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MAJOR ARTICLE

Accumulation of Neutrophil Phagocytic Antibody Features Tracks With Naturally Acquired Immunity Against Malaria in Children

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Background. Studies have demonstrated the protective role of antibodies against malaria. Young children are known to be particularly vulnerable to malaria, pointing to the evolution of naturally acquired clinical immunity over time. However, whether changes in antibody functionality track with the acquisition of naturally acquired malaria immunity remains incompletely understood.

Methods. Using systems serology, we characterized sporozoite- and merozoite-specific antibody profiles of uninfected Malian children before the malaria season who differed in their ability to control parasitemia and fever following *Plasmodium falciparum* (*Pf*) infection. We then assessed the contributions of individual traits to overall clinical outcomes, focusing on the immunodominant sporozoite CSP and merozoite AMA1 and MSP1 antigens.

Results. Humoral immunity evolved with age, with an expansion of both magnitude and functional quality, particularly within blood-stage phagocytic antibody activity. Moreover, concerning clinical outcomes postinfection, protected children had higher antibody-dependent neutrophil activity along with higher levels of MSP1-specific IgG3 and IgA and CSP-specific IgG3 and IgG4 prior to the malaria season.

Conclusions. These data point to the natural evolution of functional humoral immunity to *Pf* with age and highlight particular antibody Fc-effector profiles associated with the control of malaria in children, providing clues for the design of next-generation vaccines or therapeutics.

Keywords. malaria; clinical immunity; antibodies; neutrophils; children.

In 2021, there were approximately 247 million malaria cases worldwide with 619 000 deaths, the majority occurring among children aged <5 years in sub-Saharan Africa (World Health Organization 2022 report). Individuals who survive repeated exposures to the *Plasmodium falciparum* (*Pf*) parasite in early life can gradually acquire immunity to clinical disease [1, 2]. With the leading malaria vaccines showing incomplete protection in field studies [3] and resistance to antimalarial drugs continuing to rise [4], there is a need to better understand the basis of naturally acquired immunity to malaria to inform the development of novel strategies for reducing malaria morbidity and mortality.

Animal and human passive transfer studies have clearly demonstrated that antibodies play a key role in protection against the disease induced by blood-stage *Pf* parasites [5, 6]. Antibody functions, including opsonization [7], inhibition of infection [8, 9], antibody-dependent cellular cytotoxicity [10], and complement fixation by C1q [11], have all been implicated in protection against malaria. More recently, epidemiological studies pointed to a dramatic epitope-spreading phenomenon with age [12, 13], in which the humoral immune response evolved to target a greater diversity of malaria antigens with age, pointing to the critical need to target a broader array of antigens to prevent disease [12, 14]. However, little is known about the age-related evolution of malaria-specific antibody function, particularly as children are gradually transitioning from malaria susceptibility to partial clinical immunity [15].

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In this study, plasma samples were collected from *Pf*-negative children prior to the start of the malaria season. These children differed in their capacity to control parasitemia and fever following *Pf* infection [1]. We used an unbiased systems serology approach to explore the relationship between clinical outcomes and the preinfection functional humoral immune profile across several immunodominant *Pf* antigens. We found that both the magnitude and the functional potential of the malaria-specific humoral immune response increased with age and exposure. Moreover, we identified the presence of elevated merozoite-specific immunoglobulin G3 (IgG3) and immunoglobulin A (IgA), as well as sporozoite-specific IgG3 and immunoglobulin G4 (IgG4) in children who resisted disease, and also observed enhanced merozoite- and sporozoite-specific neutrophil phagocytic activity as a key correlate of immunity. Overall, these data point to both prehepatic and blood-stage neutrophil-activating antibodies as biomarkers of naturally acquired protection against clinical malaria in childhood.

MATERIALS AND METHODS

Human Subjects and Plasma Samples

Plasma samples and clinical data were obtained from the ongoing Kalifabougou cohort that has been described previously (for additional information, see Supplementary Materials) [16]. Plasma samples from a subset of 267 individuals spanning the entire age range of the cohort were selected to specifically investigate the impact of age on *Pf*-specific immunity (Table 1). To further characterize humoral mechanisms associated with the control of malaria disease, 109 children aged 6–11 years were selected based on their differential ability to control fever after incident parasitemia as previously described [1]. This study was approved by the Ethics Committee of the Faculty of Medicine, Pharmacy and Dentistry at the University of Sciences, Technique and Technology of Bamako; the Institutional Review Board of the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health; and Massachusetts General Brigham HealthCare/Ragon Institute.

Antigen-Specific Antibody Functions, Isotypes, Subclasses, and Fc Receptor Binding

Antigens used in this study included the following recombinant malaria proteins: *Pf* CSP (3D7), *Pf* $MSP1_{42}$, and *Pf* AMA1(FVO) (from David Narum Laboratory of Malaria Immunology and Vaccinology, NIAID). Antibody-dependent complement deposition (ADCD), cellular phagocytosis (ADCP), and neutrophil phagocytosis (ADNP) were performed with plasma samples diluted at 1:50 as previously described [17]. For ADCP and ADNP, results highlight increase over baseline bead uptake. Antigen-specific IgG1, IgG2, IgG3, IgG4, IgA, and immunoglobulin M (IgM) levels [18] and antibody binding to FcγRIIAR, FcγRIIB, FcγRIIIAF, and FcγRIIIB [19] (plasma dilution 1:100) were performed via Luminex assay. Analyses were done by flow cytometry using the IntelliCyt iQue Screener Plus (Sartorius).

Neutrophil Red Blood Cell Phagocytosis

For antibody-mediated neutrophil phagocytosis of infected red blood cells (iRBCs), iRBCs infected with 3D7 *Pf* were used and cultured synchronously as outlined previously (for additional information, see Supplementary Materials) [20].

Orthogonalized Partial Least Squares Discriminant Analysis and Orthogonalized Partial Least Squares Regression

Classification models were built to identify the impact of age, as well as to identify correlates of protection on the basis of measured antigen-specific antibody profiles. Models were built using approaches similar to those described previously [21, 22] (for additional information, see Supplementary Materials).

Time-to-Event Analysis

Time-to-event analysis was performed for (1) incident polymerase chain reaction (PCR)–confirmed parasitemia and (2) first clinical malaria (defined as having an axillary temperature >37.5°C and *Pf* parasitemia >2500 asexual parasites/μL) using enrollment as the start time, and (3) first clinical (febrile) malaria with incident parasitemia as the start time. For the latter, true time of incident parasitemia was estimated as the midpoint between the last negative *Pf*

Table 1. Cohort Characteristics

Characteristics	Delayed	Early	Immune	Not Classified
Total No.	42	41	21	181
Age, y, median (IQR)	7.48 (7.00–9.00)	7.34 (6.65–9.00)	9.00 (8.00–10.00)	4.89 (1.19–15.00)
Male sex, No. (%)	21 (50.0)	15 (36.6)	12 (57.1)	76 (46.6)
Hemoglobin genotype, No. (%)				
AA	33 (78.6)	38 (92.7)	17 (81.0)	132 (81.0)
AC	9 (21.4)	3 (7.3)	3 (14.3)	14 (8.6)
AS	0 (0.0)	0 (0.0)	0 (0.0)	16 (9.8)
SC	0 (0.0)	0 (0.0)	1 (4.8)	1 (0.6)

Abbreviation: IQR, interquartile range.

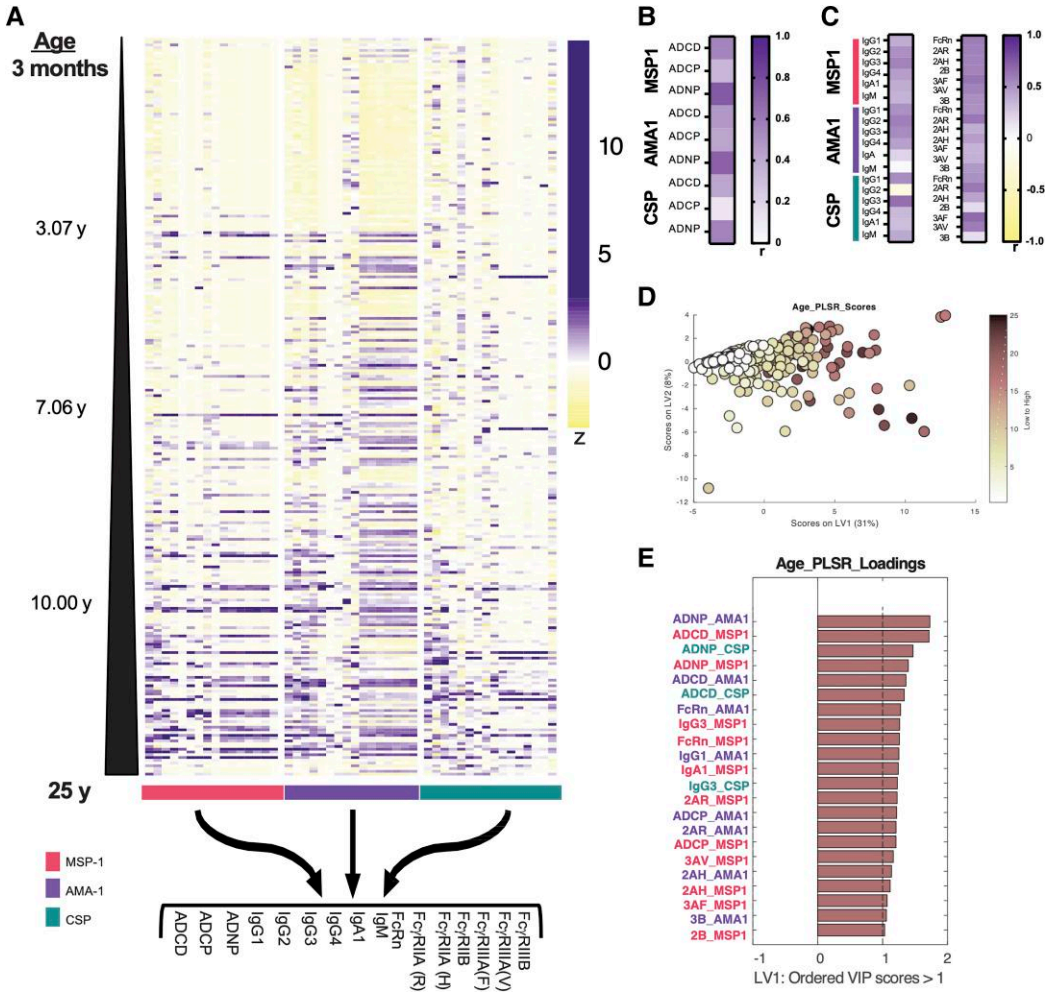

Figure 1. Malaria-specific antibody profiles increase with age. *A*, The heatmap depicts *Plasmodium falciparum*–specific antibody response ordered by age and grouped by antigen: MSP1 (pink), AMA1 (purple), and CSP (aqua). Data are z-scored across columns. Each column indicates 1 antigen-specific antibody feature. Spearman correlation (*r*) between various functions (*B*) and isotype, subclasses, and FcR binding (*C*). *D*, Regression model separating features based on age. *E*, Score plots showing separation based on age. The loading features colors are coded by antigen (same as *A*). Abbreviations: 2AR, FcγRIIAR; 2B, FcγRIIB; 3AF, FcγRIIIAF; 3B, FcγRIIIB; ADCD, antibody-dependent complement deposition; ADCP, antibody-dependent cellular phagocytosis; ADNP, antibody-dependent neutrophil phagocytosis; AMA1, apical membrane antigen; CSP, pre-erythrocytic circumsporozoite protein; LV, latent variable; MSP1, blood-stage merozoite surface protein 1; PLSR, partial least squares regression; VIP, variable importance in projection.

PCR result and the first positive *Pf* PCR result (for additional information, see Supplementary Materials).

Quantification of Parasitemia

For incident parasitemia events, parasite densities were estimated by nested quantitative PCR of genomic DNA extracted from dried blood spots using standard curves as previously described [23].

RESULTS

The Functional Malaria-Specific Humoral Immune Response Broadens With Age

We comprehensively profiled the humoral immune response in 267 young people living in the endemic region of Mali, aged 3 months to 25 years, recognizing that age is both a marker of the maturing immune system as well as a marker for exposure. Antibody response was analyzed across 3 key immunodominant malaria life-cycle antigens, including preerythrocytic circumsporozoite protein (CSP), blood-stage specific merozoite surface protein (MSP1), and merozoite apical membrane antigen (AMA1). Antibody profiles were captured prior to the onset of the malaria season in children who ultimately developed immediate disease, delayed disease, or no symptoms (immune) following infection.

As represented on Figure 1*A*, the analysis of each antibody feature showed a trend toward increasing antibody responses with increased age overall (Supplementary Figure 1). The correlation of each of the 48 antibody features with age revealed

ADNP as the most significantly correlated functional feature for all 3 antigens (Figure 1*B*). Among isotypes, subclasses, and Fc receptor (FcR) binding features, antigen-specific IgG3 most strongly correlated with age across all antigens (Figure 1*C*). However, whereas both MSP1- and AMA1-specific antibody responses strongly correlated with age across nearly all isotypes and subclasses except for AMA1 IgA and IgM, only CSP-specific IgG1 and IgG3 strongly correlated with age. Similarly, for FcR binding, there was a stronger correlation with the blood-stage antigen-specific antibodies, while CSP-specific antibody binding to FcγRIIB and FcγRIIIB exhibited a more modest correlation with age. Finally, we ran a partial least squares regression to assess the relative contributions of antibody features to variance in age (Figure 1*D* and 1*E*). The scores plot (Figure 1*D*) demonstrated a clear age-dependent maturation of the humoral immune response along the first latent variable. Moreover, the loading variable importance in projection (VIP) scores (Figure 1*E*) highlighted the specific multivariate features that evolved most tightly with age, most notably blood-stage AMA1-specific ADNP and MSP1-specifc ADCD. Overall, these data imply that it may take more exposures to fully mature the CSP-specific antibody response, while the blood-stage response matures earlier, with less exposures in time (Figure 1 and Supplementary Figure 1), pointing to further age-dependent differences in time to immunity to different stages of the *Pf* life cycle.

Antibody Neutrophil Phagocytosis in Addition to IgG and IgA Titers Are Associated With Immunity

To further understand the mechanism(s) underlying host control of malaria disease, antibody response was next analyzed across a subset of children aged 6–11 years who began the malaria season negative for PCR-detectable *Pf* parasitemia (n = 109). This age range was chosen based on the time during which the acquisition of clinical immunity to malaria develops in areas of high transmission [2]. Children were categorized into those who developed immediate fever following documented *Pf* infection (early fever group), experienced delayed fever after infection (delayed fever group), or did not experience fever despite infection (immune to fever group) [1]. ADCD and ADCP did not show differences across the clinical groups. In contrast, MSP1- and CSP-specific ADNP was significantly increased in the immune children, compared with those who experienced either delayed or early fever (Figure 2*A*). Furthermore, significant increases in highly functional MSP1-specific IgG3 and IgA titers were observed in immune children, whereas CSP-specific IgG4 was more abundant in immune children (Figure 2*B*). Conversely, no significant differences were noted in FcR binding across clinical groups (Supplementary Figure 2).

To identify the minimal antibody features that distinguish immune children from those who became febrile, we next performed a multivariate analysis using a combination of a least absolute shrinkage and selection operator (LASSO) and partial least squares discriminant analysis. Malaria-specific antibody profiles in immune individuals radiated away from the malaria-specific antibody responses in the children who developed fever (Figure 2*C*), the latter being more homogenous in their antibody profiles. The VIP plot highlighted the importance of a minimum of 3 of the total 48 features analyzed as key biomarkers that distinguished the 2 groups (Figure 2*D*), including elevated MSP1-specific IgG3 levels and CSP-specific ADNP and IgG4 responses in immune children (Figure 2*D*). Given the highly correlated nature of evolving humoral immunity, we probed the co-correlates of the LASSO-selected features (Figure 2*E*) to gain additional insights into the precise mechanisms that may evolve with immunity to malaria. Importantly, while CSP-specific IgG4 responses were narrowly associated with a diversified IgG subclass selection profile across antigens, MSP1-specific IgG3 were related to a large number of antibody features, including high levels of MSP1-specific FcR binding, pointing to a broad capacity to interact and recruit the innate immune response to this antigen. In contrast, CSP-specific ADNP was broadly correlated with several features that overlapped with MSP1-specific IgG3, but showed strongest relationships to CSP and AMA1 and particularly FcR binding profiles. Thus, these data point to broad neutrophil activation by diversified IgG subclass as potential key biomarkers of immunity against malaria-induced fever prior to infection.

Functional Antibodies Specific to MSP1 and CSP Predict Decreased Risk of Malaria

To determine whether functional antibody features specific for AMA1, CSP, or MSP1 were associated with decreased risk of the first episode of febrile malaria, we performed time-to-event analysis across the larger set of 267 children and young adults, with functional activity for each feature stratified by terciles. In the univariate analysis, the top tercile for 5 features specific for CSP (IgG3-CSP, ADNP-CSP, FcRn-CSP, FcγR3AF-CSP, and FcγR2AR-CSP) and 2 features specific for MSP1 (IgG2-MSP1, ADNP-MSP1) were significantly associated with decreased risk of febrile malaria (Figure 3; Supplementary Figure 3). When adjusted for age, sex, *Pf* PCR status at enrollment, and sickle hemoglobin (HbS), ADNP, and IgG3 against CSP remained significant at a false discovery rate (FDR) <0.20 (Supplementary Table 1). In accordance with Figure 2, these data show the strong effect that cytophilic antibodies specific to CSP, followed by MSP1-specific antibodies, have on the protection against symptomatic malaria, by enhancing neutrophil phagocytosis of the parasite.

Functional Antibodies Specific to AMA1 and MSP1 Predict Increased *Pf* Infection Risk

Time to first PCR-confirmed *Pf* infection analysis for 196 individuals who began the malaria season PCR negative for *Plasmodium* infection and had sufficient follow-up data revealed that higher levels of functional antibodies specific to

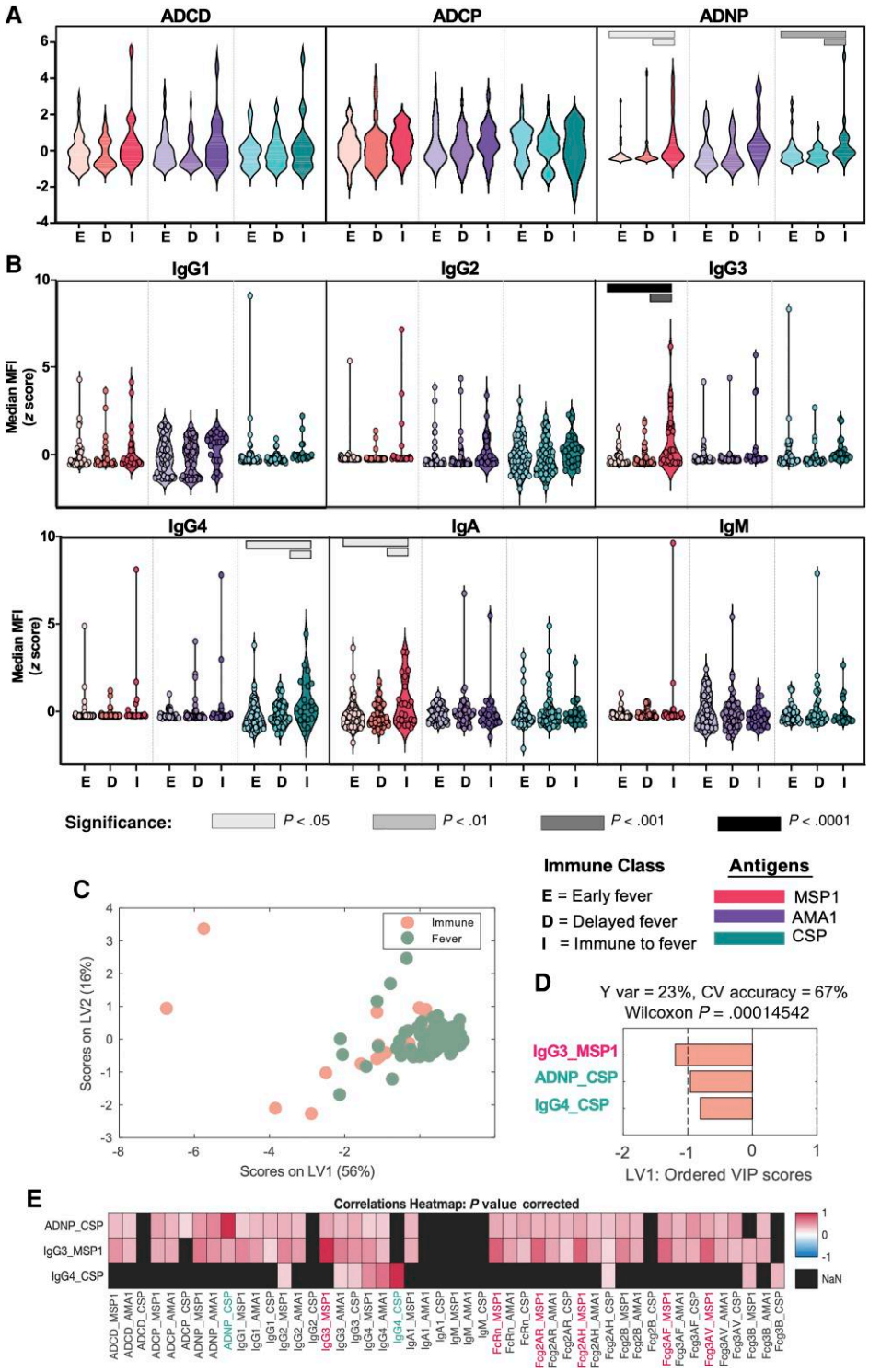

Figure 2. Immunity associated with neutrophil phagocytosis. Antibody response against MSP1 (pink), AMA1 (purple), or CSP (aqua) was analyzed in the plasma of children ages 6–11 sampled prior to the malaria season. Samples are grouped by immune class as indicated. *A*, Violin plots show the geometric mean of C3+ for antibody-dependent complement deposition, then the phagocytic score for antibody-dependent cellular phagocytosis and antibody-dependent neutrophil phagocytosis. *B*, Luminex data show median fluorescence intensity (MFI) for different antibody isotype and subclass. Significance was determined by a 1-way analysis of variance test with Tukey multiple comparisons correction. *C*, The data are visualized using a partial least squares discriminant analysis, with the model having a cross-validation Wilcoxon *P* value <.05. Features predicative of immune status are in pink, while those predictive of fever status are in green. *D*, The bar graph depicts the minimal features based on their variable importance in projection scores. *E*, Heatmap of correlation features with statistical significance ($P < .05$). Abbreviations: ADCD, antibody-dependent complement deposition; ADCP, antibody-dependent cellular phagocytosis; ADNP, antibody-dependent neutrophil phagocytosis; AMA1, apical membrane antigen; CSP, preerythrocytic circumsporozoite protein; CV, cross-validation; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; LV, latent variable; MFI, median fluorescence intensity; MSP1, blood-stage merozoite surface protein 1; VIP, variable importance in projection.

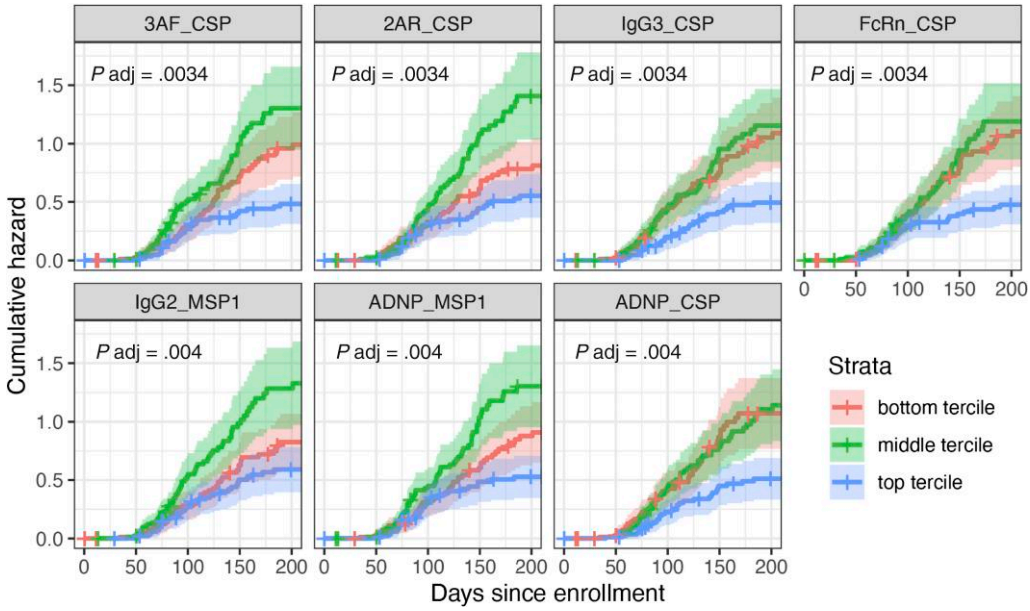

Figure 3. Functional antibodies specific against CSP and MSP1 predict decreased risk of febrile malaria. Cumulative hazards for first febrile malaria episode (axillary temperature >37.5°C and *Plasmodium falciparum* parasitemia >2500 asexual parasites/μL) over 210 d of follow-up for 267 individuals stratified by terciles of antigen-specific antibody functional activity. Significance was determined by log-rank analysis. Only functional features with a Benjamin–Hochberg adjusted *P* value (*P* adj) < .01 are shown. See Supplementary Figure 3 for all features. Abbreviations: 2AR, FcγRIIAR; 3AF, FcγRIIIAF; ADNP, antibody-dependent neutrophil phagocytosis; CSP, preerythrocytic circumsporozoite protein; IgG, immunoglobulin G; MSP1, blood-stage merozoite surface protein 1.

AMA1 and MSP1 predicted risk of incident parasitemia in both univariate analysis (Supplementary Figure 4) and when adjusted for age, sex, and HbS genotype (Supplementary Table 2). None of the significant features at FDR <20% predicted protection from *Pf* infection. The top feature ranked by significance in the adjusted analysis was FcγR2B-MSP1 (hazard ratio 2.12; FDR = 0.03) and 7 of the 10 most significant features were AMA1 features, suggesting that AMA1 and MSP1 antibody responses are strong markers of malaria transmission intensity.

FcγRIIIB-MSP1 and Cytophilic CSP IgG Predict Protection From Febrile Malaria Once Parasitemic

Analysis of time to first malarial fever once parasitemic ensures that all analyzed individuals were malaria exposed and is particularly useful for evaluating the effect of antibodies to blood-stage antigens [16, 24]. In the univariate analysis, CSP-specific IgG3, FcRn-binding levels, and IgG1 were the top 3 features ranked by significance (FDR ≤0.10; Supplementary Figure 5) and predicted protection febrile malaria once parasitemic, consistent with the time-to-clinical malaria analysis (Figure 3). When adjusted for age, sex, and HbS, only FcγRIIIB-MSP1 binding antibody levels significantly predicted protection from febrile malaria once parasitemic at FDR <20%, but CSP-specific IgG1 and IgG3 were associated with protection at a less stringent FDR cutoff of <25% (Supplementary Table 3).

Pre–Malaria Season CSP-Specific IgG3 and ADNP-CSP Predict Reduced Parasite Density at Incident Infection and Clinical Malaria, Respectively

To further assess whether baseline functional antibodies predict a reduction in parasitemia, we compared parasite densities at the first PCR-confirmed infection and the first clinical malaria episode of the season for each antibody feature stratified by terciles (Supplementary Tables 4 and 5). The top terciles of CSP-specific IgG3 and ADNP-CSP were associated with lower parasite densities at incident infection and incident clinical malaria, respectively (Supplementary Figure 6), suggesting that preexisting functional CSP-specific IgG, with enhanced functionality, mediates clinical protection by reducing parasitemia, perhaps by limiting the blood-stage inoculum.

IgG Antibodies Predominantly Drive ADNP Across Antigens

Given the selective increase in ADNP among immune children, we next aimed to determine whether particular components of the evolving polyclonal malaria-specific humoral immune response were key to promoting this robust activity, using a bead-based imaging analysis able to capture antibody-opsonized IgG complex uptake into neutrophils (Figure 4*A*). Polyclonal pools of antibodies from the immune children were then depleted of total IgG, IgG3, or IgA to define the specific subclass/isotype-specific population that may play a critical role in ADNP (Figure 4*B* and Supplementary Materials). We observed that total IgG depletion had the greatest impact on reducing ADNP and led to greater decline in blood-stage MSP1 and

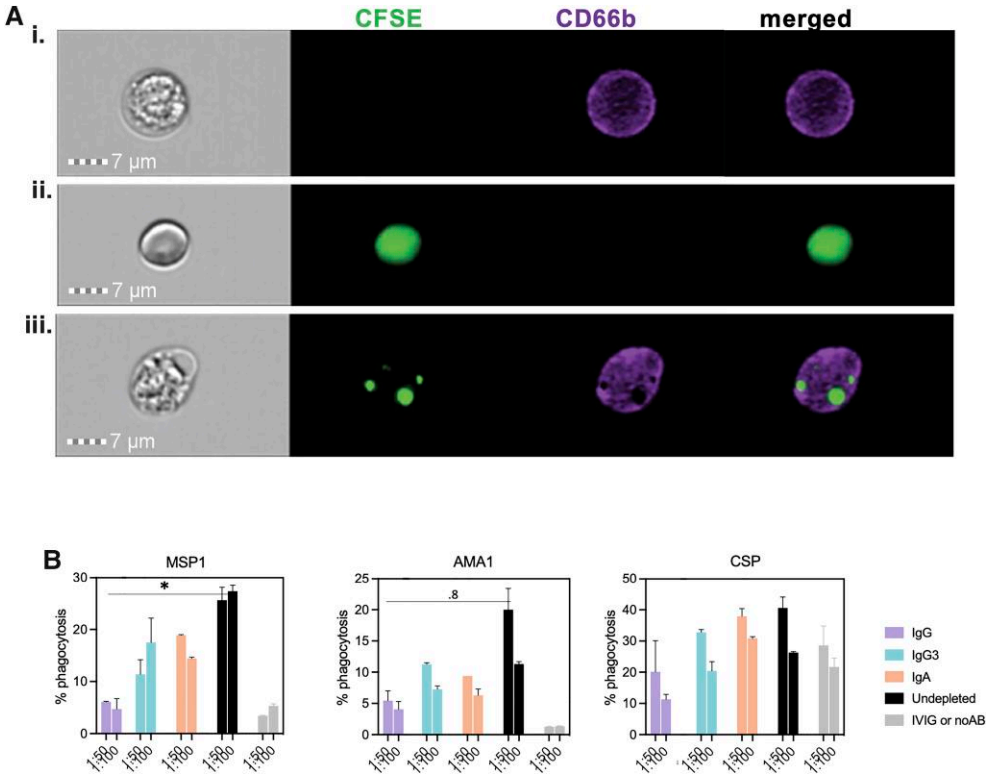

Figure 4. Neutrophil phagocytosis of infected red blood cells (iRBCs). *A*, Representative images of iRBC neutrophil phagocytosis. Green indicates CFSE-labeled iRBCs, purple indicates CD66b-labeled neutrophils. *B*, A pool of plasma from the 6- to 11-year-old cohort was generated and depleted of the following isotype: total IgG, IgG3, or IgA. Depleted plasma, along with control undepleted pool and intravenous immunoglobulin, were used in an antibody-dependent neutrophil phagocytosis assay with antigen coupled beads. Data are reported for 2 dilutions (1:50 and 1:100) performed in duplicate with 2 blood donors. $^{*}P < .05$. Abbreviations: AMA1, apical membrane antigen; CSP, preerythrocytic circumsporozoite protein; IgA, immunoglobulin A; IgG, immunoglobulin G; IVIG, intravenous immunoglobulin; MSP1, blood-stage merozoite surface protein 1; noAB, no antibodies.

AMA1-specific ADNP compared to CSP-specific immunity. However, the redundancy in isotype-mediated neutrophil phagocytosis, where neutrophils exclusively constitutively express both FcγR and FcαR receptors [25], may account for the limited impact of antibody depletion on ADNP activity. Thus, multi-isotype specific mechanisms may account for pre-hepatic control of infection, whereas IgG antibodies appear to be key to the control and clearance of blood-stage parasites.

DISCUSSION

Antibodies play a critical role in the protection against malaria [6, 11, 26, 27], yet the mechanisms by which they drive protective immunity against disease, particularly among young individuals, remain incompletely understood. Defining the co-evolution of malaria-specific antibody functions that emerge selectively with age in the context of protection against clinical malaria could help inform the development of a more effective vaccine against malaria disease. Using an unbiased systems serology approach, the evolution of the natural antibody functions in young people, prior to the onset of the malaria season, pointed to the unique evolution of neutrophil phagocytic activity as a key functional mechanism of protection against malaria disease.

Emerging data point to a critical role for the evolving breadth of the epitope-specific humoral response to malaria [12, 14, 28], as well as the functional activity of antibodies in limiting the severity of infection [8, 29]. Focusing on the immunodominant sporozoite antigen CSP and blood-stage merozoite antigens AMA1 and MSP1, changes in antigen-specific Fc profiles were comprehensively assessed in subjects from a high-transmission, malaria-endemic region of Mali prior to the malaria season, across children who exhibited a wide range of disease severities [9, 26, 27]. We observed an increase of ADCD, ADCP, and ADNP to MSP1 and AMA1 with age. Conversely, CSP-specific immunity did not develop within the same time frame and only increased after the first decade of life for ADCD and after 7 years for ADNP, with limited evolution of ADCP, pointing to differences in the evolution of sporozoite- and blood stage–specific immunity to malaria. Functional evolution was linked to increased isotype/FcR coordination across the blood-stage antigens, and a highly focused IgG1/IgG3 response to the CSP antigen with age, pointing to an

age-dependent class switch recombination. These data suggest that neutrophil phagocytic antibody activity evolved most closely with age and malaria exposure, as reported previously [30, 31] and was selectively enriched in protected children.

Multivariate modeling pointed to a critical role for both blood-stage (MSP1)– and sporozoite (CSP)–specific responses as potential biomarkers of clinical immunity. This suggests that prevention of febrile malaria may require the evolution of immunity to both phases of the infectious life cycle. Interestingly, children who went on to resist febrile malaria exhibited increasing numbers of neutrophil recruiting antibodies to both the MSP1 and CSP antigens. Given that neutrophils are the most abundant white blood cell in the systemic circulation [32] and among the first cells present at the site of infection [33], neutrophils represent a critical effector cell in the control and clearance of infection [34]. Moreover, CSP-specific ADNP activity was also linked to protection against malaria challenge in a number of controlled human challenge RTS,S vaccine studies [35]. Additionally, a delayed third dose of RTS,S vaccine conferred higher protection, which was associated with enhanced phagocytosis against both the C-terminal (Pf16) and NANP region of CSP [36]. Finally, NANP6-specific antibodies robustly restricted sporozoite invasion of primary livers in the presence of neutrophils [37, 38]. Thus, these data argue that vaccines able to elicit robust neutrophil-activating antibodies may be key to protection against malaria infection and disease.

CSP-specific IgG3 and ADNP consistently predicted protection from clinical malaria and were associated with reduction in parasitemia at incident malaria, yet did not predict protection against incident infection (ie, sterile immunity). An effective antibody response against CSP, being a preerythrocytic antigen, would be expected to confer sterile immunity. Thus, as expected, the data presented here support a mechanism whereby high levels of preexisting, functional CSP-specific antibodies confer malaria protection by limiting the initial blood-stage inoculum rather than preventing parasitemia altogether. Such incomplete protection could be attributed to genetic diversity among circulating parasites, allowing some strains to partially evade allele-specific host responses [39, 40] and requiring secondary blood-staged immunity to limit disease.

Enhanced neutrophil phagocytic activity was associated with the evolution of higher MSP1-specific IgG3 and IgA titers, as well as CSP-specific IgG3. Recent studies focusing on serum IgA in immunity to nonmucosal pathogens have shown that the production of this isotype can be induced by sporozoites at dermal inoculation sites and can reduce liver parasite burden in vivo [41]. Trials of the malaria vaccine candidate RTS,S/AS01 also showed an association between IgA responses targeting the *Pf* CSP NANP repeat region and C terminus and protection [42]. While IgG3 is the most functional IgG subclass in humans [43], emerging data suggest that IgA potently activates neutrophils via high-affinity binding to the Fcα-receptor that is constitutively expressed on neutrophils [44]. Moreover, IgA appears to drive distinct functional responses in neutrophils compared to IgG. Thus, it is plausible that the increasing IgG3 and IgA responses that we observe may mark the development of more functional and potentially affinity-matured IgG responses that may be responsible mechanistically for neutrophil activation, rather than representing the direct driver of parasite control via neutrophils. Importantly, IgG antibodies themselves may be modified posttranslationally, via altered glycosylation [45, 46], resulting in FcR affinity differences that ultimately control antibody functionality. Given that subclass selection and Fc glycosylation are likely co-regulated to drive the most functional humoral immune responses, IgG3/IgA selection may mark the generation of IgGs with a more functional Fc-glycosylation profile, able to leverage neutrophils more aggressively. While sufficient sample volumes were not available from these study participants to perform Fc-glycosylation analysis, which typically requires a total of 0.5–1 μg of purified antibody, the identification of the evolution of blood-stage and prehepatic IgG, with respect to both affinity maturation and Fc glycosylation, may provide critical insight for the design of next-generation vaccines and therapeutics able to drive enhanced protection against malaria. Moreover, while this project focuses on immunodominant *Pf* antigens (MSP1, AMA1, and CSP), the literature suggests that other antigens are also associated with protection from malaria in cohort studies (eg, PF3D7_1136200, MSP2, RhopH3, P41, or MSP11) [47]. Future work, including a broader panel of malaria antigens, capturing genetic diversity, may provide enhanced resolution on the collaboration of polyclonal functional humoral immunity and specific antibody functions involved in the prevention and control of malaria infection in children.

After birth, both adaptive and innate immune cells must coevolve to learn to respond to pathogens. Like the immature adaptive immune system, neutrophils exhibit attenuated functions in the first days and months of life, responding less effectively to pathogens. However, neutrophil activity appears to normalize in toddlers, providing a large population of innate immune cells that could potentially respond effectively to malaria infection in the presence of highly functional antibodies. While CSP-specific ADNP was associated with protection against infection in controlled human challenge studies of RTS,S [35, 38], in the endemic settings of natural infection presented here, ADNP activity is induced in an age-dependent manner. Thus, next-generation vaccine development must learn to homogenously induce robust levels of neutrophil functional antibodies but must also consider neutrophil deficiencies that may exist in the youngest children in the population as well as across particular comorbid populations.

Overall, the data presented here point to the importance of inducing immunity to both the preerythrocytic and blood stages of infection to maximize protection against clinical malaria and show particular antibody Fc-effector profiles that could guide the design of next-generation vaccines or therapeutics.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. G. A., P. D. C., T. M. T., E. A. D., N. N., and S. Dol. designed the study. E. A. D. performed system serology experiments. C. J., L. D., and J. L. provided iRBCs and helped E. A. D. and J. H. perform and analyze the phagocytosis assay. S. Dol. and T. M. T. performed the computational analysis. A. O., S. Dou, K. K., B. T., and P. D. C. designed and conducted the cohort study. T. M. T., A. O., S. Dou, and P. D. C. managed the cohort and parasitological data. N. N., E. A. D., S. Dol., T. M. T., and G. A. drafted the manuscript. J. H. and C. J. contributed to discussion surrounding the manuscript. All authors critically reviewed the manuscript.

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Potential conflicts of interest. G. A. is the founder of Seromyx Inc and Leyden Labs. G. A.'s interests were reviewed and managed by MGH and Partners HealthCare in accordance with their conflict-of-interest policies. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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