The Role of Complement Immune Response on Artemisinin Combination Therapy in a Population from Malaria Endemic Region of Western Kenya

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Abstract

BACKGROUND: Naturally acquired immunity which is characterized by protection against overt clinical disease and high parasitemia is acquired with age and transmission intensity. The role of naturally acquired immunity on the efficacy of antimalarial drugs including artemisinin combination therapies (ACTs), the first-line treatments for uncomplicated *Plasmodium falciparum* has been demonstrated. This study investigated the role of naturally acquired immunity in the response to malaria ACT drug treatment in symptomatic patients living in a malaria high-transmission area of Western Kenya.

METHODS: This study used samples obtained from a therapeutic efficacy study conducted in western Kenya, an areas of high transmission which assessed ACTs. Sera samples from malaria immune (n = 105) and naïve participants (n = 6) were assessed for *in vitro* growth inhibitory activity against 3D7 *P. falciparum* using a fluorescent-based Growth Inhibition Assay (GIA). Participants' age and parasite clearance parameters were used in the analysis.

RESULTS: The key observations of the study are: (1) Sera with intact complement displayed higher GIA activity at lower serum dilutions; (2) there was significant relationship between GIA activity, parasite clearance rate, and slope half-life; (3) Age was a confounding factor when comparing the GIA activity with parasite clearance kinetics.

CONCLUSION: Taken together, this study demonstrates for the first time there is synergy of complement, pre-existing immunity, and drug treatment in younger patients with symptomatic malaria in a high-transmission area.

Background

People living in malaria endemic areas acquire natural immunity with age, with faster rates of acquisition occurring in high compared to low transmission settings [1]. Both humoral and cell-mediated immune responses are involved in generating effective immunity [2], with individuals who possess greater breadth of immunological responses being at a lower risk of developing overt clinical symptoms [3].

Acquired immunity has been shown to influence malaria drug treatment outcomes by enhancing the efficacy of sub-optimal drug regimen and delaying the emergence of drug resistance, including artemisinin combination therapies (ACTs), the first-line treatment of *Plasmodium falciparum* in most malaria endemic countries [4]. ACTs are still highly efficacious in sub-Saharan Africa (sSA) [5], but as malaria transmission reduces, and malaria immunity at population level wanes [6], the efficacy of ACTs is likely to be impacted. It is therefore important to monitor and assess the effect of pre-existing acquired immunity on the efficacy of ACTs as part of Therapeutic Efficacy Studies (TESs).

Complement factors have been shown to play a key role in antibody-mediated immunity to malaria in humans [7]. Studies from malaria endemic areas have demonstrated that *P. falciparum* exposure is associated with growth-inhibitory activity in total [8], MSP-1 [9] or AMA-1 [10]-specific IgG fractions. Growth inhibition assays (GIA) assesses the functional activity of sera to reduce/inhibit red blood cell (RBC) invasion and/or intra-RBC growth of *P. falciparum* in vitro [11]. Using GIA, the present study aimed to assess the role of complement and

pre-existing immunity in the response to malaria drug treatment in symptomatic patients living in a malaria high-transmission area of Western Kenya.

Methods

Study Site, Population and Sample Collection

The study was conducted in Kombewa district hospital in Kisumu County, western Kenya from June 2013 through November 2014. Detailed clinical study will be reported elsewhere. Briefly, Kisumu County is a malaria holoendemic lake region with intense malaria transmission through-out the year, with annual entomological inoculation rates (EIR) of 31.1 infected bite per year [12]. This was a two-arm, randomized open-label study, where patients presenting with uncomplicated malaria at the Kombewa district hospital aged between 6 months to 65 years were recruited. Study participants were randomized to receive AL or ASMQ using block randomization schemes with varying block sizes. Venous blood samples were collected at hours 0, 4, 8, 12, 18, 24 and then every 6 hours until 2 consecutive smears became negative. Giemsa-stain films were prepared per World Health Organization (WHO) guidance and read by two independent expert microscopists. The geometric mean of the parasite count per microliter from each participant at each sampling time point was then calculated. Participants were followed for a total of 42 days. A total of 118 participants were enrolled in the study, 59 from each arm. From these, 105 blood samples from study participants (46 and 62 from individuals <5 and ≥ 5 years, respectively) were used in the GIA analysis.

P. falciparum negative venous blood samples were obtained from six male adults aged 18-65 years, with blood groups A, B and O and hemoglobin level of ≥ 13 g/dL living in a malaria non-endemic regions (Kericho and Nairobi) with no travel history to malaria endemic areas in the last six months of Kenya were used as non-immune controls for the GIA experiments.

For the maintenance of the parasite culture and performing the GIA assays, Red Blood Cells (RBCs) were obtained from blood group "O" donors, aged between 18 and 50 years, with hemoglobin level of 14 – 18g/dL for males and 12 – 16g/dL for females living in a malaria non-endemic area (Kericho and Nairobi) with no travel history to malaria endemic areas in the last six months. Briefly, 7 mL aliquots of the whole blood were added to 7 mL wash medium in 15mL centrifuge tubes, centrifuged at 800g for 10 minutes. (Eppendorf 5804R centrifuge, Eppendorf®) and buffy coat containing most White Blood Cells (WBCs) gently aspirated, followed by the entire supernatant, leaving the packed RBCs. The WBCs still remaining among the cells were removed/washed out by three-time centrifugation in wash media. For each subsequent wash, the buffy coat containing most WBCs was aspirated first followed by the entire supernatant. The packed RBCs were then gently re-suspended in 7 mL wash media and re-centrifuged at 800 g till clear of the WBCs. The cells were then suspended in equivalent wash medium at 50% hematocrit (packed cells /wash medium v/v), then stored at 2-8°C.

Parasite clearance rates calculation

The statistical models used to estimate the parasite clearance measures and lag phase duration were fitted using the Parasite Clearance Estimator (PCE) tool developed by Worldwide Antimalarial Resistance Network [13]. The following parameters were estimated: parasite clearance half-life, parasite clearance rate constant

(K/hour), and the estimated time to reduce parasitemia by 50% (PC50), 90% (PC90), 95% (PC95), and 99% (PC99). Log transformed parasite density was plotted against time in hours to generate slope half-life ($T_{1/2}$) which is defined as the time needed for parasitemia to be reduced by half [14]. This constant is independent of starting value of parasitemia. The slope half-life was calculated as follows:

$$T_{1/2} = \log_e(2)/K$$

= 0.692/K, where K is the clearance rate constant

The clearance rate K represents the rate of parasite clearance after start of drug treatment.

Culture media

P. falciparum *in vitro* culture medium (Life technologies, Carlsbad, CA) was prepared as previously described [15]. Briefly, basic media comprised of 10.4 g Roswell Park Memorial Institute (RPMI; Invitrogen, Inc., Carlsbad, California, USA) powder combined with 2 g of glucose (Sigma Inc., St Louis, Missouri, USA) and 5.95 g HEPES (Sigma, USA) dissolved to homogeneity in 1 litre of de-ionized water and sterilized with a 0.2 µM filter. RPMI 1640 tissue culture media (TCM), for all parasite culture and drug dilutions, consisted of RPMI 1640 basic media with 0.5% Albumax II (Gibco, Grand Island, NY), 3.2% (vol/vol) sodium bicarbonate (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) and 4 µg/mL hypoxanthine (Sigma Inc., St Louis, Missouri, USA). Complete RPMI 1640 media was stored at 2-8 °C.

Serum free media consisted of 1.0 mL of 1.45 mM sterile hypoxanthine to 43.4 mL of RPMI basic medium, 1.6 mL of sterile sodium bicarbonate (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA), 7.5% and sterilized with 0.2 µM filter then put in 2-8°C. 10% complete media for Control was prepared by adding 5 mL of serum to 43.4 mL of RPMI basic medium, 1.0 mL of 1.45 mM sterile hypoxanthine, 1.6 mL of sterile sodium bicarbonate, 7.5% then sterilized with 0.8 µM, 0.45 µM, 0.2 µM filters and put at 2-8°C

P. falciparum culture

P. falciparum-3D7 strain obtained through Biodefense and Emerging Infections Research Resources Repository (BEI Resources), NIAID, NIH: *falciparum*, Strain 3D7, MRA-102 were maintained in continuous culture as previously described [16]. Briefly, *P. falciparum*-3D7 were suspended in 5 mL of CMS at 6% hematocrit in tissue culture flasks (Corning Glass Works, Corning, NY). The flasks were flushed with a gas mixture consisting of 5% oxygen, 5% carbon (IV) oxide and 90% nitrogen (Air Products Corp., Allentown, PA), sealed, and incubated at 37°C. The medium was changed daily and fresh RBCs added when required. Parasite growth was monitored microscopically by counting Giemsa-stained thin blood smears. When parasitemia exceeded 3%, ring-stage parasites were synchronized by sorbitol lysis (5 % D-sorbitol (Sigma, St. Louis, MO) as follows: Parasites were mixed and transferred into a 15 mL centrifuge tube and centrifuged at 3,000 rpm for 10 min. After removing the supernatant, 5% D-sorbitol pre-warmed to 37°C was added to the RBC pellet, incubated for 10 min at 37°C, vortexed for 1 min and re-centrifuged at 3,000 rpm for 10 min. After aspirating the supernatant, and repeating the process twice using tissue culture media (TCM) ≥ 90% ring forms was achieved.

Serum preparation

Serum was obtained from blood samples as previously described [17] with minor modifications. Briefly, whole blood collected in plain (anticoagulant free) blood bags from each study participant was stored at 2-8 °C overnight to allow settling of the serum and cells portions. Serum portion was transferred into 50 mL centrifuge tubes and centrifuged at 800 g. The supernatant was transferred into 50 mL centrifuge tube to remove all cell components. This step was repeated three times and serum was stored at -65 to -80 °C until further use. The serum from the six male adults (non-immune) were pooled together forming approximately 2.0 L for use as control for subsequent experiments. Serum from each of the immune or non-immune pooled serum was divided into two equal portions. One portion was heat inactivated at 56°C for 30 minutes in a water bath as previously described [7].

Growth inhibition assay (GIA)

In vitro anti-plasmodial susceptibility assay was performed as previously described [15] with some modifications. Briefly, 10-fold dilution in TCM was done for immune serum, non-immune serum, and negative control wells. Parasitemia in the GIA assays of continuous culture $\geq 3\%$ lowered to 1% were adjusted to final 1-2% hematocrit, a ratio of 1:10 serum dilution in 100 μ L final volume was dispensed on Nalgene Nunc, 96 flat-bottom well cell cultures sterile with lid microtiter plates (Magna, Leicestershire, UK) and 10 μ L of 3D7 parasite was added in each well then incubate at 37°C for 72 hours in modular reservoirs, gassed with 90% nitrogen, 5% carbon dioxide and 5% oxygen. Parasite growth was evaluated using SYBR Green I dye, thus 4 μ L of SYBR Green I was added to 2 mL of malaria SYBR Green 1 fluorescence (MSF) lysis buffer [20 mM Tris (pH 7.5), 5 mM EDTA, 0.16% (wt/vol) saponin, and 1.6% (vol/vol) Triton X-100], 10 $\times$ SYBR green I dye concentration) and 100 μ L of a mixture of SYBR Green 1 and MSF was transferred to each well. The plates were stored in the dark at ambient temperature for 24 hours [18]. The plates were examined for relative fluorescence units (RFUs) per well using a fluorescence plate reader (Tecan™ Morrisville, North Carolina, United States). Parasite replication was quantified for each serum sample and expressed as the mean percentage growth inhibition by

$$\text{percentage growth inhibition} = \frac{\text{average RFUs in growth medium} - \text{average RFUs in patient's serum}}{\text{Average RFU in growth medium}} \times 100\%$$

Optimization was done to validate the assay and control reference ranges were established upon which the assay performance was monitored using antimalarial drugs Dihydroartemisinin (DHA) and Lumefantrine (LU) that have known IC₅₀ for the reference parasite strain. The drugs DHA and LU were used as positive control and tissue culture media (10% CMS) was used as negative control. All experiments were conducted in triplicate.

Statistical analysis

Data was analyzed using GraphPad Prism version 5.03 (GraphPad software, Inc., San Diego, CA) and Minitab, version 17 (Minitab Inc., State College, PA). Normality of the data was confirmed using Shapiro–Wilk test. Data are presented as the median with interquartile range ([IQR] 25th and 75th percentiles). Statistical difference between groups was determined using t-test and Wilcoxon matched-pairs signed rank test. Correlation

between groups was assessed using Pearson correlation coefficient test. Differences with a p -value less than 0.05 were considered statistically significant.

Results

Growth Inhibitory Activity

The *in vitro* growth inhibition of *P. falciparum* in serum from the 105 immune study participants and non-immune were compared. The percentage growth inhibition of *P. falciparum* at 10 and 1% serum concentration from the study participants was significantly higher than for the non-immune controls ($p < 0.0001$) (Figure 1), indicating parasite growth inhibition was due to the immune status. There was no statistically significant difference in percentage growth inhibition for heat inactivated (+HA) serum compared to non-heat inactivated (-HA) serum at 10% serum concentration ($p = 0.18$). Conversely, the percentage growth inhibition was significantly higher for -HA serum than +HA serum at 1% serum concentration ($p = 0.009$) (Figure 2). The results revealed the role of complement when the assay was performed at low serum concentrations where antibodies are at limiting concentrations.

Age and GIA Activity

To determine whether age or parasitemia were confounding factors in GIA, the GIA data were stratified based on individuals younger than five years (< 5 years) or five years and older (≥ 5 years). There was no age-dependence of GIA activity nor significant correlation between GIA activity and parasite density ($R^2 = 0.03$, $p = 0.95$ Pearson correlation coefficient) (Figure 3).

Impact of pre-existing immunity, GIA and treatment outcome

The main question of the present study was whether pre-existing immunity had an impact on the parasitological outcome after drug treatment and the kinetic of the response. The mean $T_{1/2}$ for both study arms combined was 2.38 h (95% confidence interval [CI], 2.23 – 2.52), the median was 2.40 h (interquartile range [IQR], 1.95 – 2.80), and the range was 0.97 – 4.22 h. The cutoff values for stratifying into the two different groups have previously been described [19, 20]. Correlations between drug treatment outcome as determined by parasite clearance parameters (slope half-life [$T_{1/2}$] and clearance rate [K]) and pre-existing GIA activity in sera were assessed (Table 1). The main observation when performing the statistical analyses was that stratifying the data based on the kinetic of the drug response revealed two factors, complement and age that were associated with a faster parasite clearance and slope half-life. Non-heat inactivated sera from study participants younger than five years showed a significant negative correlation between GIA activity and slope half-life ($R^2=0.698$, $p=0.025$ Pearson correlation) and a significant positive correlation between GIA activity and parasite clearance ($R^2=0.604$, $p=0.05$ Pearson correlation). These correlations are lost when sera were heat-inactivated clearly demonstrating the role of complement in clearing the parasite under drug treatment.

Discussion

The present study utilized samples from a TES study with a comprehensive immunological profile [20] and parasitological characterization. To complete the immune-parasitological profile, the functional activity of sera

collected prior to drug treatment was assessed in a sensitive GIA assays [15]. To determine the contribution of innate factors such as complement, sera were tested with and without heat-inactivation. The initial analysis showed that the contribution of complement to the anti-parasite activity was only notable at low serum concentrations where antimalarial antibodies are likely under limiting concentrations. This confirms previous reports demonstrating the role of complement in GIA activity [7, 21].

The next step was to determine the impact of age on the GIA activity. Contrarily to a previous study [22], we could not find an age-dependent response in the functional activity displayed by the sera. This contradiction is likely due to the fact that our study population is comprised of patients that presented with malaria symptoms while other studies investigated the role of age in asymptomatic populations.

The key observation of this study was made when the data were stratified based on the drug kinetic response of the study participants [21]. This classification revealed that the parasitological response (i.e., parasite clearance rate and slope half-life) to drug was linked to GIA activity in non-heat-inactivated sera in patients under the age of five (Table 1). This finding suggests that the role of complement varies depending on the age of the study participant, corroborating previous studies that have demonstrated the importance of age in immune acquisition [23–25]. Previous assessment of the serological antibody profile did not find differences between the age groups [21] and, therefore, our finding points towards a greater role of innate factors in younger patients. The need for complement and complement fixing antibodies has been reported to be crucial for the protection against malaria clinical disease [7], supporting our observation that heat-inactivated serum has lower GIA activity. It does not preclude that there may be differences in the levels of malaria-specific IgM which has not been assessed. This is the main antibody isotype fixing complement and there may be differences in the distribution of malaria-specific IgM and IgG in the different age groups.

In conclusion, the present study reveals differential relationships between adaptive immunity, innate immunity (mainly the role of complement), and response to drug treatment as function of age of the symptomatic study participants. Anti-parasitic activity (GIA) is significantly correlated with $T_{1/2}$ and K only in sera of younger symptomatic patients. This effect is dependent of intact (non-heat inactivated serum) indicating the importance of innate factors such as complement.

Declarations

Ethics approval and consent to participate

The study was approved by the scientific and ethics review boards of the Kenya Medical Research Institute, Nairobi Kenya (KEMRI; approved protocol # 2518) and the Institutional Review Board of the Walter Reed Army Institute of Research, Silver Spring, MD (WRAIR; approved protocol #1935). All participants and/or their legal guardians gave a voluntary written informed consent before any study procedures commenced.

Consent for publication

Not Applicable. No personal identifiable data was used in the study. All authors have provided consent to publish the findings in this study.

Availability of data and material

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

DO, BO, EK provided funding. BA provided clinical study samples, conducted clinical data analysis. CW, DO and EK designed the study protocol. CW performed laboratory assays. HA supervised and provided assays execution guidance. CW, EBL, EK, DO, GO performed data analysis and interpretation. CW, EBL, EK wrote manuscript. DO, EK, EBL, BO, BA revised, and reviewed the final manuscript draft. CW, EBL, HA, GO, BO, BA, EK approved final manuscript.

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References

1. Fowkes FJ, Boeuf P, Beeson JG: **Immunity to malaria in an era of declining malaria transmission.** *Parasitology* 2016, **143**:139-153.

2. Beeson JG, Osier FH, Engwerda CR: **Recent insights into humoral and cellular immune responses against malaria.** *Trends Parasitol* 2008, **24**:578-584.
3. Osier FH, Fegan G, Polley SD, Murungi L, Verra F, Tetteh KK, Lowe B, Mwangi T, Bull PC, Thomas AW, et al: **Breadth and magnitude of antibody responses to multiple Plasmodium falciparum merozoite antigens are associated with protection from clinical malaria.** *Infect Immun* 2008, **76**:2240-2248.
4. Ataide R, Ashley EA, Powell R, Chan JA, Malloy MJ, O'Flaherty K, Takashima E, Langer C, Tsuboi T, Dondorp AM, et al: **Host immunity to Plasmodium falciparum and the assessment of emerging artemisinin resistance in a multinational cohort.** *Proc Natl Acad Sci U S A* 2017, **114**:3515-3520.
5. Organization WH: **World Malaria Report 2017.** Geneva 2017.
6. Mugenyi CK, Elliott SR, Yap XZ, Feng G, Boeuf P, Fegan G, Osier FFH, Fowkes FJI, Avril M, Williams TN, et al: **Declining Malaria Transmission Differentially Impacts the Maintenance of Humoral Immunity to Plasmodium falciparum in Children.** *J Infect Dis* 2017, **216**:887-898.
7. Boyle MJ, Reiling L, Feng G, Langer C, Osier FH, Aspelting-Jones H, Cheng YS, Stubbs J, Tetteh KK, Conway DJ, et al: **Human antibodies fix complement to inhibit Plasmodium falciparum invasion of erythrocytes and are associated with protection against malaria.** *Immunity* 2015, **42**:580-590.
8. McCallum FJ, Persson KE, Mugenyi CK, Fowkes FJ, Simpson JA, Richards JS, Williams TN, Marsh K, Beeson JG: **Acquisition of growth-inhibitory antibodies against blood-stage Plasmodium falciparum.** *PLoS One* 2008, **3**:e3571.
9. Murhandarwati EE, Wang L, Black CG, Nhan DH, Richie TL, Coppel RL: **Inhibitory antibodies specific for the 19-kilodalton fragment of merozoite surface protein 1 do not correlate with delayed appearance of infection with Plasmodium falciparum in semi-immune individuals in Vietnam.** *Infect Immun* 2009, **77**:4510-4517.
10. Hodder AN, Crewther PE, Anders RF: **Specificity of the protective antibody response to apical membrane antigen 1.** *Infect Immun* 2001, **69**:3286-3294.
11. Crompton PD, Miura K, Traore B, Kayentao K, Ongoiba A, Weiss G, Doumbo S, Doumtabe D, Kone Y, Huang CY, et al: **In vitro growth-inhibitory activity and malaria risk in a cohort study in mali.** *Infect Immun* 2010, **78**:737-745.
12. Omukunda E, Githeko A, Ndong'a MF, Mushinzimana E, Atieli H, Wamae P: **Malaria vector population dynamics in highland and lowland regions of western Kenya.** *J Vector Borne Dis* 2013, **50**:85-92.
13. Network WAR: **Parasite Clearance Estimator.** 2012.
14. Flegg JA, Guerin PJ, White NJ, Stepniewska K: **Standardizing the measurement of parasite clearance in falciparum malaria: the parasite clearance estimator.** *Malar J* 2011, **10**:339.
15. Johnson JD, Denuil RA, Gerena L, Lopez-Sanchez M, Roncal NE, Waters NC: **Assessment and continued validation of the malaria SYBR green I-based fluorescence assay for use in malaria drug screening.** *Antimicrob Agents Chemother* 2007, **51**:1926-1933.
16. Trager W, Jensen JB: **Human malaria parasites in continuous culture.** *Science* 1976, **193**:673-675.
17. Persson KE, McCallum FJ, Reiling L, Lister NA, Stubbs J, Cowman AF, Marsh K, Beeson JG: **Variation in use of erythrocyte invasion pathways by Plasmodium falciparum mediates evasion of human inhibitory antibodies.** *J Clin Invest* 2008, **118**:342-351.

18. Cheruiyot AC, Auschwitz JM, Lee PJ, Yeda RA, Okello CO, Leed SE, Talwar M, Murthy T, Gaona HW, Hickman MR, et al: **Assessment of the Worldwide Antimalarial Resistance Network Standardized Procedure for In Vitro Malaria Drug Sensitivity Testing Using SYBR Green Assay for Field Samples with Various Initial Parasitemia Levels.** *Antimicrob Agents Chemother* 2016, **60**:2417-2424.
19. Chebon LJ, Ngalah BS, Ingasia LA, Juma DW, Muiruri P, Cheruiyot J, Opot B, Mbuba E, Imbuga M, Akala HM, et al: **Genetically Determined Response to Artemisinin Treatment in Western Kenyan Plasmodium falciparum Parasites.** *PLoS One* 2016, **11**:e0162524.
20. Odhiambo G, Bergmann-Leitner E, Maraka M, Wanjala CNL, Duncan E, Waitumbi J, Andagalu B, Jura W, Dutta S, Angov E, et al: **Correlation Between Malaria-Specific Antibody Profiles and Responses to Artemisinin Combination Therapy for Treatment of Uncomplicated Malaria in Western Kenya.** *J Infect Dis* 2019, **219**:1969-1979.
21. Odhiambo G, Bergmann-Leitner E, Maraka M, Navalayo C, Duncan E, Waitumbi J, Andagalu B, Jura W, Dutta S, Angov E, et al: **Correlation between malaria-specific antibody profiles and responses to artemisinin combination therapy for treatment of uncomplicated malaria in Western Kenya.** *J Infect Dis* 2019.
22. Dent AE, Bergmann-Leitner ES, Wilson DW, Tisch DJ, Kimmel R, Vulule J, Sumba PO, Beeson JG, Angov E, Moormann AM, Kazura JW: **Antibody-mediated growth inhibition of Plasmodium falciparum: relationship to age and protection from parasitemia in Kenyan children and adults.** *PLoS One* 2008, **3**:e3557.
23. Djimde AA, Doumbo OK, Traore O, Guindo AB, Kayentao K, Diourte Y, Niare-Doumbo S, Coulibaly D, Kone AK, Cissoko Y, et al: **Clearance of drug-resistant parasites as a model for protective immunity in Plasmodium falciparum malaria.** *Am J Trop Med Hyg* 2003, **69**:558-563.
24. Doolan DL, Dobano C, Baird JK: **Acquired immunity to malaria.** *Clin Microbiol Rev* 2009, **22**:13-36, Table of Contents.
25. Marsh K, Kinyanjui S: **Immune effector mechanisms in malaria.** *Parasite Immunol* 2006, **28**:51-60.

Table

Table 1: Correlation between parasitological response to drugs and pre-existing GIA activity

Clearance kinetic a	Age group	n subjects	T _{1/2} vs GIA ^b		K vs GIA ^b	
			-HA ^c	+ HA ^c	-HA	+ HA
< 2.02	< 5 years	9	R ² =	R ² =	R ² = 0.604	R ² = 0.302
			-0.698	-0.413	p = 0.05*	p = 0.367
			p = 0.025*	p = 0.207		
	≥ 5 years	16	R ² =	R ² =	R ² = -0.03	R ² =
			-0.181	-0.042	p = 0.915	-0.043
			p = 0.518	p = 0.886		p = 0.878
> 2.02	< 5 years	37	R ² = 0.21	R ² = 0.193	R ² =	R ² =
			p = 0.304	p = 0.335	-0.217	-0.158
					p = 0.208	p = 0.432
	≥ 5 years	43	R ² =	R ² =	R ² = 0.037	R ² = 0.266
			-0.017	-0.221	p = 0.837	p = 0.2
			p = 0.923	p = 0.217		

^a Stratification of study participants based on Slope half-life T_{1/2}¹⁷ measured in hours

^b Correlation expressed as Pearson correlation coefficient (R²); asterisk indicates statistical significance (p ≤ 0.05)

^c GIA activity assessed in sera without (-HA) or with heat-inactivation (+HA)

Figures

Figure 1

Anti-parasite activity in sera from study participants reaches saturation at a low testing concentration. Growth inhibitory activity in sera from study participants reaches saturation at a low serum concentration. Individual data plot represents in vitro growth inhibition of *P. falciparum* parasites (3D7) in the presence of 10% or 1% (v/v) serum concentration from immune patients. Circles indicate results for each study participant (n=105). A serum pool consistent of six non-immune volunteers (indicated by blue line) was tested to determine background activity of the GIA assay. Median and 95% confidence interval are indicated for each experimental conditions.

Figure 2

The contribution of complement to anti-parasite activity in sera is revealed at lower concentrations Boxplot comparing GIA activity of Sera tested at 1% (left panel) (n=105) and 10% (right panel) n=105) with (+HIA) and without (-HA) complement inactivation

Figure 3

Age is not a confounding factor in the entire patient population Boxplot showing impact of age <5 years (n=45) and ≥ 5 years (n=60) on GIA in sera from symptomatic patients