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Anti-Inflammatory Cytokines Predominate in Acute Human *Plasmodium knowlesi* Infections

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Abstract

Plasmodium knowlesi has entered the human population of Southeast Asia. Naturally acquired knowlesi malaria is newly described with relatively little available data, including data on the host response to infection. Therefore pre-treatment cytokine and chemokine profiles were determined for 94 *P. knowlesi,* and for comparison, 20, *P. vivax* and 22 *P. falciparum,* patients recruited in Malaysian Borneo. Nine, five and one patient with *P. knowlesi, P. falciparum* and *P. vivax* respectively had complicated malaria as defined by World Health Organisation. Patients with uncomplicated *P. knowlesi* had lower levels of the pro-inflammatory cytokines IL-8 and TNFα than those with complicated disease (both $p<0.05$, Dunn's post test, DPT). The anti-inflammatory cytokines IL-1ra and IL-10 were detected in all patients in the study. IL-1ra, the most abundant cytokine measured, correlated with parasitaemia in *P. knowlesi* ($r_s = 0.47$, $p = <0.0001$), *P. vivax* ($r_s = 0.61$, $p = 0.0042$) and *P. falciparum* ($r_s = 0.57$,$p = 0.0054$) malaria. IL-10 correlated with parasitaemia in both *P. knowlesi* ($r_s = 0.54$, $p = <0.0001$) and *P. vivax* ($r_s = 0.78$, $p = <0.0001$) infections. There were between group differences in soluble markers of macrophage activation (MIP-1β and MCP-1). *P. knowlesi* patients had significantly lower levels of MIP-1β than *P. falciparum* (DPT, $p = <0.01$). Uncomplicated *P. knowlesi* patients had significantly lower levels of MCP-1 than uncomplicated *P. falciparum* patients (DPT, $p = <0.001$). There was no significant difference between complicated and uncomplicated *P. knowlesi* infections. MCP-1, MIP-1β, IL-8 and TNFα increased in complicated *P. knowlesi* but decreased in complicated *P. falciparum* infections. Descriptions of human knowlesi malaria provide a comparative means to discover mediators of pathophysiology in severe *P. knowlesi* as well as *P. falciparum* malaria. Crucially, *P. knowlesi* may be the disease and experimental primate model for severe malaria.

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Introduction

Plasmodium knowlesi infection is a major cause of malaria in humans in Malaysian Borneo [1,2]. Approximately 10% of patients develop severe symptoms when classified by WHO criteria for disease severity and approximately one percent of cases have a fatal outcome [3]. Recent post mortem findings from a fatal *P. knowlesi* infection show remarkable histological similarities with *P. falciparum*, suggesting commonality of some pathophysiological processes between severe infections caused by different species of *Plasmodium* [4]. *P. knowlesi* and *P. falciparum* infections can both lead to hepatorenal dysfunction, acute respiratory distress syndrome (ARDS), metabolic disturbance and the accumulation of large numbers of heavily pigmented parasitized red blood cells in brain and other microvasculatures [4,5,6,7]. ARDS, jaundice renal dysfunction and anaemia can also occur in complicated *P. vivax* malaria [8,9,10,11]. However, despite accumulation of *P. knowlesi* infected erythrocytes in brain vessels, coma is not a feature of fatal knowlesi infection. The severity of anaemia associated with *P. knowlesi* may also be less than that seen with *P. falciparum* and *P. vivax* infection although this complication has not been studied systematically.

In fatal knowlesi malaria, progression from the onset of symptoms to the development of high parasitiaemia and death can be swift, and may be as short as 3 days [1,4]. After reviewing data from cases of severe and fatal knowlesi malaria, we observed that parasitaemia might be a better marker of disease severity than for other species of human malaria [3]. Sequestration of mature stage parasites from peripheral blood circulation in *P. falciparum* infections precludes the possibility of determining accurate estimates of asexual stage parasitaemia by microscopy and therefore of using parasitaemia as a reliable marker of disease severity in falciparum malaria [12]. Difficulty in making direct associations between parasitaemia and pathophysiology in falciparum malaria have hampered research on identifying parasite virulence, particularly in relation to estimating thresholds for the development of severe disease [13]. Within the context of host response to acute malaria, quantifiable measures of disease severity, parasitaemia and soluble mediators of immune response were measured in clinically and parasitologically well-characterised *P. knowlesi*, *P. falciparum* and *P. vivax* infections. Although falciparum and vivax patients had mostly imported malaria they were retained in the study to provide contemporaneous and other information for comparison. We test the hypothesis that soluble