Review:

IFN-γ, TNF-α and IL-10 responses in children infected with malaria parasite

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Abstract

Immunity against malaria requires early production of IFN-γ and TNF-α while excessive uncontrolled production leads to malaria complications. Severe malaria is an end result of interactions of many factors which determine the immune responses. Genetic profile, repeated infections, parasite influences and the general health are known contributors. Involvement of multiple cell types and multiple cytokines complicate the picture further. In this review the roles of cytokines IL-10, IFN-γ and TNF-α in the pathogenesis of severe malaria in children are discussed. Since severe malarial anemia and cerebral malaria represent the vast majority of the morbidity and mortality cases, the review is focused on cytokines responses and pathogenesis of these two malaria complications. Special emphasis is given to factors reported to affect the cytokines levels in children with malaria.

Introduction

In endemic areas, children younger than 5 years usually experience repeated serious attacks of malaria and the highest mortality rates. By adolescence the survivors develop partial immunity.[(1)] Older children and adults often have asymptomatic parasitemia. The vast majority of the morbidity and mortality occurs in non-immune African children, with severe malarial anemia (SMA) as the primary manifestation of severe disease in children younger than 5years.[(2)] On the other hand cerebral malaria frequently affects older children and is associated with increased morbidity and mortality.[(3, 4)] Lack of protective immunity and excessive proinflammatory cytokines responses appear to underlie the high rates of morbidity and mortality in children.[(5,6)] The role of cytokines in malaria immunity and pathogenesis is complex and less well-understood. While an early inflammatory response is needed to control malaria parasite replication, excessive levels of pro-inflammatory cytokines TNF-α and IFN-γ and low levels of anti-inflammatory cytokine IL-10 are associated with malaria complications.[(6,7,8)] Several factors are linked with changes in cytokine responses which complicate the picture more.[(9, 10)] It is of interest to note that even deficiency of trace elements was reported to change cytokines production.[(11)]

This review focuses on the role of cytokines IL-10, IFN-γ and TNF-α in immunity against malaria and the pathogenesis of severe malarial anemia and cerebral malaria as major severe malaria phenotypes. Special emphasis is given to factors reported to affect these cytokines level in children with malaria.

IFN-γ

IFN-γ in immunity to malaria: Most studies identified IFN-γ as the hallmark of immunity against malaria. Early and high production of IFN-γ is associated with protection from clinical and high-density Plasmodium falciparum infections.[(7)] IFN-γ is mainly produced by γδ T cells and αβ T cells with minor contribution from NK cells during immune responses to malaria infections.[(7)] Both CD56 (bright) and CD56 (dim) NK cells contribute to the production.[(12)]

IFN-γ responses to the liver stage malaria parasite are associated with protection against malaria and appear to require repeated infections.[(13)] CD8+ T cells

from asymptomatic adults residing in holoendemic areas produced larger amounts of IFN-γ when these cells were exposed to peptide epitopes of LSA-1 than those who were never exposed to malaria. Plasmodium sporozoite infection in mice has been shown to be suppressed by a rapid inflammatory response in the liver characterized by NK cell, IFN-γ production and activation of IFN-γ pathways.[14]

IFN-γ also plays an important role in resistance to the blood stage of malaria infection. Peripheral blood mononuclear cells from children with uncomplicated malaria (UM) produced high levels of IFN-γ when stimulated in vitro with merozoite antigens and had a lower risk of re- infection. In contrast, children with severe malaria (SM) showed lower levels of IFN-γ production by peripheral blood mononuclear cells and were more susceptible to re-infection.[15] Delayed time to re-infection with the malaria parasite in IFN-γ positive responders is also reported by Moormann et al (2013).[16]

Factors associated with changes in IFN-γ responses: IFN-γ responses appear to be associated with many factors. Age has been reported to correlate with IFN-γ responses. Winkler et al detected increased frequency of IFN-γ –producing cells in adults compared to 5 years old or more children and to those less than 5 years.[9] Our study showed that the mean IFN-γ level was significantly higher in older children.[17] This IFN-γ response appears to require repeated Plasmodium falciparum exposure which occurs with increased age and is associated with protection against clinical malaria and its complications.[13, 18]

Parasite factors such as parasite load and parasite-derived substances are also associated with IFN-γ responses. In addition to age, Winkler et al reported that CD4+ cells exclusively expressing IFN-γ were lower in patients with hyperparasitemia as well as IL-10—producing CD4+ cells. (9) Hemozoin, a product of heme digestion by the parasite, is associated with decreased circulating IFN-γ and IL-10 and increase in TNF-α level.[19, 20]

Some studies showed that heterogeneity in the magnitude of NK IFN-γ responses may reflect variation in the host capabilities to mount rapid proinflammatory responses.[21, 22] A study involving previously malaria naive subjects revealed considerable variability in the innate responses. Three identifiable groups emerged based on the strength of their inflammatory response. One group produced moderate levels of IFN-γ and IL-10 with no detectable IL-12p70. A second group produced detectable blood levels of IL-12p70 and very high levels of IFN-γ and IL-10. The third group showed no significant proinflammatory responses, but had the highest levels of TGF-β. Proinflammatory responses were associated with more rapid control of parasite growth but with development of clinical symptoms.[22] Similar heterogeneity in early IFN-γ responsiveness was observed among children residing in high-transmission areas. High levels early IFN-γ responses were associated with protection from high-density and clinical P. falciparum infections.[7]

Protective effect of IFN-γ against severe anemia:

IFN-γ production was associated with protection from severe anemia (SA).[13, 17, 23]A study in Kenyan children identified IFN-γ as a significant positive predictor of hemoglobin levels.[23] In our study IFN-γ was positively correlated with hemoglobin and children with severe anemia had the lowest level of IFN-γ. [17] This protection is associated with IFN-γ-mediated reduction of magnitude of the developing parasitemia and clinical malaria. Early and high production of IFN-γ in infected children is associated with protection from clinical and high-density Plasmodium falciparum infections.[7] In mice models, IFN-γ was found to inhibit growth of the plasmodium liver stage and may involve induction of nitric oxide which is toxic to the parasite.[14, 24, 25] Human studies had shown that liver stage parasite antigens induce IFN-γ production. T lymphocytes obtained from people living in malaria holoendemic areas, produce large amounts IFN-γ in response to LSA-1 peptides which is absent in naives.[26] During acute uncomplicated malaria infection, lymphocytes producing IFN-γ in response to LSA-1 are found in the peripheral

circulation of infected children.[27] IFN-γ responses to LSA-1 increased with increasing age and were associated with protection against clinical malaria and anemia.[13]

IFN-γ is also produced in response to malaria blood stage antigens and was associated with protection against high-density and clinical P. falciparum infections.[7] High IFN-γ responses reduced the risk of re-infection and delayed the time to re-infection.[15, 16]

It appears that IFN-γ responses to both liver and blood stages of malaria parasite are involved in protection against malarial anemia. The possible mechanism is reduction of parasitemia. Further studies examining the protective role of IFN-γ against this disease manifestation are warranted.

IFN-γ in the pathogenesis of cerebral malaria:

Excessive IFN-γ production has been linked to pathogenesis of cerebral malaria (CM). A study in Ugandan children revealed elevated IFN-γ level in children with CM than those with UM, and children with fatal CM had higher IFN-γ than children who survived.[5] Immunohistochemical staining of the human brain sections demonstrated that tissue-localized TNF-alpha, IFN-gamma, IL-I1B, and IL-10 were associated with the histopathological features of cerebral malaria.[28] Experiments in mice had shown a significant contribution of IFN-γ in the development of CM. Administration of monoclonal antibodies against IFN-γ after infection in mice prevented the development of CM together with reduction in TNF-α production.[29] IFN-γ receptor deficiency in mice infected with Plasmodium berghei ANKA resulted in protection from CM and was associated with reduced serum TNF-α level.[30] IFN-γ also acts synergistically with TNF-α to increase expression of adhesion molecules involved in sequestration of the parasite.[31] These studies revealed that IFN-γ augments both the production and action of TNF-α which has a central role in the pathogenesis of CM.[6, 30]

Other reported mechanism by which IFN-γ contribute to pathogenesis of CM is through IFN-γ-mediated enhancement of accumulation of infected RBCs and CD8T cells in the brain of infected mice.[32, 33, 34] Interestingly, high serum IFN-γ -inducible protein 10 (IP-10, CXCL10), a chemokine secreted in response to IFN-γ was found to predict increased CM mortality in Ghanaian children.[35] IP-10 is a chemoattractant for T helper1 cells. IFN-γ is also reported to promote the neurotoxic quinolinic acid pathway of tryptophan metabolism in mice infected with Plasmodium berghei ANKA.[36]

Villegas-Mendez et al showed that during experimental cerebral malaria, NK cells were the dominant source of IFN-γ response during the early stages of infection in the brain thereafter replaced by CD4+ and CD8+ T cells.[37]

TNF-α

TNF-α in immunity to malaria: TNF-α is predominantly produced by γδ T cells and CD14+ monocytes during immune responses to malaria and helps in control of parasitemia.[38,39,40] TNF-α and IFN-γ act synergistically to optimize nitric oxide production which in turn leads to parasite killing.[41] TNF-α enhances human neutrophil killing of P. falciparum.[42]Infection in mice with TNF-α receptors knock-out results in high parasitemia but it is not essential, as they clear the parasite similar to wild-type mice.[43] These studies indicate that TNF-α acts in concert with other parasite killing factors.[44]

TNF-α in the pathogenesis of CM: TNF-α plays a critical role in the pathogenesis of CM. Plasma TNF-α level in CM survivors were found twice as high as in children with uncomplicated malaria. In the fatal cases they were 10-fold higher.[6] In our work, children with CM had a significantly higher TNF- α than those without CM.[17] Central nervous system TNF α production was found to increase the risk of subsequent neurologic and cognitive morbidity.[45] Studies from both humans and mice showed an increased expression of TNF-α mRNA in brain tissue of in fatal CM.[28, 46, 47]

Studies in mice had showed the essential role of

TNF-α in CM pathogenesis. Grau et al (1992) showed that single injections of anti-TNF-α antibody ameliorated the cerebral complications.[48] TNF-α was thought to cause CM through up-regulation of the expression of the adhesion molecules involved in sequestration of infected red blood cells[49]and induction of NO production which is known to affect synaptic transmission.[50,51]

TNF-α in the pathogenesis of severe anemia: High levels of TNF-α in the serum were linked to severe anemia (SA). Othoro et al reported that TNF-α and IL-10 levels were significantly elevated in children with anemia compared with children having UM. The highest concentrations of TNF-α were found in children with malaria anemia and the mean ratio of IL-10 to TNF-α was significantly lower in children with anemia than in children with UM.[52] One of the mechanisms involved is that TNF-α synergizes with hemozoin and nitric oxide in the inhibition of erythropoeisis.[53, 54] TNF-α was also found to be associated with increased erythrophagocytosis and dyserythropoiesis.[55] Reduced prostaglandin E2 production by hemozoin is reported to lead to overproduction of TNF-α and anemia.[56]

In our work, children with SA had low TNF-α.[17] The explanation of this finding may be related to the period of symptoms in the SA group (4.46days). Children with severe anemia had significantly longer period of illness (duration of symptoms before admission to hospital) than children with uncomplicated malaria and other severe malaria manifestations.[13] It is known that during malaria immune responses proinflammatory cytokines are produced first and then the immune responses shift to anti-inflammatory cytokines. Because of delayed presentation to hospital, we might have missed the period in which TNF-α was high. Another possible alternative explanation is that this group of children may have TNF-α allele associated with low TNF-α production. Low levels of TNF-α in children with SA was reported in the presence of TNF-238A allele. [10]

Factors associated with changes in TNF-α level: Circulating TNF- α level has been linked with the genetic profile of the child. [57] May et al studied the association of allelic variants of the TNF promoter in children with severe malaria and IL-10: TNF-α ratio. They found that carriers of the wild type more frequently had an IL-10: TNF-α ratio >1. In contrast, children with a mutation at position 238 of the TNF-α promoter consistently had IL-10: TNF-α ratio <1. Children with IL-10: TNF-α ratio <1 had increased risk of SA.[10] High circulating TNF-alpha levels and the inadequate IL-10 response was also detected in the SM patients carrying TNF2 allele.[58]

Studies especially designed to link the genetic profile with the different clinical presentations of malaria are recommended so as to identify children more likely to develop excessive inflammatory response thereby malaria complications. Genetic factors may also determine the receptor population for the cytokine, thus influencing the host responses. [59]

Deficiency of trace elements may affect cytokine production. Both zinc and iron deficiencies were found to be associated with increased TNF α production from peripheral blood mononuclear cells of malaria-infected children while magnesium deficiency induced increased production of IL-10 in uninfected children. Future studies are recommended to determine whether deficiencies of these trace elements increase or decrease the risk of severe malaria. If future studies confirm increased risk, then it is wise to look for signs of trace elements deficiency in any child with severe malaria together with determination of their serum levels.[11]

Interleukin-10 (IL-10)

IL-10 is produced by large number of immune cells.[60] It has a prominent anti-inflammatory effect, limiting proinflammatory cytokines responses.[61, 62]

During malaria infection, CD4+ T cells are the major source of IL-10 while Foxp3+ CD4+ T regulatory cells make a minor contribution.[60, 63, 64] In endemic areas, repeated infections are associated with decreased parasitemia and clinical malaria.[1] The shift of the immune system responses to IL-10 production upon re-infection may contribute to

the decrease in parasitemia and clinical malaria by limiting the proinflammatory cytokine responses. This is demonstrated by Portugal et al study. [63] The study showed that before the malaria season, children's immune cells responded to infected RBCs by producing pro-inflammatory cytokines. Following a malaria infection the children's immune cells produced lower levels of pro-inflammatory cytokines and higher levels of anti-inflammatory cytokines (IL-10, TGF-β) in response to infected RBCs. The study also showed that children with asymptomatic P. falciparum infections which persisted through the six-month dry season, IL-10 was maintained partially up-regulated, suggesting that IL-10 upregulation is partially maintained by ongoing P. falciparum exposure and may contribute to protection from febrile malaria in the context of ongoing P. falciparum exposure.[63]

In line, another study in Uganda, revealed frequencies of CD4+ T cells co-producing IFNγ/ IL10 were significantly higher in children with ≥2 prior episodes/year compared to children with <2 episodes/year. In contrast, children with <2 prior episodes/year were significantly more likely to exhibit antigen-specific production of TNF-α.(64) Several human and murine studies reported low IL-10 relative to TNF-α increases susceptibility to CM and SA.

Interleukin-10 in cerebral malaria: Low IL-10: TNF-α plasma level ratio has been reported as a risk factor for both CM and SA.(10) Kossodo et al found anti IL-10 antibody administration to resistant mice induced CM in 35.7% of these mice while administration of IL-10 to susceptible mice prevents the occurrence of CM.[65] During Plasmodium chabaudi infection in IL-10 knock-out mice, there is greater parasite sequestration, more severe cerebral edema, and a high frequency of cerebral hemorrhage compared with infection of C57BL/6 mice. Anti-TNF-α treatment ameliorated both cerebral edema and hemorrhages, suggesting that proinflammatory responses contributed to cerebral complications in infected IL-10 deficient mice.[66]

Other mechanism by which IL-10 prevents CM may be through decreased platelet production. Platelets play a significant role in SM pathogenesis.[67, 68] Casals –Pascaul et al reported that thrombocytopenia in children with severe malaria strongly correlates with high levels of IL-10.[69]

Interleukin-10 in severe anemia: Low level of IL-10 was also linked to SA. Children with SA were found to have lower levels of IL-10.[52, 70] Nussenblatt et al found that in older children higher levels of TNF-α were significantly associated with higher IL-10 levels unlike younger children who had low IL-10. These data suggest that younger children do not maintain IL-10 production in response to the inflammatory process.[71]

The beneficial effect of IL-10 is demonstrated in children with malaria and helminthes co-infection. Cytokine profile from co-infected children showed an increase in IL-10 production and a decrease in proinflammatory cytokines. [72] Such immune response was found to protect co-infected children from anemia.[73]

A study in children with severe anemia concluded that Low production of IL-10 in children with SA is not an inherent incapability to produce IL-10 but may rather reflect specific cytokine dysregulation in malaria. Data from this study revealed that monocyte and T cells isolated from children with SA produce IL-10 readily when stimulated by lipopolysaccharide or phytohemagglutinin respectively.[74]

Low IL-10 may also be a reflection of the genetic profile of the child. Ouma et al reported that the -1,082G/-819C/-592C (GCC) haplotype was associated with protection against SA and increased IL-10 production. Individuals with the -1,082A/-819T/-592A (ATA) haplotype had an increased risk of SA and reduced circulating IL-10 levels. The IL-10: TNF-α ratio was higher in the GCC group and lower in individuals with the ATA haplotype. [75]

Conclusions

In an attempt to control the parasite growth, the host releases an array of pro- and anti-inflammatory cytokines. Depending on the magnitude and timing of inflammatory mediator release, the immune responses to malaria can result in either successful

control of the parasite growth or alternatively, an inappropriate balance in the inflammatory milieu that can induce malaria complications. Genetic profile, repeated infections, parasite influences and the general health influence the immune responses and predict malaria complications. Involvement of multiple cell types and the cytokines is one of the indicators of development of SM in children.

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