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Cord blood IgG and the risk of severe *Plasmodium falciparum* malaria in the first year of life

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ABSTRACT

Young infants are less susceptible to severe episodes of malaria but the targets and mechanisms of protection are not clear. Cord blood antibodies may play an important role in mediating protection but many studies have examined their association with the outcome of infection or non-severe malaria. Here, we investigated whether cord blood IgG to *Plasmodium falciparum* merozoite antigens and antibody-mediated effector functions were associated with reduced odds of developing severe malaria at different time points during the first year of life. We conducted a case-control study of well-defined severe *falciparum* malaria nested within a longitudinal birth cohort of Kenyan children. We measured cord blood total IgG levels against five recombinant merozoite antigens and antibody function in the growth inhibition activity and neutrophil antibody-dependent respiratory burst assays. We also assessed the decay of maternal antibodies during the first 6 months of life. The mean antibody half-life range was 2.51 months (95% confidence interval (CI): 2.19–2.92) to 4.91 months (95% CI: 4.47–6.07). The rate of decline of maternal antibodies was inversely proportional to the starting concentration. The functional assay of antibody-dependent respiratory burst activity predicted significantly reduced odds of developing severe malaria during the first 6 months of life (Odds ratio (OR) 0.07, 95% CI: 0.007–0.74, $P = 0.007$). Identification of the targets of antibodies mediating antibody-dependent respiratory burst activity could contribute to the development of malaria vaccines that protect against severe episodes of malaria in early infancy.

1. Introduction

Plasmodium falciparum is a leading cause of childhood morbidity and mortality with approximately 214 million cases and 438,000 deaths reported globally in the year 2015 (World Health Organization, 2015). A disproportionate number of the malaria-related deaths occur in sub-Saharan Africa with children under the age of 5 years being at the highest risk of severe and life-threatening malaria. Severe malaria in children manifests in three overlapping clinical syndromes: severe anemia, impaired consciousness and respiratory distress (Marsh et al., 1995). The presentation of these clinical features varies with host age and the level of malaria transmission (Snow et al., 1994, 1997; Roca-Feltrer et al., 2010). In high transmission areas, severe anemia is predominant and affects mainly children aged less than 24 months, while in low-moderate transmission areas cerebral malaria is the main clinical manifestation in older children (O'Meara et al., 2008; Roca-Feltrer et al., 2010), causing high mortality despite appropriate intervention. A significant proportion of those who recover develop long-term neurological and cognitive deficits (Idro et al., 2005).

Young infants in malaria endemic countries are relatively resistant to severe malaria (Snow et al., 1998). Cord blood antibodies are thought to confer protection against clinical episodes of malaria (Edozien et al., 1962) but the evidence is far from clear (Riley et al., 2001; Dobbs and Dent, 2016). Although passively transferred cord blood IgG was shown to reduce parasitemia and

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clinical symptoms in one study (Edozien et al., 1962), the targets of such antibodies have yet to be identified (Hviid and Staalsoe, 2004). Importantly, although many studies have investigated maternal antibodies in relation to the risk of infection, clinical or febrile malaria, none have focused on severe malaria as the end-point of interest.

We designed a case-control study of severe malaria nested within a longitudinal birth cohort of infants who were monitored for episodes of well-characterised severe malaria (Lundblom et al., 2013; Murungi et al., 2016). We identified the sub-group of infants for whom a cord blood sample was available. We measured cord blood plasma total IgG levels against five recombinant *P. falciparum* merozoite antigens and its functional activity in the growth inhibition activity (GIA) and antibody-dependent respiratory burst (ADRB) assays (Llewellyn et al., 2015; Murungi et al., 2016). We investigated factors that were likely to have an influence on these antibody measures and assessed the decay of antigen-specific cord blood IgG over the first 6 months of life. Finally we investigated whether antibody levels and function in cord blood were associated with reduced odds of developing severe *falciparum* malaria at different time points during the first year of life when maternal antibodies are likely to persist.

2. Materials and methods

2.1. Study site and population

The study was conducted in Kilifi County, on the Kenyan coast. The area experiences two seasonal peaks in malaria transmission (May to August and October to November). The study setting and study population are described in detail elsewhere (Lundblom et al., 2013; Murungi et al., 2016). Briefly, following informed consent, infants born to mothers who delivered at Kilifi County Hospital (KCH) or those attending the immunisation clinic during the first month of life were recruited into a birth cohort (Kilifi Birth Cohort, KBC) (n = 5,949) set up between 2001 and 2008 to study the risk factors of invasive pneumococcal disease in young children. As the study was primarily set up to study pneumococcal disease, malaria-specific indices such as intermittent preventive treatment during pregnancy (iPTp) and bed net usage were not recorded. The children were followed up quarterly at the Outpatient Department of KCH until 2 years of age. During the quarterly visits, a blood sample was collected and thick and thin blood smears prepared for detection of parasites by microscopy. In the event of an illness outside the scheduled 3-monthly visits, parents were advised to seek care at KCH and the children were treated according to national guidelines. Children who were admitted to hospital were identified using a unique number that linked their clinical, demographic and laboratory information.

2.1.1. Study design

We designed a matched case-control study of well-defined severe malaria cases that included all infants enrolled into the KBC and longitudinally monitored as described in Section 2.1. We included cases admitted to hospital between April 2002 and January 2010. Cases were individually matched to a maximum of three controls by age, date of sample collection and area of residence. Controls were selected from KBC participants who did not present to KCH with severe malaria during the 8-year monitoring period. A total of 61 severe malaria cases were identified and these were individually matched to 161 controls (Lundblom et al., 2013; Murungi et al., 2016). The data presented here are drawn from the subset of these children who were recruited at birth and had a 2 ml venous blood sample taken from the umbilical vein (n = 130). Following informed consent, baseline information (age of the mother, number of previous pregnancies, antenatal clinic attendance, gestation period, birth weight and gender of the infant) and a cord blood sample were obtained (Table 1). We also analyzed samples collected at 3 and 6 months of age from the cases and controls to determine the dynamics of decay of maternal antibodies.

2.1.2. Severe malaria case definition

Inclusion criteria for severe malaria cases were admission to hospital between April 2002 and January 2010 with detectable parasites by microscopy and one of the following symptoms: (i) impaired consciousness (Blantyre Coma Score <5), (ii) chest indrawing or deep breathing or (iii) severe anemia (Hb <5 g/dL).

2.1.3. Detection of asymptomatic infections

Detection of malaria parasites in the samples collected every 3 months was performed retrospectively by microscopy and PCR as previously described (Lundblom et al., 2013). Briefly, thick and thin blood films were stained with Giemsa and examined by light microscopy. Parasite densities were determined as the number of parasites per 8,000 white blood cells per µl of blood. The prevalence of submicroscopic infections was determined by PCR amplification of the polymorphic block 3 region of the merozoite surface protein 2 (*msp2*) gene followed by capillary electrophoresis (Liljander et al., 2009).

2.2. Recombinant P. falciparum merozoite antigens

We measured total IgG titres to a panel of five recombinant merozoite antigens that are currently being assessed in clinical, pre-clinical, animal model and in vitro studies as potential blood-stage malaria vaccine candidates. Reactivity to schizont extract was used as a marker of previous exposure to infection. Full-length apical membrane antigen (AMA)1 (3D7 *P. falciparum* strain)

Table 1
Baseline characteristics of the mothers and their infants at enrolment in the study.

	Cases (n = 32)	Controls (n = 98)	P value[a]
Median maternal age in years (range)[b]	27.8 (14.9–44.0)	25.4 (14.7–48.0)	0.26
Median no. of previous pregnancies (range)[c]	5 (0–8)	4 (0–13)	0.25
Gender female, ***n***/Total (%)	15/32 (46.8%)	47/98 (47.9%)	0.91
Median gestation weeks[d] (range)[e]	38 (23–42)	38 (28–50)	0.82
Median birth weight in kilograms (range)[f]	2.7 (1.8–4.3)	2.8 (1.6–3.8)	0.68
Antenatal clinic attendance, *n*/Total recorded (%)	12/13 (92.3%)	43/45 (95.5%)	0.64
Year of birth, *n* (%)			
2002	15 (46.8%)	40 (40.8%)	0.56
2003	9 (28.1%)	21 (21.4%)	
2004	6 (18.7%)	31 (31.6%)	
2005	2 (6.2%)	4 (4.0%)	
2006	0 (0%)	2 (2.0%)	
Season of birth			
Dry season[g]	20 (62.5%)	58 (59.1%)	0.74
Rainy season[h]	12 (37.5%)	40 (40.8%)	

[a] The Mann–Whitney *U* and chi-square tests were applied for comparisons of continuous variables and proportions, respectively, among the cases and controls.
[b] Data were available from mothers of 32 (100%) severe malaria cases and 96 (97%) controls.
[c] Data were recorded from mothers of 22 (68%) cases and 74 (75%) controls.
[d] Gestation age was assessed based on the date of last menstrual period (LMP) or symphyseal-fundal height of the uterus, converted to weeks using dating charts.
[e] Data were recorded from mothers of 13 (40%) cases and 45 (45%) controls.
[f] Data were available for 16 (50%) children who developed severe malaria and 50 (51%) controls.
[g] The dry season occurs in January–March.
[h] The rainy season occurs in May–August and October–November.

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