EMBO Molecular Medicine

Molecular and functional properties of human Plasmodium falciparum CSP C-terminus antibodies

Opeyemi Oludada, Giulia Costa, Clare Burn Aschner, Anna Obraztsova, Katherine Prieto, Caterina Canetta, Stephen Hoffmann, Peter Kremsner, Benjamin Mordmueller, Rajagopal Murugan, Jean-Philippe Julien, Elena Levashina, and Hedda Wardemann
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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

13th Feb 2023

Dear Dr. Wardemann,

Thank you for the submission of your manuscript to EMBO Molecular Medicine. We have now received feedback from the three reviewers who agreed to evaluate your manuscript. As you will see from the reports below, all referees support publication of the study, but also raise important concerns that should be addressed in a revision of the current manuscript. No additional experiments are required. If you would like to discuss further the points raised by the referees, I am available to do so via email or video. Let me know if you are interested in this option.

Addressing the reviewers' concerns in full will be necessary for further considering the manuscript in our journal. EMBO Molecular Medicine encourages a single round of revision only and therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript. For this reason, and to save you from any frustrations in the end, I would strongly advise against returning an incomplete revision.

In addition, please amend the following:
1) Figures: Please upload separate, high-resolution main Figure files. Please check "Author Guidelines" for more information: https://www.embopress.org/page/journal/17574684/authorguide#figureformat
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- In M&M, please include statement that the informed consent was obtained from the human subject and that in addition to the principles set out in the WMA Declaration of Helsinki the experiments also conformed the Department of Health and Human Services Belmont Report.
- Please rename "Competing Interest Statement" to "Disclosure Statement & Competing Interests". We updated our journal's competing interests policy in January 2022 and request authors to consider both actual and perceived competing interests. Please review the policy https://www.embopress.org/competing-interests and update your competing interests if necessary.
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8) Please provide a separate point-by-point response to my comments (as Word file).

We would welcome the submission of a revised version within three months for further consideration. Please let us know if you require longer to complete the revision.

I look forward to receiving your revised manuscript.

Yours sincerely,

Zeljko Durdevic

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Editor
EMBO Molecular Medicine

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Could you identify some relevant ones and provide such information as well? Some examples are patient associations, relevant databases, OMIM/proteins/genes links, author's websites, etc...

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***** Reviewer's comments *****

Referee #1 (Comments on Novelty/Model System for Author):

This is an important and novel study, made particularly relevant and potentially impactful by the recent endorsement of the poorly protective RTS,S vaccine and the need to design new vaccines with enhanced efficacy. The study was made possible by access to samples collected from malaria-naïve individuals who underwent immunization with Sanaria's PfSPZ vaccine, consisting of radiation-attenuated Pf sporozoites, which constitutes a most appropriate model system to conduct this study.

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The Introduction is thorough and well-structured, the figures are informative, and the conclusions are supported by the data. The Discussion is very well organized and very clear. Overall, I have no objections to the publication of the manuscript in its current form, except for one minor suggestion, regarding the last sentence of the Abstract and, indeed, the author's take home message summarized by that statement, which reads "The data provide novel insights in the human anti-C-linker and anti-α-TSR antibody response that support exclusion of the PfCSP C terminus from malaria vaccine designs". In my opinion, the statement that these data "support exclusion of the PfCSP C terminus from malaria vaccine designs" overlooks the fact that this region of CSP contains T-cell epitopes that may be key to an effective protection against infection and disease. As such, I urge the authors to revise this statement or at least to discuss the importance of the C-terminal region of CSP for T-cell immunity and protective efficacy. As it is, the statement completely overlooks this aspect of immunity and, in my opinion, is misleading.

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We would like to thank the reviewer for raising this important point, which we now address as follows in the discussion:
“Future studies will need to determine whether efforts to design a second generation PfCSP vaccine might benefit from suppressing or even abrogating the humoral response against C-CSP, e.g. by boosting the anti-repeat and junction response. However, exclusion of the complete domain, especially of the highly immunodominant α-TSR, would eliminate the main T helper cell epitopes with likely strong negative effects on the quality and strength of the humoral response against the potent repeat and junction epitopes. Inclusion of linear peptide epitopes rather than the complete C-CSP may be sufficient to provide efficient T cell help without inducing non-protective humoral responses (Wahl et al., 2022). Alternatively, non-PfCSP T cell epitopes could substitute for the loss of T cell help and promote affinity maturation of the PfCSP-specific response. “

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The reviewer raises a very interesting point. To the best of our knowledge, it is unclear whether the PfSPZ antigens are presented differently to the immune system than the antigens of mosquito-transmitted sporozoites. The overall weak response induced by natural parasites and strong differences in the number of transmitted parasites in the field vs. PfSPZ vaccination and different routes of parasite injection (s.c. for mosquito-bites vs. i.v. for PfSPZ vaccination) make direct quantitative comparisons difficult.

2. In the same vein, I wondered whether the authors had considered doing labelling experiments with Pf sporozoites, rather than Pb-PfCSP sporozoites - in case their is a difference in their heterologous presentation on Pb sporozoites, or indeed if any labelling had been trialled on PfSPZ (cryo-preserved) sporozoites? It might have been nice to see some images of these labelled to partner the flow data.

We used transgenic Pb-PfCSP parasites for quantification of antibody binding capacity to live sporozoites because they express mCherry, a red fluorescence marker that allows us to accurately gate single sporozoites for flow cytometry analyses. We have used live Pf and Pb-PfCSP sporozoites in IFA analyses and did not detect differences in the staining pattern, however, this method has low resolution for detection of fine differences related to heterologous expression. The reviewer raised an interesting question about potential changes in antibody binding to irradiated or cryo-preserved parasites. However, to the best of our knowledge, this question has not yet been quantitatively assessed, likely due to the lack of a *Pf* fluorescence reporter line compatible with *A. gambiae* mosquitoes.

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wondered whether this and Figure 5 could be simplified slightly just to make them easier for a non-structural biologist (but avid vaccinologist) to follow?

We appreciate the complexity of the structural data, and have included some additional context with the results. No changes have been made to the figures, however, additional guidance on interpreting the figures has been provided in the results, and the important take-home messages from the structural data are emphasized in the discussion.

4. Figure 4 took me several reads to realise that Panel A had a separate panel - just wondered if this could be either divided into a new A/B or made distinct from Panel E below.

We agree with the reviewer that Panel A was difficult to read and have modified Fig. 4 accordingly. For clarity, we now show the data for each antibody separately and indicate the respective affinity in Panel A. The raw data panel has been moved to extended view figure 4.

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For clarity, we modified the legend as follows:
"Capacity of the passively-transferred indicated antibodies (100 µg) to protect mice (n=10 for mAb 317 (Oyen et al., 2017), n=8 for mAb 1961, and n=9 for mAb 1710 (Scally et al., 2018)) from parasitemia after the bite of three PbPfCSP(mCherry)-infected mosquitoes."

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The reviewer raises a very valid point. We would like to clarify that we fully agree that most of the protective capacity comes from anti-repeat antibodies and that C-CSP specific antibodies alone would not show any parasite inhibitory effect. Indeed, the C-CSP antibodies might simply be a marker of exposure and of the overall strength of the anti-parasite response. To clarify this point, we have modified the discussion as follows:
“In response to RTS,S/AS01 immunization, anti-NANP and anti-C-terminus antibody titers correlate with protection (Chaudhury et al, 2021; Chaudhury et al, 2016; Dobano et al, 2019). To what degree the C-CSP-reactive serum antibodies recognize C-CSP specifically or cross-react with the repeat and junction has not been determined. Therefore, it is unclear whether protection is associated with C-CSP-specific or cross-reactive antibodies or whether C-CSP antibodies are simply a marker of the overall strength of the anti-parasite response whereas protection is mediated by anti-repeat antibodies. “

23rd Mar 2023

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Zeljko Durdevic

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Reporting Checklist for Life Science Articles (updated January 2022)
This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: 10.31222/osf.io/9sm4x). Please follow the journal's guidelines in preparing your manuscript.
Please note that a copy of this checklist will be published alongside your article.

Abridged guidelines for figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n<5, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements.
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Please complete ALL of the questions below.
Select "Not Applicable" only when the requested information is not relevant for your study.

Materials

Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
New materials and reagents need to be available; do any restrictions apply?	Not Applicable	

Antibodies	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
For **antibodies** provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number - Non-commercial: RRID or citation	Yes	Materials, Methods, results and figures Section: Commercially available antibodies used are: mouse anti-human CD27-PE (Clone ID: M-T271; Cat. No: 555441), CD38-BV605 (Clone ID: HB7; Cat. No: 562665), IgG-BV510 (Clone ID: G18-145; Cat. No: 563247), IgM-BV421 (Clone ID: G20-127; Cat. No: 562618), IgD- APC-H7 (Clone ID: IA6-2; Cat

DNA and RNA sequences	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Short novel DNA or RNA including primers, probes: provide the sequences.	Not Applicable	

Cell materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Cell lines: Provide species information, strain. Provide accession number in repository **OR** supplier name, catalog number, clone number, and/**OR** RRID.	Yes	Materials and Methods Section: FreeStyle™ 293-F Cells (Thermo Fischer Scientific, Cat#R79007); HC-04, BEI Resources (MRA-975).
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Not Applicable	
Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Yes	Materials and Methods Section: All cell lines were tested negative for *Mycoplasma* contamination.

Experimental animals	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository **OR** supplier name, catalog number, clone number, **OR** RRID.	Yes	Materials and Methods Section: Female C57BL/6J mice (8 weeks old), female CD-1 mice (7-12 weeks old), *Anopheles coluzzii* (Ngousso strain) (Harris et al. 2010), transgenic *Anopheles gambiae* (7b strain) (Pompon & Levashina, 2015)
Animal observed in or captured from the field: Provide species, sex, and age where possible.	Not Applicable	
Please detail **housing and husbandry conditions**.	Yes	Materials and Methods Section: Animals were bred in the MPIIB Experimental Animal Facility (Marienfelde, Berlin) and housed in a pathogen-free animal

Plants and microbes	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Plants: provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens).	Not Applicable	
Microbes: provide species and strain, unique accession number if available, and source.	Yes	Materials and Methods Section: Bacteria. MAX efficiency DH10B™ (ThermoFisher scientific; Cat. No: 18297010) competent cell was used for DNA transformation. Parasites: transgenic *Plasmodium berghei* ANKA strain

Human research participants	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If collected and within the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants.	Not Applicable	These demographics are listed in recent publication: Mordmüller et al. A PfSPZ vaccine immunization regimen equally protective against homologous and heterologous controlled human malaria infection. NPJ Vaccines. 2022

Core facilities	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Yes	Acknowledgement: This work benefited from core facility support.

Design

Study protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)

If study protocol has been **pre-registered, provide DOI in the manuscript**. For clinical trials, provide the trial registration number **OR** cite DOI.	Not Applicable	The clinical trial is described in recent publication: Mordmüller et al. A PfSPZ vaccine immunization regimen equally protective against homologous and heterologous controlled human malaria infection. NPJ Vaccines. 2022 doi: 10.1038/s41541-022-00510-z. The manuscript is cited in the respective
Report the **clinical trial registration number** (at ClinicalTrials.gov or equivalent), where applicable.	Not Applicable	The samples used in this study were collected from the clinical trial registered with ClinicalTrials.gov, NCT02704533. The clinical trial registration number is also mentioned in recent publication: Mordmüller et al. A PfSPZ vaccine

Laboratory protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Provide DOI OR other citation details if **external detailed step-by-step protocols** are available.	Yes	Detailed protocols for cloning and expression of monoclonal antibodies can be found in our recent publication: Wardemann H, Busse CE. Expression Cloning of Antibodies from Single Human B Cells. *Methods Mol Biol*.

Experimental study design and statistics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Include a statement about **sample size** estimate even if no statistical methods were used.	Not Applicable	
Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. **randomization procedure**)? If yes, have they been described?	Yes	Materials and Methods Section: Mice were randomly assigned to their respective experimental groups from the same batches.
Include a statement about **blinding** even if no blinding was done.	Yes	Materials and Methods Section: Investigators were not blinded to group assignment during the experiment and/or when evaluating the outcome.
Describe **inclusion/exclusion criteria** if samples or animals were excluded from the analysis. Were the criteria pre-established? If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.	Yes	Figure legends: The pre-established inclusion criteria for mice in challenge experiment was the presence of detectable levels of mAbs at the time of the challenge. Two mice were excluded from the mAb 1961 immunized group and one mouse was excluded from the mAb 1710 immunized group because their mAb serum concentrations were not detectable. No samples were excluded from the analyses. Number of mice with detectable serum IgG per immunized
For every figure, are **statistical tests** justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Yes	Materials and Methods Section: Statistical analysis was done with GraphPad Prism (version 9.12) using the two-tailed Mann-Whitney assuming a non-normally distributed data and the two-tailed Mantel-Cox log-rank test for the *in vivo* experiments.

Sample definition and in-laboratory replication	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
In the figure legends: state number of times the experiment was **replicated** in laboratory.	Yes	All experimental results were reproduced at least twice and the number of replicates are indicated in the corresponding figure legends.
In the figure legends: define whether data describe **technical or biological replicates**.	Yes	All technical or biological replicates are indicated in the figure legends

Ethics

Ethics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Studies involving **human participants**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval.	Not Applicable	Human participants were not directly involved in thus study. However, details of authority granting ethics are described in a recent publication: Mordmüller et al. A PfSPZ vaccine immunization regimen equally protective against homologous and heterologous controlled human malaria infection. NPJ Vaccines. 2022 doi: 10.1038/s41541-022-00510-z. The manuscript is cited in the respective results and methods sections.
Studies involving **human participants**: Include a statement confirming that **informed consent** was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	Not Applicable	Statement confirming informed consent is found in a recent publication: Mordmüller et al. A PfSPZ vaccine immunization regimen equally protective against homologous and heterologous controlled human malaria infection. NPJ Vaccines. 2022 doi: 10.1038/s41541-022-00510-z. The manuscript is cited in the respective results and methods sections.
Studies involving **human participants:** For publication of **patient photos**, include a statement confirming that consent to publish was obtained.	Not Applicable	
Studies involving experimental **animals**: State details of **authority granting ethics approva**l (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations.	Yes	Materials and Methods Section: Mice were handled in accordance with the German Animal Protection Law (§8 Tierschutzgesetz) and approved by the Landesamt für Gesundheit und Soziales (LAGeSo), Berlin, Germany (project numbers 368/12 and H0335/17).
Studies involving **specimen and field samples:** State if relevant **permits** obtained, provide details of authority approving study; if none were required, explain why.	Not Applicable	

Dual Use Research of Concern (DURC)	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Could your study fall under dual use research restrictions? Please check biosecurity documents and list of select agents and toxins (CDC): https://www.selectagents.gov/sat/list.htm	Not Applicable	
If you used a select agent, is the security level of the lab appropriate and reported in the manuscript?	Not Applicable	
If a study is subject to dual use research of concern regulations, is the name of the **authority granting approval and reference number** for the regulatory approval provided in the manuscript?	Not Applicable	

Reporting

The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.

Adherence to community standards	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
State if relevant guidelines or checklists (e.g., **ICMJE, MIBBI, ARRIVE, PRISMA**) have been followed or provided.	Not Applicable	
For **tumor marker prognostic studies,** we recommend that you follow the **REMARK** reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	Not Applicable	
For **phase II and III randomized controlled trials**, please refer to the **CONSORT** flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	Not Applicable	

Data Availability

Data availability	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Have **primary datasets** been deposited according to the journal's guidelines (see 'Data Deposition' section) and the respective accession numbers provided in the Data Availability Section?	Yes	Materials and Methods Section: Primary datasets or material generated in this study can be obtained from the corresponding authors under standard transfer agreements. Datasets are deposited on ENA with accession ID:PRJEB60486, Zenodo DOI: 10.5281/zenodo.7708746
Were **human clinical and genomic datasets** deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Not Applicable	
Are **computational models** that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Not Applicable	
If publicly available data were reused, provide the respective **data citations in the reference list**.	Not Applicable	