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## MINIREVIEWS

# Cytokines in the Pathogenesis of and Protection against Malaria

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Malaria is caused by the protozoan *Plasmodium*, transmitted to humans by *Anopheles* mosquitoes. The most dangerous of the plasmodia infecting humans is *Plasmodium falciparum*. Most of the clinical signs of this disease are caused by the parasite at stages in which it multiplies asexually in red blood cells. *P. falciparum* infection is most severe in children. However, only a small proportion of infected children develop severe complications; in nonimmune individuals these can cause severe and life-threatening disease. The reasons for these differences are not fully understood, but it is likely that host genetic, immune, and social and geographic factors play a role. Malaria is the world's most prevalent parasitic disease and against which effective control measures are urgently needed. Many attempts have been made by using several *Plasmodium* antigens both in model systems and in humans to develop a malaria vaccine. However, the results, although encouraging, are far from satisfactory (36, 73). One of the difficulties hindering the design of a successful vaccine against *Plasmodium* is our current incomplete knowledge of protective immunity and how it can be induced. Moreover, the pathogenesis of two of the most severe complications of *P. falciparum* malaria, cerebral malaria (CM) and severe malarial anemia (SA), both appear to involve dysregulation of the immune system (56). Therefore, a greater appreciation of the mechanisms of protective immunity on the one hand and of immunopathology on the other would provide crucial clues as to how manipulation of the immune system may best be achieved in order to reach the goal of better vaccines.

The purpose of this review is to summarize recent work on the role of cytokines in antiparasitic immune responses and immunopathology caused by blood-stage parasites. Although, other points in the biological cycle of *Plasmodium* offer attractive sites for prophylactic intervention, all clinical symptoms are provoked by this parasitic stage.

### INTERACTION OF PLASMODIA WITH THE IMMUNE SYSTEM

Considerable effort has been devoted to the study of the characteristics of immune responses against *Plasmodium* spp. Of course, much of our knowledge is based on murine experimental systems. First, there is wide agreement that both cell-

mediated and antibody-dependent immunity are required for adequate protection, likely encompassing different mechanisms finely tuned in time (58). Besides, innate immunity is thought to play a crucial role in clearing *Plasmodium* from parasitized hosts. Most of the elimination occurs in the spleen under normal circumstances, although the liver has been shown to function as an alternative clearing site (20). The splenic response is complex and involves extraordinary tissuer changes that provoke dramatic alterations in blood flow through the organ (3). These changes prevent the access of infected erythrocytes to splenic tissues in which the immune response is going on until armed effector cells are produced. In general, most of the evidence supports the hypothesis that cells from the monocyte-macrophage lineage are more effective than neutrophils at phagocytosing parasitized erythrocytes (93). Even among cells from the monocytic lineage there appear to be subsets of cells especially well prepared for the clearance of parasites. Splenic marginal zone macrophages, a phagocytic subset of macrophages at the interface between red and white pulp, seem not to be involved in eradicating infected erythrocytes. This task is apparently assumed by macrophages of the red pulp (102). Less is known about the role of other innate cellular and humoral mechanisms that are likely to interact with the above-mentioned systems. Recent work has shown that *P. chabaudi* infection in  $\gamma/\delta$  T-cell-deficient mice have exacerbated early and chronic parasitemias (83). These results would fit well with data ascribing early production of gamma interferon (IFN- $\gamma$ ) and tumor necrosis factor alpha (TNF- $\alpha$ ) both to splenic  $\gamma/\delta$  T lymphocytes and to natural killer (NK) cells (12). This redundancy might explain why in other studies depletion of NK1.1<sup>+</sup> cells did not affect parasitemia in a model of infection by *P. berghei* XAT (attenuated variant of *P. berghei* NK65) (106).

Both the cellular and humoral arms of the adaptive immune system are pivotal elements in the eradication of *Plasmodium* from the body, and both are critically dependent on  $\alpha/\beta$  CD4<sup>+</sup> lymphocytes. It has been firmly established that CD4<sup>+</sup> T cells are comprised of at least two functionally different subsets, distinguished on the basis of lymphokine secretion in Th1 (IFN- $\gamma$ -producing) and Th2 (interleukin-4 [IL-4]/IL-5-producing) cells (reviewed in reference 22). Of particular interest is the fact that CD4<sup>+</sup> T cells, of either Th1 or Th2 type, also have regulatory functions in human *P. falciparum* malaria. Both Th1 and Th2 responses seem to be required to control the infection, but they need to be adequately tuned in intensity and time (96, 97). Thus, both Th1 and Th2 are activated during infection with *P. yoelii* 17XL in C57BL/6 mice, but early activation of

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TABLE 1. Characterization of genetically modified mice

Gene knocked out	Status <sup>a</sup>			
	Parasitemia	Anemia	CM	Mechanism
IL-12	↑		↓	↓ IFN-γ
GM-CSF	↑	=		
TNF-RI	=, ↑?	↑	=	↓ Memory
TNF-RII	↑?		↓	
IRF-1	=		↓	↑ IL-10
IL-10	=	↑ Pathology		
IL-18	↑			↓ IFN-γ
IFN-γ	↑↑			↓ NO
IFN-γR	=, ↑ (second wave)	↑ Leukocytes	↓	↓ ICAM-1
TNF-α/β			↓	↓ ICAM-1
TNF-α	↑	=, Pathology	↓?	
IL-6	=		=	

<sup>a</sup> Summary of phenotype data from knockout mice (see the text for details). =, No effect; ↑, increase; ↓, decrease; ?, to be confirmed.

Th2 cells is deleterious, conferring susceptibility to infection (34). In addition, in *P. chabaudi chabaudi* AS infection there is a shift from Th1 to Th2 during peak parasitemia that is important for clearance of parasites (27). In contrast, the effectiveness of CD8<sup>+</sup> T cells against the asexual blood stage of *Plasmodium* appears to be limited, although CD8<sup>+</sup> T lymphocytes might contribute to the fight against the parasite through secretion of cytokines as IFN-γ.

### ROLE OF CYTOKINES IN EARLY PROTECTION

Here, we will briefly review the most recent data obtained with genetically deficient mice (summarized in Table 1) and more conventional approaches assessing the effect of recombinant cytokines or anti-cytokine monoclonal antibodies in order to elucidate the role of cytokines in malarial infection (summarized in Table 2). As a general rule, the variety of animal models utilized and the high variability of plasmodial strains have made it not difficult to find contradictory data on specific issues, even for tests in similar models. However, in spite of these limitations, much relevant knowledge has been gathered that will form the basis for the development of successful therapeutic and prophylactic strategies against malaria.

Taken as a whole, the data available are consistent with a

TABLE 2. Role of cytokines in protection and pathology (mice)

Cytokine	Status <sup>a</sup>		
	Parasitemia	Anemia	CM
IL-12	↓	↓	↑
IFN-γ	↓	=	↑
TNF-α	↑	=	↑
IL-10	=, ↓ (late phase)	=	=, ↓
IL-4	=, ↓ (late phase)		=, ↓
TGF-β	↑, ↓		↓
IL-1	↓	=	↑?
IL-6	=	=	=
IL-18	↑		
MIF	-	↑?	

<sup>a</sup> Inferred role of the cytokines, derived from data from cytokine administration, neutralization with antibodies or clinical correlations (see text for details). =, No effect; ↑, increase; ↓, decrease; ?, to be confirmed.

requirement for early production of IFN-γ to give resistance against infection. In support of this view, analysis of IFN-γR<sup>-/-</sup> mice infected with *P. chabaudi chabaudi* reveals a critical role of IFN-γ in immunity against this pathogen, although the production of parasite-specific immunoglobulins was not affected (23). Interestingly, Tan et al. (91) reported that IFN responsive factor (IRF-1)<sup>-/-</sup> mice infected with *P. berghei* showed lower mortality than wild-type mice, although they produced no IFN-γ or NO. These animals suffered a later onset of disease and a reduced peak of parasitemia. These results may therefore indicate that IFN-γ and/or other molecules under the control of IRF-1 can actually have a pathological role. Of note concerning the latter aspect, a striking lack of correlation between changes in parasitemia and clinical symptoms has been repeatedly observed (15, 76). To illustrate this point, in C57BL/6 mice infected with *P. yoelii* 17XL and treated with anti-IFN-γ (exacerbating effect) or anti-IL-10 (protective effect) there were severe consequences on survival with no apparent changes in parasitemia (33).

It is likely that there are mechanisms of resistance independent of IFN-γ and NO. In this regard, it is interesting that treatment in vivo with anti-IFN-γ exacerbates *P. yoelii* 17XL infection in C57BL/6 because mice treated with antibody die earlier (33). In contrast, treatment with aminoguanidine, an irreversible inhibitor of NO production, has no effect (33). Consistently, mice lacking inducible nitric oxide synthase (iNOS<sup>-/-</sup>) cleared *P. berghei* XAT (an attenuated variant of *P. berghei* NK65) as effectively as did wild-type animals. In this case, resistance was dependent on IFN-γ, since its in vivo blocking provoked progression of parasitemia and death (106). Again, the evidence supports an absence of strict dependency between IFN-γ and NO.

In order to interpret these findings it is important to keep in mind that a relevant source of variability may be introduced by the use of subtle different experimental conditions. In fact, it has clearly been shown that the dose of inoculum may have dramatic effects on the responses obtained. Hence, after infection of IFN-γR α-chain<sup>-/-</sup> mice (129Sv/eV background) with *P. berghei* ANKA it was found that the dose of parasite was a determinant of the differences found. When 10<sup>6</sup> parasitized erythrocytes were inoculated, knockout mice had higher parasitemias and died earlier than wild-type animals (whether or not the animals had CM), whereas with 10<sup>5</sup> parasitized erythrocytes there were no differences between strains (5).

An important role for IL-12 in early responses against *Plasmodium* has been proposed, and although it may have a role in making mice resistant or susceptible, it is also possibly involved in the pathology itself (1, 76, 77, 108). IL-12 appears to be critically linked to or to act through IFN-γ production, thereby allowing an early and sustained Th1 response (19, 76, 109). Thus, after infection of C57BL/6 GKO (IFN-γ<sup>-/-</sup>) with *P. chabaudi* AS, higher levels of parasitemia during acute infection and severe mortality were found. These phenomena were associated with reduced amounts of IL-12p70, TNF-α, and NO (89). In contrast, in addition to its role in resolving primary infection, IL-12 has been shown to be required for the production of a protective immunoglobulin G2a (IgG2a) antibody (90). This finding suggests that the immunoregulatory activity of IL-12 extends to the antibody responses against *Plasmodium*.

Many evidences indicate that IL-18 enhances Th1 immune

responses depending on IL-12, but it can also potentiate Th2 immune responses when IL-12 is not available (64). Concerning the role of IL-18 on early immunity against *Plasmodium*, recent results have suggested that IL-18 plays a protective role by enhancing IFN- $\gamma$  production in vivo. Hence, it has been shown that IL-18 knockout mice were more susceptible to *P. berghei* ANKA than wild-type mice. Besides, administration of neutralizing IL-18 antibody exacerbated infection in wild-type mice C57BL/6 (86). Noteworthy, IL-18 has been found not to be absolutely required for hepatotoxicity produced after infection with *P. berghei*, which is actually dependent on IL-12 production (1). Therefore, although much of the evidence available suggests that both IL-18 and IL-12 act, at least in part, through induction of IFN- $\gamma$ , these two proinflammatory cytokines do play different specific roles in responses against *Plasmodium*.

A similar picture to that depicted for IFN- $\gamma$  emerges for the role of TNF- $\alpha$  in early responses against *Plasmodium*. Treatment with anti-TNF- $\alpha$  monoclonal antibody results in a tendency toward longer times for parasite clearance. Interestingly, this effect is associated with reduced levels of IFN- $\gamma$  (43). Supporting this general view, an association between the ability to produce high levels of TNF- $\alpha$  and an accelerated cure and improved prognosis has been reported in humans (61). Similarly, TNF- $\alpha^{-/-}$  mice infected with a nonlethal parasite have significantly higher levels of parasitemia than controls (85). However, experiments with knockout mice indicate that the requirement may not be absolute. Evidence reported by Sam et al. (78) shows that TNFR1/R2 $^{-/-}$  mice infected with *P. chabaudi* AS overcome primary infection, as well as wild-type mice. It is noteworthy that in these experiments knockout mice showed increased TNF- $\alpha$  and IL-10 production with respect to wild-type mice, whereas levels of IL-12, IFN- $\gamma$ , IL-4, or NO were similar. Confirming these findings, Li et al. (41) have reported that signaling through TNFR1 was not absolutely required for overcoming primary infections, although TNFR1 $^{-/-}$  mice had increased parasitemia and recrudescences. However, this mutation severely affected B-cell-dependent antibody responses in the immunity to reinfection; a fact that possibly reflects the lack of germinal center formation in these mutant mice (67). The overall conclusion that can be drawn from this is that the role of a particular cytokine is likely to be different at different stages of the infectious process.

A prominent role in switching from Th1 to Th2 responses is attributed to IL-10. Therefore, it is probably involved in controlling the adequate timing of antiparasitic responses. Early IL-10 production has been associated with susceptibility to infection (34, 107), and it is thought that this cytokine has a prominent anti-inflammatory effect, limiting in some way the damage inflicted on normal tissues by an excessive Th1 response. This idea is supported by experiments in which IL-10 $^{-/-}$  mice were infected with *P. chabaudi chabaudi* AS. Linke et al. (42) reported more severe signs of disease in these animals, and this was apparently not due to overwhelming parasitemias. Conversely, these remarkable symptoms were related to enhanced Th1 responses in the acute phase that persisted into the chronic phase. Consistent with the above report, Li et al. (40) found enhanced pathology in a comparable system, and Kobayashi et al. (33) showed that an anti-IL-10 antibody used

to treat C57BL/6 mice infected with *P. yoelii* 17XL in vivo increased survival times with no detectable changes in parasitemia.

Studies with transforming growth factor  $\beta$  (TGF- $\beta$ ), another anti-inflammatory and immunoregulatory cytokine, have also yielded contradictory results. This cytokine may have a critical role, most likely by regulating the activation of different effector mechanisms. On the one hand, positive effects of TGF- $\beta$  on immune responses against *Plasmodium* have been described. For example, Omer and Riley (65) found that anti-TGF- $\beta$  worsened *P. berghei* infection and conferred susceptibility to *P. chabaudi* but not to *P. yoelii*. On the other hand, resistant C57BL/10 mice became susceptible to *P. chabaudi* upon administration of recombinant TGF- $\beta$ , whereas susceptible BALB/c mice became resistant upon administration of anti-TGF- $\beta$ , with concomitant increases in IFN- $\gamma$  and NO production (98).

Administration of cytokines has been used to work out the role of cytokines in early immune responses against *Plasmodium*, and protective effects and deleterious effects have been reported for a number of cytokines. For example, recombinant human IL-12 conferred sterile protection in monkeys infected with *P. cynomolgi*. This protective response was associated with increased production of IFN- $\gamma$  and possibly involved the liver (29). In mice, administration of recombinant IL-12 increased protection in infected A/J mice (88), even at low doses when used in combination with chloroquine (57). Interestingly, several reports agree that this protective effect is dependent on IFN- $\gamma$  and, at least partially, on NO production (82, 88, 91). Administration of IL-18 plays a protective role during blood-stage infection by murine malaria by enhancing IFN- $\gamma$  production (86). Consistent with these results, administration of recombinant IFN- $\gamma$  or TNF- $\alpha$  reduced the extent of parasitemia caused by *P. chabaudi adami*, possibly through a mechanism involving free radicals (13). However, it is likely that these actions occur, at least in the case of TNF- $\alpha$ , through pleiotropic activities on different immune cells (62). Moreover, injection of human recombinant TNF- $\alpha$  reduced parasitemia in mice infected with *P. berghei* K-173 (72). Human recombinant TNF- $\alpha$  afforded protection to A/J susceptible mice infected with *P. chabaudi* AS but did not alter the course of the infection in resistant C57 BL/6 mice. Therefore, signaling through TNFR1 seems to be sufficient to induce a marked protective effect (87). Similarly, recombinant IL-1 reduced parasitemia via a mechanism dependent on T cells (16). However, in overt contrast to all of the data presented above, the injection of recombinant IL-2 reduced parasitemia but did not alter mortality (45). More recently, Haque et al. (26) have also shown that DBA/2 mice resistant to *P. yoelii* are made susceptible by injection of recombinant IL-2. This treatment was followed by the development of vascular inflammatory lesions enriched in  $\gamma\delta$  T cells expressing CD25 and CD54. In this way, a delicate balance between the elements of immune responses has been revealed.

#### CYTOKINES IN THE IMMUNOPATHOLOGY OF MALARIA

The pathogenesis of malaria is complex and most likely entails immunologic and nonimmunologic mechanisms (56). In general, it is now accepted that severe malaria is the consequence of alterations in many tissues and organs. These dys-

functions often lead to metabolic acidosis and localized ischemia. In this section, we focus on the involvement of cytokines in the immunologic mechanisms. It is evident that parasite factors can contribute to the severity of disease, as is clear from their ability to infect a high percentage of erythrocytes (11) or to induce production of proinflammatory cytokines. In particular, much evidence has been accumulated that points to glycosylphosphatidylinositols from *Plasmodium* as important pathogenic factors due to their ability to induce TNF- $\alpha$  and IL-1 (80). This view is strongly supported by the fact that the toxicity of malarial parasite extracts can be neutralized with monoclonal antibodies against this moiety in experimental models (81). It is noteworthy that recent work suggests that the presence of anti-glycosylphosphatidylinositol antibodies in the serum of patients may provide protection against clinical symptoms of malaria (63). Therefore, cytokines, viewed as potential pathogenic elements, can contribute either directly or indirectly to many pathological processes (14). Of these, control of CM and SA are critical for patient care. Although both *P. falciparum* and *P. vivax* can cause SA, only *P. falciparum* causes the many complications associated with CM.

**CM.** CM is characterized by a coma situation in patients with *P. falciparum* infection that is often accompanied by metabolic acidosis, seizures, and hypoglycemia (56). Animal models of malaria have provided convincing evidence of the important role of inflammatory processes in the development of CM (7). Monkey, rat, and mouse models for CM have been developed, although none of them completely duplicates the situation in humans. The most complete information has been obtained from experiments in mice. Lou et al. (44) have recently reviewed this issue, emphasizing the role of adhesion molecules and platelets in immune-mediated damage of vascular endothelium of the brain.

Not all proinflammatory cytokines are equally relevant for the development of CM. The best-documented evidence implicates IFN- $\gamma$ , TNF- $\alpha$ , and IL-12, whereas no evidence has been found for IL-6 (25). In the case of IL-12 and/or IFN- $\gamma$ , strong support for their involvement in the pathogenesis of CM comes from studies with knockout mice infected with *P. berghei* ANKA. Consistently, experiments with IFN- $\gamma^{-/-}$  or IFN- $\gamma R^{-/-}$  have revealed an essential requirement of this cytokine in the development of CM (5, 75, 103). Overall, an inhibition of TNF- $\alpha$  production and ICAM-1 expression (5, 75) or IL-12 production (75) was found in experiments with these animals. Interestingly, NO seems not to be involved in the pathogenesis of CM (5). In addition, IRF-1 $^{-/-}$  mice infected with *P. berghei* are resistant to CM (84), a result consistent with a major pathological role for IL-12 and IFN- $\gamma$ .

Less clear is the role of anti-inflammatory cytokines in the control of CM. Although data based on knockout mice indicate that IL-4 and IL-10 are not required for development of CM (103), in models in which IL-12 and IFN- $\gamma$  display a dominant pathological role a protective effect has been demonstrated for IL-10. Tan et al. (92) found that infected wild-type mice mounted an early Th1 response that shifted to a late Th2 response, whereas in infected IRF-1 $^{-/-}$  animals an early protective Th2 was found that avoids Th1-dependent pathological damage. Moreover, neutralization of IL-10 in vivo has been demonstrated to increase the percentage of mice with CM in a CM-resistant strain (35). Interestingly, sex-related determi-

nants might play a role in determining the relative importance of IL-10. Thus, infection of IL-10 $^{-/-}$  mice with *P. chabaudi chabaudi* (AS) led to exacerbated pathology in female mice. In that study hypoglycemia, hypothermia, and loss in body weight were significantly greater in female IL-10 $^{-/-}$  mice than in male knockout mice and all wild-type mice during the acute phase of infection (40).

The role of TNF- $\alpha$  in CM seems to be well established. Thus, transgenic mice expressing high levels of soluble TNFR-1-FcIgG3 under the control of the  $\alpha_1$ -antitrypsin gene promoter are markedly protected from CM caused by *P. berghei* (24). However, apparently contradictory findings were reported by Shear et al. (85), who used TNF- $\alpha^{-/-}$  mice infected with nonlethal *P. yoelii*. These animals had slightly higher levels of infected erythrocytes, but their susceptibility to death from this infection was unaffected. It was therefore concluded that TNF- $\alpha$  was not absolutely required for death. A possible explanation could lie in the time and site of cytokine production. In this regard it has been shown that TNF- $\alpha$ , produced locally in the central nervous system, has an essential role in the development of CM (55). Moreover, TNFR2 $^{-/-}$  mice but not TNFR1 $^{-/-}$  mice were considerably protected from CM. This protection was associated with a lack of upregulation of ICAM-1. It has been suggested that membrane-TNF- $\alpha$ -TNFR2 interactions are critical for CM because of the requirement for TNF- $\alpha$  in the upregulation of ICAM-1 in cerebral vascular endothelium (46). In any case, other factors also seem to play a decisive role in the development of CM. Thus, despite a production of TNF- $\alpha$  similar to that in wild-type animals, CD40 $^{-/-}$  and CD40L $^{-/-}$  mice infected with *P. berghei* ANKA were protected from CM and survived. In addition to the unchanged systemic production of TNF- $\alpha$ , the expression of TNF- $\alpha$  in the brains of these mutants did not correlate with local macrophage sequestration, and circulating but not cerebral TNF- $\alpha$  is sufficient to induce upregulation of CD54 (ICAM-1) in brain endothelial cells (71).

Although there is convincing evidence implicating TNF- $\alpha$  in the development of CM, there are some important discrepancies. CM caused by *P. berghei* in CBA mice is not clearly related with TNF- $\alpha$  levels or histopathological findings (10). Moreover, systemic administration of rhTNF- $\alpha$  protects against CM, although this has been indirectly related to its ability to decrease parasitemia (72). It is noteworthy that the administration of murine IL-1, another proinflammatory cytokine, also protects from CM (16).

An important question that arises is whether data on human CM are compatible with the picture that emerges from studies done in mice. In some reports, histopathological features found in CM in humans are associated with local production of TNF- $\alpha$ , IFN- $\gamma$ , and IL-1 $\beta$  but also of IL-10 (50). Similar results have been found in a monkey model of CM (95). However, other authors have found that the mRNA of proinflammatory (TNF- $\alpha$  and IL-1 $\beta$ ) cytokines, detected in postmortem samples of patients suffering CM, does not correlate with the density of parasitized erythrocytes. (8). Interestingly, patients treated with neutralizing anti-TNF- $\alpha$  antibodies show a faster resolution of clinical symptoms (43). In addition, a focal accumulation of TGF- $\beta$ 1, -2, and -3 during reorganization of brain parenchyma was found in patients with CM, suggestive of endothelial activation and immunologic dysfunction (18). Wild

isolates obtained from children suffering from CM tend to induce higher amounts TNF- $\alpha$  than isolates from patients with uncomplicated disease (4).

Analysis of genetic polymorphisms of the TNF- $\alpha$  promoter provides suggestive evidence for an important role of immunological responses in CM. In African populations, mutations located at positions -308 and -376 are associated with CM (32, 54), and similar studies in Asian populations have identified other mutations (TNF- $\alpha$  promoter D) (99). Interestingly, in all those cases evidence has been obtained that suggests that the explanation for this association is an enhanced ability to produce TNF- $\alpha$  (28, 101).

**Anemia.** Among the physiopathological processes underlying severe malaria, anemia remains as one of the most intriguing phenomena. Many mechanisms affecting erythrocyte integrity have been proposed. However, none of them provide a satisfactory explanation for the observed severity. It has been suggested that host-related factors are the main responsible for malarial anemia (49). In vivo neutralizing studies have provided evidences indicating that IFN- $\gamma$ , TNF- $\alpha$ , and IL-1 $\beta$  are not the key cytokines involved in the inhibition of erythropoiesis (104, 105). These data are essentially consistent with findings from knockout models. In one such study, *P. chabaudi* AS infection in TNFR1<sup>-/-</sup> mice led to a slightly greater loss of erythrocytes than observed in wild-type animals (41). In another, IFN- $\gamma$ R<sup>-/-</sup> mice infected with *P. berghei* ANKA, although protected from CM, died of severe anemia (75).

Other factors involved in hematopoiesis have been shown not to be involved in the development of anemia. Granulocyte-macrophage colony-stimulating factor (GM-CSF)<sup>-/-</sup> mice, for example, have anemia comparable to wild-type animals (74). Other hematological alterations do not to involve changes in levels of stem cell factor or IL-3 (9). However, in mice infected with blood-stage *P. chabaudi*, anemia has been associated with elevated IL-3 levels and an expansion of IL-3-responsive, IL-4-producing non-B, non-T cells (27).

So far, most of the evidence supporting an immunological involvement in malarial anemia comes from data related to the role of IL-12 in this pathology. The levels of IL-12, a cytokine that boosts erythropoiesis, are correlated with anemia (59). Furthermore, administration of recombinant IL-12 is able to ameliorate anemia in A/J mice infected with *P. chabaudi* (60). Recently, production of macrophage inhibitory factor, another factor produced by macrophages, has been correlated with the development of anemia (51). This cytokine is able to inhibit erythropoiesis in vitro and has some of the characteristics proposed for a putative soluble inhibitory factor detected in serum of infected mice. In addition, alteration in platelets could be related to the overproduction of cytokines. Thus, elevated levels of M-CSF in severe malaria might cause a platelet disorder (thrombocytopenia) by enhancing macrophage phagocytic activity (39).

Studies in humans have also highlighted the importance of immune mechanisms in the development of severe anemia in malaria. Neopterin accumulation in serum is correlated with the degree of anemia (6, 94), and in agreement with this, Luty et al. (48) found an association of the highest levels of TNF- $\alpha$  in serum with severe anemia. However, this is not a universal finding. In another study, low levels of this cytokine were detected in the serum of Ghanaian children with SA (2). This

discrepancy might be explained by previous work indicating that the ratio of IL-10 to TNF- $\alpha$  in serum is a more accurate determinant of the severity of anemia. Patients with severe anemia showed low levels of IL-10, this amount being insufficient to counteract the proinflammatory activity of high concentrations of TNF- $\alpha$  (37). Other studies support these findings. Low IL-10/TNF- $\alpha$  ratios are associated with severe anemia, suggesting that IL-10 might play a role in preventing the adverse effects of TNF- $\alpha$  on hematopoiesis. In contrast, higher ratios have been found in children with uncomplicated malaria (66).

#### CYTOKINES IN THE DIAGNOSIS AND PROGNOSIS OF MALARIA

An emerging view is that pathogenesis of malaria is a complex process in which a common outcome might be reached by different routes (56). This idea emphasizes the relevance of diagnostic and prognostic parameters in predicting the specific risks associated with different clinical characteristics.

The polarization of immune responses has been investigated to address this point. Skewed responses to Th2 profiles have been reported in humans, with elevated levels of IgE being found in the blood of malaria patients, presumably due to the predominance of Th2 cells over Th1 helper cells. This polarization was significantly higher in the case of patients suffering from severe malaria (70). Reinforcing this view, several studies support the notion that Th1 responses are important for clearance of *P. falciparum* malaria. In accordance with this, nonimmune children with severe *P. falciparum* malaria showed lower levels of IL-12 and IFN- $\gamma$  in serum and had a reduced capacity to produce them after in vitro stimulation. It is interesting that children with severe anemia had the highest levels of TNF- $\alpha$  (48). Consistent with this, it has been reported that children with prior mild malaria showed an enhanced ability to express iNOS in vitro over children with prior severe malaria (68). Furthermore, Luty et al. (47) found that peripheral blood mononuclear cells of patients with mild malaria produced IFN- $\gamma$  in response to malarial antigens, whereas those with severe malaria did not. However, no associations were found with TNF- $\alpha$  production.

Of relevance to diagnosis and prognosis is the perception that specific clinical conditions might have distinguishable immunological features. It has been suggested that a marked imbalance in cytokines found in serum might be used as a marker of progression to a fatal outcome. Thus, studies on Ghanaian children showed that only patients with uncomplicated malaria had a positive correlation with levels of TNF- $\alpha$  and soluble TNF- $\alpha$ R1 and TNF- $\alpha$ R2 in serum. In the same study, children with CM had high levels of TNF- $\alpha$  (2), and although TNF- $\alpha$  levels were associated with fever no differences were observed in soluble TNF- $\alpha$  receptors. Interestingly, children with fever and detectable parasitemia, but not afebrile parasitized patients, had elevated levels of TNF- $\alpha$  (53). In this regard, patients who died from *P. falciparum* malaria had higher amounts of IL-6, IL-10, and TNF- $\alpha$  in serum than did the patients who survived. In the present study, IL-6, IL-10, and IFN- $\gamma$  were associated with hyperparasitemia, jaundice, and shock. Moreover, TNF- $\alpha$  was associated with renal failure. Surprisingly, lower levels of cytokines were found in CM. In an important finding, a relative deficiency of IL-10 was detected

as death approached (17). In other studies, IL-6 and IL-6R were found to be elevated, both in CM patients and in patients with renal failure. These levels dropped 24 h after the initiation of therapy (100). In agreement with this, Jakobsen et al. (30) reported that excessive production of IL-6 may predispose to CM, whereas  $\beta$ 2-glycoprotein I might protect against a fatal outcome in this disease. Thuma et al. (94) reported increased levels of neopterin, IL-6, and IL-4 in children with CM. In this case, IL-6 was associated with parasitemia, and IL-4 was associated with the duration of fever prior to admission.

So, are these laboratory findings of potential use to management of malaria? In support of a positive answer, TNF- $\alpha$  levels in serum can predict a fatal outcome in cases of CM. This parameter was independent of parasitemia and glucose concentrations. In the present study, patients suffering from CM who died were found to have TNF- $\alpha$  concentrations in serum up to 10 times higher than patients with uncomplicated malaria. Survivors, on the other hand, had only a twofold increase in this cytokine (38). Jakobsen et al. (31) suggested that soluble IL-2R is a useful marker of the severity of disease. Children with severe malaria showed much higher amounts of soluble IL-2R than those suffering from mild malaria. However, it has been suggested that severe malaria may be related to an inflammatory cascade characterized by high levels of TNF- $\alpha$  and an inhibition of the potential beneficial effects of TGF- $\beta$ 1 or/and IL-12, whose levels were found to be decreased in patients with severe malaria (characterized as high parasitemia and anemia) (69). Recent results suggest that the best potentially useful parameter could be the ratio of anti-inflammatory (IL-10) to inflammatory (TNF- $\alpha$ ) cytokines either in serum (52) or produced by leukocytes of the patients after mitogen or antigen stimulation (21). After therapy of *falciparum* malaria, surviving patients showed a rapid decline in TNF- $\alpha$ , IL-6, IL-10, and IL-2 soluble receptor. However, this decline was slow in dying patients, and the level of TNF- $\alpha$ R1 remained elevated (79).

### CONCLUDING REMARKS

In summary, in the last years a lot of evidence has been accumulated that has somewhat modified our perception of malaria and the role played by the immune system both in protection and pathogenesis. First, both type 1 and type 2 cytokines are both required for adequate protection, likely encompassing different mechanisms finely tuned in time and intensity. Type 1 cytokines are important in controlling early parasitemia, although they need to be counterbalanced later in the infection by a type 2 response which leads to antibody production. Second, pathogenesis of malaria is a complex process in which a common outcome might be reached by different routes. For example, various proinflammatory cytokines that clearly play a role in CM may be redundant, making it difficult to unequivocally assign to them a pathogenic role in all clinical situations. Less clear is the role of cytokines in SA. Third, although animal models, most notably knockout mice, have been paramount to our understanding of the role of cytokines in malaria by providing much valuable information, it is still controversial whether they can reproduce all of the features of human malaria. Other factors, such as concomitant infection, may explain some of the discrepancies found between animal models and the human situation. Finally, routine detection of some of the cytokines may be relevant to diagnosis and prognosis of the var-

ious clinical conditions that might have distinguishable immunological features.

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