SHORT REPORT: HIGHER PRODUCTION OF PERIPHERAL BLOOD MACROPHAGE MIGRATION INHIBITORY FACTOR IN HEALTHY CHILDREN WITH A HISTORY OF MILD MALARIA RELATIVE TO CHILDREN WITH A HISTORY OF SEVERE MALARIA

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Abstract. Plasmodium falciparum malaria is one of the leading causes of childhood morbidity and mortality in sub-Saharan Africa. The host immune response to P. falciparum is a critical determinant of malarial pathogenesis and disease outcomes. Macrophage migration inhibitory factor (MIF) is a central regulator of innate immune responses to bacterial and parasitic infections. Our recent investigations demonstrated that peripheral blood MIF production was suppressed in children with severe malaria. Because examination of MIF production in children with active disease does not account for the inherent ability of the host to generate MIF, basal circulating MIF and peripheral blood mononuclear cell (PBMC) MIF transcript levels were determined in healthy children with a history of either mild or severe malaria. Children with prior mild malaria had higher plasma MIF levels and PBMC MIF transcripts than children with an identical number of previous episodes of severe malaria. These results suggest that increased basal MIF production may be important in generating immune responses that protect against the development of severe malaria.

More than one million people in sub-Saharan Africa die each year from Plasmodium falciparum malaria, with most deaths occurring in children less than five years of age. The clinical manifestations of pediatric P. falciparum malaria vary from asymptomatic infection to severe life-threatening complications, such as hyperparasitemia, hypoglycemia, cerebral malaria, severe anemia, respiratory distress, and hyperlactatemia. A number of important factors appear to influence disease severity, including host genetic variation, age of first exposure, and rate of exposure (endemicity). In addition, it is well established that the host immune response to P. falciparum is an important determinant of the development and outcomes of childhood malaria.

As part of our ongoing studies examining the role of the host immune response in conditioning the outcomes of pediatric malaria, we have been investigating the role of macrophage migration inhibitory factor (MIF) in the pathogenesis of malaria. Many studies have identified MIF as a central regulator of innate and adaptive immune responses that could mediate both protection and enhanced pathogenesis of bacterial and parasitic infections. Although previous studies in placental malaria and in murine models of malaria have suggested a pathogenic role for MIF in malaria, our recent studies demonstrate that increased circulating MIF production is associated with protection from severe childhood malaria.

To further clarify the role of MIF in malarial immunity, we examined MIF protein levels in the circulation and MIF mRNA levels in peripheral blood mononuclear cells (PBMCs) from a group of healthy children (2–8 years of age, mean age = 6.3 years) enrolled in a longitudinal prospective study previously conducted at the Albert Schweitzer Hospital in Lambaréné, Gabon, a hyperendemic area for P. falciparum transmission. In this region, hyperparasitemia and severe anemia are the predominant complications of severe malaria. Study participants selected for investigation were in the convalescent phase of malaria and were free of malaria parasites and any other detectable diseases for four or more months based on bi-monthly malaria parasitemia determinations and clinical evaluations. A more detailed description of this study cohort is provided in our previous publication.

Based on their previous malaria disease history, children were divided into two groups: prior mild malaria (PMM, n = 15, 8 boys and 7 girls, mean age = 6 years 3 months) or prior severe malaria (PSM, n = 10, 6 boys and 4 girls, mean age = 6 years 4 months). Definitions of malaria disease severity during the acute illness were based on World Health Organization criteria, with severe malaria defined as > 200,000 parasites/μL and/or a hemoglobin (Hb) level ≤ 5.0g/dL and mild malaria defined as < 100,000 parasites/μL, Hb level > 5.0g/dL, and without any signs or symptoms of severe malaria. None of the study participants had a prior episode of cerebral malaria. Children in the PMM and PSM groups were matched so that the mean number of previous malaria episodes was identical in the two groups. Moreover, children selected for the PPM group had no prior history of severe malaria, and those in the PSM group had no prior history of mild malaria. Participation in the study was completely voluntary and written informed consent was obtained from the parents/guardians of the pediatric participants. The study was reviewed and approved by the ethics committee of the International Foundation of the Albert Schweitzer Hospital, Duke University Medical Center Investigational Review Board, and the University of Pittsburgh Institutional Review Board.

Venous blood (< 5 mL) was collected into EDTA-containing vials, plasma was separated, and PBMCs were iso-

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lated according to our previous methods. At the time of sampling, a physical and clinical evaluation was performed to verify that the children were healthy and a thick blood smear was prepared to confirm that the study participants were free of malaria parasites. Plasma MIF concentrations were measured using a commercially available enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). The MIF transcripts were determined in ex vivo PBMCs by a Taqman® real time reverse transcription–polymerase chain reaction (Applied Biosystems, Foster City, CA) and normalized to β-actin according to our previous methods.

Healthy children were selected for investigation because differences in MIF production during an active infection may reflect the overall response to the pathogen, and baseline measurements in disease-free children in the convalescent phase of disease likely reflects either inherent genetic differences and/or adaptation to prior malaria episodes. As shown in Figure 1, children with PMM (median = 1.704 [interquartile range = 1.161–2.497]) had significantly higher circulating MIF levels than children with PSM (1.007 [887–1.230]) (\( P < 0.005 \)). In addition, MIF transcripts measured in ex vivo PBMC demonstrated that children with PMM had 2.4-fold higher MIF mRNA levels than children with PSM (\( P = 0.2 \), Figure 1B).

Unlike most cytokines, MIF is constitutively produced in significant quantities, and is further augmented by inflammatory stimuli. Therefore, baseline MIF production may be important for determining the nature and magnitude of the host immune response to an invading pathogen such as Plasmodium. Because samples examined in this study were taken from healthy children who had fully resolved their malarial infection, the observed levels of circulating MIF and PBMC MIF transcripts represent basal MIF production.

Our results show that children with prior episodes of mild malaria produce significantly higher baseline levels of MIF than children who previously experienced severe disease. Given the pivotal role of MIF in mediating innate immunity and regulation of pro-inflammatory cytokine production, we propose that elevated baseline MIF levels may protect against the development of severe malaria by promoting a rapid and potent innate immune response that could result in more efficient control over the initial phases of parasitemia. For example, adequate MIF production is required for induction of interleukin-12, tumor necrosis factor-α, and nitric oxide (NO) all of which are important mediators of the innate immune response to malaria. In addition, because MIF is important in adaptive immunity through promotion of T cell and B cell activation and proliferation, and antibody production, adequate MIF concentrations may be required for an efficient antigen-specific immune response to malaria. Conversely, elevated MIF production in children with PMM may be related to the phenomenon of malarial tolerance in which increased MIF levels may provide negative-feedback mechanisms that help control over-expression of pro-inflammatory cytokines that could cause enhanced pathologic effects.

Our previous studies in the same cohort of healthy, malaria-exposed children showed that NO production was significantly higher in the PMM group. This finding, along with results presented here, are consistent with our hypothesis that elevated levels of regulatory inflammatory mediators in children with prior malaria exposure may condition tolerance to malaria and reduce susceptibility to severe disease. Alternatively, differences in host genetics may explain our current findings. Two polymorphisms in the MIF promoter (MIF-173 G/C and MIF-794 CAAT) have been associated with functional changes in MIF production in a number of inflammatory diseases. In addition, we recently demonstrated a significant association between circulating MIF levels and MIF-173 G/C variability in Kenyan children. Although polymorphic variability was not determined here, we are currently investigating the impact of MIF promoter polymorphisms on baseline and malaria-induced MIF production in pediatric populations.

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