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Acquisition of Fc-afucosylation of PfEMP1-specific IgG is age-dependent and associated with clinical protection against malaria

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Protective immunity to malaria depends on acquisition of parasite-specific antibodies, with *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) being one of the most important target antigens. The effector functions of PfEMP1-specific IgG include inhibition of infected erythrocyte (IE) sequestration and opsonization of IEs for cell-mediated destruction. IgG glycosylation modulates antibody functionality, with increased affinity to FcγRIIIa for IgG lacking fucose in the Fc region (Fc-afucosylation). We report here that selective Fc-afucosylation of PfEMP1-specific IgG1 increases with age in *P. falciparum*-exposed children and is associated with reduced risk of anemia, independent of the IgG levels. A similar association was found for children having PfEMP1-specific IgG1 inducing multiple effector functions against IEs, particularly those associated with antibody-dependent cellular cytotoxicity (ADCC) by NK cells. Our findings provide new insights regarding protective immunity to *P. falciparum* malaria and highlight the importance of cell-mediated destruction of IgG-opsonized IEs.

P lasmodium falciparum remains the leading cause of severe malaria and malaria-associated mortality, particularly affecting young children and pregnant women in sub-Saharan Africa[1], with anemia as the main clinical manifestation of severe malaria among children[2]. The virulence of *P. falciparum* can be attributed partly to the unique ability of infected erythrocytes (IEs) to adhere to specific vascular host receptors (cytoadhesion/sequestration). This allows IEs to escape spleen-mediated clearance but also causes tissue inflammation, circulatory disturbances, and organ dysfunction that can be life-threatening. The *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family of parasite adhesins is the key ligand mediating IE sequestration[3,4]. PfEMP1 variants categorized as Group A and B/A have been associated with severe malaria in children, whereas parasites expressing VAR2CSA-type PfEMP1 cause placental malaria[3,4].

The development of protective immunity against *P. falciparum* malaria following infection by the parasites primarily relies on antibody-mediated mechanisms to asexual-stage antigens[5], especially the variant surface antigens expressed on IEs[6,7] with PfEMP1 being the

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dominant target[8,9]. Group A-specific IgG is acquired early in life, whereas VAR2CSA-specific IgG is only acquired following *P. falciparum*-exposed pregnancies[10,11]. Antibodies to merozoite antigens such as glutamate-rich protein (GLURP), merozoite surface protein 1 (MSP1), and reticulocyte-binding protein homolog 5 (PfRh5) also contribute to naturally acquired protection[12–15]. The effector functions of PfEMP1-specific IgG include neutralization (blocking IE cytoadhesion and rosetting)[16,17] and opsonization of IEs for cell-mediated destruction via antibody-dependent cellular phagocytosis (ADCP)[17–19] and antibody-dependent cellular cytotoxicity (ADCC)[20–22].

ADCP and ADCC require binding of the target-bound IgG to Fcγ receptors (FcγR) on the effector cells, and the affinity of IgG1 and IgG3 to FcRγIIIa/b is affected by the composition of the biantennary glycan at asparagine 297 (N297) in the Fc region[23]. In total human plasma IgG, this glycan typically contains fucose (~94%), is partly galactosylated (~40%), carries in part bisecting GlcNAc (~10-30%), and contains little sialic acid (~4%)[24,25]. However, IgG specific for certain antigens can be dominated by distinctive IgG glycovariants[23]. For instance, IgG specific for enveloped viruses and some alloantigens are markedly Fc-afucosylated[26], resulting in enhanced ADCC due to the up to 20-fold increased affinity of afucosylated IgG for FcγRIIIa[27,28].

Galactosylation can further increase this affinity two-fold[28] and enhance complement binding and activation[28,29]. The increased affinity can result in an even larger increase of effector functions from absent to considerable[21,27,28] and can lead to better antiviral control in HIV infections[30], but is also associated with immunopathology in dengue hemorrhagic fever[31] and SARS-CoV-2 infections[32].

Recently, we reported that PfEMP1-specific IgG1 in Ghanaian women exposed to *P. falciparum* is strongly Fc-afucosylated[21]. Uniform and marked Fc-afucosylation was observed for IgG specific for the Group A-type PfEMP1 HB3VAR06, whereas the degree of Fc-afucosylation of IgG specific for the VAR2CSA-type PfEMP1 antigen IT4VAR04 exhibited greater variation. This variation was associated with parity and exposure to the antigen, in contrast to HB3VAR6-specific IgG, a non-pregnancy-restricted PfEMP1 variant representative of this family. Those findings led us to hypothesize that the uniformly Fc-afucosylated HB3VAR06-specific IgG in the studied women was due to their repeated exposure to that antigen in childhood. We further hypothesized that Fc-afucosylation of the antigen-specific IgG is a stable phenotype once acquired and a determinant of clinical protection against malaria. Here, we investigate these hypotheses by assessing the Fc-glycosylation of antibodies in the plasma from children naturally exposed to *P. falciparum*, its impact on antibody effector functions in vitro, and its relation to clinical protection against malaria-related morbidity.

Results

Acquisition of P. falciparum-specific IgG is age-dependent and dominated by IgG1 and IgG3

We analyzed IgG levels and IgG1-Fc glycosylation features of 210 plasma samples collected from Ghanaian children between 2 and 15 years-of-age[33] (Fig. 1 and Supplementary Table 1). None of the children had any clinical symptoms of malaria, but 21% carried PCR-detectable *P. falciparum* infections[33]. Two-thirds of the children were anemic, but only nine children had severe anemia. Levels of *P. falciparum*-specific IgG increase with age among individuals living in areas with stable transmission of these parasites, likely due to increased cumulative parasite exposure. In agreement with this, we found that levels of IgG specific for the merozoite protein GLURP[34] and for the Group A PfEMP1 antigen HB3VAR06[35] increased with age (Fig. 2a) and were significantly higher among children with current *P. falciparum* infection (Fig. 2b). In agreement with previous studies[14,19,36], the cytophilic (FcγR-binding) subclasses IgG1 and IgG3 dominated the PfEMP1-specific antibody response (Fig. 2c, d) and levels increased with age (Fig. 2e).

PfEMP1-specific IgG is selectively Fc-afucosylated

In addition to the IgG subclass, the glycan composition at position N297 in the Fc region also affects IgG affinity for FcγR and, thus, its effector functions[28]. HB3VAR06-specific IgG1 was Fc-afucosylated (median 66% [interquartile range: 49% to 85%]; $p < 0.001$) (Fig. 3), although to a lesser and more variable degree than previously observed among adults[21]. In contrast, GLURP-specific IgG in the children was fully Fc-fucosylated (Fig. 3), as also reported for adults previously[21]. These findings support the hypothesis that Fc-afucosylation occurs in response to foreign antigens expressed on host membranes[32]. Both GLURP- and HB3VAR06-specific IgG1 were more galactosylated and sialylated at N297 than total IgG, similar to adults[21], and contained less bisecting GlcNAc than total IgG (Fig. 3). There were no differences between children with and without PCR-detectable *P. falciparum* infection at the time of sample collection (Supplementary Fig. 1a) or among children with different Hb phenotypes (Supplementary Fig. 1b) regarding the patterns mentioned above.

Acquisition of Fc-afucosylation of PfEMP1-specific IgG is age-dependent

HB3VAR06-specific IgG1 Fc-fucosylation correlated negatively with age (Fig. 4a), and this association remained after adjusting for levels of HB3VAR06-specific IgG and Hb phenotype ($\beta = -2.83$; $p < 0.001$). Galactosylation increased with age while bisecting GlcNAc decreased. Including our previously published data from adults[21], it appears that the Fc-fucosylation of HB3VAR06-specific IgG1 stabilizes at low levels

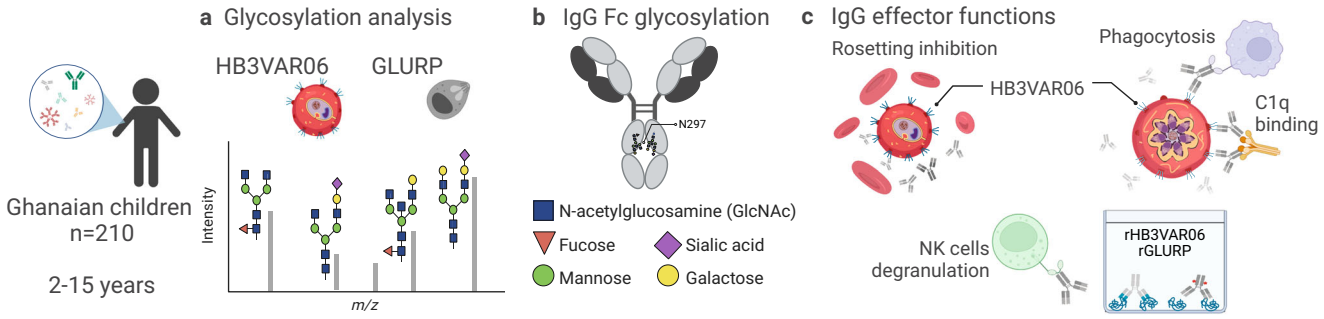

Fig. 1 | Study workflow. We analyzed antibodies specific for the merozoite antigen GLURP and HB3VAR06, a Group A PfEMP1 protein expressed on the surface of IEs, which has been associated with severe malaria in children[35]. **a** Glycosylation profiles of antigen-specific IgG were analyzed by liquid chromatography-mass spectrometry (LC-MS) as described before[21]. **b** Schematic representation of IgG1 Fc-glycosylation at position N297 with color code. **c** IgG effector functions were assessed by flow cytometry using IEs expressing HB3VAR06: rosetting inhibition, C1q binding, and phagocytosis. ADCC by NK cells was assessed using HB3VAR06 or GLURP recombinant proteins. Panels (**a**–**c**) created in BioRender. Lopez-Perez, M. (2023) https://BioRender.com/x61f738.

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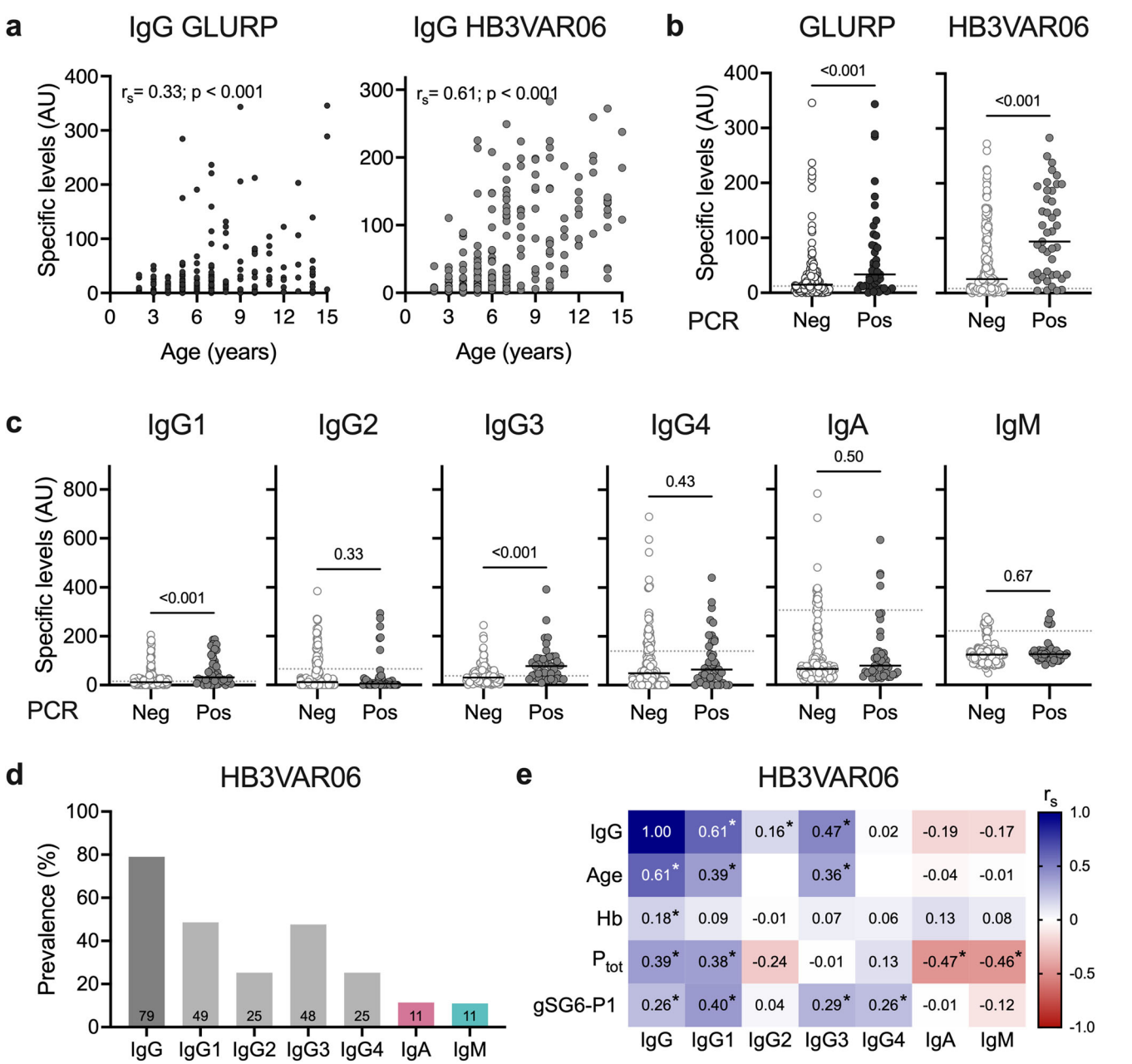

Fig. 2 | IgG1 and IgG3 dominate antibody response to PfEMP1. a Correlation between GLURP- or HB3VAR06-specific IgG levels and age. Spearman's rank correlation (r_s) and p values are shown. **b** Specific IgG levels against recombinant GLURP and HB3VAR06 in plasma samples from children ($n = 210$) determined by ELISA. **c** HB3VAR06-specific levels of IgG1, IgG2, IgG3, IgG4, IgA, and IgM in plasma samples from children ($n = 210$). **b**, **c** Values are expressed as arbitrary units (AU) in children with PCR-negative (open symbols; $n = 167$) and -positive (closed symbols; $n = 43$) for *P. falciparum* infection. Medians and *p*-values using the two-tailed Mann-Whitney test are shown. Negative cut-off values obtained from adults without malaria exposure (NIP) are shown as dotted lines and used to calculate seroprevalence. **d** Seroprevalence of HB3VAR06-specific antibody isotypes (IgG, IgM, and IgA) and subclasses (IgG1, IgG2, IgG3, and IgG4). **e** Correlation matrix of antibody response to HB3VAR06 and children metadata. Positive correlations using Spearman's rank correlation (r_s) are displayed in blue and negative correlations in red (r_s color key). *Indicates a *p*-value < 0.05. gSG6-P1, exposure marker; Hb, hemoglobin; P_{tot}, total parasite biomass.

in young adults (Fig. 4b), while fucosylation of GLURP-specific IgG1 (Fig. 4c) and total IgG1 is only slightly reduced (Fig. 4d). Although sialylation of HB3VAR06-specific IgG1 was not modified by age (Fig. 4a), sialylation of GLURP-specific IgG1 was significantly higher early in life (Fig. 4c).

Fc-glycosylation of HB3VAR06-specific IgG3 was also evaluated. As IgG3-glycopeptides in the African population have an identical mass to IgG4, we cannot formally distinguish between the two[37,38]. However, since IgG1 and IgG3 dominated the HB3VAR06-specific response, we consider that the IgG3/4 fraction is likely dominated by IgG3. Fc-afucosylation of HB3VAR06-specific IgG3/4 was only marginally lower than total IgG3/4 (92% vs. 96%; $p < 0.001$; Supplementary Fig. 2), but it did correlate negatively with age ($r_s = -0.44$; $p < 0.001$), as was seen for IgG1. Specific age-related changes were observed with respect to Fc-glycosylation of GLURP-specific IgG1 (Fig. 4c) but not for IgG3/4. Overall, the Fc-glycosylation patterns of IgG3/4 mirrored those observed for IgG1 (Supplementary Fig. 2).

The above data, in combination with our previously published data from adults[21], show that both the levels and the degree of Fc-afucosylation of PfEMP1-specific IgG increase gradually as protective

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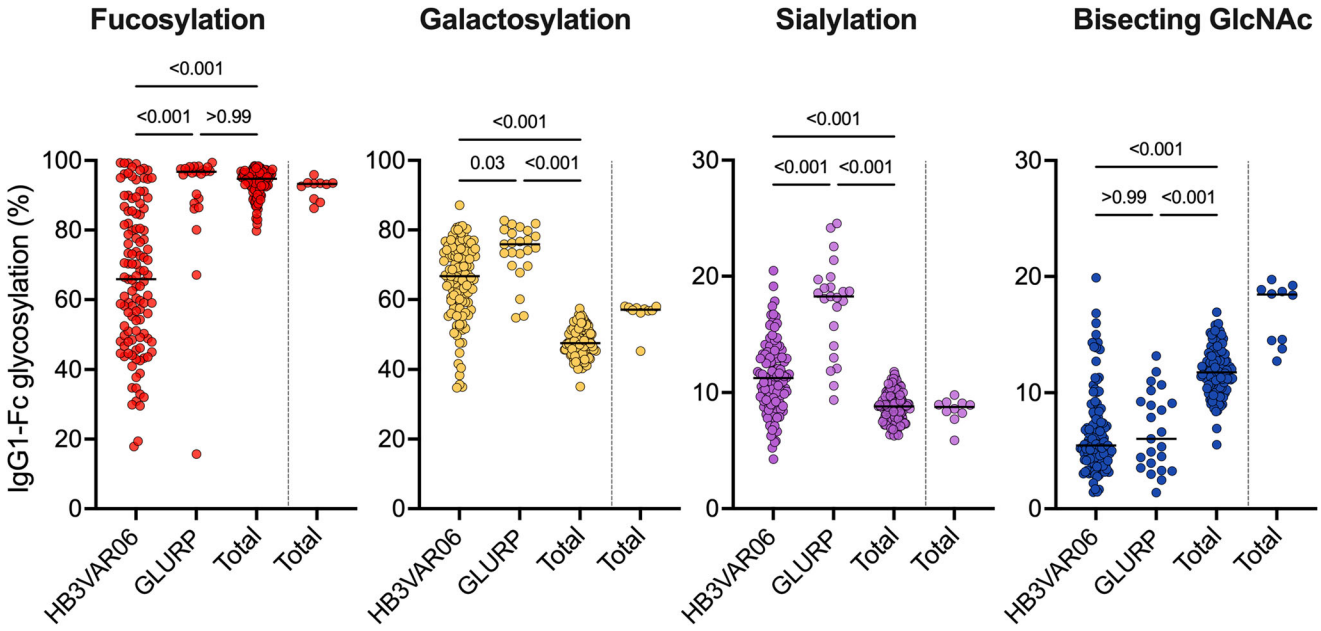

Fig. 3 | Fc-glycosylation pattern of total and specific IgG1. Glycoform abundance of total ($n = 120$), GLURP ($n = 23$), and HB3VAR06-specific ($n = 115$) IgG1 in children. Medians and p-values using the Kruskal-Wallis test, followed by Dunn's multiple comparison tests are shown. Total plasma IgG1 Fc-glycosylation in unexposed Dutch adults ($n = 10$) is included for comparison (left panel).

immunity to malaria is acquired in childhood and adolescence, followed by a plateau (high IgG levels and marked Fc-afucosylation) in adulthood.

Fc-afucosylation of PfEMP1-specific IgG and risk of anemia

PfEMP1-specific IgG is generally thought to function mainly by inhibiting the adhesion of IEs to host vascular receptors. However, the levels of Hb in the children studied correlated negatively with the degree of Fc-afucosylation of HB3VAR06-specific IgG1, suggesting the clinical relevance of PfEMP1-specific IgG Fc-afucosylation. As both IgG levels and the percentage of Fc-afucosylation of HB3VAR06-specific IgG varied with age, we categorized the children into three groups based on tertiles of Fc-afucosylation of their HB3VAR06-specific IgG1. Children with markedly Fc-afucosylated (lower tertile) HB3VAR06-specific IgG1 had a significantly reduced risk of anemia compared to children in the upper tertile after adjusting for age, Hb phenotype, HB3VAR06-specific IgG levels, and Fc-afucosylation of HB3VAR06-specific IgG3/4 (adjusted odds ratio aOR = 0.15 [95% confidence interval: 0.02 to 0.76]; $p = 0.03$).

HB3VAR06-specific IgG and inhibition of rosette formation

Protective immunity to *P. falciparum* malaria is likely to involve a combination of neutralizing and non-neutralizing PfEMP1-specific antibodies, and the above data clearly point to an important role for IEs-opsonizing IgG mediating FcRγIIIa-dependent destruction. We, therefore, assessed the relative importance of neutralization and opsonization for different effector functions.

Rosetting is an adhesive phenotype that is possibly a marker of endothelial IE sequestration[39], and that has repeatedly been associated with severe malaria[40]. Furthermore, neutralizing IgG that inhibits rosettes has been reported to protect against severe malaria[41]. HB3VAR06 mediates rosetting[35], and we observed that plasma from the children studied here significantly inhibited it compared to plasma from unexposed control donors (13% [2.4% to 35%]; $p < 0.001$). Inhibitory levels were significantly higher in children with PCR-detectable *P. falciparum* infection (Fig. 5a), and relative rosette size was marginally impacted by immune plasma (96% [90% to 105%]) compared to plasma from unexposed control donors ($p = 0.05$). The ability to inhibit rosette formation was positively correlated with age ($r_s = 0.42$; $p < 0.001$) and with levels of HB3VAR06-specific IgG ($r_s = 0.55$, $p < 0.001$).

HB3VAR06-specific IgG and C1q binding

Plasma IgG from the studied children induced moderately increased C1q binding on IEs compared to non-immune plasma (1.34 vs 0.93; $p < 0.001$; Fig. 5b), but less so than plasma from *P. falciparum*-exposed women (Supplementary Fig. 3a). Levels of C1q binding were significantly higher in *P. falciparum*-positive children (Fig. 5b). Moreover, the levels of C1q binding were positively correlated with anti-HB3VAR06 IgG levels ($r_s = 0.25$; $p < 0.001$) and with age ($r_s = 0.39$; $p < 0.001$) when data from children and adults were combined. The levels of hemoglobin in supernatants after incubation with immune plasma were marginally but significantly higher than after incubation with non-immune plasma (Fig. 5c), and C1q binding and IEs lysis were weakly but significantly correlated ($r_s = 0.21$, $p = 0.01$). We also observed low C5-9 binding to plasma opsonized IEs (Supplementary Fig. 3b, c), suggesting a limited formation of membrane attack complex (MAC) and lysis of IEs, as reported recently for IEs expressing VAR2CSA-type PfEMP1[42].

HB3VAR06-specific IgG and ADCP/ ADCC

IEs opsonized by immune plasma from children induced higher ADCP by undifferentiated THP-1 cells than IEs opsonized by non-immune plasma (59.8% vs 10.1%; $p < 0.001$), with slightly higher levels in infected children (Fig. 5d). Plasma from older children was more efficient in inducing phagocytosis ($r_s = 0.40$; $p < 0.0001$). The ability of HB3VAR06-specific antibodies to induce ADCC by NK cells, measured by CD107a expression varied markedly among samples (Fig. 5e). NK-cell production of IFNγ alone (Fig. 5f) or in addition to expression of CD107a (1.1% [0.01% to 3.6%]) was low but variable. Plasma from children with *P. falciparum* infection induced significantly higher levels of NK-cell CD107a expression, IFNγ production, and secretion of perforin than PCR-negative children (Fig. 5e, g). CD107a expression increased with levels of HB3VAR06-specific IgG and age (Fig. 6a). Moreover, levels of CD107a expression positively correlated with IFNγ production ($r_s = 0.92$; $p < 0.0001$) and perforin secretion ($r_s = 0.40$; $p < 0.0001$). Although IgG effector functions triggered by HB3VAR06-specific IgG

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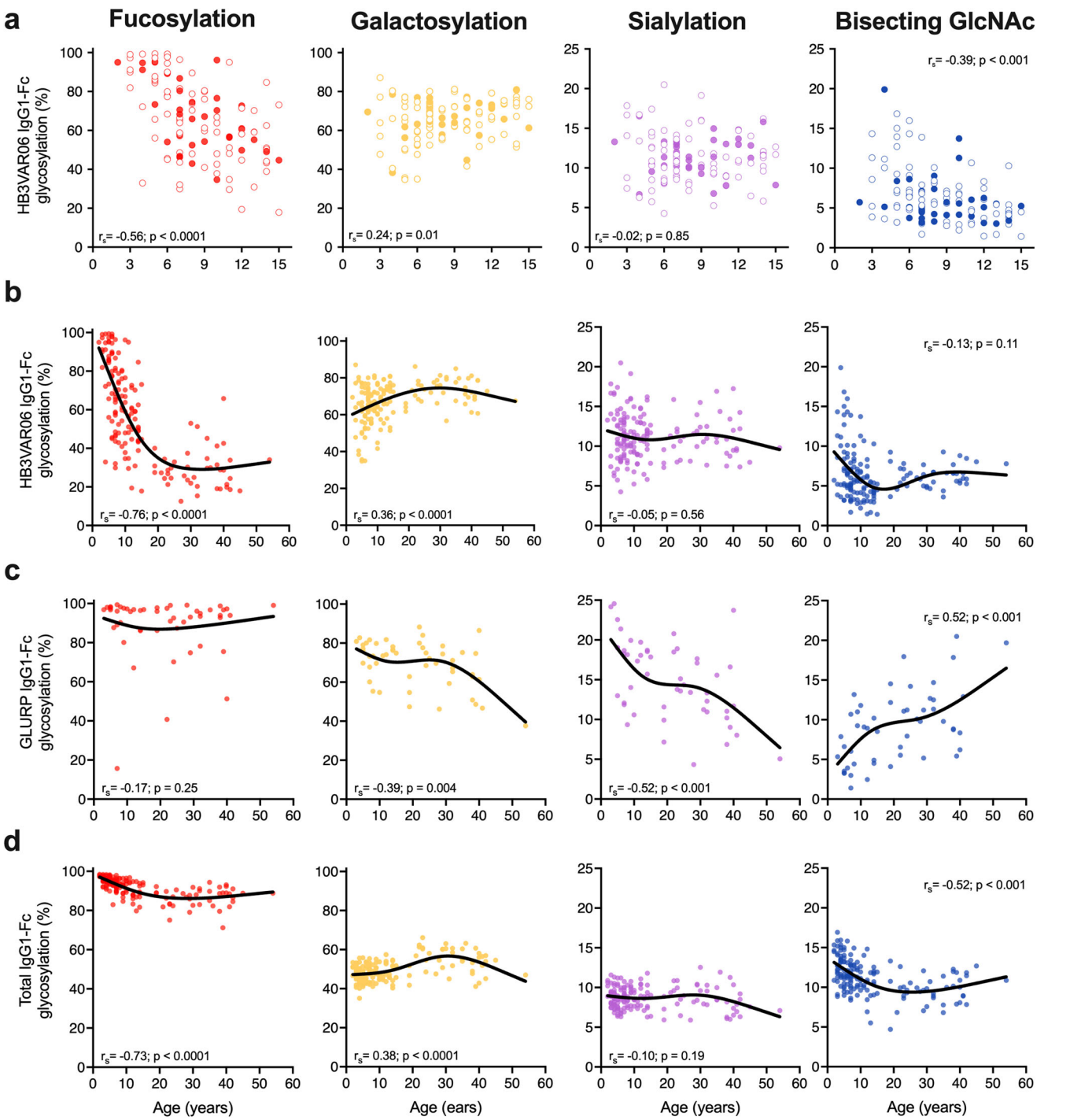

Fig. 4 | Fc-glycosylation pattern of HB3VAR06-specific IgG1 is age-dependent. **a** Correlation between glycoform abundance for HB3VAR06-specific IgG1 and age in children. Data for children with PCR-negative (open symbols; $n = 81$) and -positive (closed symbols; $n = 34$) for *P. falciparum* infection are shown. **b**–**d** Correlation between age and glycoform abundance for (**b**) HB3VAR06-specific IgG1 (children, $n = 115$ and non-pregnant adults, $n = 42$[21]). **c** GLURP-specific IgG1 (children, $n = 23$ and non-pregnant adults, $n = 28$[21]) and (**d**) total IgG1. Age (2–54 years) was fitted using a restricted cubic spline with four internal knots and 1004 points. Spearman's rank correlation (r_s) and *p*-values are also shown in all panels.

were highly variable among the samples (Fig. 5h), cumulative *z*-scores increased with age. In contrast, GLURP-specific antibodies were less effective at inducing ADCC by NK cells (Supplementary Fig. 4). Together, these results highlight the ability of HB3VAR06-specific IgG to bind complement and engage FcRγ of immune cells.

Effector functions and the risk of anemia

Afucosylation and elevated galactosylation in the Fc region of the HB3VAR06-specific IgG1 increased NK-cell mediated ADCC (Fig. 6a, c, d), as did afucosylation of HB3VAR06-specific IgG3/4 (Fig. 6e), likely due to their improved binding affinity for FcγRIIIa[28]. Moreover, C1q-binding and lysis of IEs increased with Fc-galactosylation levels in IgG1 and IgG3, respectively. Although ADCP by THP-1 cells was negatively correlated with fucosylation levels in IgG1, the association was no longer significant after adjusting for specific IgG antibody levels, supporting FcγRI and FcγRII as important contributors to ADCP. IgG breadth scores, defined as the sum of effector functions in each child, correlated positively with age, levels of Hb, HB3VAR06-specific IgG,

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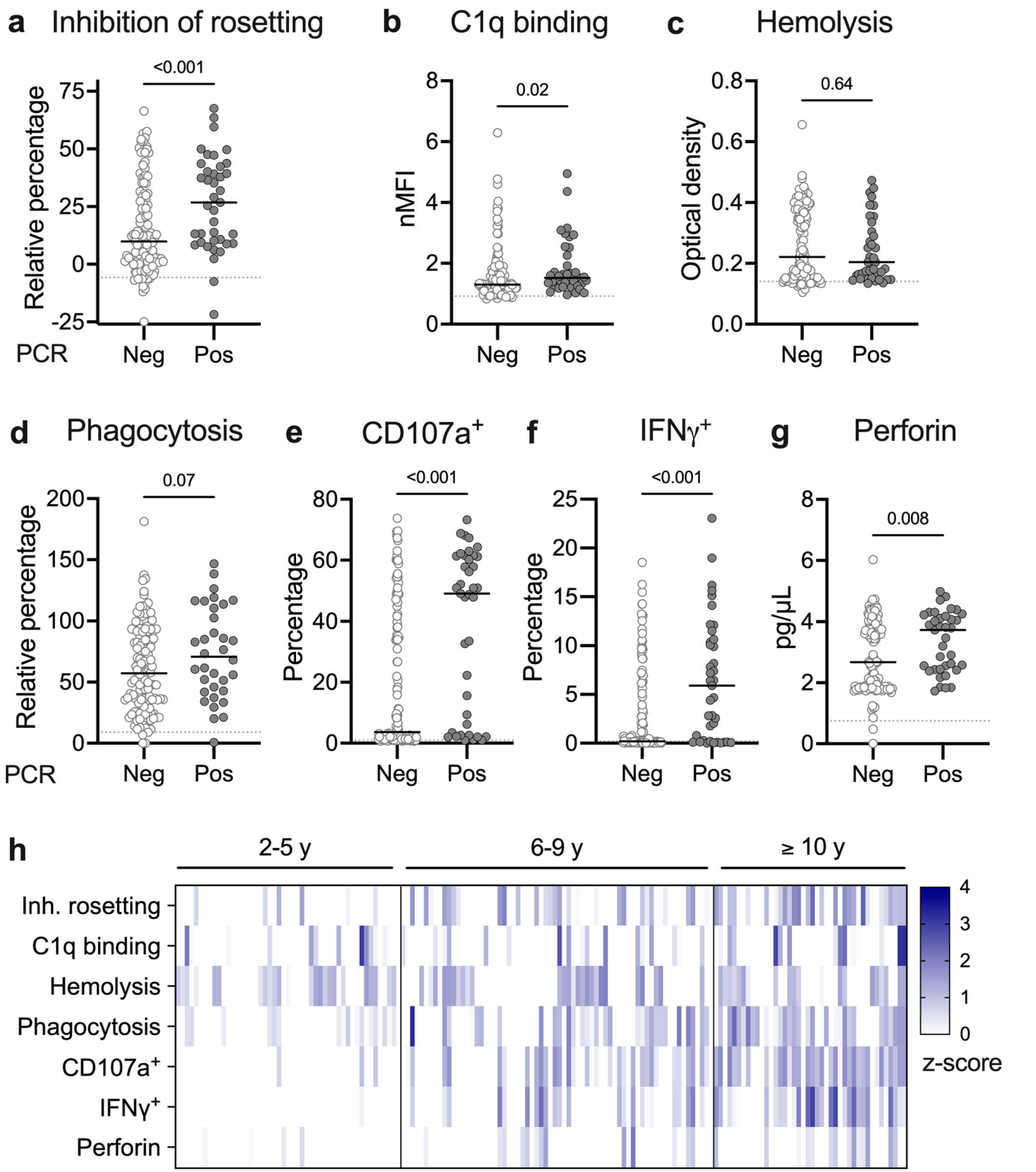

Fig. 5 | Effector functions triggered by HB3VAR06-specific IgG. a Rosette disruption by plasma ($n = 172$) was measured as the rosetting rate of IEs expressing HB3VAR06 relative to the absence of specific PfEMP1 antibodies using Danish adults without malaria exposure (zero inhibition). Values are expressed as relative percentages for PCR-negative (open symbols) and -positive (closed symbols) for *P. falciparum* infection. **b** C1q binding to opsonized HB3VAR06 IEs and (**c**) antibody-mediated complement lysis by plasma ($n = 172$). **d** Phagocytosis by THP-1 cells of HB3VAR06 IEs opsonized with plasma from children (n = 162). Percentages are relative to the positive control (100% phagocytosis). **e**–**g** ADCC by FcγRIIIa-expressing NK92 cell line incubated with immobilized HB3VAR06-specific IgG from children ($n = 172$): CD107a expression (**e**), IFNγ production (**f**), and perforin release (**g**). Medians and *p*-values using the two-tailed Mann-Whitney test are shown for (**a**–**g**) panels. Dotted line: median with plasma from Danish adults without malaria exposure. **h** Heatmap showing *z*-score (data centered and scaled to have zero mean and a standard deviation of 1) for each antibody feature ordered by age.

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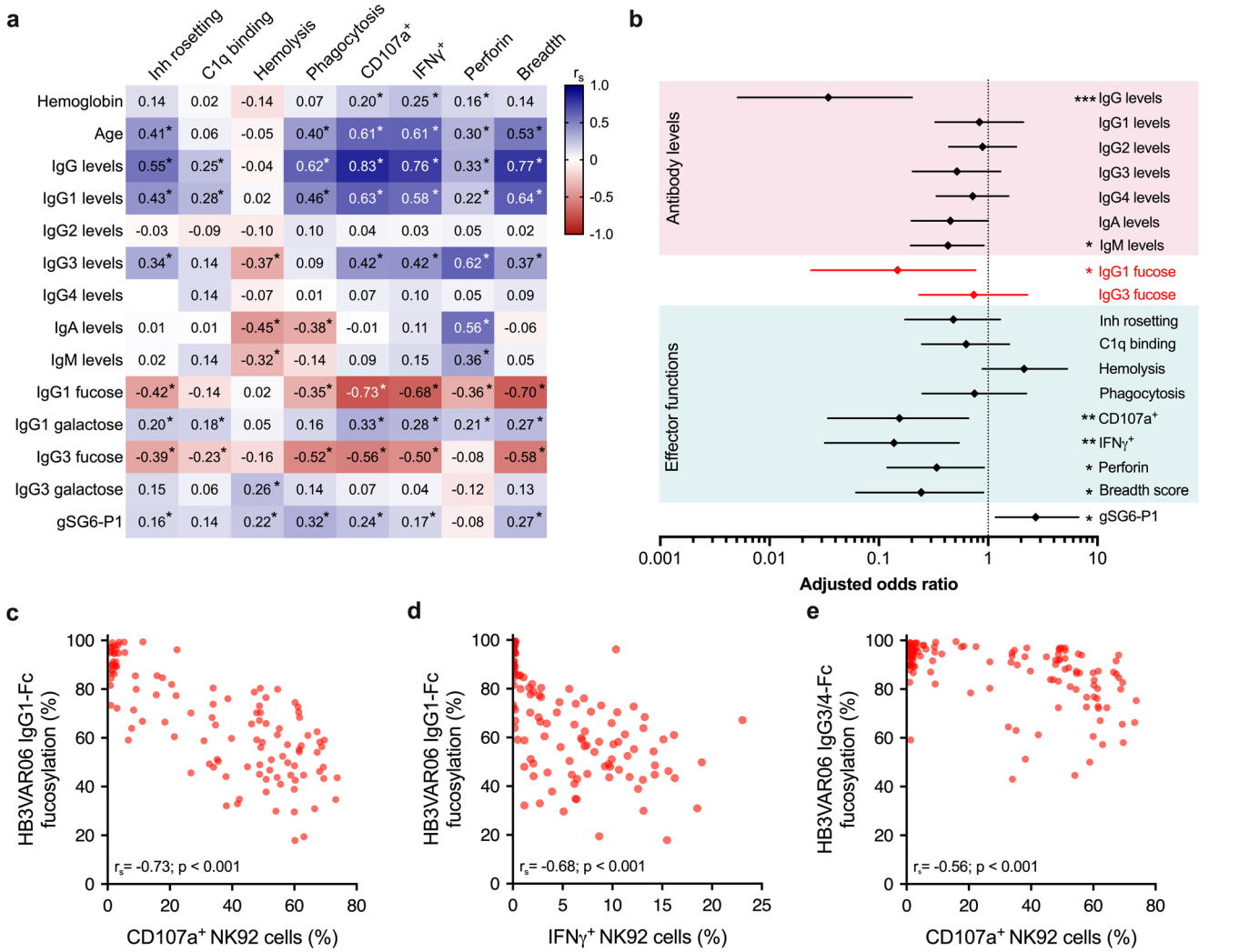

Fig. 6 | Association among features of HB3VAR06-specific antibodies and protection from anemia. a Correlation matrix of antibody response to HB3VAR06 and effector functions. Positive correlations using Spearman's rank correlation (r_s) are displayed in blue and negative correlations are displayed in red (r_s color key). *Indicates a p-value < 0.05. **b** Risk of anemia according to HB3VAR06-specific antibody levels and effector functions ($n = 115$). Multiple logistic regression model comparing low to high responders (defined as having high antibody levels, high breadth score, or low fucose levels) was used to calculate odds ratios (ORs; center), 95% confidence intervals (error bars), and p values. The model was adjusted for age and Hb phenotype for antibody levels. For effector functions, fucosylation, and exposure (gSG6-P1), the model was further adjusted for HB3VAR06-specific IgG levels. The dotted line (OR = 1) indicates no association with protection from anemia. *p-value < 0.05, **p < 0.01, ***p < 0.001. **c**, **d** Correlation between Fc-afucosylation of HB3VAR06-specific IgG1 and CD107a expression (**c**) and IFNγ production (**d**) by FcγRIIIa-expressing NK92 cell line. **e** Correlation between Fc-afucosylation of HB3VAR06-specific IgG3/4 and CD107a expression. Spearman's rank correlation (r_s) and p-values are shown.

IgG1 and IgG3, and HB3VAR06-specific IgG1 Fc-galactosylation, and negatively with Fc-fucosylation of HB3VAR06-specific IgG1 and IgG3 (Fig. 6a). Moreover, children who appeared to have been highly exposed to mosquito bites (high levels of gSG6-P1-specific IgG) had higher IgG breadth scores.

Using anemia as a marker of malaria risk in children (Fig. 6b), we found that in addition to Fc-afucosylation of HB3VAR06-specific IgG1, those with high levels of HB3VAR06-specific IgG and IgM, NK-cell mediated ADCC, and IgG breadth scores were less likely to be anemic. In contrast, malaria risk was not affected by Fc-afucosylation (OR 0.67; [0.06 to 6.43], $p = 0.73$) or high levels of GLURP-specific IgG (OR 1.06;0.26 to 4.13]; $p = 0.93$). Together, these findings indicate that multiple features of HB3VAR06-specific IgG contribute to clinical protection against malaria in children.

Discussion

Protective immunity against malaria acquired in response to *P. falciparum* infection relies on IgG antibodies to asexual-stage antigens[5], among which PfEMP1 dominates[3,4,8,9]. However, the relationship between levels of specific IgG per se and protection is not straightforward[19,43,44]. Indeed, the comprehensive profiling of 16 features of such antibodies in plasma from *P. falciparum*-exposed children presented here indicates that a broad range of IgG effector functions combine as critical determinants of clinical protection. Of particular significance is our observation that children with selectively Fc-afucosylated HB3VAR06-specific IgG1 and children with many IgG-dependent effector functions are at reduced risk of anemia.

Glycosylation contributes to the structural integrity and conformation of the Fc region and allows interaction of the IgG Fc region with FcγR[45]. Thus, the Fc-glycan composition modulates IgG effector functions. During childhood, the overall Fc-fucosylation of plasma IgG decreases slightly but remains almost complete (~94%) even in adults[46]. Despite this, IgG specific for particular antigens can be markedly Fc-afucosylated among adults[21,32]. Here, we report that IgG specific for the *P. falciparum* PfEMP1 antigen HB3VAR06 in Ghanaian children is at least as Fc-afucosylated as virus-specific IgG among

adults[32] and similar as reported for Rh D-specific IgG in allo-immunized women[47] or for HB3VAR06-specific IgG in Ghanaian adults[21]. Recently, we have observed a parity-dependent Fc-afucosylation of IgG specific for VAR2CSA-type PfEMP1 involved in the pathogenesis of placental malaria[21,48] and uniformly low Fc-fucosylation of HB3VAR06-specific IgG1[21]. We, therefore, hypothesized that Fc-afucosylation of PfEMP1-specific IgG is a function of repeated antigen exposure. That hypothesis is supported by the evidence presented here. HB3VAR06 belongs to the Group A-type PfEMP1 variants associated with severe malaria in childhood, and specific-IgG response to this group is acquired early in life[10,17,49]. The new data presented here also support our hypothesis that Fc-afucosylation of PfEMP1-specific IgG is a persistent phenotype once it has been acquired[21,48], pointing toward long-lived plasma cells as its cellular origin. Finally, the present data further support the hypothesis that Fc-afucosylation is restricted to IgG specific for foreign antigens expressed on surface membranes of host cells[32]. Admittedly, Fc-afucosylation of GLURP-specific IgG was occasionally observed here and among a few adults in the earlier study[21], which does not seem to immediately conform to this hypothesis, as GLURP is a merozoite-specific antigen. We speculate that it results from GLURP deposited on the erythrocyte surface during invasion, as has been described for other merozoite-specific antigens[50], but this needs future validation. The lower levels of sialylation of GLURP-specific IgG1 in older individuals, particularly females, agree with previous studies reporting a decrease of sialyation with age in adult females[23], although we have previously reported that sialylation on human IgG1 had no or little effect on FcγR binding[28]. This, together with higher levels of GLURP-specific IgG in females, warrants further analysis of the impact of sex in specific malaria responses, but that is beyond the scope of this study.

Antibodies targeting epitopes involved in the binding of PfEMP1 to their host receptors inhibit the IEs adhesion to endothelial cells, uninfected erythrocytes, or placental syncytiotrophoblast. Efforts to develop PfEMP1-based vaccines have, therefore, primarily focused on such neutralizing antibodies[51–53]. However, non-neutralizing IgG can also be protective by functioning as opsonins. Consistent with this, the cytophilic subclasses IgG1 and IgG3 were the dominant HB3VAR06-specific subclasses identified in our cohort of *P. falciparum* exposed children, confirming previous findings with other PfEMP1 variants[19].

Almost two-thirds of Africa's children under the age of five years are anemic, according to the Global Health Observatory, and malaria is a leading cause. Our finding that the level of HB3VAR06-specific IgG is associated with protection from anemia is consistent with PfEMP1 being a clinically important target of acquired immunity to malaria. More specifically, the association between Fc-afucosylation of HB3VAR06-specific IgG1 and protection from anemia reported here clearly indicates that this immunity does not solely rely on antibody-mediated neutralization. Even more so, it strongly implicates IE destruction by FcγRIIIa-expressing effector cells as an important protective mechanism. In contrast, C1q-mediated opsonization and lysis of IEs seem to be less important, probably due to the patchy distribution of PfEMP1 on the IE surface that prevents the on-target IgG hexamerization required for the efficient complete activation[29,54].

Although our analysis offers insights into the intricate interplay of antibody effector functions and specific IgG targeting PfEMP1 proteins, it also has some limitations. For instance, a longitudinal study would help dissect the clinical protection against severe malaria while simultaneously controlling for confounding factors not evaluated here. Finally, this work indicates that while specific IgG levels are important, they are not the most significant predictors of protection, as other antibody features, such as neutralizing activity and Fc-glycosylation profiles augment clinical protection in malaria. These findings call for a revision of the current focus of PfEMP1-based vaccine development on inhibition of IE sequestration.

Methods

Ethics statement

The samples used here were collected as part of earlier studies, approved by the Ethics Review Committee of the Ghana Health Service (GHS; GHS-ERC 008/07/19), by the Institutional Review Board of Noguchi Memorial Institute for Medical Research (NMIMR-IRB CPN 006/19 and 038/10-11), by the Regional Research Ethics Committees, Capital Region of Denmark (protocol H-4-2013-083), and by the Academic Medical Center Institutional Medical Ethics Committee of the University of Amsterdam. A declaration of free willingness to participate in the study and written informed consent were obtained from all participants or guardians before enrollment.

Study participants

We used 210 plasma samples collected in a cross-sectional community-based study in children (2 to 15 years of age) conducted during the rainy season in Northern Ghana[33] (Fig. 1 and Supplementary Table 1). Since structural variants in hemoglobin (Hb) such as HbC and HbS affect susceptibility to malaria[55] and PfEMP1-specific immunity[17], we selected samples with the three main Hb phenotypes (HbAA, HbAS, HbAC; determined by isoelectric focusing electrophoresis[56]). Infection by *P. falciparum* was determined by PCR[33], and total parasite biomass was calculated from PfHRP2 plasma levels (Quantimal™ CELISA kit; Cellabs, Australia)[17]. Levels of hemoglobin (g/dL) were used to define anemia: Hb < 11.0 g/dL for children < 5 years-of-age; < 11.5 g/dL for children 5–11 years-of-age; < 12.0 g/dL for children 12–14 years-of-age; and < 8.0 g/dL severe anemia[57]. Plasma samples from Ghanaian adults with previous *P. falciparum* infection were included as positive controls. Plasma samples from healthy Dutch ($n = 10$) and Danish ($n = 17$) adults without malaria exposure, referred to as non-immune plasma (NIP), were used as negative controls.

Recombinant proteins

The full-length ectodomain of the Group A PfEMP1 protein HB3VAR06 was produced in baculovirus-transfected Sf9 insect cells[58]. The amino-terminal, non-repetitive R0 region of glutamate-rich protein (GLURP) was produced in *Escherichia coli*[59]. The peptide gSG6-P1 of *An. gambiae* saliva[60] was used as a biomarker for exposure to *Anopheles* mosquito bites and malaria transmission[61].

Enzyme-linked immunosorbent assay (ELISA)

IgG reactivity against recombinant proteins was measured by ELISA[62]. Briefly, plasma samples were added to 96-well flat-bottom microtiter plates (Nunc MaxiSorp; Thermo Fisher Scientific) previously coated with recombinant protein (100 ng/well) in phosphate-buffered saline (PBS). After blocking, horseradish peroxidase-conjugated rabbit anti-human IgG (1:3,000; Dako), mouse anti-human IgG1, IgG2, IgG3, IgG4 (1:1,000; Invitrogen), goat anti-human IgM, or IgGA (1:4,000; Invitrogen) were added. Bound antibodies were detected by adding TMB PLUS2 (Eco-Tek), and the reaction stopped with 0.2 M H_2SO_4. The absorbance was read (HiPo MPP-96 microplate reader; Molecular Devices), and the specific antibody levels were calculated in arbitrary units (AU)[62]. Negative cut-off values were calculated as the mean AU values plus two standard deviations (SD) obtained with the Danish NIP samples.

Purification of IgG from plasma samples

Total IgG was purified with Protein G-coupled cartridges using the AssayMAP Bravo platform (Agilent Technologies, Santa Clara, USA)[21]. Briefly, 1 μL of plasma diluted in PBS was applied to the cartridges, followed by washes with PBS and LC-MS pure water. IgG antibodies were then eluted with 1% formic acid. Antigen-specific IgG was purified from plasma samples diluted in PBS supplemented with Tween20® (0.05%; PBS-T) using 96-well flat-bottom microtiter plates (Nunc Maxisorp, Thermo Fisher Scientific) pre-coated with HB3VAR6

(100 ng/well) or GLURP (50 ng/well) in PBS (4 °C; ON). The diluted plasma samples were incubated in the coated plates (1 h; RT; shaking), followed by three washes with PBS-T, two washes with PBS, and two washes with ammonium bicarbonate (50 mM). Specific IgG was then eluted with formic acid (100 mM; 5 min; shaking)[63].

Mass spectrometric IgG Fc glycosylation analysis

Eluates containing either antigen-specific antibodies or total IgG we collected and dried by vacuum centrifugation followed by mass spectrometry analysis as described before[21,63]. Briefly, the dried samples were dissolved in a reduction and alkylation buffer containing sodium deoxycholate (0.4%), TCEP (10 mM), chloroacetamide (40 mM), and TRIS (100 mM, pH8.5), followed by proteolytic cleavage using trypsin (5 μg/mL) in ammonium bicarbonate (50 mM). After overnight incubation, the digestion was terminated by adding 2% formic acid. Analysis of IgG Fc glycosylation was performed with nanoLC reverse phase-electrospray-mass spectrometry on an Impact quadrupole-time-of-flight mass spectrometer (Bruker Daltonics, Bremen, Germany), and data processed with Skyline software (version 4.2.0.19107). The level of fucosylation and bisection were calculated as the sum of the relative intensities of glycoforms containing the respective glycotraits. Galactosylation and sialylation levels were calculated as antenna occupancy. Details on analyzed glycopeptides are in the supplementary information (Supplementary Tables 2 and 3).

Malaria parasite culture and PfEMP1 selection

The HB3 clone of *P. falciparum* was maintained in serum-free RPMI-1640 medium as described[64]. The genotypic identity of the parasites and the absence of *Mycoplasma* contamination in the cultures were regularly verified. Late-stage HB3 IEs were selected for surface expression of HB3VAR06 using protein A-coupled DynaBeads coated with a specific rabbit antiserum[58], as described previously[65]. IE surface expression of the HB3VAR06 protein was monitored by flow cytometry using a specific rabbit antiserum (1:20)[65]. A CytoFLEX S (Beckman Colter Life Sciences) flow cytometer was used for data acquisition, and data were analyzed with FlowLogic software version 8.3 (Inivai Technologies, Australia). Median fluorescence intensities (MFI) data were normalized to the MFI on Danish NIP.

Rosetting assays

The ability of plasma samples to disrupt rosettes was tested by flow cytometry as described before[17,66]. Briefly, late-stage IEs of HB3VAR06 maintained in 10% human serum RPMI-1640 medium were incubated for 1 h at 37 °C with plasma samples (1:10). Parasite nuclei were stained with Hoechst 33342 (10 μg/mL; Invitrogen) and dihydroethidium (5 μg/mL; Invitrogen). Late-stage IEs (Hoechst and DHE positive) were gated using FSC-A vs. FSC-H to determine the percentage of multiplets (rosettes) and the mean SSC-A (rosette size). Data acquisition and analysis were done as described above (Supplementary Fig. 5). Rosetting disruption and rosette sizes were calculated relative to values obtained with plasma from Danish NIP (zero inhibition).

Antibody-dependent complement deposition

Antibody-dependent complement deposition was measured by C1q and C5-9 binding on the IEs surface by flow cytometry as described before[42]. Late-stage IEs of HB3VAR06 at ~13% parasitemia and 0.5% hematocrit (freshly collected erythrocytes) were incubated with 25% non-immune serum plus 10% NIP or immune plasma in VBS^{++} buffer (Complement Technology) for 1 h at 37 °C. Samples were centrifuged, and the supernatant was collected to determine hemoglobin release as a proxy for complement-mediated lysis of IEs. After washing with 0.5% BSA in PBS, C1q and C5-9 in IEs were detected by staining with FITC-conjugated rabbit polyclonal anti-human C1q (1:150; Abcam) or C5-9 (1:150; biorbyt) and Hoechst 33342 (10 μg/mL, Invitrogen). Data acquisition and analysis were done as described above (Supplementary Fig. 6). MFI data were normalized to the MFI on Danish NIP.

Antibody-dependent THP-1 cell phagocytosis (ADCP) assay

High throughput flow cytometry-based ADCP assays were performed as previously described[17], using undifferentiated THP-1 cells expressing FcγRI and FcγRII but not FcγRIIIa[67]. In brief, nuclei of purified late-stage IEs of HB3VAR06 were stained with ethidium bromide (10 μg/ml; EtBr). For opsonization, stained IEs were incubated for 30 min at 37 °C with plasma samples (1:10), followed by co-incubation with THP-1 cells (10:1 ratio, IEs to THP-1) for 40 min at 37 °C in a 5% CO_2 atmosphere to allow phagocytosis. Non-phagocytized IEs were removed using an ammonium chloride lysing solution. Data acquisition and analysis were performed as described above (Supplementary Fig. 7). The percentage of THP-1 cells that phagocytosed IEs ($EtBr^+$ THP-1 cells) was calculated relative to the phagocytosis obtained with plasma from Ghanaian adults with previous *P. falciparum* infection (100% phagocytosis).

Antibody-dependent cellular cytotoxicity (ADCC) assay

NK92 cells stably expressing FcγRIIIa and GFP[68] were used to evaluate CD107a surface expression, IFNγ production, and perforin release with an antigen-IgG-plate bound assay, as described previously[21]. In brief, 96-well flat-bottom microtiter plates (Nunc MaxiSorp™; Thermo Fisher Scientific) coated with recombinant HB3VAR06 or GLURP proteins (100 ng/well in PBS) were opsonized with plasma samples (1:20) for 1 h at 37 °C. After washing, ~1.6×10^5 NK92 cells were added to each well together with anti-human CD107a-PE (1:40; H4A3 clone; BD Biosciences), brefeldin A (10 μg/mL; Sigma-Aldrich), and monensin (2 μM, Sigma-Aldrich) followed by 4 h incubation at 37 °C in 5% CO_2. The supernatants were stored at −80 °C for later detection of perforin secretion by ELISA (Mabtech, Sweden) following the manufacturer's instructions, and the cells were stained with near-IR fixable Live/Dead dye (Invitrogen). After fixation and permeabilization, cells were intracellularly stained with anti-human IFNγ-APC (1:200; clone B27; BD Biosciences), followed by data acquisition as described above (Supplementary Fig. 8). Wells with antigen and NK cells but without antibodies were included in all experiments to compensate for antibody-independent activation.

Statistical analysis

Data were analyzed and plotted using GraphPad Prism version 10.3.1 (GraphPad Software, San Diego, California, USA). The sample size and specific statistic tests are indicated in the text and figures. All tests were two-tailed, and p-values < 0.05 were considered statistically significant.

Antibody levels and effector functions (rosetting disruption, C1q binding, hemolysis, ADCP, CD107a surface expression, IFNγ production, and perforin release) were normalized using a z-score (data centered and scaled to have zero mean and a standard deviation of 1) and used to create a heatmap. Each individual's z-scores for effector functions were summed up to generate the IgG breadth scores. Antibody levels, percentage of glycosylation, and breadth scores were categorized into three groups (low, medium, and high) based on tertiles with models comparing low and high responders. Multiple regression models adjusted for age, Hb phenotype, and HB3VAR06-specific IgG levels were used to evaluate the effect of potential confounders on the association between antibody levels and effector functions and relevant independent factors.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The data generated in this study are provided in the Supplementary Information/Source Data file. Source data are provided in this paper.

References

1. WHO. *World Malaria Report*. (2023).
2. WHO. Severe malaria. *Trop. Med. Int. Health.* **19**, 7–131 (2014).
3. Hviid, L. & Jensen, A. T. PfEMP1 - A parasite protein family of key importance in *Plasmodium falciparum* malaria immunity and pathogenesis. *Adv. Parasitol.* **88**, 51–84 (2015).
4. Hviid, L., Jensen, A. R. & Deitsch, K. W. PfEMP1 and var genes - Still of key importance in *Plasmodium falciparum* malaria pathogenesis and immunity. *Adv. Parasitol.* **125**, 53–103 (2024).
5. Cohen, S., Mc, G. I. & Carrington, S. Gamma-globulin and acquired immunity to human malaria. *Nature* **192**, 733–737 (1961).
6. Marsh, K. & Howard, R. J. Antigens induced on erythrocytes by *P. falciparum*: expression of diverse and conserved determinants. *Science* **231**, 150–153 (1986).
7. Bull, P. C. et al. Parasite antigens on the infected red cell surface are targets for naturally acquired immunity to malaria. *Nat. Med.* **4**, 358–360 (1998).
8. Chan, J. A. et al. Targets of antibodies against *Plasmodium falciparum*-infected erythrocytes in malaria immunity. *J. Clin. Invest.* **122**, 3227–3238 (2012).
9. Tessema, S. K. et al. Protective immunity against severe malaria in children is associated with a limited repertoire of antibodies to conserved PfEMP1 variants. *Cell Host Microbe* **26**, 579–590.e575 (2019).
10. Cham, G. K. et al. Hierarchical, domain type-specific acquisition of antibodies to *Plasmodium falciparum* erythrocyte membrane protein 1 in Tanzanian children. *Infect. Immun.* **78**, 4653–4659 (2010).
11. Salanti, A. et al. Evidence for the involvement of VAR2CSA in pregnancy-associated malaria. *J. Exp. Med.* **200**, 1197–1203 (2004).
12. Conway, D. J. et al. A principal target of human immunity to malaria identified by molecular population genetic and immunological analyses. *Nat. Med.* **6**, 689–692 (2000).
13. Douglas, A. D. et al. The blood-stage malaria antigen PfRH5 is susceptible to vaccine-inducible cross-strain neutralizing antibody. *Nat. Commun.* **2**, 601 (2011).
14. Villasis, E. et al. Anti-*Plasmodium falciparum* invasion ligand antibodies in a low malaria transmission region, Loreto, Peru. *Malar. J.* **11**, 361 (2012).
15. Kana, I. H. et al. Naturally acquired antibodies target the glutamate-rich protein on intact merozoites and predict protection against febrile malaria. *J. Infect. Dis.* **215**, 623–630 (2017).
16. Ghumra, A. et al. Immunisation with recombinant PfEMP1 domains elicits functional rosette-inhibiting and phagocytosis-inducing antibodies to *Plasmodium falciparum*. *PLoS ONE* **6**, e16414 (2011).
17. Oleinikov, A. V. et al. Profiling the *Plasmodium falciparum* erythrocyte membrane protein 1-specific immunoglobulin G response among Ghanaian children with hemoglobin S and C. *J. Infect. Dis.* **229**, 203–213 (2024).
18. Chan, J. A. et al. Antibody targets on the surface of *Plasmodium falciparum*-infected erythrocytes that are associated with immunity to severe malaria in young children. *J. Infect. Dis.* **219**, 819–828 (2019).
19. Suurbaar, J. et al. ICAM-1-binding *Plasmodium falciparum* erythrocyte membrane protein 1 variants elicits opsonic-phagocytosis IgG responses in Beninese children. *Sci. Rep.* **12**, 12994 (2022).
20. Arora, G. et al. NK cells inhibit *Plasmodium falciparum* growth in red blood cells via antibody-dependent cellular cytotoxicity. *Elife* **7**, e36806 (2018).
21. Larsen, M. D. et al. Afucosylated *Plasmodium falciparum*-specific IgG is induced by infection but not by subunit vaccination. *Nat. Commun.* **12**, 5838 (2021).
22. Ty, M. et al. Malaria-driven expansion of adaptive-like functional CD56-negative NK cells correlates with clinical immunity to malaria. *Sci. Transl. Med.* **15**, eadd9012 (2023).
23. Nimmerjahn, F., Vidarsson, G. & Cragg, M. S. Effect of posttranslational modifications and subclass on IgG activity: from immunity to immunotherapy. *Nat. Immunol.* **24**, 1244–1255 (2023).
24. Bakovic, M. P. et al. High-throughput IgG Fc N-glycosylation profiling by mass spectrometry of glycopeptides. *J. Proteome Res.* **12**, 821–831 (2013).
25. Selman, M. H. et al. Fc specific IgG glycosylation profiling by robust nano-reverse phase HPLC-MS using a sheath-flow ESI sprayer interface. *J. Proteom.* **75**, 1318–1329 (2012).
26. Oosterhoff, J. J., Larsen, M. D., van der Schoot, C. E. & Vidarsson, G. Afucosylated IgG responses in humans - structural clues to the regulation of humoral immunity. *Trends Immunol.* **43**, 800–814 (2022).
27. Shields, R. L. et al. Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human FcγRIII and antibody-dependent cellular toxicity. *J. Biol. Chem.* **277**, 26733–26740 (2002).
28. Dekkers, G. et al. Decoding the human immunoglobulin G-glycan repertoire reveals a spectrum of Fc-receptor- and complement-mediated-effector activities. *Front. Immunol.* **8**, 877 (2017).
29. van Osch, T. L. J. et al. Fc galactosylation promotes hexamerization of human IgG1, leading to enhanced classical complement activation. *J. Immunol.* **207**, 1545–1554 (2021).
30. Ackerman, M. E. et al. Natural variation in Fc glycosylation of HIV-specific antibodies impacts antiviral activity. *J. Clin. Invest.* **123**, 2183–2192 (2013).
31. Wang, T. T. et al. IgG antibodies to dengue enhanced for FcγRIIIA binding determine disease severity. *Science* **355**, 395–398 (2017).
32. Larsen, M. D. et al. Afucosylated IgG characterizes enveloped viral responses and correlates with COVID-19 severity. *Science* **371**, eabc8378 (2021).
33. Seidu, Z. et al. *Plasmodium falciparum* infection and naturally acquired immunity to malaria antigens among Ghanaian children in northern Ghana. *Parasite Epidemiol. Control* **22**, e00317 (2023).
34. Borre, M. B. et al. Primary structure and localization of a conserved immunogenic *Plasmodium falciparum* glutamate rich protein (GLURP) expressed in both the preerythrocytic and erythrocytic stages of the vertebrate lifecycle. *Mol. Biochem. Parasitol.* **49**, 119–132 (1991).
35. Ghumra, A. et al. Induction of strain-transcending antibodies against Group A PfEMP1 surface antigens from virulent malaria parasites. *PLoS Pathog.* **8**, e1002665 (2012).
36. Megnekou, R., Staalsoe, T., Taylor, D. W., Leke, R. & Hviid, L. Effects of pregnancy and intensity of *Plasmodium falciparum* transmission on immunoglobulin G subclass responses to variant surface antigens. *Infect. Immun.* **73**, 4112–4118 (2005).
37. Loveslati, B. Y. et al. A study of Gm allotypes and immunoglobulin heavy gamma IGHG genes in Berbers, Arabs and sub-Saharan Africans from Jerba Island, Tunisia. *Eur. J. Immunogenet.* **28**, 531–538 (2001).
38. Wuhrer, M. et al. Glycosylation profiling of immunoglobulin G (IgG) subclasses from human serum. *Proteomics* **7**, 4070–4081 (2007).
39. Wang, C. W. & Hviid, L. Rifins, rosetting, and red blood cells. *Trends Parasitol.* **31**, 285–286 (2015).
40. Doumbo, O. K. et al. High levels of *Plasmodium falciparum* rosetting in all clinical forms of severe malaria in African children. *Am. J. Trop. Med. Hyg.* **81**, 987–993 (2009).
41. Carlson, J. et al. Human cerebral malaria: association with erythrocyte rosetting and lack of anti-rosetting antibodies. *Lancet* **336**, 1457–1460 (1990).
42. Opi, D. H. et al. Reduced risk of placental parasitemia associated with complement fixation on *Plasmodium falciparum* by antibodies among pregnant women. *BMC Med.* **19**, 201 (2021).
43. Cutts, J. C. et al. Pregnancy-specific malarial immunity and risk of malaria in pregnancy and adverse birth outcomes: a systematic review. *BMC Med.* **18**, 14 (2020).

44. Aitken, E. H. et al. Developing a multivariate prediction model of antibody features associated with protection of malaria-infected pregnant women from placental malaria. *Elife* **10**, e65776 (2021).
45. Krapp, S., Mimura, Y., Jefferis, R., Huber, R. & Sondermann, P. Structural analysis of human IgG-Fc glycoforms reveals a correlation between glycosylation and structural integrity. *J. Mol. Biol.* **325**, 979–989 (2003).
46. de Haan, N., Reiding, K. R., Driessen, G., van der Burg, M. & Wuhrer, M. Changes in healthy human IgG Fc-glycosylation after birth and during early childhood. *J. Proteome Res.* **15**, 1853–1861 (2016).
47. Kapur, R. et al. Low anti-RhD IgG-Fc-fucosylation in pregnancy: a new variable predicting severity in haemolytic disease of the fetus and newborn. *Br. J. Haematol.* **166**, 936–945 (2014).
48. Lopez-Perez, M. et al. Fc-afucosylation of VAR2CSA-specific immunoglobulin G and clinical immunity to placental *Plasmodium falciparum* malaria. *J. Infect. Dis.* **25**, jiae529 (2024).
49. Obeng-Adjei, N. et al. Longitudinal analysis of naturally acquired PfEMP1 CIDR domain variant antibodies identifies associations with malaria protection. *JCI Insight* **5**, e137262 (2020).
50. Awah, N. W., Troye-Blomberg, M., Berzins, K. & Gysin, J. Mechanisms of malarial anaemia: potential involvement of the *Plasmodium falciparum* low molecular weight rhoptry-associated proteins. *Acta Trop.* **112**, 295–302 (2009).
51. Jensen, A. R., Adams, Y. & Hviid, L. Cerebral *Plasmodium falciparum* malaria: The role of PfEMP1 in its pathogenesis and immunity, and PfEMP1-based vaccines to prevent it. *Immunol. Rev.* **293**, 230–252 (2020).
52. Mordmuller, B. et al. First-in-human, randomized, double-blind clinical trial of differentially adjuvanted PAMVAC, a vaccine candidate to prevent pregnancy-associated malaria. *Clin. Infect. Dis.* **69**, 1509–1516 (2019).
53. Sirima, S. B. et al. PRIMVAC vaccine adjuvanted with Alhydrogel or GLA-SE to prevent placental malaria: a first-in-human, randomised, double-blind, placebo-controlled study. *Lancet Infect. Dis.* **20**, 585–597 (2020).
54. Diebolder, C. A. et al. Complement is activated by IgG hexamers assembled at the cell surface. *Science* **343**, 1260–1263 (2014).
55. Taylor, S. M., Parobek, C. M. & Fairhurst, R. M. Haemoglobinopathies and the clinical epidemiology of malaria: a systematic review and meta-analysis. *Lancet Infect. Dis.* **12**, 457–468 (2012).
56. Lamptey, H. et al. Impact of haemoglobinopathies on asymptomatic *Plasmodium falciparum* infection and naturally acquired immunity among children in Northern Ghana. *Front. Hematol.* **2**, https://doi.org/10.3389/frhem.2023.1150134 (2023).
57. WHO. *Haemoglobin Concentrations for the Diagnosis of Anaemia and Assessment of Severity. Vitamin and Mineral Nutrition Information System*. (ed. WHO/NMH/NHD/MNM/11.1) (World Health Organization, Geneva, 2011).
58. Stevenson, L. et al. Investigating the function of Fc-specific binding of IgM to *Plasmodium falciparum* erythrocyte membrane protein 1 mediating erythrocyte rosetting. *Cell Microbiol.* **17**, 819–831 (2015).
59. Theisen, M., Vuust, J., Gottschau, A., Jepsen, S. & Hogh, B. Antigenicity and immunogenicity of recombinant glutamate-rich protein of *Plasmodium falciparum* expressed in *Escherichia coli*. *Clin. Diagn. Lab Immunol.* **2**, 30–34 (1995).
60. Londono-Renteria, B. et al. Biomarker of *Anopheles* exposure in Ghanaian children with hemoglobin S and C. *Acta Trop.* **249**, 107043 (2024).
61. Kearney, E. A. et al. *Anopheles* salivary antigens as serological biomarkers of vector exposure and malaria transmission: A systematic review with multilevel modelling. *Elife* **10**, e73080 (2021).
62. Lopez-Perez, M. et al. IgG responses to the *Plasmodium falciparum* antigen VAR2CSA in Colombia are restricted to pregnancy and are not induced by exposure to *Plasmodium vivax*. *Infect. Immun.* **86**, e00136–00118 (2018).
63. Falck, D. & Wuhrer, M. GlYcoLISA: antigen-specific and subclass-specific IgG Fc glycosylation analysis based on an immunosorbent assay with an LC-MS readout. *Nat. Protoc.* **19**, 1887–1909 (2024).
64. Lopez-Perez, M. & Seidu, Z. Establishing and maintaining in vitro cultures of asexual blood stages of *Plasmodium falciparum*. *Methods Mol. Biol.* **2470**, 37–49 (2022).
65. Lopez-Perez, M. & Olsen, R. W. Immunomagnetic selection of *Plasmodium falciparum*-infected erythrocytes expressing particular PfEMP1 variants. *Methods Mol. Biol.* **2470**, 69–78 (2022).
66. Hedberg, P. et al. Red blood cell blood group A antigen level affects the ability of heparin and PfEMP1 antibodies to disrupt *Plasmodium falciparum* rosettes. *Malar. J.* **20**, 441 (2021).
67. Forrester, M. A. et al. Similarities and differences in surface receptor expression by THP-1 monocytes and differentiated macrophages polarized using seven different conditioning regimens. *Cell Immunol.* **332**, 58–76 (2018).
68. Snyder, K. M. et al. Expression of a recombinant high affinity IgG Fc receptor by engineered NK cells as a docking platform for therapeutic mAbs to target cancer cells. *Front. Immunol.* **9**, 2873 (2018).

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Author contributions

M.L.P. and L.H. conceptualized the study. M.L.P., G.V., M.F.O., and L.H., acquired funding. Z.S. and M.F.O. collected clinical samples and data in Ghana. M.L.P. generated the laboratory data. M.D.L., W.W., J.N., and M.W. processed and analyzed mass spectrometry data. M.L.P. analyzed the data and wrote the original draft. All authors edited and approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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