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BRIEF REPORT

RTS,S/AS02A Malaria Vaccine-Induced IgG Responses Equally Recognize Native-Like Fucosylated and Nonfucosylated *Plasmodium falciparum* Circumsporozoite Proteins

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The RTS,S/AS02A malaria vaccine is based on the *Plasmodium falciparum* circumsporozoite protein (PfCSP), which is *O*-fucosylated on the sporozoite surface. We determined whether RTS,S/AS02A-induced immunoglobulin G (IgG) antibodies recognize vaccine-like nonfucosylated PfCSP better than native-like fucosylated PfCSP. Similar to previous vaccine trials, RTS,S/AS02A vaccination induced high anti-PfCSP IgG levels associated with malaria protection. IgG recognition of nonfucosylated and fucosylated PfCSP was equivalent, suggesting that PfCSP fucosylation does not affect antibody recognition.

Clinical Trials Registration. NCT00197041.

Keywords. RTS,S; antibodies; immunogenicity; *Plasmodium falciparum*; circumsporozoite protein (PfCSP); fucosylation; malaria.

RTS,S/AS01E is the first malaria vaccine recommended for widespread use by the World Health Organization in African children. The vaccine has a favorable safety profile and reduces episodes of both clinical and severe malaria in children [1]. Data from the phase 3 trial showed that vaccine efficacy against clinical malaria is modest 12 months after a 3-dose primary vaccination, estimated at 55.8% (97.5% confidence interval [CI], 50.6%–60.4%) in children aged 5–17 months [1], and 31.3% (97.5% CI, 23.6%–38.3%) in infants aged 6–12 weeks [2], and the duration of protection wanes over time [1, 2]. The development of a more efficacious vaccine remains a high priority for malaria control and elimination. Multiple factors may contribute to the suboptimal efficacy of adjuvanted recombinant protein subunit vaccines like RTS,S/AS01E, and the absence of native glycosylation residues may be one important element [3]. The vaccine, produced by GlaxoSmithKline Biologicals (Belgium), contains the hepatitis B virus surface antigen (HBsAg) genetically fused to a fragment of the *Plasmodium falciparum* circumsporozoite protein (PfCSP) including the last 18 NANP repeats of the central domain and its C-terminal end, and is formulated as virus-like particles with a liposome-based adjuvant (AS01E) [4]. We have previously shown that the avidity of antibodies to the C-terminus of PfCSP correlates with protection against malaria following vaccination with RTS,S/AS01E [5].

The PfCSP C-terminus comprises a domain with homology to the thrombospondin type-1 repeat superfamily (TSR), which in its native form contains an *O*-fucosylation motif [6], and a fucose monosaccharide modification was recently identified on PfCSP from salivary gland sporozoites [7]. *O*-fucosylation is a simple posttranslational modification that facilitates protein folding and trafficking and may affect antigenicity [6]. Recent evidence suggests that *O*-fucosylation of TSR domains in *P. falciparum* proteins is important for liver infection and may also be relevant in the mosquito stages of the life cycle [8]. Moreover, the glycosylation profile on *P. falciparum* surface proteins such as PfCSP has the potential to influence parasite-specific humoral and cellular immune responses. We, therefore, hypothesized that the antibodies induced by the RTS,S vaccine, in which PfCSP is not fucosylated, might not properly recognize the native fucosylated PfCSP antigen on the sporozoite, which could compromise the overall protective efficacy. To test this hypothesis, we used immune sera from young children enrolled in the pivotal Mozambique phase 2b trial of the RTS,S vaccine formulated with a previous version of the adjuvant (AS02A) [9], and compared the levels of vaccine-induced immunoglobulin G (IgG) that bound to fucosylated versus nonfucosylated PfCSP.

METHODS

Study Participants

Participants in this phase 2b clinical trial of the malaria vaccine candidate RTS,S/AS02A in the Manhiça District, southern

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Mozambique (ClinicalTrials.gov registry No. NCT00197041) were children aged 1–4 years at first vaccination. The samples analyzed in this study were from children in the Manhiça cohort 1 (n = 708) (Supplementary Figure 1). The clinical trial definitions, study design and population, procedures, and interventions have been described elsewhere [9].

Ethics

The clinical trial research protocols were approved by the National Mozambican Ethics Review Committee, the Hospital Clínic of Barcelona Ethics Review Committee in Spain, and the PATH-Malaria Vaccine Initiative Research Ethics Committee, and all parents or legal guardians of the participants provided written informed consent.

Antigens

The recombinant full-length PfCSP was expressed using the *Pichia pastoris* expression system, and the TSR C-terminal domain of PfCSP was expressed in *Escherichia coli*. Both PfCSP TSR and full-length PfCSP fucosylated domains were produced using the human protein *O*-fucosyltransferase 2 (huPoFUT2). The details of the antigens production, purification and sequences can be found in Supplementary Materials and Methods and Supplementary Figure 2.

Antibody Assays

An in-house multiplex bead-based antibody assay was performed to assess IgG responses against fucosylated and nonfucosylated versions of the PfCSP full-length and the C-terminal TSR domain (Supplementary Materials and Methods and Supplementary Figure 2). The assay applied the xMAP technology (Luminex Corporation), previously standardized and optimized [10]. Briefly, antigens were coupled to magnetic beads, multiplexed, and incubated with 50 µL of the test, negative control, or positive control serum samples. Next, beads were washed and incubated with a biotinylated secondary antibody, washed again, and incubated with streptavidin-R-phycoerythrin. Finally, using a Luminex xMAP 100/200 analyzer, at least 50 microspheres per analyte were acquired per well. The IgG levels were measured as median fluorescence intensity [10] and transformed to levels in arbitrary units using the modelled calibration curves from serial dilutions of a positive reference sample (Supplementary Figure 3).

Statistical Analysis

Antibody levels were compared with boxplots showing geometric means, medians, and interquartile ranges. Fold-changes in IgG level geometric means between time points or between vaccination groups were estimated by calculating average differences in $\log_{10}$-transformed measurements. The 95% CI and *P* values were obtained from 2-tailed 2-sample *t* tests for comparisons between vaccination groups and 2-tailed paired *t* tests for comparisons between time points. Through exponentiation of the $\log_{10}$-transformed level differences and their corresponding 95% CI, we obtained the desired IgG level fold-change estimates.

To study the relationship between the levels of IgG binding to fucosylated and nonfucosylated antigens, we used scatter plots to capture the bivariate distribution, acknowledging that the closer the points to the diagonal, the closer the relationship to that of perfect identity. We also calculated the Pearson correlation coefficients to assess the strength of this relationship. Statistics and plots were always conducted or shown in $\log_{10}$-transformed levels or scales to stabilize the variance.

RESULTS

The baseline characteristics comparing vaccination groups (Supplementary Table 1) and prevaccination antibody levels (month 0) were similar for both fucosylated and nonfucosylated versions of PfCSP full-length (Figure 1*A*) and C-terminus TSR domain-only antigens (Figure 1*B*). Primary vaccination (month 3) anti-PfCSP IgG reactivity was strong, with fold-increases in geometric mean levels >1000 for RTS,S vaccinees as compared to the comparator group. These data are well aligned with previous estimates using enzyme-linked immunosorbent assay (ELISA) from a larger longitudinal study of the same clinical trial [11] and with other RTS,S clinical trials measuring anti-NANP IgG [12]. Anti-TSR IgG responses were somewhat weaker than the full-length PfCSP IgG responses, with increase estimates between vaccination groups at month 3 that were 5- to 6-fold lower for TSR. Also, as expected, PfCSP full-length and TSR antibody levels waned between month 3 and month 8 (reductions by 5- and 3.5-fold, respectively), closely replicating previous estimates of IgG decay rates [11]. Contrary to our expectation, we observed nearly identical responses when we compared IgG levels bound to fucosylated versus nonfucosylated PfCSP full-length and TSR antigens. Figure 1*C* and 1*D* shows anti-PfCSP fucosylated versus not fucosylated IgG levels, yielding a bivariate distribution that approached perfect correlation.

Next, we examined the children's characteristics affecting increases in IgG antibody levels reacting to fucosylated PfCSP constructs relative to prevaccination. We observed no significant differences in the rise of IgG geometric means between pre- and postvaccination time points against the fucosylated PfCSP proteins between age groups or sexes in the RTS,S vaccinees. However, we observed a lower increase of IgG to fucosylated TSR in older children (2–4 years) compared to younger (1–2 years) after RTS,S vaccination (month 3) ($P = .02$; Table 1). As expected, higher increases in anti-PfCSP full-length and TSR IgG levels were associated with protection from disease. Thus, RTS,S-vaccinated children who did not develop clinical malaria within the first year of follow-up had significantly higher anti-PfCSP IgG increases from month 0 to month 3 ($P = .042$; Table 1) as well as a trend towards higher anti-TSR IgG increases ($P = .2$). Differences were larger and

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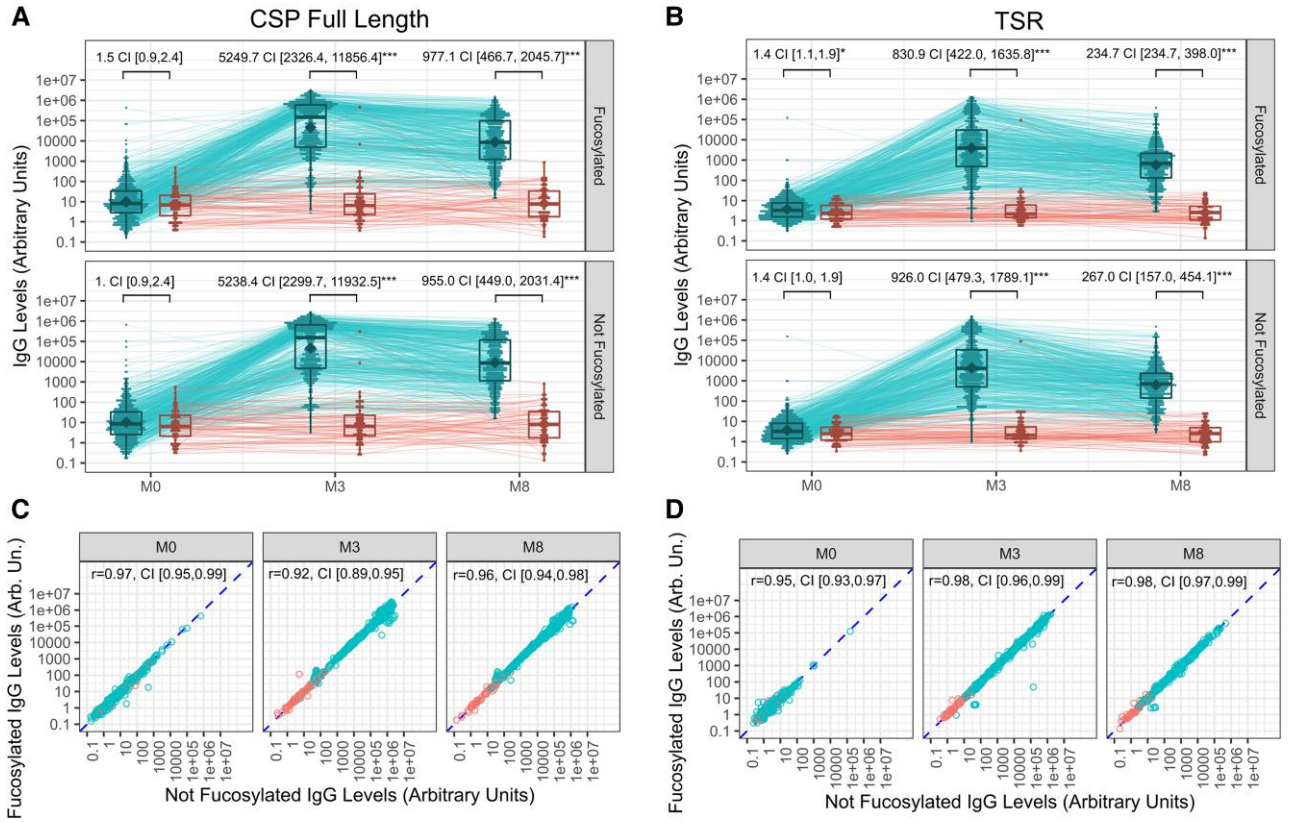

Figure 1. RTS,S/AS02A induces strong IgG responses that equally recognize fucosylated and nonfucosylated *Plasmodium falciparum* CSP and C-terminal TSR antigen constructs. *A* and *B,* Levels of IgG antibodies binding to fucosylated (y-axis) and nonfucosylated (x-axis) PfCSP full-length (*A*) and C-terminus TSR (*B*) at different time points (month 0, 3, and 8). Boxplots represent the geometric mean (diamond), median (central lines), quartiles (box limits), and estimated range (whiskers extending 1.5 times the interquartile range). IgG levels from the same individual across time points are linked with thin lines (longitudinal trajectories). Vaccination groups were compared through a 2-sample *t* test on the $\log_{10}$-transformed levels and statistical significance indicated by asterisks (*$P < .05$; **$P < .01$, ***$P < .001$). *C* and *D,* Scatter plots comparing IgG antibody levels binding to the fucosylated antigen (y-axis) versus those binding to the nonfucosylated equivalent (x-axis). The comparison is repeated for all time points (month 0, 3, and 8) for PfCSP full-length (*C*) and TSR (*D*). The Pearson correlation coefficient is computed across all observations. Abbreviations: AU, arbitrary unit; CI, 95% confidence interval; CSP, circumsporozoite protein; IgG, immunoglobulin G; PfCSP, *Plasmodium falciparum* circumsporozoite protein; TSR, thrombospondin type-1 repeat.

more clearly significant for anti-PfCSP IgG level increases from month 0 to month 8 for both full-length ($P = .0004$) and TSR ($P = .005$) (Table 1). Associations between IgG levels against nonfucosylated PfCSP full-length and TSR antigens with age, sex, and clinical malaria were identical to the results reported for fucosylated antigens (Supplementary Table 2).

To determine whether malaria endemicity could influence the binding of vaccine or naturally induced IgG to fucosylated versus nonfucosylated PfCSP antigens, another set of samples (n = 124) from Ilha Josina, a trial site of higher transmission intensity (cohort 2) [9], was also tested. Again, no difference was noted in recognition of fucosylated compared to nonfucosylated PfCSP antigens (Supplementary Figure 4), suggesting that the binding is independent of malaria exposure.

DISCUSSION

To our knowledge, no previous study has directly tested whether RTS,S vaccine-induced IgG antibodies bind less to a native-like fucosylated PfCSP (present on *P. falciparum* sporozoites) [7] than to nonfucosylated PfCSP (as present in the RTS,S vaccine). Of note, while our results show that RTS,S-induced antibodies recognize similarly fucosylated and nonfucosylated PfCSP constructs, we observed the same phenomenon in prevaccination and comparator postvaccination samples. This suggests that even IgG induced by natural exposure, presumably to fucosylated PfCSP on sporozoites, efficiently recognized the target antigen epitopes regardless of fucosylation.

Because our assay was multiplexed and tested the same sample for each individual against 2 slightly different PfCSP presentations, potential differences in the binding of any relatively important subpopulation of polyclonal IgG would have implied a divergence in the paired antigen-specific IgG levels, which was not observed.

Nevertheless, we cannot rule out a potential benefit of an alternative RTS,S vaccine containing fucosylated PfCSP. To establish this, fucosylated and nonfucosylated PfCSP-based

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Table 1. Factors Affecting Increases in IgG Antibody Levels Reacting to Fucosylated PfCSP Constructs

	Month 3/Month 0		Month 8/Month 0	
Compared Conditions	Fold Difference (95% CI)	*P* Value	Fold Difference (95% CI)	*P* Value
Antigen PfCSP full length				
Increase ratio estimate	5079.1 (3833.1–6730.01)	<.0001***	829.2 (631.9–1088.2)	<.0001***
Old/young	0.65 (.34–1.25)	.20	0.61 (.32–1.14)	.12
Female/male	1.04 (.59–1.83)	.89	0.83 (.48–1.43)	.48
P. falciparum not infected/infected	1.96 (1.02–3.75)	.042*	3.07 (1.64–5.73)	.0004**
Antigen PfCSP TSR				
Increase ratio estimate	778.4 (625.7–968.3)	<.0001***	153.3 (128.0–183.5)	< .0001***
Old/young	0.56 (.34–.92)	.02*	0.7 (.46–1.06)	.09
Female/male	1.1 (.71–1.7)	.68	0.85 (.59–1.21)	.36
P. falciparum not infected/infected	1.39 (.84–2.31)	.2	1.8 (1.18–2.72)	.005*

Associations are reported as ratio estimates of month 3/month 0 or month 8/month 0 between groups of interest (compared conditions).

Abbreviations: CI, confidence interval; PfCSP, *Plasmodium falciparum* circumsporozoite protein; TSR, thrombospondin type-1 repeat.

*$P < .05$; **$P < .01$, ***$P < .001$.

vaccines should be tested head-to-head to compare overall immunogenicity (humoral and cellular responses) and vaccine efficacy. The concentration and quality of immunoglobulins might differ when induced by a fucosylated PfCSP, particularly IgG subclasses, their avidity, and their neutralizing and non-neutralizing functional capacities. It is also possible that a fucosylated PfCSP protein-based vaccine could lead to broader glycan-dependent T helper and cytotoxic cell responses, as it can be presented on both MHC-I and MHC-II molecules, and the resulting complex can be recognized by T cells. Recent advances in human immunodeficiency virus (HIV-1) [13] and hepatitis C virus (HCV) [14] vaccine development have been stimulated by a better understanding of the HIV-1 envelope spike and HCV E1 and E2 glycan composition and their interactions with the human immune responses. These advances highlight increasing evidence that glycans are important antigenic determinants in immune responses to various pathogens. Their relevance for prophylactic and therapeutic vaccine design and development is worth exploring in more depth.

Limitations of our study include the lack of data on IgG subclasses, which have different affinities for cellular Fc receptors, thus mediating different antiparasite effector functions, and functional antibody measures. In addition, we used PfCSP, recombinantly expressed in *P. pastoris* instead of native PfCSP, which might replicate better the complexity of the sporozoite surface. However, obtaining sufficient quantities of native PfCSP poses significant technical challenges and the potential divergent outcomes remain uncertain, considering our robust data that show a similar recognition of fucosylated and nonfucosyltated protein. In contrast, recombinant PfCSP can be readily overexpressed in *P. pastoris*, its production has been optimized, and it is well characterized [15], possibly mirroring native PfCSP conformation.

In conclusion, our data suggest that posttranslational modification by *O*-fucosylation in PfCSP, absent in the RTS,S vaccine, does not affect antibody-antigen binding both for IgG induced by vaccination (not fucosylated) and natural infections (fucosylated). This negative result does not weigh in favor of the hypothesis that a fucosylated PfCSP-based vaccine could lead to improved vaccine efficacy. However, this result alone is insufficient to rule it out and further experiments are required.

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

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Author contributions. R. R. D., G. M., L. I., and C. D. contributed conceptualization. C. J., J. P. T.-Y., L. M. and M. V. performed experimental work. R. A., C. D., G. M. supervised laboratory work. D. M. and R. S. performed data curation and analysis. J. S. coordinated the clinical trial. C. J., D. M., R. A., G. M., L. I., and C. D. wrote the original draft. R. H.-G. A., K. R., D. L. N., B. L.-G., T. H., and R. R. D. performed antigen expression and modification. All authors critically read, revised, and approved the final manuscript.

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Data availability. The datasets generated and/or analyzed in the current investigation are available from the corresponding author upon reasonable request.

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Potential conflicts of interest. All authors: No reported 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.

References

1. RTS,S Clinical Trials Partnership; Agnandji ST, Lell B, Soulanoudjingar SS, et al. First results of phase 3 trial of RTS,S/AS01 malaria vaccine in African children. N Engl J Med **2011**; 365:1863–75.
2. RTS,S Clinical Trials Partnership; Agnandji ST, Lell B, et al. A phase 3 trial of RTS,S/AS01 malaria vaccine in African infants. N Engl J Med **2012**; 367:2284–95.
3. Goddard-Borger ED, Boddey JA. Implications of *Plasmodium* glycosylation on vaccine efficacy and design. Future Microbiol **2018**; 13:609–12.
4. Kaslow DC, Biernaux S. RTS,S: toward a first landmark on the malaria vaccine technology roadmap. Vaccine **2015**; 33:7425–32.
5. Dobaño C, Sanz H, Sorgho H, et al. Concentration and avidity of antibodies to different circumsporozoite epitopes correlate with RTS,S/AS01E malaria vaccine efficacy. Nat Commun **2019**; 10:2174.
6. Holdener BC, Haltiwanger RS. Protein *O*-fucosylation: structure and function. Curr Opin Struct Biol **2019**; 56: 78–86.
7. Swearingen KE, Lindner SE, Shi L, et al. Interrogating the *Plasmodium* sporozoite surface: identification of surface-exposed proteins and demonstration of glycosylation on CSP and TRAP by mass spectrometry-based proteomics. PLoS Pathog **2016**; 12:e1005606.
8. Lopaticki S, Yang ASP, John A, et al. Protein *O*-fucosylation in *Plasmodium falciparum* ensures efficient infection of mosquito and vertebrate hosts. Nat Commun **2017**; 8:561.
9. Alonso PL, Sacarlal J, Aponte JJ, et al. Efficacy of the RTS,S/AS02A vaccine against *Plasmodium falciparum* infection and disease in young African children: randomised controlled trial. Lancet **2004**; 364:1411–20.
10. Ubillos I, Aguilar R, Sanz H, et al. Analysis of factors affecting the variability of a quantitative suspension bead array assay measuring IgG to multiple *Plasmodium* antigens. PLoS One **2018**; 13:e0199278.
11. Aide P, Dobã No C, Sacarlal J, et al. Four year immunogenicity of the RTS,S/AS02 A malaria vaccine in Mozambican children during a phase IIb trial. Vaccine **2011**; 29:6059–67.
12. White MT, Verity R, Griffin JT, et al. Immunogenicity of the RTS,S/AS01 malaria vaccine and implications for duration of vaccine efficacy: secondary analysis of data from a phase 3 randomised controlled trial. Lancet Infect Dis **2015**; 15:1450–8.
13. Crispin M, Ward AB, Wilson IA. Structure and immune recognition of the HIV glycan shield. Annu Rev Biophys **2018**; 47:499–523.
14. Sepulveda-Crespo D, Resino S, Martinez I. Hepatitis C virus vaccine design: focus on the humoral immune response. J Biomed Sci **2020**; 27:78.
15. Herrera R, Anderson C, Kumar K, et al. Reversible conformational change in the *Plasmodium falciparum* circumsporozoite protein masks its adhesion domains. Infect Immun **2015**; 83:3771–80.

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