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Associations between IgG reactivity to *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) antigens and Burkitt lymphoma in Ghana and Uganda case-control studies

Andriy Derkach^a, Isaac Otim^b, Ruth M. Pfeiffer^a, Olusegun O. Onabajo^c, Ismail D. Legason^b, Hadijah Nabalende^b, Martin D. Ogwang^d, Patrick Kerchan^e, Ambrose O. Talisuna^f, Leona W. Ayers^g, Steven J. Reynolds^h, Francis Nkrumahⁱ, Janet Neequaye^j, Kishor Bhatia^a, Thor G. Theander^k, Ludmila Prokunina-Olsson^c, Louise Turner^k, James J. Goedert^a, Thomas Lavstsen^k, Sam M. Mbulaiteye^{a,*}

^a Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

^b EMBLEM Study, African Field Epidemiology Network, Kampala, Uganda

^c Laboratory of Translational Genomics, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

^d EMBLEM Study, St. Mary's Hospital, Lacor, Gulu, Uganda

^e EMBLEM Study, Kuluva Hospital, Arua, Uganda

^f World Health Organization, Regional Office for Africa, Brazzaville, Congo

^g Department of Pathology, The Ohio State University, Columbus, OH, USA

^h Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

ⁱ Noguchi Memorial Institute, Accra, Ghana

^j Department of Child Health, Korle Bu University Teaching Hospital in Accra, Ghana

^k Centre for Medical Parasitology, Department of International Health, Immunology & Microbiology, University of Copenhagen and Department of Infectious Diseases, Rigshospitalet, Copenhagen, Denmark

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ABSTRACT

Background: Endemic Burkitt lymphoma (eBL) is an aggressive childhood B-cell lymphoma linked to *Plasmodium falciparum* (Pf) malaria in sub-Saharan Africa. We investigated antibody reactivity to several human receptor-binding domains of the Pf erythrocyte membrane protein 1 (PfEMP1) that play a key role in malaria pathogenesis and are targets of acquired immunity to malaria.

Methods: Serum/plasma IgG antibody reactivity was measured to 22 Pf antigens, including 18 to PfEMP1 CIDR domains between cases and controls from two populations (149 eBL cases and 150 controls from Ghana and 194 eBL cases and 600 controls from Uganda). Adjusted odds ratios (aORs) for case-control associations were estimated by logistic regression.

Findings: There was stronger reactivity to the severe malaria associated CIDR α 1 domains than other CIDR domains both in cases and controls. eBL cases reacted to fewer antigens than controls (Ghana: $p = 0.001$; Uganda: $p = 0.03$), with statistically significant lower ORs associated with reactivity to 13+ antigens in Ghana (aOR 0.39, 95% CI 0.24–0.63; $p_{\text{heterogeneity}} = 0.00011$) and Uganda (aOR 0.60, 95% CI 0.41–0.88; $p_{\text{heterogeneity}} = 0.008$). eBL was inversely associated with reactivity, coded as quartiles, to group A variant CIDR δ 1 ($p_{\text{trend}} = 0.035$) in Ghana and group B CD36-binding variants CIDR α 2.2 ($p_{\text{trend}} = 0.006$) and CIDR α 2.4 ($p_{\text{trend}} = 0.033$) in Uganda, and positively associated with reactivity to SERA5 in Ghana ($p_{\text{trend}} = 0.017$) and Uganda ($p_{\text{trend}} = 0.007$) and group A CIDR α 1.5 variant in Uganda only ($p_{\text{trend}} = 0.034$).

Interpretation: eBL cases reacted to fewer antigens than controls using samples from two populations, Ghana and Uganda. Attenuated humoral immunity to PfEMP1 may contribute to susceptibility to low-grade malaria and eBL risk.

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1. Introduction

Burkitt lymphoma (BL) is an aggressive B-cell lymphoma characterized by chromosomal translocation involving c-MYC oncogene and immunoglobulin gene regions [1]. An endemic form of BL (eBL) occurs

* Corresponding author at: 9609 Medical Center Dr, Rm. 6E118 MSC 9706, Bethesda, MD 20892-9704, USA.

E-mail address: mbulais@mail.nih.gov (S.M. Mbulaiteye).

Research in context

Evidence before this study

Endemic Burkitt lymphoma (eBL) is a germinal centre B-cell tumour that was described in African children >50 years ago and linked to *Plasmodium falciparum* (*Pf*) malaria infection because of similar geographical distributions. This association was confirmed in studies measuring IgG reactivity to malaria schizont antigens. However, those results provided limited insights about malaria-related immunological predisposition because the malaria schizont antigens tested are not known to play a role in protective immunity against malaria. Immunity to malaria is thought to be mediated through acquisition of antibodies targeting and inhibiting human receptor binding by the highly polymorphic malarial *Pf* erythrocyte membrane protein 1 (*PfEMP1*). *PfEMP1* is exported to the erythrocyte surface where it binds endothelial receptors, thereby allowing the parasites to escape blood circulation and destruction in the spleen. To escape immune detection, the *PfEMP1* protein family has diversified into four main types of *PfEMP1*: VAR2CSA *PfEMP1*, expressed by parasites binding chondroitin sulphate A in the placenta; and three types of *PfEMP1* characterized by presence either a CIDR α 1, CIDR α 2–6 or CIDR β / δ / γ domains that bind to EPCR, CD36 or an unknown receptor, respectively, in endothelial tissues. Infection with parasites expressing EPCR-binding *PfEMP1* is associated with severe malaria, whereas acquisition of antibodies to CIDR α 1 correlates with development of immunity to severe malaria. We searched PUBMED with terms (“Burkitt lymphoma” AND “malaria”) to identify studies that have evaluated immune responses to *PfEMP1* antigens in relation to eBL. We found only two papers that reported some data about IgG reactivity to *PfEMP1* antigens in eBL cases and controls. One study tested two *PfEMP1* antigens (DBL- α and VAR2CSA) as part of a 15-malaria antibody Luminex panel, while the other study focused only on reactivity to VAR2CSA. Thus, the relationship between eBL and IgG reactivity to *PfEMP1* antigens, which represent the main functional diversification of *PfEMP1* and mediate severe childhood malaria, remains open for investigation.

Added value of this study

We report the first quantitative and qualitative relationships between eBL and IgG reactivity to 13 *PfEMP1* and 3 non-*PfEMP1* antigens reliably tested using case-control data from Ghana and Uganda. We observed lower IgG reactivity to 13 *PfEMP1* antigens in eBL cases compared to controls — 0.39-fold in Ghana and 0.60-fold in Uganda. These results are not readily explained by selection bias, confounding or reverse causality, as eBL is associated with elevated IgG reactivity to other antigens, particularly Epstein-Barr virus. Our study highlights the potential to untangle the predisposition to eBL through study of immune responses to malaria antigens that play a central role in pathogenesis of severe malaria.

Implications of all the available evidence

Endemic BL develops in a small fraction of children 4–6 years after the general peak age of severe malaria. Our findings of a pattern of attenuated IgG reactivity to *PfEMP1* antigens in eBL children than controls prompt two opposing questions: a) does clinical eBL decrease reactivity to *PfEMP1* antigens and, if so, do children with eBL become more susceptible to malaria infection? or b) is the attenuated reactivity to *PfEMP1* antigens a pattern that precedes eBL and predisposes to recurrent low-grade malaria and eBL risk

in malaria-exposed children. Further research focused on eBL cases and children exposed to heavy malaria may uncover a profile of susceptibility to severe malaria or a profile of attenuated IgG reactivity pattern associated with resistance to severe malaria but susceptibility to recurrent low-grade malaria and eBL risk.

in sub-Saharan African countries, including Ghana and Uganda [2]. The similar distribution of eBL with *Plasmodium falciparum* (*Pf*) malaria infection suggested etiological linkage [3]. A Mendelian randomization analysis using the sickle cell trait as an instrumental variable supported a causal role of malaria in eBL [4]. *Pf* infection increases eBL risk by stimulating polyclonal expansion of B-cells, including those with chromosomal translocations [1].

Although *Pf* causes lethal severe malaria, starting at a median age of 10 months with a peak in children aged 2–3 years old [5], the relationship between immunity to malaria and eBL, which occurs 4–6 years after the peak age of severe malaria, is poorly defined. Severe malaria pathogenesis is closely tied to *Pf* parasite's capacity to sequester infected red blood cells (iRBCs) to the endothelium of the microvasculature [6]. This sequestration allows iRBCs to escape destruction in the spleen and is mediated by *Pf* erythrocyte membrane protein 1 (*PfEMP1*) binding to the host endothelial receptors. *PfEMP1*-derived antigens are targets of acquired immunity to malaria [6], and as a result, the *PfEMP1*-encoding *var* genes have expanded to ~60 different *var* genes, bestowing each parasite a similar repertoire of *PfEMP1* phenotypes. *PfEMP1*s have diversified to four mutually exclusive adhesion phenotypes, associated with different complications of malaria: parasites expressing VAR2CSA *PfEMP1* cause pregnancy-associated malaria by binding placental chondroitin sulfate A (CSA) [7]; CIDR α 1 domain-containing *PfEMP1* bind to endothelial protein C receptor (EPCR) [8] and are expressed by parasites causing severe malaria [9]; *PfEMP1* with CIDR α 2–6 domains bind the leukocyte differentiation antigen (CD36) [10] and are frequently detected in parasites causing mild or asymptomatic malaria infections [11]; and *PfEMP1* with CIDR β / δ / γ domains bind unknown receptors but have not been associated with any specific malaria complication. In addition to blood congestion and local inflammation due to sequestration, parasites expressing the EPCR-binding CIDR α 1 type *PfEMP1*s are thought to aggravate pathogenesis by inhibiting EPCR binding to activated protein C leading to dysfunctional regulation of inflammation, coagulation and endothelial cell integrity [12].

Acquisition of anti-*PfEMP1* antibodies also reflects the association of EPCR-binding *PfEMP1* with severe malaria, as individuals living in malaria endemic areas acquire antibodies to CIDR α 1 antigens before antibodies to other CIDR α variants, i.e., early in life when risk of severe malaria is highest [13]. Because eBL develops at a median age of 6–9 years, after the peak age for severe malaria [2], study of IgG reactivity to *PfEMP1* subsets or to *PfEMP1* in general may shed light on both the nature of malaria immunity and eBL risk. We characterized the repertoire of IgG reactivity to 13 *PfEMP1*, as well as 3 non-*PfEMP1* antigens (Supplementary Table 1) reliably tested, and associations of this repertoire to eBL in children from Ghana and Uganda.

2. Materials and methods

2.1. Study design and participants

We analyzed archival serum/plasma samples from 150 eBL cases and 150 eBL-free controls aged 0–15 years from Ghana (enrolled 1965–1994) [14] and 194 eBL cases and 600 eBL-free controls from Uganda (enrolled 2010–2015) exposed to malaria from birth [4]. The Ghana cases were enrolled at Korle Bu Hospital in Accra and the controls were enrolled contemporaneously from the same village as the cases [14]. Age, sex, and date of enrollment of participants were

recorded and pre-treatment venous samples were collected in serum separation tubes and serum aliquots were prepared and stored at -70°C until testing [15]. The Uganda cases and controls were from the Epidemiology of Burkitt Lymphoma in East African Children and Minors (EMBLEM) in malaria and eBL endemic regions of Northern Uganda [4,16]. The eBL cases were enrolled at two hospitals with facilities to diagnose and treat eBL in Northern Uganda (St. Mary's Hospital, Lacor, in Gulu, and Kuluva Hospital in Arua district). Awareness messages about eBL were disseminated to increase case spotting and referral and diagnosis and treatment of eBL. The controls were healthy children in the same age range as the eBL cases enrolled from 100 random villages in the study area, as described previously [16]. Cases and controls were regular residents of Northern Uganda (at least 4 months prior to enrollment). The season of enrollment and the rural or urban status or proximity to water of the village was captured to enable adjustment for unmeasured factors correlated with climatic or micro-geographical factors [16].

Age, sex, address, and detailed information about lifetime inpatient or outpatient malaria treatment (never, 0–12 months, or >13 months ago) and other household exposures were captured using structured questionnaires administered to both cases and controls. Pre-treatment venous blood specimens were collected in EDTA tubes for research (10 ml) and clinical tests (4 ml). Clinical specimens were immediately tested for malaria using microscopy and malaria-rapid diagnostic tests (malaria-RDTs) [16]. Research specimens were transported in cold boxes to field laboratories within two hours of collection, and centrifuged for 15 min at 1300g to separate plasma, buffy coat, and red cell fractions and stored at -80°C .

2.2. Ethical issues

The Office of Human Subject Research at the National Institutes of Health gave ethical approval to study the anonymized Ghana samples. Ethical approval for the EMBLEM study was given by the Uganda Virus Research Institute Research Ethics Committee, the Uganda National Council for Science and Technology, and the National Cancer Institute Special Studies Institutional Review Board. Written informed consent was obtained from the child's parents and assent from children aged ≥ 7 years old.

2.3. Laboratory procedures

IgG reactivity to 22 *Pf*-derived recombinant antigens was measured in previously unfrozen or minimally thawed serum/plasma samples by a multiplex Luminex bead assay. Tetanus toxoid and bovine serum albumin (BSA) antigens were included in the assay to assess non-malaria-related reactivity. The *Pf* antigens included 18 functional but genetically diverse 3D7 *Pf*EMP1 CIDR domains [8] and 4 non-*Pf*EMP1 proteins (Supplementary Table 1: glutamate-rich protein (GLURP), 6NANP protein, SE36, a recombinant antigen of serine repeat antigen 5 (SERA5), and histidine rich protein-2 (HRP-2)) [14]. The selected *Pf*EMP1 variants represent the functional and sequence diversification of *Pf*EMP1, including 9 EPCR-binding phenotype CIDR α 1 domains with antigens representing all sequence sub-groups CIDR α 1-1.8 domain classes; 6 group B CD36-binding phenotype CIDR α 2.2-3.3 domains; and 3 CIDR δ 1 domains with unknown receptor binding phenotype. The CIDR proteins were produced in a Baculovirus expression system and functionally validated for receptor binding as previously described [10,17,18].

Each protein was covalently coupled to particular fluorescence-coded, carboxylated Luminex microspheres (beads) according to the manufacturer's protocol [17]. Before multiplexing the assay, antigen-bead coupling was validated using standard control serum and Tag-specific antibodies. IgG reactivity of each sample to each antigen was measured using a 96-well plate format Luminex instrument, mixing

beads and plasma diluted 1:80 in Assay Buffer E (ABE; 0.1% BSA, 0.05% Tween-20, 0.05% sodium azide in PBS, pH 7.4) as previously described [17].

Standard reference sera from 40 malaria-naïve individuals from Denmark (DK controls) were included in all runs. IgG reactivity per antigen was reported as median fluorescent intensity (MFI) readings for 100 read beads. Blinded quality control (QC) duplicate samples from 25 Ghanaian children were included to assess variability within and between plates. The case-control and QC status and the country of origin of the samples were masked during testing.

2.4. Statistical analysis

Intra-class correlation coefficients (ICCs) of MFI values were calculated using 24 of 25 successfully tested technical replicates. Antigens with ICCs >70% were included in the analyses. Results for 5 *Pf*EMP1 antigens with ICCs <70% (Supplementary Table 1) and for HRP-2 (total of 6 antigens), whose reactivity in the cases and controls was lower than in the DK controls, were excluded from further analysis. MFI values for the remaining 16 antigens (13 *Pf*EMP1 and 3 non-*Pf*EMP1) were transformed using the natural logarithm before analysis. The threshold of reactivity for each antigen was defined separately for Ghana and Uganda samples using two methods: a) the mean + 2 times the standard deviation of the DK controls [17]; and b) using a flexible two-component mixture models [19] fitted to the MFI values from the controls and the BL-free controls for the respective country. This model assumes that the MFI values for each antigen reflect distributions from two subpopulations with distinct malaria infection experience, but makes no assumptions about normality of the distributions, and maximizes the sum of sensitivity and specificity of the threshold [19]. The models for SE36 did not converge for the Uganda data so the threshold obtained from the Ghana data was used for the Ugandan samples. The two methods yielded similar patterns except for six antigens (Supplementary Table 2), whose model-determined IgG threshold resulted in a prevalence 30–50% higher than that determined by the SD-based method in one or both populations. Model-determined results were considered more realistic for children from these populations, who have been exposed to malaria since birth, and are presented hereafter.

The Ghana and Uganda studies were analyzed separately. IgG reactivity according to basic epidemiological variables was verified separately for cases and controls (results for the controls only are presented: Supplementary Tables 3 and 4). These confirmed no sex differences but higher reactivity with older age, with malaria antigenemia (anti-HRP-2 antibodies in Ghana [14] and RDT in Uganda [16], and without the sickle cell trait in Uganda and Ghana [4]. Chi-squared tests were used to assess differences in reactivity for categorical variables. Odds ratios (ORs) and 95% confidence intervals (CIs) for case-control associations were calculated using logistic regression models with IgG reactivity to malaria antigens coded as a binary variable, as quartiles based on cutoffs determined from the controls, or breadth of reactivity (number of antigens with reactivity above threshold). The basic models were adjusted for age group and year of enrollment for Ghana, and for age group, proximity of village to surface water (near/far), rural/urban status of village [16], and assay plate number for Uganda. We assessed independent effects by including all the antigens with either a marginal two-sided *p*-value for heterogeneity or trend < 0.05 in the same logistic model. For the given sample sizes the study had 80% power to detect an OR of 2.4 for the highest compared to the lowest quartiles of IgG reactivity with an alpha level of 0.05 in Ghana and an OR of 1.9 for the highest compared to the lowest quartile in Uganda. Because our study was conducted to generate hypotheses about the antibody reactivity against *Pf*EMP1 and selected non-*Pf*EMP1 antigens with eBL, we did not formally adjust for multiple comparisons. Two-sided *p* < 0.05 was considered statistically significant.

Table 1
Characteristics of cases and controls in Ghana and the Uganda EMBLEM study.

Characteristics	Ghana			Uganda		
	Controls (%) ^{a,b}	Cases (%) ^a	p value	Controls (%) ^a	Cases (%) ^a	p value
All subjects	149	150		600	194	
Sex			1			0.222
Female	54 (36%)	55 (37%)		271 (45%)	76 (39%)	
Male	95 (64%)	95 (63%)		329 (55%)	115 (59%)	
Age group, years			0.759			0.731
0–2	5 (3%)	2 (1%)		33 (6%)	7 (4%)	
3–5	26 (17%)	29 (19%)		73 (12%)	27 (14%)	
6–8	54 (36%)	62 (41%)		162 (27%)	48 (25%)	
9–11	43 (29%)	36 (24%)		191 (32%)	60 (31%)	
12–14	13 (9%)	13 (9%)		129 (22%)	49 (25%)	
15+	8 (5%)	8 (5%)		12 (2%)	3 (2%)	
Enrolment years			0.042			
1965–1974	53 (36%)	46 (31%)		–	–	
1975–1984	45 (30%)	66 (44%)		–	–	
1985–1994	51 (34%)	38 (25%)		–	–	
2010–2012	–	–		60 (10%)	104 (54%)	
2013–2015	–	–		540 (90%)	90 (46%)	<0.0001
Enrollment season						<0.0001
Dry	–	–		443 (74%)	89 (46%)	
Wet	–	–		157 (26%)	105 (54%)	
Rural/urban						0.840
Rural	–	–		380 (63%)	115 (59%)	
Urban	–	–		220 (37%)	70 (36%)	
Proximity to surface water						<0.0001
Near	–	–		166 (28%)	25 (13%)	
Far	–	–		434 (72%)	160 (82%)	
Region						1
Northwest	–	–		134 (22%)	43 (22%)	
North-central	–	–		466 (78%)	151 (78%)	
Outpatient malaria						<0.0001
In past 12 months	–	–		407 (68%)	88 (45%)	
>12 months ago	–	–		55 (9%)	43 (22%)	
No	–	–		134 (22%)	60 (31%)	
Inpatient malaria						0.0461
In past 12 months	–	–		83 (14%)	15 (8%)	
>12 months ago	–	–		116 (19%)	47 (24%)	
No	–	–		396 (66%)	129 (66%)	
Malaria infection			1			<0.0001
Positive	102 (68%)	103 (69%)		330 (55%)	68 (35%)	
Negative	47 (32%)	47 (31%)		270 (45%)	124 (64%)	
Sickle cell genotype						0.013
AA	–	–		490 (82%)	166 (86%)	
AS/SS	–	–		110 (18%)	19 (10%)	

^a Percentages do not add up to 100% for covariates missing data for certain categories.

^b Quality control failed for IgG results from one control subject, so this subject was excluded from all analyses.

3. Results

3.1. Baseline characteristics of the study participants

As detailed in Table 1, the eBL cases and controls did not differ with respect to sex, and age-group. Qualitatively, IgG reactivity seemed generally stronger in Ghana than in Uganda. IgG reactivity to tetanus toxoid was much lower in Ghana and Ugandan subjects than DK controls (MFI range: 146–2286 versus 752–26,622), likely reflecting higher known tetanus vaccination rates in DK controls compared to African children, or perhaps malaria-related immunosuppression. In Uganda, more cases were enrolled during 2010–2012, whereas most controls were enrolled in 2013–2015 ($P_{\text{heterogeneity}} < 0.0001$). Cases were less likely to be malaria-RDT positive at enrollment ($p < 0.0001$) and to have the sickle cell trait ($p = 0.013$) [4].

3.2. IgG reactivity to PfEMP1-derived antigens in the control children

Among controls only, IgG reactivity to Pf-derived antigens was unrelated to sex but increased significantly with older age, was positively associated with malaria antigenemia, and was inversely associated with carriage of the sickle cell trait (Supplementary Table 4). EPCR-binding

CIDR α 1 antigens (associated with severe malaria) were more often recognized than other CIDR variants. Although children who reported no lifetime inpatient or outpatient malaria treatment may be considered to have been exposed lightly or not at all, we found strong reactivity against EPCR-binding PfEMP1 CIDR α 1 antigens in the controls (Supplementary Table 4), suggesting that these children in Uganda were indeed exposed to malaria and the sparseness of symptoms likely reflects anti-disease immunity (capacity to tolerate parasite infections without developing a fever).

3.3. Association between eBL and IgG reactivity to PfEMP1-derived antigens

As detailed in Table 2, IgG reactivity to 11 of the PfEMP1-derived antigens, and to the 6NANP antigen was lower in eBL cases compared to controls in Ghana. Similarly, Ugandan eBL cases had lower IgG reactivity to three PfEMP1-derived antigens, two of which matched those found in Ghana. Consistently, significant inverse associations between eBL and IgG reactivity, as a binary variable, were observed for all functional types of CIDR, suggesting that reduced IgG reactivity in eBL is not linked to PfEMP1 function. As noted for controls, cases also recognized CIDR α 1 antigens (associated with severe malaria) more commonly than other CIDR domain types. By

Table 2
Association between endemic Burkitt lymphoma and serum IgG reactivity to 16 *P. falciparum* malaria antigens successfully tested in Ghana and Uganda EMBLEM study.

Domain	Binding phenotype	Antigen	Ghana				Uganda			
			% Controls positive ^a	% Cases positive ^a	OR (95% CI) ^b	p value	% Controls positive ^a	% Cases positive ^a	OR (95% CI) ^b	p value
CIDRα1·1	EPCR	<i>Pf</i> EMP1_IT4var06	83%	73%	0.53 (0.30–0.95)	0.031	81%	83%	1.00 (0.62–1.63)	0.987
CIDRα1·1	EPCR	<i>Pf</i> EMP1_raj116_var8	83%	71%	0.50 (0.28–0.89)	0.016	73%	75%	1.00 (0.65–1.54)	0.992
CIDRα1·8a	EPCR	<i>Pf</i> EMP1_GA026	84%	73%	0.50 (0.28–0.90)	0.019	70%	70%	0.83 (0.55–1.25)	0.371
CIDRα1·6b	EPCR	<i>Pf</i> EMP1_GA019	86%	73%	0.41 (0.22–0.76)	0.004	74%	78%	1.02 (0.66–1.56)	0.941
CIDRα1·5	EPCR	<i>Pf</i> EMP1_1965–2	82%	65%	0.39 (0.22–0.70)	0.001	65%	76%	1.68 (1.10–2.56)	0.014
CIDRδ	Unknown	<i>Pf</i> EMP1_HB3var05	79%	60%	0.35 (0.20–0.60)	0.000	59%	59%	0.86 (0.60–1.25)	0.434
CIDRδ	Unknown	<i>Pf</i> EMP1_IT4var02	86%	70%	0.36 (0.20–0.66)	0.001	72%	74%	0.96 (0.63–1.45)	0.836
CIDRα2·2	CD36	<i>Pf</i> EMP1_IT4var24	81%	65%	0.45 (0.26–0.77)	0.003	78%	62%	0.41 (0.28–0.61)	0.000
CIDRα3·3	CD36	<i>Pf</i> EMP1_IT4var26	80%	67%	0.52 (0.30–0.90)	0.018	76%	80%	1.20 (0.77–1.87)	0.418
CIDRα2·9	CD36	<i>Pf</i> EMP1_IT4var30	77%	61%	0.48 (0.29–0.81)	0.006	73%	75%	0.99 (0.65–1.51)	0.955
CIDRα2·4	CD36	<i>Pf</i> EMP1_IT4var33	79%	65%	0.55 (0.32–0.93)	0.026	68%	60%	0.59 (0.40–0.85)	0.006
CIDRα2·9	CD36	<i>Pf</i> EMP1_IT4var45	77%	65%	0.59 (0.35–1.00)	0.051	71%	71%	0.81 (0.54–1.20)	0.297
CIDRα2·9	CD36	<i>Pf</i> EMP1_IT4var61	79%	69%	0.59 (0.34–1.02)	0.056	70%	74%	1.14 (0.75–1.73)	0.544
non- <i>Pf</i> EMP1	–	GLURP_R2	79%	79%	1.06 (0.59–1.88)	0.850	54%	50%	0.70 (0.49–1.00)	0.050
non- <i>Pf</i> EMP1	–	SE36	47%	56%	1.60 (0.99–2.59)	0.053	20%	24%	1.24 (0.82–1.86)	0.310
non- <i>Pf</i> EMP1	–	6NANP	75%	59%	0.47 (0.28–0.78)	0.003	69%	70%	0.90 (0.60–1.36)	0.626
No of antigens with reactivity ^c										
		0–7	15%	23%	Ref		22%	19%	Ref	
		8–12	19%	35%	1.07 (0.52–2.22)	0.856	32%	44%	1.37 (0.83–2.27)	0.215
		13–15	40%	23%	0.34 (0.17–0.69)	0.003	39%	31%	0.76 (0.44–1.31)	0.322
		15+	26%	19%	0.51 (0.24–1.09)	0.083	6%	6%	0.83 (0.35–1.95)	0.667
		X ² trend (P-value)			0.71 (0.56–0.90)	0.004			0.83 (0.66–1.04)	0.108
		X ² Heterogeneity (3df)				0.001				0.034
		IgG reactivity to 13+ antigens ^d	66%	42%	0.39 (0.24–0.63)	0.000	45%	37%	0.60 (0.41–0.88)	0.008

^a IgG reactivity defined as being above the MFI threshold from the mixture model (See Methods). Results for 5 antigens (*Pf*EMP1_1994–2, *Pf*EMP1_HB3var03, *Pf*EMP1_IT4var22, *Pf*EMP1_2083–1, *Pf*EMP1_HB3var35, and HRP-2) that had low reliability results or were unexpectedly low were excluded from analysis (See Methods).

^b Case-control associations with IgG reactivity were determined using logistic regression models, controlling for age group and calendar year enrollment period in Ghana and age group, proximity to water, rural urban status, and plate number in Uganda. Chi-square results are provided assuming linear relationship across categories, based on quartiles of MFI values, or heterogeneity of associations across the quartiles (See Methods).

^c The groupings for the numbers of antigens with reactivity above threshold were determined to assess trends.

^d The grouping of the numbers of antigens with reactivity above threshold was chosen to assess effects among highly reactive children.

country, significant but opposite associations were observed between eBL and IgG reactivity to group A EPCR-binding variant CIDRα1·5, being inverse in Ghana (OR = 0.39, 95% CI 0.22–0.70; $p_{\text{heterogeneity}} = 0.0012$) and positive in Uganda (OR = 1.68, 95% CI 1.10–2.56; $p_{\text{heterogeneity}} = 0.014$). IgG reactivity to GLURP was the most intense, but it was not associated with eBL.

The overall breadth of IgG reactivity to the antigens was lower in the eBL cases than controls in Ghana ($p_{\text{heterogeneity}} = 0.001$) and in Uganda ($p_{\text{heterogeneity}} = 0.034$) (Table 2). IgG reactivity to 13 or more antigens (median among all children) was inversely associated with eBL in Ghana (OR 0.39, 95% CI 0.24–0.63; $p_{\text{heterogeneity}} = 0.0011$) and in Uganda (OR 0.60, 95% CI 0.41–0.88; $p_{\text{heterogeneity}} = 0.008$). Breadth of IgG reactivity was also decreased within groups of CIDR antigens representing EPCR-, or CD36-, or unknown-binding phenotypes in Ghana (CIDRα1: aOR = 0.30, 95% CI 0.14–0.63; $p_{\text{heterogeneity}} = 0.0011$; and CIDRα2·2–2·9: aOR = 0.35, 95% CI 0.17–0.70; $p_{\text{heterogeneity}} = 0.0025$; and CIDRδ: aOR = 0.28, 95% CI 0.14–0.53; $p_{\text{heterogeneity}} \leq 0.0001$, respectively), and with a similar trend for the CD36-binding-phenotypes in Uganda (CIDRα2·2–2·9: aOR = 0.59, 95% CI 0.33–1.06; $p_{\text{heterogeneity}} = 0.077$). Reactivity to SE36 was positively associated with eBL in Ghana ($p_{\text{trend}} = 0.017$) and Uganda ($p_{\text{trend}} = 0.007$).

Finally, in models that included all antigens with univariate eBL associations (13 CIDR types and 2 non-*Pf*EMP1 in Ghana, four CIDR antigens and 2 non-*Pf*EMP1 in Uganda; Tables 3 and 4, last columns), IgG reactivity level, coded as quartiles of MFI, against 14 antigens, including all evaluated CIDR types in Ghana and three antigens in Uganda, was inversely associated with eBL. However, we also found positive associations between eBL and IgG reactivity to one antigen in Ghana (Table 3) and two antigens in Uganda (Table 4), but with no specific association to functional CIDR domain type.

4. Discussion

We investigated the breadth and intensity of IgG reactivity to 13 *Pf*EMP1- and 3 non-*Pf*EMP1-derived antigens reliably tested in children with eBL and similar-age, geographically-matched healthy controls exposed to malaria from birth in Ghana and Uganda. Serum from eBL cases and controls more often reacted on the *Pf*EMP1 CIDRα1 domains associated with severe malaria. Counterintuitively, compared to controls, serum from eBL cases in both Ghana and Uganda showed lower breadth and intensity of IgG reactivity to *Pf*EMP1 CIDR domains. This finding mirrors our previous observation of decreased IgG reactivity to VAR2CSA in a different set of samples from the Ghana study [20]. The lower breadth and intensity of IgG reactivity to *Pf*EMP1-derived antigens affected all *Pf*EMP1 functional domain types in Ghana and Uganda; thus, the reduced IgG reactivity is unrelated to *Pf*EMP1 functional phenotype. We hypothesize that the observed IgG hypo-reactivity is a pattern that presents pre-eBL and this attenuated immunity to malaria predisposes children to breakthrough *Pf* infections, with eBL as a rare complication.

IgG antibodies to *Pf*EMP1 are significantly higher in patients with uncomplicated malaria compared to those with severe malaria [21]. The reduced diversity of *Pf*EMP1 antibodies in our eBL patients suggests that these patients should become more susceptible to severe malaria. However, eBL patients were less likely to have had inpatient or outpatient malaria in the past year, thus the attenuated IgG reactivity to *Pf*EMP1 antigens is not associated with increased risk of severe malaria. Risk of severe malaria is not known to be increased in eBL children. Because children in eBL areas are continually exposed to malaria parasites from birth, their low risk of development of severe malaria could mask the fact that these young children are heavily exposed and maintain a more chronic, but uncomplicated type of malaria with smoldering inflammation [12]. This would be sufficient to maintain a high B cell

Table 3
Association between endemic Burkitt lymphoma and IgG reactivity to *P. falciparum* malaria antigens successfully tested in Ghana.

PfEMP group	Domain class	Binding phenotype	PfEMP1 assay	Ghana			
				Controls (%)	Cases (%)	OR (95% CI) ^a	Adjusted OR (95% CI) ^b
A	CIDRαCIDRα1.1	EPCR	PfEMP1_IT4var06				
			Q1	24%	41%	Ref.	Ref.
			Q2	25%	25%	0.60 (0.32–1.12)	0.80 (0.3–2.18)
			Q3	25%	18%	0.44 (0.23–0.85)	0.76 (0.22–2.62)
			Q4	26%	17%	0.37 (0.19–0.72)	0.41 (0.1–1.72)
			X ² trend (P-value)	.	.	0.001	0.232
A	CIDRαCIDRα1.1	EPCR	PfEMP1_raj116_var8				
			Q1	24%	40%	Ref.	Ref.
			Q2	25%	27%	0.65 (0.35–1.22)	1.13 (0.4–3.17)
			Q3	25%	17%	0.39 (0.20–0.77)	0.86 (0.23–3.22)
			Q4	26%	16%	0.37 (0.19–0.73)	0.9 (0.18–4.4)
			X ² trend (P-value)	.	.	0.001	0.832
A	CIDRαCIDRα1.8a	EPCR	PfEMP1_GA026				
			Q1	24%	37%	Ref.	Ref.
			Q2	25%	28%	0.67 (0.36–1.26)	1.1 (0.44–2.73)
			Q3	25%	17%	0.45 (0.23–0.89)	1.6 (0.48–5.36)
			Q4	26%	18%	0.46 (0.24–0.9)	2.14 (0.53–8.71)
			X ² trend (P-value)	.	.	0.009	0.276
A	CIDRαCIDRα1.6b	EPCR	PfEMP1_GA019				
			Q1	24%	46%	Ref.	Ref.
			Q2	25%	20%	0.41 (0.22–0.79)	0.8 (0.28–2.28)
			Q3	25%	19%	0.41 (0.22–0.79)	0.69 (0.22–2.17)
			Q4	26%	15%	0.29 (0.15–0.57)	0.55 (0.14–2.08)
			X ² trend (P-value)	.	.	0.000	0.373
A	CIDRαCIDRα1.5	EPCR	PfEMP1_1965–2				
			Q1	24%	45%	Ref.	Ref.
			Q2	25%	23%	0.48 (0.25–0.91)	0.57 (0.22–1.46)
			Q3	25%	14%	0.27 (0.13–0.55)	0.42 (0.14–1.26)
			Q4	26%	17%	0.33 (0.17–0.66)	0.66 (0.2–2.18)
			X ² trend (P-value)	.	.	0.000	0.495
A	CIDRδ	Unknown	PfEMP1_HB3var05				
			Q1	24%	43%	Ref.	Ref.
			Q2	25%	20%	0.42 (0.22–0.8)	0.46 (0.18–1.17)
			Q3	25%	24%	0.48 (0.25–0.91)	0.63 (0.22–1.84)
			Q4	26%	13%	0.24 (0.11–0.49)	0.22 (0.06–0.73)
			X ² trend (P-value)	.	.	0.000	0.035
A	CIDRδ	Unknown	PfEMP1_IT4var02				
			Q1	24%	40%	Ref.	Ref.
			Q2	25%	25%	0.60 (0.32–1.12)	1.09 (0.4–2.96)
			Q3	25%	21%	0.49 (0.26–0.94)	0.86 (0.29–2.58)
			Q4	26%	14%	0.31 (0.16–0.62)	0.85 (0.23–3.13)
			X ² trend (P-value)	.	.	0.001	0.687
B	CIDRα2.2	CD36	PfEMP1_IT4var24				
			Q1	24%	37%	Ref.	Ref.
			Q2	25%	20%	0.51 (0.26–0.98)	0.52 (0.19–1.41)
			Q3	25%	29%	0.83 (0.45–1.55)	2.76 (0.8–9.52)
			Q4	26%	15%	0.38 (0.19–0.75)	1.04 (0.23–4.8)
			X ² trend (P-value)	.	.	0.029	0.455
B	CIDRα3.3	CD36	PfEMP1_IT4var26				
			Q1	24%	40%	Ref.	Ref.
			Q2	25%	27%	0.64 (0.35–1.2)	1.44 (0.53–3.95)
			Q3	25%	14%	0.34 (0.17–0.68)	0.46 (0.15–1.46)
			Q4	26%	19%	0.47 (0.24–0.9)	0.79 (0.24–2.58)
			X ² trend (P-value)	.	.	0.006	0.328
B	CIDRα2.9	CD36	PfEMP1_IT4var30				
			Q1	24%	39%	Ref.	Ref.
			Q2	25%	27%	0.63 (0.34–1.18)	0.88 (0.32–2.37)
			Q3	25%	17%	0.44 (0.23–0.87)	1.12 (0.36–3.46)
			Q4	26%	16%	0.40 (0.20–0.78)	0.93 (0.27–3.22)
			X ² trend (P-value)	.	.	0.003	0.976
B	CIDRα2.4	CD36	PfEMP1_IT4var33				
			Q1	24%	43%	Ref.	Ref.
			Q2	25%	26%	0.65 (0.35–1.2)	0.52 (0.18–1.45)
			X ² Heterogeneity (3df) (P-value)	.	.	0.025	0.958
			X ² trend (P-value)	.	.	0.001	0.410
			X ² Heterogeneity (3df) (P-value)	.	.	0.001	0.410

(continued on next page)

Table 3 (continued)

PfEMP1 group	Domain class	Binding phenotype	PfEMP1 assay	Ghana			
				Controls (%)	Cases (%)	OR (95% CI) ^a	Adjusted OR (95% CI) ^b
B	CIDRα2-9	CD36	Q3	25%	14%	0.35 (0.17–0.69)	0.25 (0.07–0.89)
			Q4	26%	17%	0.41 (0.21–0.8)	0.75 (0.17–3.41)
			X ² trend (P-value)	.	.	0.002	0.481
			X ² _{Heterogeneity} (3df) (P-value)	.	.	0.008	0.057
			PfEMP1_IT4var45				
			Q1	24%	35%	Ref.	Ref.
			Q2	25%	28%	0.79 (0.42–1.49)	1.35 (0.43–4.27)
			Q3	25%	23%	0.69 (0.36–1.32)	2.4 (0.62–9.3)
			Q4	26%	13%	0.38 (0.19–0.78)	1.06 (0.21–5.43)
			X ² trend (P-value)	.	.	0.010	0.779
B	CIDRα2-9	CD36	X ² _{Heterogeneity} (3df) (P-value)	.	.	0.059	0.313
			PfEMP1_IT4var61				
			Q1	24%	38%	Ref.	Ref.
			Q2	25%	29%	0.75 (0.41–1.4)	0.86 (0.32–2.34)
			Q3	25%	18%	0.45 (0.23–0.88)	1.07 (0.34–3.37)
			Q4	26%	15%	0.43 (0.21–0.85)	0.97 (0.24–3.86)
			X ² trend (P-value)	.	.	0.004	0.911
			X ² _{Heterogeneity} (3df) (P-value)	.	.	0.034	0.966
			GLURP_R2				
			Q1	24%	23%	Ref.	–
–	–	–	Q2	25%	33%	1.44 (0.75–2.76)	–
			Q3	25%	18%	0.70 (0.34–1.43)	–
			Q4	26%	26%	1.09 (0.56–2.13)	–
			X ² trend (P-value)	.	.	0.677	–
			X ² _{Heterogeneity} (3df) (P-value)	.	.	0.211	–
			SE36				
			Q1	24%	29%	Ref.	Ref.
			Q2	25%	9%	0.31 (0.14–0.67)	0.30 (0.11–0.79)
			Q3	25%	38%	1.43 (0.76–2.67)	2.31 (1.01–5.3)
			Q4	26%	25%	0.92 (0.48–1.77)	1.77 (0.69–4.53)
–	–	–	X ² trend (P-value)	.	.	0.391	0.017
			X ² _{Heterogeneity} (3df) (P-value)	.	.	0.001	0.000
			6NANP				
			Q1	24%	39%	Ref.	Ref.
			Q2	25%	27%	0.64 (0.34–1.19)	0.92 (0.32–2.69)
			Q3	25%	18%	0.44 (0.23–0.86)	0.53 (0.15–1.86)
			Q4	26%	16%	0.38 (0.19–0.76)	0.59 (0.16–2.12)
			X ² trend (P-value)	.	.	0.002	0.342
			X ² _{Heterogeneity} (3df) (P-value)	.	.	0.022	0.658

^a Case-control associations with IgG reactivity to malaria antigens determined using logistic regression. IgG reactivity is coded as quartiles 0, 1, 2, and 3 estimated from MFI values in the country-specific controls, and the results are controlled for age group and calendar year enrollment period in Ghana (minimally adjusted models).

^b Case-control associations with IgG reactivity to malaria antigens determined using logistic regression with additional adjustment for antigens associated with eBL at a $p < 0.05$ based on trend or heterogeneity analysis in the minimally adjusted models.

proliferative index in the germinal center reaction and increase the load of proliferating B cells with primary deleterious IG-MYC translocations. There is some evidence that PfEMP1 on infected iRBC membranes activates lytic EBV replication [22], a known carcinogen of eBL. However, as outlined in a later paper from same authors [23], it was not clear which PfEMP1 type was expressed by the parasites activating EBV, and thus if the EBV activation was specific to any specific type of PfEMP1. Regardless of which actual protein is involved, EBV and Pf appear to synergistically deregulate adenosine-induced cytosine deaminase (AICDA) expression, which plays a key role in generating aberrant somatic hypermutation to generate DNA changes that contribute to progression of B-cells with IG-MYC translocations to eBL. Repeated cycles of parasite-driven inflammation and germinal center reactions could also lead to increased PD-1 expression on T follicular helper cells, a form of T cell exhaustion [24]. This can lead to hyperproliferative B cell phenotypes including hypergammaglobulinemia [25], a condition characterized by increased poly-specific IgG but lack of antigen-specific IgG, probably due to defective formation of memory B cells [26]. Interestingly, PD-1-dependent exhaustion of CD8+ T cells has been associated with chronic malaria [24].

Because malaria is deadly and eBL occurs in older children, another hypothesis is that eBL occurs in children who are more resistant to severe malaria, possibly manifested by the attenuated IgG reactivity observed in eBL children. We found a report on Tanzanian children without eBL who never developed patent parasitemia during follow-

up from birth [5]. These malaria-resistant children were similar to those who were non-resistant for seroprevalence of sporozoite stage antibody responses and, similar to our eBL cases, had lower IgG reactivity to three non-PfEMP1 antigens [5]. Our finding that eBL cases were less likely to have malaria-antigenemia supports the idea that eBL cases can control patent malaria. We found similar reports from a study in Kenya [27] and a prospective study in Uganda [28], with results being dismissed by the author's assumption that eBL cases were more likely to have been treated with anti-malarial drugs prior to admission. Our results are based on malaria-RDT, which remains positive for approximately 35–42 days after treatment of symptomatic malaria due to persistent malaria antigenemia [29], and were supplemented by questionnaires data, which show a lower frequency of inpatient and outpatient malaria treatment in the cases one year before eBL onset.

The reduced diversity of PfEMP1 antibodies in malaria-resistant (or eBL) children may be due to dysfunction in the T cell compartment as a result of chronic malaria infection [24]. The reduced diversity of PfEMP1 antibodies raises the question whether protection against malaria disease in the eBL children is less dependent on antibodies. Those children may have stronger immunity against liver stage Pf forms, thereby reducing the load of liver-stage merozoites released into the blood stream. Another possibility is that they utilize non-humoral mechanisms to control blood-stage infection. Such mechanisms may include soluble EPCR [12], CD36 on platelets [10], altered regulation of cytokines released by Natural Killer (NK) cells [30], or $\gamma\delta$ T cells [31].

Table 4

Association between endemic Burkitt lymphoma and IgG reactivity to *P. falciparum* malaria antigens successfully tested in Uganda EMBLEM study.

PjEMP group	Domain class	Binding phenotype	PjEMP1 assay	Uganda			
				Controls (%)	Cases (%)	OR (95% CI) ^a	Adjusted OR (95% CI) ^b
A	CIDRαCIDRα1.1	EPCR	PjEMP1_IT4var06				
			Q1	25%	24%	Ref.	–
			Q2	25%	24%	0.86 (0.52–1.43)	–
			Q3	25%	27%	1.02 (0.62–1.67)	–
			Q4	25%	25%	0.91 (0.55–1.51)	–
			X ² trend (P-value)	.	.	0.915	–
A	CIDRαCIDRα1.1	EPCR	X ² Heterogeneity (3df) (P-value)	.	.	0.893	–
			PjEMP1_raj116_var8				
			Q1	25%	23%	Ref.	–
			Q2	25%	24%	0.97 (0.58–1.64)	–
			Q3	25%	26%	1.02 (0.6–1.73)	–
			Q4	25%	27%	1.15 (0.68–1.93)	–
A	CIDRαCIDRα1.8a	EPCR	X ² trend (P-value)	.	.	0.540	–
			X ² Heterogeneity (3df) (P-value)	.	.	0.910	–
			PjEMP1_GA026				
			Q1	25%	24%	Ref.	–
			Q2	25%	28%	1 (0.61–1.66)	–
			Q3	25%	24%	0.81 (0.48–1.38)	–
A	CIDRαCIDRα1.6b	EPCR	Q4	25%	25%	0.83 (0.49–1.41)	–
			X ² trend (P-value)	.	.	0.363	–
			X ² Heterogeneity (3df) (P-value)	.	.	0.762	–
			PjEMP1_GA019				
			Q1	25%	22%	Ref.	–
			Q2	25%	28%	1.05 (0.64–1.72)	–
A	CIDRαCIDRα1.5	EPCR	Q3	25%	29%	1.04 (0.62–1.73)	–
			Q4	25%	21%	0.8 (0.47–1.37)	–
			X ² trend (P-value)	.	.	0.414	–
			X ² Heterogeneity (3df) (P-value)	.	.	0.686	–
			PjEMP1_1965–2				
			Q1	25%	12%	Ref.	Ref.
A	CIDRδ	Unknown	Q2	25%	40%	2.74 (1.56–4.8)	3.91 (2.08–7.34)
			Q3	25%	25%	1.95 (1.07–3.54)	3.0 (1.5–6.01)
			Q4	25%	23%	1.78 (0.96–3.28)	3.47 (1.63–7.36)
			X ² trend (P-value)	.	.	0.544	0.034
			X ² Heterogeneity (3df) (P-value)	.	.	0.003	0.000
			PjEMP1_HB3var05				
A	CIDRδ	Unknown	Q1	25%	23%	Ref.	–
			Q2	25%	24%	1.04 (0.63–1.72)	–
			Q3	25%	28%	1.06 (0.64–1.76)	–
			Q4	25%	25%	0.91 (0.53–1.53)	–
			X ² trend (P-value)	.	.	0.731	–
			X ² Heterogeneity (3df) (P-value)	.	.	0.918	–
A	CIDRδ	Unknown	PjEMP1_IT4var02				
			Q1	25%	22%	Ref.	–
			Q2	25%	31%	1.18 (0.71–1.97)	–
			Q3	25%	25%	0.92 (0.54–1.58)	–
			Q4	25%	22%	0.86 (0.49–1.5)	–
			X ² trend (P-value)	.	.	0.349	–
B	CIDRα2.2	CD36	X ² Heterogeneity (3df) (P-value)	.	.	0.575	–
			PjEMP1_IT4var24				
			Q1	25%	39%	Ref.	Ref.
			Q2	25%	25%	0.58 (0.37–0.93)	0.58 (0.34–0.98)
			Q3	25%	19%	0.42 (0.26–0.68)	0.48 (0.26–0.88)
			Q4	25%	17%	0.37 (0.22–0.61)	0.38 (0.19–0.77)
B	CIDRα3.3	CD36	X ² trend (P-value)	.	.	0.000	0.006
			X ² Heterogeneity (3df) (P-value)	.	.	0.000	0.038
			PjEMP1_IT4var26				
			Q1	25%	20%	Ref.	–
			Q2	25%	32%	1.55 (0.95–2.53)	–
			Q3	25%	22%	0.94 (0.55–1.61)	–
B	CIDRα2.9	CD36	Q4	25%	26%	1.1 (0.63–1.89)	–
			X ² trend (P-value)	.	.	0.698	–
			X ² Heterogeneity (3df) (P-value)	.	.	0.152	–
			PjEMP1_IT4var30				
			Q1	25%	25%	Ref.	–
			Q2	25%	22%	0.8 (0.48–1.33)	–
B	CIDRα2.4	CD36	Q3	25%	27%	0.93 (0.56–1.55)	–
			Q4	25%	26%	0.93 (0.55–1.56)	–
			X ² trend (P-value)	.	.	0.955	–
			X ² Heterogeneity (3df) (P-value)	.	.	0.847	–
			PjEMP1_IT4var33				
			Q1	25%	34%	Ref.	Ref.
B	CIDRα2.4	CD36	Q2	25%	25%	0.62 (0.39–1)	0.56 (0.31–1.01)

(continued on next page)

Table 4 (continued)

PfEMP1 group	Domain class	Binding phenotype	PfEMP1 assay	Uganda			
				Controls (%)	Cases (%)	OR (95% CI) ^a	Adjusted OR (95% CI) ^b
B	CIDRα2-9	CD36	Q3	25%	24%	0.51 (0.31–0.84)	0.52 (0.26–1.03)
			Q4	25%	18%	0.4 (0.23–0.68)	0.41 (0.19–0.89)
			X ² trend (P-value)	.	.	0.000	0.033
			X ² Heterogeneity (3df) (P-value)	.	.	0.005	0.121
			PfEMP1_IT4var45				
			Q1	25%	25%	Ref.	Ref.
			Q2	25%	32%	0.99 (0.61–1.59)	1.37 (0.76–2.46)
			Q3	25%	21%	0.62 (0.37–1.05)	1.36 (0.65–2.82)
			Q4	25%	22%	0.66 (0.39–1.12)	1.8 (0.8–4.04)
			X ² trend (P-value)	.	.	0.041	0.189
B	CIDRα2-9	CD36	X ² Heterogeneity (3df) (P-value)	.	.	0.129	0.521
			PfEMP1_IT4var61				
			Q1	25%	22%	Ref.	–
			Q2	25%	24%	0.97 (0.57–1.63)	–
			Q3	25%	24%	0.95 (0.56–1.61)	–
			Q4	25%	30%	1.41 (0.83–2.39)	–
			X ² trend (P-value)	.	.	0.183	–
			X ² Heterogeneity (3df) (P-value)	.	.	0.310	–
			GLURP_R2				
			Q1	25%	26%	Ref.	–
–	–	–	Q2	25%	27%	0.88 (0.55–1.42)	–
			Q3	25%	24%	0.79 (0.48–1.3)	–
			Q4	25%	23%	0.66 (0.39–1.09)	–
			X ² trend (P-value)	.	.	0.096	–
			X ² Heterogeneity (3df) (P-value)	.	.	0.420	–
			SE36				
			Q1	25%	20%	Ref.	Ref.
			Q2	25%	21%	1.12 (0.67–1.9)	1.14 (0.65–2)
			Q3	25%	27%	1.47 (0.88–2.43)	1.69 (0.98–2.92)
			Q4	25%	32%	1.61 (0.98–2.65)	1.92 (1.1–3.36)
X ² trend (P-value)	.	.	0.032	0.007			
–	–	–	X ² Heterogeneity (3df) (P-value)	.	.	0.190	0.054
			6NANP				
			Q1	25%	22%	Ref.	Ref.
			Q2	25%	34%	1.23 (0.75–2)	0.94 (0.52–1.68)
			Q3	25%	28%	1.09 (0.65–1.83)	0.94 (0.49–1.82)
			Q4	25%	17%	0.62 (0.35–1.12)	0.54 (0.26–1.15)
			X ² trend (P-value)	.	.	0.084	0.109
			X ² Heterogeneity (3df) (P-value)	.	.	0.056	0.199

^a Case-control associations with IgG reactivity to malaria antigens determined using logistic regression. IgG reactivity is coded as quartiles 0, 1, 2, and 3 estimated from MFI values in the country-specific controls, and the results are controlled for age group, proximity to water, rural urban status, and plate number in Uganda (minimally adjusted models).

^b Case-control associations with IgG reactivity to malaria antigens determined using logistic regression with additional adjustment for antigens associated with eBL at a $p < 0.05$ based on trend or heterogeneity analysis in the minimally adjusted models.

Parasites could also down-regulate antibody response through expression of inhibitory receptors, such as RIFINs, to dampen detrimental B-cell-mediated immune response and render the host more hospitable to parasites [32]. Finally, co-infections, especially Epstein-Barr virus (EBV), which has been reported to induce suppression of humoral immunity to malaria [33], may down-modulate malaria-related inflammation in a manner beneficial to the host, parasite and virus.

We considered alternative explanations, including whether eBL cases were exposed to malaria to the same degree as the controls, or whether eBL is associated with impaired humoral immunity. While the case-control design does not allow assessment of temporality of the associations, it is an efficient design, and we closely matched the eBL cases and controls geographically. We found that the Ugandan eBL cases were more likely than their matched controls to be exposed to malaria, based on enrolment during the wet season and being more likely to report recent (one year before onset of symptoms) outpatient (22% versus 9%) and inpatient (24% versus 19%) malaria treatment. Whether eBL is associated with decrease in plasma IgG reactivity, due to increased catabolism or loss in urine, cannot be excluded. There are multiple reports of lower levels of total and malaria-specific IgG, IgM and IgA antibodies in eBL cases than controls in Uganda, Ghana, and Nigeria, and lower IgG response to measles in Uganda (reviewed in [14]). However, the reported lower levels of IgG, IgA, and IgM did not change when the cases were monitored before treatment and through long-term remission in Uganda and Ghana. We also note that eBL cases are more likely to have

higher IgG reactivity to EBV capsid antigens [28] and to other malaria antigens (*Pf* whole schizont extract and HRP-2 protein [14]). Behavior change after eBL diagnosis, such as hospitalizations associated with treatment for malaria or mosquito bed-net usage may reduce exposure to malaria and loss of immunological memory response, but this explanation is not supported by our finding that recent inpatient and outpatient malaria treatment was less frequent in eBL cases than controls; overall, all biases cannot be eliminated. However, we adjusted for other factors, including age, geographical variables (season, rural-urban status, proximity of village to water) and sickle cell trait, malaria antigenemia in sensitivity analyses, to increase the validity of our findings [4]. The strengths of this study are the use of a broad panel of antigen targets of immunity to malaria representing all main functional types of PfEMP1 variants and the utilization of well-matched samples from two geographically distinct studies. The use of samples from two populations is a strength because the consistent findings, despite apparent unexplained differences in sample variability (Ghana samples seemed to have less variability than the Ugandan samples), suggest that the observed patterns are likely valid. Our findings for SE36, a recombinant protein comprising the N-terminal of SERA5, conflict with those from our previous study in Ghana in which antibody levels to SE36 was found to be lower in eBL cases compared to controls [14]. This might be due to differences in the antigens used (*Pf* Honduras 1 versus 3D7 *Pf*).

In conclusion, we found significant inverse associations between eBL and a narrower breadth and intensity of reactivity to PfEMP1-derived

antigens in Ghana and Uganda. These findings suggest that attenuated humoral immunity to Pf may be an eBL risk factor for children who remain exposed to malaria.

Data sharing

The de-identified data, including individual participant data and a data dictionary defining each field can be requested from the authors via the request for collaboration link on the EMBLEM website: <https://www.emblem.cancer.gov/>.

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Declaration of interests

We declare no competing interest

Author contributors

SMM and TL conceived the idea, designed the study and supervised the work. IO, IDL, HN, MDO, KB, SJR and JJG conducted and monitored EMBLEM field work. AOT and LWA consulted in study design and diagnostic aspects of the fieldwork. FN and JN conducted fieldwork in Ghana. TGT, LT, and TL conducted laboratory studies of PfEMP1 and interpreted data. LPO conducted genetic studies of sickle cell genotypes in EMBLEM and Ghana. AD and RMP conducted statistical analysis and interpreted data. SMM drafted the manuscript; JJG, OOO and LPO helped with critical editing. All authors contributed to the manuscript and read and approved the final manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ebiom.2018.12.020>.

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