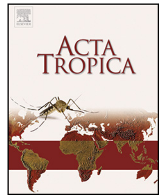
Acta Tropica 128 (2013) 149–153

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica



Transplacentally transferred functional antibodies against *Plasmodium falciparum* decrease with age

Patrick T. Wilson[a,*], Indu Malhotra[a], Peter Mungai[a,b], Christopher L. King[a], Arlene E. Dent[a]

[a] Center for Global Health and Diseases, Case Western Reserve University, Cleveland, OH, USA
[b] Division of Vector Borne and Neglected Tropical Diseases, Nairobi, Kenya

ARTICLE INFO

Article history:
Received 12 February 2013
Received in revised form 17 July 2013
Accepted 21 July 2013
Available online 30 July 2013

Keywords:
Malaria
Plasmodium falciparum
Functional antibodies
Growth inhibition assay

ABSTRACT

Transplacental transfer of antibodies from clinically malaria immune pregnant women to their fetuses is thought to provide passive protection against malaria during infancy. However, the presences and duration of functional antibodies against *Plasmodium falciparum* (Pf) in newborns has not been described. We used growth inhibition assays (GIA) to measure total anti-malaria functional antibodies present at birth and over the following year. Samples were drawn from cord blood ($n = 86$) and in infants at six and 12 months of life ($n = 86$ and 65 respectively). Three laboratory Pf strains (D10, W2mef, 3D7) and a field isolate (Msambweni 2006) were used in the assays. Median (ranges) GIA levels for cord plasma differed between laboratory parasite strains: D10, 0% (0–81); W2mef, 6% (0–80); 3D7, 18% (0–88); Msambweni 2006, 6% (0–43) ($P < 0.001$, Wilcoxon signed-rank test). GIA levels against all Pf strains were found to decline in infants from birth to six months ($P < 0.01$, Wilcoxon, signed-rank test). Functional antibodies as measured by GIA are transferred to the fetus and wane in the infants over time. Infant protection from clinical malaria disease may in part be mediated by these functional anti-malaria antibodies.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Although malaria is a leading cause of death in children, it rarely causes clinically significant disease in those less than six to eight months of age. A leading hypothesis for this observation is infants are protected by maternal antibodies (Macdonald, 1950; McGuinness et al., 1998). Previous studies have attempted to test this hypothesis by examining cord blood for the presence of malaria-specific antibodies by ELISA. Antibodies detected by this method have not correlated with protection (Ahlborg et al., 2000; Dodoo et al., 2008; Zhou et al., 2002). It has been demonstrated however, that such ELISA assays may not always detect functional antibodies, which can inhibit merozoite invasion or growth.

Antibodies likely protect infants from clinical malaria by multiple mechanisms including the ability to bind to merozoite surface proteins inhibiting erythrocyte invasion and intraerythrocytic maturation (Marsh and Kinyanjui, 2006). Such antibodies would be expected to result in invasion/growth inhibition of blood stage *Plasmodium falciparum* (Pf) parasite in vitro, as measured by a growth inhibition assay (GIA). This assay quantifies antibody-mediated activity against blood stage parasites by measuring parasite growth in the presence of malaria exposed plasma in comparison with non-malaria exposed control plasma and thought to be mediated primarily by IgG (Crompton et al., 2010; Miura et al., 2008). GIA has been used in vaccine studies (Darko et al., 2005; Singh et al., 2003, 2006; Dicko et al., 2007; Thera et al., 2006; Withers et al., 2006) and found in persons with naturally acquired malaria immunity (Bolad et al., 2003; Dent et al., 2008; Perraut et al., 2005).

This is of importance since transplacental transport is restricted to IgG isotype and the efficiency of transplacental transport varies by subclass with IgG1 = IgG4 > IgG3 > IgG2 (Costa-Carvalho et al., 1996). Thus if functional antibodies are not IgG1 or IgG4 or are primarily IgG3 or IgG2 subclasses, transplacental transfer of putatively protective antibodies may be diminished relative to total. Because many malaria antigens show antigen-specific subclass distribution (Dodoo et al., 2008) antibodies to certain antigens may be underrepresented in transplacental IgG. Other factors important in transplacental antibody transfer are maternal antibody levels and gestational age (Palmeira et al., 2012). Most transplacentally transferred IgG has a half-life of 21 days and is undetectable by six months of age, but the duration of functional activity exerted by these antibodies against malaria is unknown.

* Corresponding author. Present address: Columbia University Medical Center, 3959 Broadway, New York, NY 10023, USA. Tel.: +1 212 305 8458; fax: +1 212 342 2293.
E-mail addresses: ptw2107@columbia.edu, patrick_164@hotmail.com (P.T. Wilson).

0001-706X/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.actatropica.2013.07.018

We hypothesized that functional antibodies would be demonstrable in cord blood of infants born to women in a malaria endemic area using growth inhibition assays, and that they would wane over time. We investigated this hypothesis by examining functional antibodies in cord blood, and at six and 12 months of life.

2. Materials and methods

2.1. Study participants

Healthy pregnant women from the area served by the Msambweni District Hospital on the South Coast of Kenya were recruited from 2005 to 2007 as part of a larger study (Malhotra et al., 2009) that was approved by the Case Western Reserve University Institutional Review Board and the Kenyan Medical Research Institute/National Ethical Review Committee. At the time of delivery, cord blood was collected and infants were followed every six months of age with a clinical assessment and venous blood draw. Only HIV negative women with term deliveries (37 weeks gestation or later) were included in the study. The average age of mothers in our cohort was 25.5 years with an average parity of 2.3. Any participants with malaria infections were treated according to Kenya Ministry of Health guidelines.

2.2. Treatment of plasma samples

All plasma samples were stored at −80 °C with minimal freeze/thaw cycles. 300 μl of each plasma sample was dialyzed with two buffer exchanges of sterile PBS and 100,000 molecular-weight-cutoff dialysis tubes (Spectrum Lab, Rancho Dominguez, CA) at 4 °C then reconstituted to the original 300 μl starting volume using 100 kDa molecular-weight-cutoff centrifugal concentration tubes (Pall Corporation, Ann Arbor, MI) to retain antibodies and remove drugs or other potential factors that could augment or inhibit parasite growth (Sy et al., 1990). Non-malaria exposed negative control cord plasma was obtained from four North American neonates at University Hospitals in Cleveland, Ohio, USA that were then pooled and dialyzed as described above.

2.3. Growth inhibition assays

Laboratory strains of Pf (W2Mef, D10, 3D7) were maintained in 10 ml plastic Petri dishes at 4% hematocrit of O + erythrocytes in RPMI-HEPES medium with 0.2% sodium bicarbonate supplemented with 200 mM hypoxanthine, 200 mM L-glutamine, 10% Albumax, and 50 mg/ml gentamicin (Dent et al., 2008; McCallum et al., 2008; Persson et al., 2006). Parasite strains were cultured at 1% O_2, 5% CO_2, and 95% nitrogen atmosphere and at 37 °C (Beeson et al., 1999). Parasites were synchronized at the ring stage with pre-warmed 5% D-Sorbitol (Sigma, St Louis, MO) two times per week. No genotypic analysis was performed to verify the identity of the different parasites used at different times. 10 μl of test plasma and 40 μl of complete culture media were added to the 96-well flat bottom plates (Falcon; Becton Dickinson, Franklin Lakes, NJ) in duplicate. 50 μl of parasite culture at the mature trophozoite stage (confirmed by microscopy) was added to each well. The final plasma dilution was 1:10. Starting parasitemias were between 0.3% and 0.8%. Plates were covered, gassed, and incubated at 37 °C until one invasion cycle was completed as observed by microscopy of parallel cultures (approximately 24 h). 25 μl of re-suspended cultures were removed and fixed with 0.25% gluteraldehyde in PBS for 45 min and parasitemia measured with a BD LSRII flow cytometer (Becton-Dickinson, Franklin Lakes, NJ; Dent et al., 2008) using 10X Sybr Green (Invitrogen, Eugene, OR) and analyzed with FlowJo software (Tree Star, Inc., Ashland, OR) by gating on 50,000 red blood cells using forward and side scatter then determining the number of Sybr Green staining ring invaded red blood cells (Bei et al., 2010; Izumiyama et al., 2009; Johnson et al., 2007). Difference in parasite growth between the test plasma and non-malaria exposed control plasma is reported as percent inhibition attributable to functional antibodies.

2.4. Msambweni 2006 field isolate

Blood was obtained from a pediatric patient admitted to Msambweni District Hospital for treatment of severe malaria. Parasitemia was confirmed by microscopy and red blood cells separated using Histopaque (Sigma, St. Louis, MO) density gradient, centrifuged for 30 min at 400 × g and washed three times with RPMI complete media. The red blood cell pellet was frozen in glycerolyte (Diggs et al., 1975), placed at −80 °C for 24 h then transferred to liquid nitrogen. The isolate was shipped to the United States, thawed, established in laboratory culture for approximately two weeks prior to use in the GIA described above.

2.5. Maternal Pf infection status

Maternal venous blood, intervillous placental blood, and cord blood were examined for malaria infection status by PCR/ligase detection reaction-fluorescent microsphere assay specific for Pf (Kasehagen et al., 2006).

2.6. Statistical analysis

Wilcoxon signed-rank test was used to compare differences in GIA levels of cord blood with four different Pf strains and changes in GIA from birth to six months to one year. A linear mixed model with random intercept was used to estimate rate of decline of GIA levels. Spearman's rank coefficient (r_s) was used to measure GIA correlation between parasite strains. Mann–Whitney *U* test was used to compare GIA levels between infected and non-infected mothers at time of birth. Analyses were conducted with R-Commander, SAS and GraphPad Prism 4.

3. Results

3.1. GIA are transferred to the fetus and levels vary against different Pf strains

Growth inhibitory antibody responses likely target merozoite surface antigens, proteins involved in invasion and those necessary for intraerythrocytic growth. These pathways vary among Pf strains thus making it important to examine GIA responses to multiple strains. Therefore we examined GIA against strains representing sialic acid dependent (W2Mef) and sialic acid independent (3D7 and D10) erythrocyte invasion pathways as well as strains that vary considerably on their surface proteins, 3D7 and D10. We also examined GIA to a parasite strain isolated from the endemic area referred to as Msambweni 2006. The median (ranges) GIA levels of cord blood were: D10, 0% (0–81); W2mef, 6% (0–80); 3D7, 18% (0–88) inhibition for $N = 270$ samples (a composite of three different experiments: $N = 104$, $N = 54$, and $N = 112$) and significantly differed among the laboratory lines ($P < 0.001$, Wilcoxon signed-rank test) (Fig. 1A). The prevalence of positive responders (≥5% inhibition) for D10, W2Mef, and 3D7 was 46%, 55% and 64% respectively. GIA levels correlated between the sialic acid independent and dependent strains; W2Mef and D10 $r_s = 0.54$, W2Mef and 3D7 $r_s = 0.83$ and less between the sialic independent strains 3D7 and D10 $r_s = 0.52$, although many individuals had antibodies that inhibited only one Pf strain. Thus transplacental transfer of growth inhibitory antibodies occurs against both sialic-acid independent and dependent invasion pathways in Kenyan mothers. Of note, maternal infection

Download English Version:

https://daneshyari.com/en/article/6127850

Download Persian Version:

https://daneshyari.com/article/6127850

Daneshyari.com