The Journal of Infectious Diseases

BRIEF REPORT

Declining Antibody Affinity Over Time After Human Vaccination With a *Plasmodium falciparum* Merozoite Vaccine Candidate

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Maintaining high-affinity antibodies after vaccination may be important for long-lasting immunity to malaria, but data on induction and kinetics of affinity is lacking. In a phase 1 malaria vaccine trial, antibody affinity increased following a second vaccination but declined substantially over 12 months, suggesting poor maintenance of high-affinity antibodies.

Clinical Trials Registration. Australian New Zealand Clinical Trials Registry ACTRN12607000552482.

Keywords. antibody affinity; antibody; malaria; malaria vaccine; affinity maintenance.

There remains an ongoing need for malaria vaccines with high efficacy to accelerate malaria control and elimination [1]. Greater understanding of protective immune mechanisms and their kinetics are needed to achieve highly efficacious and long-lasting vaccines in the future. Antibodies are a key mediator of immunity for many malaria vaccine candidates, including the RTS, S and R21 vaccines that were recently recommended by the World Health Organization for implementation in young children in malaria-endemic regions [1, 2]. Antibody affinity refers to the strength of interaction between an antibody and antigen and may influence the functional activity of antibodies and protective immunity. Affinity increases with somatic hypermutation of immunoglobulin G (IgG), whereby higher affinity typically reflects an immune response that has undergone affinity maturation following infection or vaccination. High IgG affinity is associated with vaccine efficacy for some bacterial diseases [3, 4], and generating high affinity is often a focus of vaccine development. The importance of antibody affinity in malaria immunity has not been established, but an association between antibody affinity and RTS, S vaccine efficacy was reported in vaccinated children [5]. There is a paucity of data on the induction of affinity and whether it is maintained over time. Knowledge of the kinetics of affinity after vaccination is important given that current malaria vaccines have substantial waning of efficacy within 12 months [6].

In the current study, we quantified vaccine-induced antibody affinity over time in a phase 1 trial of a *Plasmodium falciparum* vaccine based on merozoite surface protein 2 (MSP2-C1). MSP2 is an abundant merozoite surface protein and a target of naturally acquired immunity [7]. Our earlier study found that naturally acquired, high-affinity antibodies to MSP2 were associated with protection from infection in a cohort of children and adults [8]. Although anti-MSP2 antibodies have a weak ability to directly inhibit host-cell invasion, they can function through antibody complement-dependent inhibition of invasion, opsonic phagocytosis, and antibody-dependent cellular inhibition [9, 10]. MSP2 is polymorphic and can be grouped into 2 major allelic families, 3D7 and FC27. The MSP2-C1 vaccine described in the current study contained both the 3D7 and FC27 isoforms to cover diversity present in populations [10].

METHODS

A double-blinded, placebo-controlled phase 1 clinical trial was performed in healthy, malaria-naive Australian adults (Australian New Zealand Clinical Trials Registry ACTRN12607000552482, registered 26 October 2007) [10]. Adult participants (aged 18–45 years) were vaccinated with equal amounts of the 3D7 and FC27 isoforms of recombinant MSP2 expressed in *Escherichia coli* (10 μg or 40 μg of protein) formulated with Montanide ISA720 as the adjuvant (n = 7 and n = 10 completed vaccination in the 10- and 40-μg dose group), or adjuvant alone as a placebo (n = 4). Three doses were given at 12-week intervals for the 10-μg cohort, while the 40-μg cohort was given the vaccine on 2 occasions with a 12-week interval. Blood samples were drawn at days 0, 28, 112, and 336 (and at day 196 for the 10-μg dose group). Plasma samples were stored at −80°C until use in immunoassays [10]. Ethics approval was obtained from Queensland Institute of

Received 23 February 2024; editorial decision 06 May 2024; accepted 08 May 2024; published online 9 May 2024

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The Journal of Infectious Diseases® 2024;230:e753–7

https://doi.org/10.1093/infdis/jiae259

Medical Research Human Research Ethics Committee and Alfred Health Human Research Ethics Committee, Australia.

Plasma samples were evaluated in immunoassays using the same recombinant 3D7 and FC27 MSP2 proteins used in the vaccine. Antibody affinities were estimated using dissociation rates (k_d values) in a Biacore 3000 measuring surface plasmon resonance (SPR) under flow [8, 11]. Recombinant MSP2 proteins, 0.01 mg/mL in 10 mM NaAc pH4.8, were bound to CM5 sensor chips (Biacore, GE Health Care) using N-terminal amine coupling (Amine coupling kit, Biacore, N-hydroxysuccinimide (NHS)/1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and ethanolamine) at 5 µL/minute. Plasma samples were diluted in running buffer (HBSEp, Biacore; 0.01 M Hepes pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.0005% Surfactant P20) and used at 2 different plasma dilutions (1:7.5 and 1:15), flow 30 µL/minute, and we confirmed that similar k_d values were obtained irrespective of concentration (see [8, 11] for further details on methodology, including examples of sensorgrams). The correlation between K_d values for samples measured at the different concentrations was very high; $r = 0.972$ ($P < .001$). The average variance between the 2 measurements was 2.1%. Evaluation of antibody affinity to tetanus toxoid among the same subjects was performed for comparison.

Antigen-specific IgM and IgG subclasses were quantified using established enzyme-linked immunosorbent assay (ELISA) [12] plasma diluted 1:100. Individuals who received the placebo vaccine were tested in SPR and ELISA but did not show significant MSP2-specific antibody responses and were therefore not included in this analysis.

Statistical analyses were performed using Graphpad Prism and differences between paired and unpaired samples were evaluated using the Wilcoxon signed rank test and Mann-Whitney *U* test, respectively. Correlations between ELISA and SPR were evaluated using Spearman rank correlation.

RESULTS

Vaccination with MSP2-C1 induced peak IgG responses to MSP2 after the second vaccine dose, measured on day 112 of the study [10]. IgG1 was the predominant antibody response (Figure 1*A*). There was also significant induction of IgG2 and IgG3 to both isoforms of MSP2, apart from anti-3D7 IgG2 ($P = .195$) and IgG3 ($P = .055$) in the 10-µg dose group ($P < .01$ for all other tests; Figure 1*B* and 1*C*). There was no induction of antigen-specific IgG4 (Supplementary Figure 1*A*), and marginal levels of IgM were detected after 2 vaccinations (Supplementary Figure 1*B*). The 10-µg dose group was given a third vaccination, but there were no substantial changes in antibody magnitude measured on day 196. Responses tended to be higher in the 40-µg dose group compared to the 10-µg group, especially for IgG1 ($P = .044$) and IgG2 ($P = .044$)

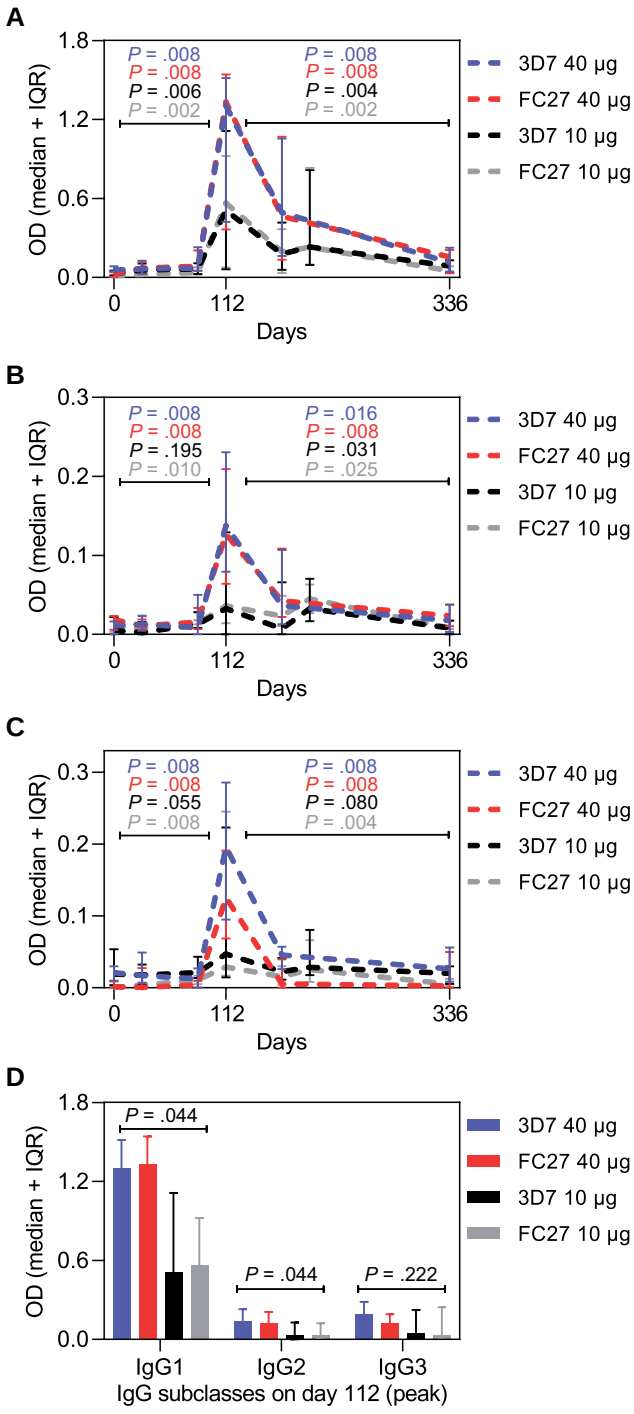

Figure 1. IgG subclass responses to MSP2. Individuals received two 40-µg doses (n = 10) or three 10-µg doses (n = 7) of the bivalent MSP2 vaccine. Serum samples collected on days 0, 28, 112, and 336 of the study (and day 196 for the 10-µg dose group) were tested for IgG subclasses including IgG1 (*A*), IgG2 (*B*), and IgG3 (*C*). There was no substantial induction of MSP2-specific IgG4 or IgM (Supplementary Figure 1). The group median and IQR are shown (values are OD) and reactivity between paired samples collected at days 0 and 112, and days 112 and 336 were compared using the Wilcoxon signed rank test. *D*, IgG subclasses to each MSP2 isoform at days 28, 112, and 336 were compared between the vaccine dosing groups using the Kruskal-Wallis test (median and IQR are shown). Abbreviations: IgG, immunoglobulin G; IQR, interquartile range; MSP2, merozoite surface protein 2; OD, optical density.

(Figure 1*D*). Notably, vaccine-induced IgG1, IgG2, and IgG3 had significantly decreased in magnitude by the end of the study at day 336 compared to the peak response ($P < .05$ for all tests; Figure 1*A*–*C*). The vaccine also induced low levels of IgA, measured at day 112, which was previously reported [9].

Antibody affinity (measured as the dissociation constant, k_d) was determined using SPR whereby a lower k_d value represents higher affinity. In the 40-μg dose group, IgG affinity to MSP2 3D7 and FC27 significantly increased after the second vaccine dose ($P = .031$ and $P = .031$, respectively; Figure 2*A* and 2*B*). Of note, antibody affinity subsequently declined significantly from day 112 to the end of the study at day 336 in both dose groups and for both MSP2 isoforms ($P = .016$ to .031; Figure 2*A*). IgG affinity to 3D7 and FC27 MSP2 was higher in the 40-μg dose group than in the 10-μg group at day 112 ($P = .077$) and day 336 ($P = .012$; Figure 2*B*). To account for potential changes in antibody affinity over time that was not specific to MSP2 responses, we also measured affinity of antibodies to the tetanus toxoid antigen as all participants had received this vaccine and were likely to have stable antibody responses. In contrast to the declining affinity of antibodies to MSP2, there were no changes in antibody affinity to tetanus toxoid over time (Supplementary Figure 1*C*).

We evaluated the relationship between peak IgG affinity and antibody magnitude to the 3D7 and FC27 MSP2 isoforms measured on day 112 (Figure 2*C* and 2*D*). IgG affinity correlated most strongly with the magnitude of IgG1 (3D7, ρ −0.765; FC27, ρ −0.800) and IgG2 (3D7, ρ −0.741; FC27, ρ −0.684; $P < .01$ for all tests).

DISCUSSION

We found that affinity of vaccine-induced antibodies significantly increased after the second vaccine dose compared to the first with the higher dose of 40 μg, and the higher vaccine dose generated higher antibody affinity. Such knowledge is valuable for understanding how to maximize affinity of antibodies induced by vaccines. However, it was surprising that antibody affinity subsequently substantially declined over time postvaccination, which may be relevant to how vaccine efficacy decays over time. There were no differences between the 2 MSP2 isoforms in antibody levels and affinity, supporting the concept of using a multiple antigen vaccine formulation. Our findings further suggest that 2 doses of this vaccine are at least as good as 3 doses, which has potential advantages in terms of implementation and coverage. Analyzing a phase 1 trial conducted in malaria-naive donors enabled the evaluation of antibody affinity without any confounding that may be introduced by malaria exposure prior to vaccination or during the follow-up period.

It is unclear why antibody affinity declined over time. Antibodies in serum are produced by plasma cells (and other antibody secreting cells) generated after vaccination. There was an overall decline in IgG magnitude over time that likely

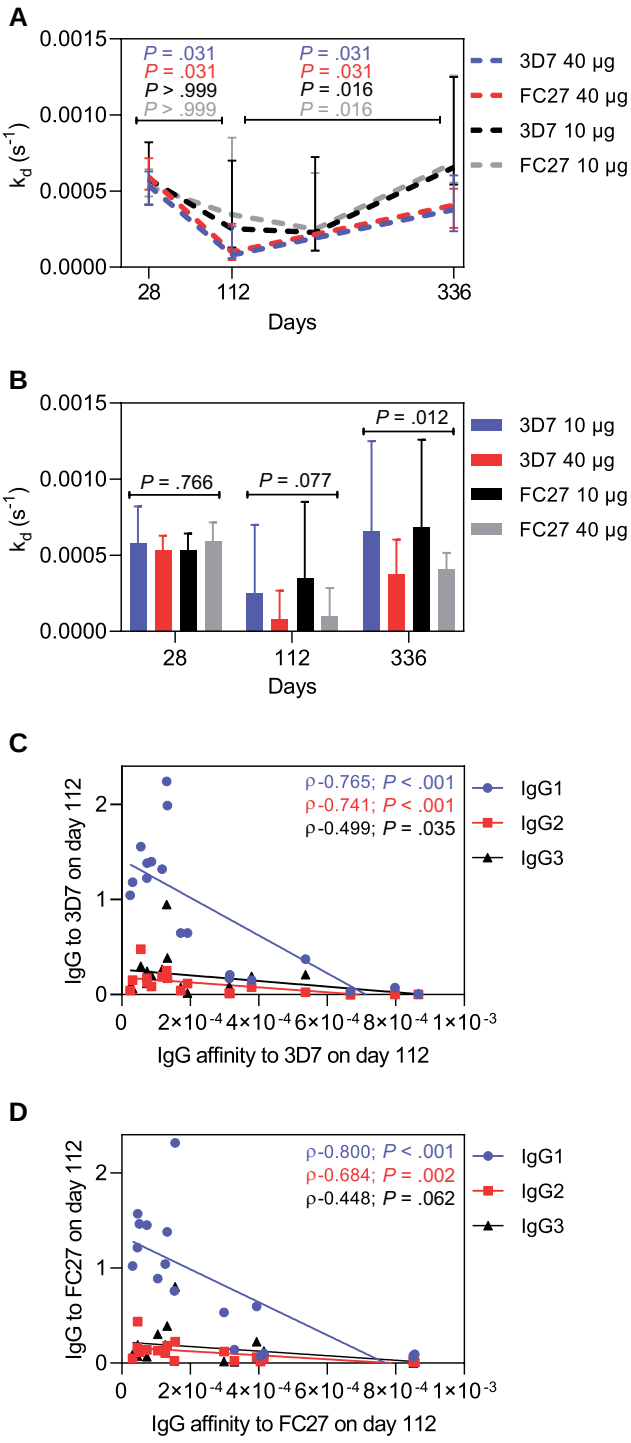

Figure 2. Affinity of IgG induced by vaccination. *A*, Serum samples collected on days 28, 112, and 336 of the study (and day 196 for the 10-μg dose group) were tested for IgG affinity (data shown as $1/k_d$ values); higher $1/k_d$ values represent higher affinity. The group median and IQR are shown and reactivity between paired samples collected at days 28 and 112, and days 112 and 336 were compared using the Wilcoxon signed rank test. *B*, IgG affinity measured to each MSP2 isoform at days 28, 112, and 336 were compared between the vaccine dosing groups using the Kruskal-Wallis test (median and IQR are shown). *C* and *D*, Correlations between antibody affinity and magnitude of IgG1, IgG2, and IgG3 to the 3D7 (*C*) and FC27 (*D*) isoforms of MSP2, measured at day 112 (peak response). The Spearman correlation coefficient (ρ) and linear line of best fit are shown. Abbreviations: IgG, immunoglobulin G; IQR, interquartile range; k_d, dissociation rate; MSP2, merozoite surface protein 2.

reflects an overall loss of IgG-secreting plasma cells over time. However, the decline in IgG affinity suggests that there is a greater loss of plasma cells that secrete high-affinity antibodies over time, or perhaps other changes impact on the affinity of antibodies being produced over time. Why this occurs is unclear. In contrast to our findings, a recent study of the kinetics of naturally acquired immunity in a population following a rapid decline in malaria transmission found that antibody avidity (measured using an ELISA-based method) increased over time [13], suggesting a loss of low-avidity antibodies. The kinetics of affinities of antibodies acquired through natural exposure versus vaccination may differ. Changes in affinity over time have not been reported for malaria vaccines. We also observed that vaccine-induced IgG magnitude correlated with affinity, which suggests that subjects that generate the highest IgG responses also tend to generate higher affinity responses.

Estimating antibody affinity may be influenced by the method used. In an ELISA-based method, which has been widely used, proteins often bind to the plate with hydrophobic interactions, and estimation of affinity is influenced by antibody and chaotrope concentration. In SPR, proteins are bound covalently and antibody k_d is measured as an indicator of affinity that is independent of antibody concentration. SPR has been widely used for analysis of monoclonal antibodies, and we recently reported its utility for quantifying affinity of polyclonal antibodies against *Plasmodium* antigens [8]. For another merozoite antigen, a range of dissociation constants (K_D) measured for different naturally acquired antibodies was mainly due to variations in dissociation rates (k_d) [14], indicating that the SPR method is useful in measuring affinity of antibody mixtures.

Our results contribute important new knowledge of the kinetics of antibody affinity after vaccination in humans and inform malaria vaccine development. Further studies of the kinetics of antibody affinity are needed for other vaccines in clinical trials to better understand the maintenance of antibody affinity over time after vaccination. Failure to maintain high-affinity antibodies over time may be one contributing factor to the difficulty in developing long-lasting malaria vaccines.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank Alex Umbers for manuscript editing, Nadia Cross for data compiling and analysis, and staff who contributed to conducting the clinical trial.

Author contributions. J. B. and K. P. designed the study. K. P. and J. H. conducted experiments. K. P., J. B., J. H., and L. K. analyzed data. J. M., R. A., and J. B. provided clinical samples and data, and specific reagents. All authors contributed to writing the manuscript.

Data availability. Data are available on reasonable request from the corresponding author, pending any ethical or regulatory clearances.

Financial support. This work was supported by the National Health and Medical Research Council, Australia (grant numbers 1173046 and 1092789 to J. B.); and Avtal om Läkarutbildning och Forskning (Region Skåne/Lund University to K. P.). Funding to pay the open access publication charges for this article was provided by Burnet Institute.

Potential conflicts of interest. All authors: No reported conflicts of interest. 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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