Primed Innate Immune Responses in Monocytes from Kenyan Children with Uncomplicated Falciparum Malaria

Katherine Dobbs¹, Paula Embury¹, John Vulule², Peter Sumba Odada², Bruce Rosa³, Makedona Mitreva³, James Kazura², Arlene Dent²

¹ Center for Global Health and Diseases, Case Western Reserve University, Cleveland, OH, USA ²Kenya Medical Research Institute, Kisumu, Kenya ³McDonnell Genome Institute, Washington University, St. Louis, MO, USA

Abstract

Background: Monocytes are innate immune cells that play a key role in host protection and pathogenesis during malaria. We sought to determine whether uncomplicated falciparum malaria in children modulates Toll-like receptor (TLR) responsiveness in monocytes.

Methods: Freshly isolated monocytes were obtained from 8 children in western Kenya at presentation with acute uncomplicated malaria and 6 weeks following treatment and from 4 malaria-naive North Americans (NAM). Monocytes were cultured for 18 hours with media alone, a TLR4 agonist (LPS), or a TLR2/TLR4 agonist (Pam3CSK4). Supernatant cytokine concentrations were measured using a magnetic bead-based immunoassay. DNA methylation analysis was performed using the MethylationEPIC array. Gene expression profiles were analyzed in another 6 acute-recovery pairs and 5 NAM using a targeted digital RNA sequencing panel.

Results: Both acute and recovery monocytes showed robust and equivalent responses to LPS and Pam3CSK4, with markedly increased production over media alone of IL-10, IL-6, IL-1, IL-12p40, and TNF (Friedman test P < 0.05). Compared to NAM, acute and recovery monocytes showed a greater magnitude of responses to LPS and Pam3CSK4, especially for IL-6, IL-12p40, and TNF (Kruskal-Wallis test P < 0.05). Monocyte gene expression for the cytokines IL-1α, IL-1β, IL-8, IL-6, and TNF was not different between acute and recovery, though these genes were significantly upregulated in acute and recovery monocytes compared to NAM (FDR adjusted P < 0.01). DNA methylation analysis showed significant differential methylation in acute vs. recovery monocytes in the promoter regions for IL1A, IL1B, IL10, CCR5, and CCL5 (FDR adjusted P < 0.05).

Conclusions: These data suggest that uncomplicated malaria in children has a priming effect on innate immune responses that is maintained several weeks after clinical recovery, which may be mediated in part by epigenetic changes such as altered DNA methylation patterns.

Methods

Study population: Participants included children from western Kenya aged 1-10 years with acute uncomplicated malaria. Cases were treated with artesunate/amodiaquine and a recovery venous blood sample was collected 6 weeks after treatment.

Monocyte TLR responses: Monocytes were isolated from whole blood via negative selection with the RosetteSep Human Monocyte Enrichment Cocktail (StemCell Technologies). Cells were stimulated with LPS 10 ng/ml, and Pam3CSK4 100 ng/ml, and compared to a media-alone control. Cells were cultured for 18 hrs at 37°C in 5% CO₂ on an orbital shaker. A multiplex magnetic bead-based immunoassay was used to simultaneously measure cytokines of IL-10, IL-6, IL-8, IL-12p40, and TNF (Millipore) in the supernatant.

Targeted digital RNA sequencing: Monocytes were negatively selected from PBMC over a magnetic column. Targeted digital RNA sequencing was performed to analyze expression of 508 genes involved in inflammation and immunity (Quagen). DESeq² differential gene expression analysis identified significantly upregulated and downregulated genes for three different comparisons (AM vs. media; AM vs. NAM; NAM vs. NAM).

DNA methylation array: Monocyte genomic DNA was bisulfite-treated and applied to the MethylationEPIC (Enhancer and Promoter Informed Content) array (Illumina). Methylation values at CpG sites are indicated by β-values, where β = (Max(M)-Min(U))/(Max(M)+Min(U)+100), where 1 corresponds to complete methylation and 0 corresponds to no methylation. Differential methylation analysis was performed using a threshold for CpG selection at Delta β scores > 0.1 at FDR-adjusted P < 0.05.

Pathway enrichment analysis was performed for differentially methylated promoter regions using Ingenuity Pathway Analysis software (Quagen).

Results

Figure 1. Monocytes from children during acute malaria and 6 weeks following treatment are highly responsive to stimulation with TLR ligands. Monocytes were negatively selected from fresh venous blood samples from children during acute malaria and 6 weeks following treatment (∼8 pairs). Healthy North American adult controls (∼4) were used as experimental controls. Cells were cultured for 18 hours with media alone, LPS 10 ng/ml, or Pam3CSK4 (PSC) 100 ng/ml, and cytokine concentrations were measured in culture supernatants. *P < 0.05, **P < 0.01, as compared to controls.

Figure 2. Monocytes from children with uncomplicated malaria display a distinct inflammatory gene expression profile. Targeted digital RNA sequencing of 508 genes was performed in children with malaria and comparison groups. DESeq² differential gene expression analysis identified significantly upregulated and downregulated genes for three different comparisons (AM vs. NAM; AM vs. NAM; NAM vs. NAM). DNA methylation array: Monocyte genomic DNA was bisulfite-treated and applied to the MethylationEPIC array (Enricher and Promoter Informed Content) array (Illumina). Methylation values at CpG sites are indicated by β-values, where β = (Max(M)-Min(U))/(Max(M)+Min(U)+100), where 1 corresponds to complete methylation and 0 corresponds to no methylation. Differential methylation analysis was performed using a threshold for CpG selection at Delta β scores > 0.1 at FDR-adjusted P < 0.05.

Pathway enrichment analysis was performed for differentially methylated promoter regions using Ingenuity Pathway Analysis software (Quagen).

Conclusions

• Uncomplicated malaria in children has a priming effect on monocyte TLR responses.
• Enhanced TLR responses were observed in monocytes obtained from children 6 weeks after curative treatment.
• Monocytes from children with uncomplicated malaria have a distinct, regulated inflammatory gene expression profile.
• Innate immune priming may be mediated in part by epigenetic changes such as altered DNA methylation patterns.
• Studies are ongoing to compare DNA methylation profiles in malaria-naïve and -immune populations and to further evaluate the effect of acute malaria on innate immune memory phenotypes.

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