- [103] Musumeci M, Malaguarnera L, Simporè J, Messina A, Musumeci S. Modulation of immune response in *Plasmodium falciparum* malaria: role of IL-12, IL-18 and TGF- β . *Cytokine* 2003; 21: 172-8.
- [104] Wipasa J, Elliott S, Xu H, Good MF. Immunity to asexual blood stage malaria and vaccine approaches. *Immunol Cell Biol* 2002; 80: 401-14.
- [105] Malaguarnera L, Musumeci S. The immune response to *Plasmodium falciparum* malaria. *Lancet Infect Dis* 2002; 2: 472-8.
- [106] Othoro C, Lal AA, Nahlen B, Koech D, Orago ASS, Udhayakumar V. A low interleukin-10 tumor necrosis factor- α ratio is associated with malaria anemia in children residing in a holoendemic malaria region in Western Kenya. *J Infect Dis* 1999; 179: 279-82.
- [107] Li C, Sanni LA, Omer F, Riley E, Langhorne J. Pathology of *Plasmodium chabaudi chabaudi* infection and mortality in interleukin-10 deficient mice are ameliorated by anti-tumor necrosis factor alpha and exacerbated by anti-transforming growth factor β antibodies. *Infect Immun* 2003; 71: 4850-6.
- [108] Perkins DJ, Weinberg JB, Kremsner PG. Reduced interleukin-12 and transforming growth factor- β 1 in severe childhood malaria: relationship of cytokine balance with disease severity. *J Infect Dis* 2000; 182: 988-92.
- [109] Malaguarnera L, Imbesi RM, Pignatelli S, Simpore J, Malaguarnera M, Musumeci S. Increased levels of interleukin-12 in *Plasmodium falciparum* malaria: correlation with severity of disease. *Parasite Immunol* 2002; 24: 387-9.
- [110] Malaguarnera L, Pignatelli S, Musumeci M, Simpore J, Musumeci S. Plasma levels of interleukin-18 and interleukin-12 in *Plasmodium falciparum* malaria. *Parasite Immunol* 2002; 24: 489-92.
- [111] Malaguarnera L, Pignatelli S, Simpore J, Malaguarnera M, Musumeci S. Plasma levels of interleukin-12 (IL-12), interleukin-18 (IL-18) and transforming growth factor beta (TGF- β) in *Plasmodium falciparum* malaria. *Eur Cytokine Netw* 2002; 13: 425-30.
- [112] Chaisavaneeyakorn S, Othoro C, Shi YP, Otieno J, Chaiyaroj SC, Lal AA, Udhayakumar V. Relationship between plasma interleukin-12 (IL-12) and IL-18 levels and severe malarial anemia in an area of holoendemicity in Western Kenya. *Clin Diagn Lab Immunol* 2003; 10: 362-366.
- [113] Luty AJF, Perkins DJ, Lell B, Schmidt-Ott R, Lehman LG, Luckner D, Greve B, Matousek P, Herbich K, Schmid D, Weinberg JB, Kremsner PG. Low interleukin-12 activity in severe *Plasmodium falciparum* malaria. *Infect Immun* 2000; 68: 3909-15.
- [114] Omer FM, Souza JB, Riley EM. Differential induction of TGF- β regulates proinflammatory cytokine production and determines the outcome of lethal and nonlethal *Plasmodium yoelii* infections. *J Immunol* 2003; 171: 5430-6.
- [115] Omer FM, Riley EM. Transforming growth factor β production is inversely correlated with severity of murine malaria infection. *J Exp Med* 1998; 188: 39-48.
- [116] Stevenson MM, Su Z, Sam H, Mohan K. Modulation of host responses to blood-stage malaria by interleukin-12: from therapy to adjuvant activity. *Microbes Infect* 2001; 3: 49-59.
- [117] Omer FM, Kurtzhals JA, Riley EM. Maintaining the immunological balance in parasitic infections: a role for TGF- β ? *Parasitol Today* 2000; 16: 18-23.
- [118] Kurtzhals JAL, Akanmori BD, Goka BQ, Adabayeri V, Nkrumah FK, Behr C, Hviid L. The cytokine balance in severe malaria anemia. *J Infect Dis* 1999; 180: 1753-4.
- [119] MacPherson GG, Warrel MJ, White NJ, Looareesuwan S, Warrel DA. Human cerebral malaria. A quantitative ultra-structural analysis of parasitized erythrocyte sequestration. *J Pathol* 1985; 119: 385-401.
- [120] Ho M, White NJ. Molecular mechanisms of cytoadherence in malaria. *Am J Physiol* 1999; 276: C1231-42.
- [121] Chen Q, Schlichtherle M, Wahlgren M. Molecular aspects of severe malaria. *Clin Microbiol Rev* 2000; 13: 439-450.
- [122] Grau GE, Pointaire P, Piguet PF, Vesin C, Rosen H, Stamenkovic I, Takei F, Vassalli P. Late administration of monoclonal antibody to leukocyte function-antigen 1 abrogates incipient murine cerebral malaria. *Eur J Immunol* 1991; 21: 2265-7.
- [123] Falanga PB, Butcher EC. Late treatment with anti-LFA-1 (CD11a) antibody prevents cerebral malaria in a mouse model. *Eur J Immunol* 1991; 21: 2259-63.
- [124] Combes V, Rosenkranz AR, Redard M, *et al*. Pathogenic role of P-selectin in experimental cerebral malaria. Importance of the endothelial compartment. *Am J Pathol* 2004; 164: 781-6.
- [125] Wassmer SC, Combes V, Grau GE. Pathophysiology of cerebral malaria: role of host cells in the modulation of cytoadhesion. *Ann N Y Acad Sci* 2003; 992: 30-8.
- [126] Newton CRJC, Taylor TE, Whitten RO. Pathophysiology of fatal *falciparum* malaria in African children. *Am J Trop Med Hyg* 1998; 58: 673-83.
- [127] Silamut K, Phu NH, Whitty C, *et al*. A quantitative analysis of the microvascular sequestration of malaria parasites in the human brain. *Am J Pathol* 1999; 155: 395-410.
- [128] Taylor TE, Fu WJ, Carr RA *et al*. Differentiating the pathologies of cerebral malaria by postmortem parasite counts. *Nat Med* 2004; 10: 143-5.
- [129] Turner GDH, Morrison H, Jones M, *et al*. Evidence for widespread endothelial activation and a potential role for intercellular adhesion molecule-1 in cerebral sequestration. *Am J Pathol* 1994; 145: 1057-69.
- [130] Sherman IW, Eda S, Winograd E. Cytoadherence and sequestration in *Plasmodium falciparum*: defining the ties that bind. *Microbes Infect* 2003; 5: 897-909.
- [131] Cooke BM, Mohandas N, Cowman AF, Coppel RL. Cellular adhesive phenomena in apicomplexan parasites or red blood cells. *Vet Parasitol* 2005; 132: 273-95.
- [132] Lucas R, Lou J, Morel DR, Ricou B, Suter PM, Grau GE. TNF receptors in the microvascular pathology of acute respiratory distress syndrome and cerebral malaria. *J Leukoc Biol* 1997; 61: 551-8.
- [133] Dondorp A, Pongponratn E, White NJ. Reduced microcirculatory flow in severe *falciparum* malaria: pathophysiology and electron-microscopic pathology. *Acta Trop* 2004; 89: 309-17.
- [134] Favre N, Laperousaz C, Ryyffel NA, Imhof BA, Rudin W, Lucas R, Piguet PF. Role of ICAM-1 (CD54) in the development of murine cerebral malaria. *Microbes Infect* 1999; 1: 961-8.
- [135] Grau GE, Tacchini-Cottier F, Vesin C, Milon G, Lou JN, Piguet PF, Juillard P. TNF-induced microvascular pathology: active role for platelets and importance of the LAF-1/ICAM-1 interaction. *Eur Cytokine Netw* 1993; 4: 415-9.
- [136] Grau GE, Mackenzie CD, Carr RA, *et al*. Platelet accumulation in brain microvessels in fatal pediatric cerebral malaria. *J Infect Dis* 2003; 187: 461-6.
- [137] Wassmer SC, Lépolard C, Traoré B, Pouvelle B, Gysin J, Grau GE. Platelets reorient *Plasmodium falciparum*-infected erythrocyte cytoadhesion to activated endothelial cells. *J Infect Dis* 2004; 189: 180-9.

- [138] Ehrhardt C, Kneuer C, Bakowsky U. Selectins- an emerging target for drug delivery. *Adv Drug Deliv Rev* 2004; 56: 527-49.
- [139] Hviid L, Theander TG, Elhassan IM, Jensen JB. Increased plasma levels of soluble ICAM-1 and ELAM-1 (E-selectin) during acute *Plasmodium falciparum* malaria. *Immunol Letters* 1993; 36: 51-8.
- [140] Jakobsen PH, Morris-Jones S, Ronn A, Hviid L, Theander TG, Elhassan IM, Bygbjerg IC, Greenwood BM. Increased plasma concentration of sICAM-1, sVCAM-1 and sELAM-1 in patients with *Plasmodium falciparum* or *P. vivax* malaria and association with disease severity. *Immunology* 1994; 83: 665-9.
- [141] Santos-Neto L, Muniz-Junqueira MI, Tosta CE. Severe cases of *Plasmodium falciparum* malaria show increased plasma level of both E-selectin and tumor necrosis factor- α . *Clin Res* 1994; 42: 283A.
- [142] Wenisch C, Varijanonta S, Looareesuwan S, et al. Soluble intercellular adhesion molecule 1 (ELAM-1), and tumor necrosis factor receptor (55kDa TNF-R) in patients with acute *Plasmodium falciparum* malaria. *Clin Immunol Immunopathol* 1994; 71: 344-8.
- [143] Diamond M, Staunton DE, Fougerolles AR, et al. ICAM-1 (CD54): a counter receptor for Mac-1 (CD11b/CD18). *J Cell Biol* 1990; 111: 3129-39.
- [144] Craig AG, Pinches R, Khan S, Roberts DJ, et al. Failure to block adhesion of *Plasmodium falciparum*-infected erythrocytes to ICAM-1 with soluble ICAM-1. *Infect Immun* 1997; 65: 4580-5.
- [145] Chakravorty SJ, Craig A. The role of ICAM-1 in *Plasmodium falciparum* cytoadherence. *Eur J Cell Biol* 2005; 84: 15-27.
- [146] Clark IA, Awburn MM, Harper CG, Liomba NG, Molyneux ME. Induction of HO-1 in tissue macrophages and monocytes in fatal *falciparum* malaria and sepsis. *Malar J* 2003; 2: 41-53.
- [147] McCormick CJ, Craig A, Roberts D, Newbold CI, Berendt AR. Intercellular adhesion molecule-1 and CD36 synergize to mediate adherence of *Plasmodium falciparum*-infected erythrocytes to cultured human microvascular endothelial cells. *J Clin Invest* 1997; 100: 2521-9.
- [148] Luscinskas FW, Ma S, Nusrat A, Parkos CA, Sunil K, Shawa SK. Leukocyte transendothelial migration: A junctional affair. *Semin Immunol* 2002; 14, 105-13.
- [149] Kikuchi M, Looareesuwan S, Ubalee R, et al. Association of adhesion molecule PECAM-1/CD31 polymorphism with susceptibility to cerebral malaria in Thais. *Parasitol Int* 2001; 50: 235-39.
- [150] Heddini A. Malaria pathogenesis: a jigsaw with an increasing number of pieces. *Int J Parasitol* 2002; 32: 1587-98.
- [151] Treutiger CJ, Heddini A, Fernández V, Müller WA, Wahlgren M. PECAM/CD31, an endothelial receptor for binding *Plasmodium falciparum*-infected erythrocytes. *Nat Med* 1997; 3: 1405-8.
- [152] Brown H, Turner G, Rogerson S, et al. Cytokine expression in the brain in human cerebral malaria. *J Infect Dis* 1999; 180: 1742-6.
- [153] Ockenhouse CF, Tegoshi T, Maeno Y, et al. Human vascular endothelial cell adhesion receptors for *Plasmodium falciparum*-infected erythrocytes: roles for endothelial leukocyte adhesion molecule 1 and vascular cell adhesion molecule 1. *J Exp Med* 1992; 176: 1183-9.
- [154] Heddini A, Pettersson F, Kai O, et al. Fresh isolates from children with severe *Plasmodium falciparum* malaria bind to multiple receptors. *Infect Immun* 2001; 69: 5849-56.
- [155] Weitz-Schmidt G. Statins as anti-inflammatory agents. *Trends Pharmacol Sci* 2002; 23: 482-6.
- [156] Sherer Y, Shoenfeld Y. Immunomodulation for treatment and prevention of atherosclerosis. *Autoimmun Rev* 2002; 1: 21-7.
- [157] Muniz-Junqueira MI, Karnib SR, Paula-Coelho VN, Junqueira Jr LF. Effects of pravastatin on the *in vitro* phagocytic function and hydrogen peroxide production by monocytes of healthy individuals. *Int Immunopharmacol* 2006; 6: 53-60.
- [158] Lefer DJ. Pharmacology of selectin inhibitors in ischemia/reperfusion states. *Ann Rev Pharmacol Toxicol* 2000; 40: 283-94.
- [159] Tucker GC. Inhibitors of integrins. *Curr Op Pharmacol* 2002; 2: 394-402.
- [160] Vanderslice P, Biediger RJ, Woodside DG, Berens KL, Holland GW, Dixon RAF. Development of cell adhesion molecule antagonists as therapeutics for asthma and COPD. *Pulm Pharmacol Ther* 2004; 17: 1-10.
- [161] González-Amaro R, Mittelbrunn M, Sánchez-Madrid F. Therapeutic anti-integrin ($\alpha 4$ and αL) monoclonal antibodies: two-edged swords? *Immunology* 2005; 116: 289-96.
- [162] Simmons DL. Anti-adhesion therapies. *Curr Op Pharmacol* 2005; 5: 389-404.
- [163] Dockrell HM, Playfair JH. Killing of blood-stage murine malaria parasites by hydrogen peroxide. *Infect Immun* 1983; 39: 456-9.
- [164] Ockenhouse CF, Shear HL. Oxidative killing of the intraerythrocytic malaria parasite *Plasmodium yoelii* by activated macrophages. *J Immunol* 1984 132, 424-431.
- [165] Thumwood CM, Hunt NH, Cowden WB, Clark IA. Antioxidants can prevent cerebral malaria in *Plasmodium berghei*-infected mice. *Br J Exp Pathol* 1989; 70: 293-303.
- [166] Stevenson MM, Huang DY, Podoba JE, Nowotarski ME. Macrophage activation during *Plasmodium chabaudi* AS infection in resistant C57BL/6 and susceptible A/J mice. *Infect Immun* 1992; 60: 1193-201.
- [167] Gyan B, Troye-Blomberg M, Perlmann P, Björkman A. Human monocytes cultured with and without interferon-gamma inhibit *Plasmodium falciparum* parasite growth *in vitro* via secretion of reactive nitrogen intermediates. *Parasite Immunol* 1994; 16: 371-5.
- [168] Kremsner PG, Winkler S, Wilding E, Prada J, Bienzle U, Graninger W, Nüssler AK, 1996. High plasma levels of nitrogen oxides are associated with severe disease and correlate with rapid parasitological and clinical cure in *Plasmodium falciparum* malaria. *Trans R Soc Trop Med Hyg* 1996; 90: 44-7.
- [169] Odeh, M. The role of tumour necrosis factor- α in the pathogenesis of complicated *falciparum* malaria. *Cytokine* 2001; 14: 11-8.
- [170] Becker K, Tilley L, Vennerstrom JL, Roberts D, Rogerson S, Ginsburg H. Oxidative stress in malaria parasite-infected erythrocytes: host-parasite interactions. *Int J Parasitol* 2004; 34: 163-89.
- [171] Rockett KA, Targett GAT, Playfair JHL, 1988. Killing of blood-stage *Plasmodium falciparum* by lipid peroxides from tumor necrosis serum. *Infect Immun* 1988; 56: 3180-3.
- [172] Das BS, Patnaik JK, Mohanty S. Plasma antioxidants and lipid peroxidation products in *falciparum* malaria. *Am J Trop Med Hyg* 1993; 49: 720-25.
- [173] Malhotra K, Salmon D, Le-Bras J, Vilde JL. Susceptibility of *Plasmodium falciparum* to peroxidase-mediated oxygen-dependent microbicidal system. *Infect Immun* 1988; 56: 3305-09.
- [174] Descamps-Latscha B, Lunel-Fabiani F, Kara-Binis A, Druilhe P. 1987. Generation of reactive oxygen species in whole blood from patients with acute *falciparum* malaria. *Parasite Immunol* 1987; 9: 275-9.
- [175] Clark, I.A., Chaudhri, G., Cowden, W.B. Some roles of free radicals in malaria. *Free Radic Biol Med* 1989; 6, 315-321.
- [176] Thuma PE, Mabeza GF, Biemba G, et al. Effect of iron chelation therapy on mortality in Zambian children with cerebral. *Trans R Soc Trop Med Hyg* 1998; 92: 214-8.
- [177] Watt G, Jongsakul K, Ruangvirayuth R. A pilot study of N-acetylcysteine as adjunctive therapy for severe malaria. *Q J Med* 2002; 95: 285-90.
- [178] Sobolewski P, Gramaglia I, Frangos J, Intaglietta M, van der Heyde HC. Nitric oxide bioavailability in malaria. *Trends Parasitol* 2005; 21: 415-22.
- [179] Rockett KA, Awburn MM, Cowden WB, Clark IA. Killing of *Plasmodium falciparum* *in vitro* by nitric oxide derivatives. *Infect Immun* 1991; 59: 3280-3.
- [180] Prada J, Kremsner PG. Enhanced production of reactive nitrogen intermediates in human and murine malaria. *Parasitol Today* 1995; 11: 409-410.
- [181] Weiss G, Thuma PE, Biemba G, Mabeza G, Werner ER, Gordeuk VR. 1998. Cerebral fluid levels of biopterin, nitric oxide metabolites, and immune activation markers and the clinical course of human cerebral malaria. *J Infect Dis* 1998; 177: 1064-8.
- [182] Maneerat Y, Viriyavejakul P, Punpoowong B, et al. Inducible nitric oxide synthase expression is increased in the brain in fatal cerebral malaria. *Histopathology* 2000; 37: 269-77.
- [183] Warrel DA. Management of severe malaria. *Parassitologia* 1999; 41: 287-94.