RESEARCH ARTICLE

Blood Interferon Signatures Putatively Link Lack of Protection Conferred by the RTS,S Recombinant Malaria Vaccine to an Antigen-specific IgE Response [version 1; peer review: 1 approved, 1 approved with reservations]

Darawan Rinchai¹, Scott Presnell², Damien Chaussabel ¹

¹Sidra Medical and Research Center, Doha, Qatar

²Benaroya Research Institute, Seattle, WA, USA

V1 First published: 29 Sep 2015, 4:919
<https://doi.org/10.12688/f1000research.7093.1>
 Latest published: 18 Jul 2017, 4:919
<https://doi.org/10.12688/f1000research.7093.2>

Abstract

Malaria remains a major cause of mortality and morbidity worldwide. Progress has been made in recent years with the development of vaccines that could pave the way towards protection of hundreds of millions of exposed individuals. Here we used a modular repertoire approach to re-analyze a publically available microarray blood transcriptome dataset monitoring the response to malaria vaccination. We report the seminal identification of interferon signatures in the blood of subjects on days 1, 3 and 14 following administration of the third dose of the RTS,S recombinant malaria vaccine. These signatures at day 1 correlate with protection, and at days 3 and 14 to susceptibility to subsequent challenge of study subjects with live parasites. In addition we putatively link the decreased abundance of interferon-inducible transcripts observed at days 3 and 14 post-vaccination with the elicitation of an antigen specific IgE response in a subset of vaccine recipients that failed to be protected by the RTS,S vaccine.

Keywords

Transcriptome , Blood , Malaria , Vaccination , IgE , Humoral , Bioinformatics , Modular repertoires

Open Peer Review

Approval Status

	1	2
version 2 (revision) 18 Jul 2017		view
version 1 29 Sep 2015	view	view

1. **Geneviève Milon**, Institut Pasteur, Paris, France
2. **Adrian J. F. Luty** , French Research Institute for Development (IRD), Paris, France

Any reports and responses or comments on the article can be found at the end of the article.

This article is included in the **Sidra Medicine** gateway.

Corresponding author: Damien Chaussabel (dchaussabel@sidra.org)

Competing interests: No competing interests were disclosed.

Grant information: This work was made possible through funding support from NIH (U01AI082110, U19AI089987, U19AI08998 and U19AI057234) to DC and SP, and the Qatar Foundation to DR and DC.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2015 Rinchai D *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Rinchai D, Presnell S and Chaussabel D. **Blood Interferon Signatures Putatively Link Lack of Protection Conferred by the RTS,S Recombinant Malaria Vaccine to an Antigen-specific IgE Response [version 1; peer review: 1 approved, 1 approved with reservations]** F1000Research 2015, 4:919 <https://doi.org/10.12688/f1000research.7093.1>

First published: 29 Sep 2015, 4:919 <https://doi.org/10.12688/f1000research.7093.1>

Introduction

About 3.4 billion people, nearly half of the world's population, live in areas at risk of malaria transmission¹. Malaria infection resulted in an estimated 198 million cases in 2013 that may have caused between 367,000 and 755,000 deaths, according to the World Health Organization². Recent concerns have been caused by rise in parasite resistance to artemisinin, which is the last effective monotherapy available for the treatment of malaria³. Cases of artemisinin resistance have been reported from much of Southeast Asia and now appear likely to reach the Indian subcontinent, with potentially dire consequences⁴. But significant advances over the past years have been made towards the development of an effective malaria vaccine⁵. Most notably this includes successful testing of a live vaccine consisting of radiation-attenuated sporozoites⁶, and this year licensure by regulatory authorities of the first malaria vaccine, the recombinant adjuvanted vaccine developed by global pharmaceutical GSK, called RTS,S also known by its commercial name, Mosquirix[®]. This is a highly significant landmark but unfortunately the efficacy of the vaccine for unknown reasons, and despite optimization attempts, remains suboptimal^{7,8}. Thus identification of mechanisms underlying protection conferred by this vaccine, or lack thereof, may be key to the development of a broadly effective prophylactic vaccine against malaria. Unbiased “systems approaches”, consisting in profiling all the elements constitutive of a given biological system, have recently been implemented to investigate responses to vaccines⁹. Such an approach consisting in measuring blood transcript abundance on a genome-wide scale has been adopted for the serial profiling of responses to the influenza, pneumococcal, yellow fever or malaria vaccines^{10–15}. In 2010, Vahey *et al.* reported results from a study investigating changes in transcript abundance in blood following administration of the malaria RTS,S vaccine¹⁵. In this report we share the results of a re-analysis of the data made available by Vahey *et al.* upon publication of their findings. We employed an innovative approach developed earlier; including by other team members part of previous publications which consists in identification of modular transcriptional repertoires – collections of co-clustered gene sets – in order to carry out modular level “fingerprinting analyses”¹⁶. This re-analysis led to original findings, with the identification of an interferon transcriptional signature at day 1 post-vaccination, correlating with protection as well as a second interferon signature at days 3 and 14 post-vaccination correlating this time with lack of protection of study subjects from subsequent challenges with the malaria parasite.

Methods

Construction of the modular repertoire framework

The methodology for constructing modular transcriptional repertoires has been described earlier^{16,17}. The particular framework employed in this re-analysis has been described in an earlier study investigating responses to influenza and pneumococcal vaccines¹². Briefly, nine datasets were used as input, including blood transcriptome profiles generated from patients with HIV, tuberculosis, sepsis, systemic lupus erythematosus, systemic arthritis, and liver transplant. Each dataset was clustered independently using Hartigan's k-means clustering, using the elbow criterion to determine the optimal number of clusters for each dataset. Cluster

membership information for each gene across the nine datasets was used to build a table recording the number of co-clustering events for each possible gene pair. This table was used in turn to build a weighted co-clustering network where each node is a gene and edges indicate co-clustering events with weight ranging from 1 (pair of genes belonging to the same cluster in 1 out of 9 datasets) to 9 (pair of genes belonging to the same cluster in 9 out of 9 datasets). The module selection process consisted in the identification within this large network of cliques, which are densely connected subnetworks. A principled approach was used starting in the first round with the selection of the largest subnetworks carrying the highest weight (co-clustering in 9 out of 9 datasets; corresponding to the M1 modules), followed by identification and removal from the selection pool of the next largest subnetwork and so on (with minimum clique size set at 10). When no additional modules could be identified for a given round of selection the stringency of the selection criteria was progressively relaxed (e.g. co-clustering occurring for 8 out of 9 datasets in the second round of selection, corresponding to the M2 modules; in 7 out of 9 datasets in the third round of selection, corresponding to the M3 modules, etc...). The datasets used for module construction have been deposited in NCBI's Gene Expression Omnibus: [GSE30101](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE30101).

Functional characterization of the blood modular repertoire

Functional analyses were carried out systematically for each module using commercial as well as publically available tools (primarily MetaCore™ version 5.0 and DAVID version 6.7¹⁸) and results are reported on a wiki page: http://www.biir.net/public_wikis/module_annotation/V2_Trial_8_Modules. A complete list of the genes forming the modules is also available from the wiki.

Module-level analyses

The top six rounds of modules defined by this approach (M1–M6, a total of 62 modules) were used as a framework to analyze and interpret the datasets generated in the context of the Vahey *et al.* study: i.e. rather than carrying out analyses at the individual gene level, which assume that changes in transcript abundance for each gene occur independently from that of other genes, we performed analyses at the modular level, where changes are assessed for sets of co-clustered genes. Thus we summarize “modular response” as a single value, the percent of responsive genes for a given module. In earlier analyses the average fold change per module was also used to demonstrate that high level of concordance could be observed across microarray platforms at the modular level but not at the gene level¹⁷. For determining changes for individual subjects post-vaccination a cutoff is set against which individual genes constitutive of a module are tested. If the gene meets the set criteria it is considered “responsive”. “Module-level” data is subsequently expressed as a % value representing the proportion of responsive transcripts for a given module.

Statistical analyses

Mann Whitney tests were performed on individual module response values expressed as percentages comparing protected and non-protected groups using GraphPad Prism software version 6 (GraphPad Software, San Diego, CA).

Results

Study design and reanalysis using modular repertoires

The design of the vaccine trial is described in detail by Vahey *et al.* and in an earlier publication^{15,19}. Briefly, study subjects received the RTS,S vaccine, which consists of sequences of the *Plasmodium falciparum* Circum Sporozoite Protein (CSP) expressed in hepatitis B surface antigen and formulated with the proprietary adjuvant systems AS01/AS02²⁰. Challenge was performed with a homologous 3D7 strain of *P. falciparum* delivered by 5 bites from infected mosquitoes. Samples were obtained from study participants at study entry (36 samples); on the day of the third vaccination (44 samples); at day 1 (43 samples), day 3 (43 samples), and day 14 (37 samples) thereafter; and at day 5 post-challenge (39 samples). Whole blood transcriptome profiles were generated using commercial Affymetrix HG-U133 chips. Data processing and normalization methodologies are described in the original publication. Data are available publically from the NCBI Gene Expression Omnibus (GSE18323). Only the blood transcriptional profiles generated on the day of the third vaccination and at day 1, day 3 and day 14 post-third vaccination were used in our re-analysis.

We employed a “modular repertoire approach” first described in 2008 in a research paper¹⁷, and more recently in a review¹⁶. Briefly, this approach consists in a priori identifying relationships among constituents of a given biological system, which in our case is the blood transcriptome. This makes it in turn possible to analyze transcriptional profiles as functionally interpretable gene sets rather than independent genes. Modular repertoires are established in an entirely data-driven process through the recording of co-clustering patterns of transcripts across a wide range of immune-related diseases. A collection of datasets encompassing infectious as well as autoimmune disorders and primary immune deficiency was used as input in order to capture a wide variety of immune signatures. The module construction process and modular analyses are described in detail in the Methods section.

In the original analysis of this dataset Vahey *et al.* report the identification: 1) of a transient signature at 24 hours post-vaccination that was not observed at subsequent time points. This signature is described as being associated with inflammatory processes elicited by the vaccine and was not associated with outcome of the infectious challenge; 2) of a signature at 5 days post-challenge that distinguish vaccinated from non-vaccinated individuals, thus directly reflecting and demonstrating the effect of vaccination; 3) of a signature at 14 days post-vaccination correlating with protection conferred by the vaccine. This 393-gene signature was identified using high resolution Gene Set Enrichment Analysis (GSEA) and consisted in transcripts belonging to the immunoproteasome pathway associated with the processing of major histocompatibility complex class I peptides.

Increased interferon module response at day 1 correlates with protection

In our re-analysis we first assessed changes in transcript abundance at the modular level. The percentage of responsive transcripts constitutive of a given module was determined for each individual at days 1, 3 and 14 following administration of the third vaccine dose in comparison to the levels obtained in samples collected just prior

to that injection (see Methods for details). Hierarchical clustering was then performed at each time point to group modules (rows) and subjects (columns) based on patterns of changes in blood transcript abundance represented by the percent module response values (day 1, Figure 1A). Modules were filtered to only retain those with changes >15% in at least one subject. This analysis is unsupervised since it does not take knowledge of outcome of the infectious challenge into account. We observed nonetheless that samples tended to segregate based on whether or not the vaccine conferred protection (Figure 1A). Three modules associated with induction by interferon appeared to be the main elements driving the clustering of study subjects, with higher abundance levels being observed in subjects protected from subsequent infectious challenge. We demonstrated in our previous work that those three interferon modules represent distinct signatures that can be used for stratification of subjects with systemic lupus erythematosus²¹. Thus, we used in turn the same M1.2, M3.4 and M5.12 modules to stratify malaria vaccine recipients. Hierarchical clustering using only this subset of modules contributed to further separation of subjects based on the outcome of the infectious challenge (Figure 1B). The difference in % module responsiveness between protected and non-protected subjects was also statistically significant for M1.2 ($p=0.0094$, Mann Whitney test) (Figure 1C). M3.4 tended to be elevated compared to pre-vaccination baseline in both protected and non-protected individuals but was not different between those two groups. Abundance of M5.12 transcripts did not change following vaccination.

Decreased interferon module response at days 3 and 14 correlate with lack of protection

We next used a similar approach to classify subjects at days 3 and 14 post-third vaccination. Subjects once again segregated based on whether or not protection is conferred by the vaccine (Figures 2A & 2B). Notably, however, at these time points the signature showed a decrease in levels of transcript abundance in comparison to baseline pre-vaccine samples in subjects that were not protected. Thus conversely with the signature described at day 1, signatures at days 3 and 14 correlated with lack of protection by the vaccine. Differences between protected and non-protected groups were highly significant for M1.2 (Figure 2C, day 3 $p<0.0001$, day 14 $p<0.0001$, Mann Whitney test). M3.4 and M5.12 did not show significant differences between those groups. Notably, we found that the genes constitutive of M1.2 do not overlap with the day 14 immunoproteasome signature described by Vahey *et al.* Taken together results of our reanalysis of the Vahey dataset using a modular repertoire framework led to an original finding, by demonstrating the association between diverging day 1 and days 3 and 14 interferon signatures and protection conferred by the RTS,S vaccine.

Putative model accounting for the contrasted interferon signatures observed in response to RTS,S vaccination

We have shown in an earlier work that the three interferon modules that were described above tend to become elevated sequentially in patients with systemic lupus and may be associated with differential induction of type I and type II interferon in this disease²¹. Furthermore lupus disease severity was found to correlate significantly with M5.12 levels. We have also shown that an interferon

Figure 1. Increased modular interferon response at day 1. Blood transcriptional responsiveness to malaria vaccination was determined at the modular level in subjects one day following administration of the third dose of RTS,S. **A.** The percentage of responsive transcripts was determined for each module (co-clustered gene set) and represented by a colored spot on a heatmap where modules are arranged in rows and samples in columns (using a custom web; manuscript describing this resource is in preparation). Increases in transcript abundance compared to baseline pre-third vaccine sample are shown in red and decreases in transcript abundance in blue. Modules and samples are arranged by hierarchical clustering based on patterns of module responsiveness. **B.** Grouping of samples based on patterns of responsiveness of interferon modules is shown here. **C.** Responsiveness of the three interferon modules on day 1 is shown on a plot.

Figure 2. Decreased modular interferon response at days 3 and 14. Blood transcriptional responsiveness to malaria vaccination was determined at the modular level in subjects 3 and 14 days following administration of the third dose of RTS,S. **A.** As is the case of [Figure 1](#) the percentage of responsive transcripts was determined for each module (co-clustered gene set) and represented by a colored spot on a heatmap where modules are arranged in rows and samples in columns. Increases in transcript abundance compared to baseline pre-third vaccine sample are shown in red and decreases in transcript abundance in blue. Modules and samples are arranged by hierarchical clustering based on patterns of module responsiveness. **B.** Similar description as in **A**, this time applied to day 14 data. **C.** Responsiveness of module M1.2 at days 3 and 14 post vaccination are represented on a plot showing also differences between the protected and non-protected groups.

response dominated by M1.2 and M3.4 was transiently increased 1 day following vaccination with the trivalent influenza virus¹². O’Gorman *et al.* recently demonstrated that this transient interferon response is mediated by flu antigen-specific IgG immune complexes rather than engagement of pathogen-associated molecular pattern receptors²². The day 1 interferon response observed in the context of malaria vaccination could similarly be the result of engagement of CSP-specific IgG immune complexes since it occurs following administration of the third dose of RTS,S, at a time when a pre-existing humoral response would have been elicited by the first two doses.

But most peculiar is the fact that this increased modular interferon response in protected individuals at day 1 is followed by a persistent decrease in abundance of M1.2 transcripts below the pre-vaccination baseline in individuals that were not protected by the RTS,S vaccine. Indeed, in over 10 years of investigating blood transcriptome responses in a wide range of clinical and experimental settings the authors have not encountered a single instance of such a sustained and uniform decrease in abundance of interferon-inducible transcript. What is especially striking is the clear cut association between lack of protection conferred by RTS,S with the decrease in abundance of M1.2 transcripts seen in [Figure 2C](#). This implies that the immunological mechanism underlying this suppressed interferon signature may be key to overcoming current limitations of sub-unit malaria vaccination.

Here we putatively attribute this decrease in abundance of interferon-inducible transcripts and subsequent lack of protection to the elicitation by the vaccine of an antigen-specific IgE response. This assertion is based on an array of converging evidence, as outlined below:

Engagement of the high affinity IgE receptor, FCER1, mediates decreased responsiveness to interferon-inducing stimuli. Gill *et al.* have shown that constitutively plasmacytoid dendritic cells (pDCs) isolated from patients with allergic asthma produce reduced levels of interferon alpha in response to the influenza virus *in vitro* when compared with pDCs isolated from non-asthmatic controls²³. They also demonstrated that production of interferon alpha by pDC stimulated *in vitro* with the virus is significantly decreased upon cross-linking of the FCER1 receptor²³. Similar findings have been reported more recently in PBMCs exposed to Human Rhinovirus (HRV)²⁴. This is to our knowledge the only immune-mediated mechanisms of suppression of interferon responses that may explain the decrease in M1.2 observed following RTS,S

vaccination. Thus we hypothesize that the suppression by RTS,S of levels of interferon inducible transcripts results from formation of IgE-CSP immune complexes, with anti-CSP IgE being elicited in earlier rounds of vaccination ([Figure 3](#)). IgE-antigen immune complexes would cause cross-linking and downstream signaling through the FCER1 that is expressed at the surface of leukocytes of the myeloid lineage. While IgG levels have recently been correlated with protection conferred by RTS,S²⁵, to our knowledge the elicitation of IgE responses by this vaccine has thus far not been reported. Furthermore, our hypothesis is supported by evidence independently linking IgE responses, and specifically engagement of the FCER1, to susceptibility to malaria. Perlmann *et al.* identified IgE as a pathogenic factor in malaria, with immune complexes contributing to excess TNF induction in peripheral blood mononuclear cells *in vitro*²⁶. Furthermore, mice deficient for the high affinity IgE receptor showed increased resistance to malaria infection, specifically implicating FCER1 expressing neutrophils as pathogenic mediators²⁷. A more recent study has established a link between asthma and atopic dermatitis and delayed development of clinical immunity to *P. falciparum*²⁸. Notably, in addition to shifting cytokine balance by promoting IL10 and TNF production, engagement of high affinity IgE receptors has been reported to critically impair phagocytic function of monocytes, a mechanism that is essential for the control of malaria infection²⁹.

Conclusions

Gaining an understanding of immunological mechanisms that confer protection via immunization with the RTS,S malaria vaccine, or conversely that prevent it, can help address decisively the global health challenges caused by malaria infection. In this report we identify a candidate blood transcriptional signature correlating with protection following subsequent infectious challenge. Furthermore we establish a potential link between the peculiar decrease in abundance of interferon-inducible transcripts observed at days 3 and 14 following administration of the third dose of the vaccine and the possible elicitation of an IgE response in a subset of individuals that subsequently fail to be protected by vaccination. The validity of the model that we are proposing here can easily be tested by groups having ready access to samples obtained from subjects enrolled in the RTS,S vaccine trials. If this model holds true it would also open the possibility through the choice of appropriate antigens or adjuvants, or other immune modulating agents, to design strategies aiming at preventing, suppressing or skewing the development of IgE responses and thus confer high rates of protection against malaria infection through prophylactic immunization.

Figure 3. Proposed immunological mechanism determining protection – or lack thereof - conferred by the RTS,S malaria vaccine. Our model infers that the interferon signatures observed on days 1, 3 and 14 post-vaccination correlating with outcome of the infectious challenge are the result of engagement of Fc receptors by immune complexes. According to our model no interferon signatures should be observed following administration of the first vaccine dose in absence of pre-existing immunity to the Circum Sporozoite Protein (CSP). The injection of the first two doses of vaccines should elicit a humoral response, which in non-protected individuals is dominated by IgE rather than IgG. Ig-CSP immune complexes should form when the third dose of vaccine, which contains the CSP antigen, is administered. The transient interferon response elicited in individuals who develop a protective response and that we observed at Day 1 could be mediated engagement of the FCGR by IgG-CSP immune complexes as has been described earlier in the context of influenza vaccination²². Our model predicts that IgE-CSP complexes form in non-protected individuals and cross-link the high affinity IgE receptor FCER1 at the surface of leukocytes of the myeloid lineage. FCER1 engagement would in turn mediate reduction in levels of IFN-inducible (IFI) transcripts that is observed on days 3 and 14. This suppression of constitutive levels of IFN inducible genes in the non-protected group would be countered at least partially on Day 1 by residual IgG-IC response in subjects displaying mixed IgE/IgG humoral responses. If this is indeed the case the levels of M1.2 suppression on Day 1 in individuals displaying IgE response should be inversely correlated with levels of CSP-specific IgG. Given the transient nature of the interferon signature that was observed and that we tentatively attribute to IgG ICs this partial reversal of IgE mediated suppression would not be observed at later time point, which would account for the sustained decrease in abundance of interferon-inducible transcripts that was observed at days 3 and 14. Engagement and crosslinking of FCER1 may also result in altered phagocytic and parasite killing abilities thus contributing to lack of resistance to infection in cases where the humoral response to RTS,S vaccination is dominated by IgE²⁹.

Author contributions

DR: data analysis & interpretation, manuscript preparation; SP: software & database development; DC: data analysis & interpretation; manuscript preparation.

Competing interests

No competing interests were disclosed.

Grant information

This work was made possible through funding support from NIH (U01AI082110, U19AI089987, U19AI08998 and U19AI057234) to DC and SP, and the Qatar Foundation to DR and DC.

I confirm that the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

- Loitto VM, Forslund T, Sundqvist T, *et al.*: **Neutrophil leukocyte motility requires directed water influx.** *J Leukoc Biol.* 2002; **71**(2): 212–222.
[PubMed Abstract](#)
- Malaria Fact sheet N°94.** 2015.
[Reference Source](#)
- Ashley EA, Dhorda M, Fairhurst RM, *et al.*: **Spread of artemisinin resistance in *Plasmodium falciparum* malaria.** *N Engl J Med.* 2014; **371**(5): 411–423.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Tun KM, Imwong M, Lwin KM, *et al.*: **Spread of artemisinin-resistant *Plasmodium falciparum* in Myanmar: a cross-sectional survey of the K13 molecular marker.** *Lancet Infect Dis.* 2015; **15**(4): 415–421.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hoffman SL, Vekemans J, Richie TL, *et al.*: **The march toward malaria vaccines.** *Vaccine.* 2015; pii: S0264-410X(15)01070-1.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Seder RA, Chang LJ, Enama ME, *et al.*: **Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine.** *Science.* 2013; **341**(6152): 1359–1365.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ockenhouse CF, Regules J, Tosh D, *et al.*: **Ad35.CS.01-RTS,S/AS01 Heterologous Prime Boost Vaccine Efficacy against Sporozoite Challenge in Healthy Malaria-Naïve Adults.** *PLoS One.* 2015; **10**(7): e0131571.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- RTS,S Clinical Trials Partnership: **Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial.** *Lancet.* 2015; **386**(9988): 31–45.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Hagan T, Nakaya HI, Subramaniam S, *et al.*: **Systems vaccinology: Enabling rational vaccine design with systems biological approaches.** *Vaccine.* 2015; **33**(40): 5294–5301.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Dunachie S, Hill AV, Fletcher HA: **Profiling the host response to malaria vaccination and malaria challenge.** *Vaccine.* 2015; **33**(40): 5316–5320.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Gaucher D, Therrien R, Kettaf N, *et al.*: **Yellow fever vaccine induces integrated multilineage and polyfunctional immune responses.** *J Exp Med.* 2008; **205**(13): 3119–3131.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Obermoser G, Presnell S, Domico K, *et al.*: **Systems scale interactive exploration reveals quantitative and qualitative differences in response to influenza and pneumococcal vaccines.** *Immunity.* 2013; **38**(4): 831–844.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Querec TD, Akondy RS, Lee EK, *et al.*: **Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans.** *Nat Immunol.* 2009; **10**(1): 116–125.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Tsang JS, Schwartzberg PL, Kotliarov Y, *et al.*: **Global analyses of human immune variation reveal baseline predictors of postvaccination responses.** *Cell.* 2014; **157**(2): 499–513.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Vahey MT, Wang Z, Kester KE, *et al.*: **Expression of genes associated with immunoproteasome processing of major histocompatibility complex peptides is indicative of protection with adjuvanted RTS,S malaria vaccine.** *J Infect Dis.* 2010; **201**(4): 580–589.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Chaussabel D, Baldwin N: **Democratizing systems immunology with modular transcriptional repertoire analyses.** *Nat Rev Immunol.* 2014; **14**(4): 271–280.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Chaussabel D, Quinn C, Shen J, *et al.*: **A modular analysis framework for blood genomics studies: application to systemic lupus erythematosus.** *Immunity.* 2008; **29**(1): 150–164.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Huang da W, Sherman BT, Lempicki RA: **Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources.** *Nat Protoc.* 2009; **4**(1): 44–57.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Kester KE, Cummings JF, Ofori-Anyinam O, *et al.*: **Randomized, double-blind, phase 2a trial of falciparum malaria vaccines RTS,S/AS01B and RTS,S/AS02A in malaria-naïve adults: safety, efficacy, and immunologic associates of protection.** *J Infect Dis.* 2009; **200**(3): 337–346.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Lee S, Nguyen MT: **Recent advances of vaccine adjuvants for infectious diseases.** *Immune Netw.* 2015; **15**(2): 51–57.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Chiche L, Jourde-Chiche N, Whalen E, *et al.*: **Modular transcriptional repertoire analyses of adults with systemic lupus erythematosus reveal distinct type I and type II interferon signatures.** *Arthritis Rheumatol.* 2014; **66**(6): 1583–1595.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- O’Gorman WE, Huang H, Wei YL, *et al.*: **The Split Virus Influenza Vaccine rapidly activates immune cells through Fcγ receptors.** *Vaccine.* 2014; **32**(45): 5989–5997.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Gill MA, Bajwa G, George TA, *et al.*: **Counterregulation between the FcεpsilonRI pathway and antiviral responses in human plasmacytoid dendritic cells.** *J Immunol.* 2010; **184**(11): 5999–6006.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Durrani SR, Montville DJ, Pratt AS, *et al.*: **Innate immune responses to rhinovirus are reduced by the high-affinity IgE receptor in allergic asthmatic children.** *J Allergy Clin Immunol.* 2012; **130**(12): 489–495.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Ajua A, Lell B, Agnandji ST, *et al.*: **The effect of immunization schedule with the malaria vaccine candidate RTS,S/AS01_E on protective efficacy and anti-circumsporozoite protein antibody avidity in African infants.** *Malar J.* 2015; **14**: 72.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Perlmann P, Perlmann H, Flyg BW, *et al.*: **Immunoglobulin E, a pathogenic factor in *Plasmodium falciparum* malaria.** *Infect Immun.* 1997; **65**(1): 116–121.
[PubMed Abstract](#) | [Free Full Text](#)
- Porcherie A, Mathieu C, Peronet R, *et al.*: **Critical role of the neutrophil-associated high-affinity receptor for IgE in the pathogenesis of experimental cerebral malaria.** *J Exp Med.* 2011; **208**(11): 2225–2236.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Herrant M, Loucoubar C, Bassène H, *et al.*: **Asthma and atopic dermatitis are associated with increased risk of clinical *Plasmodium falciparum* malaria.** *BMJ Open.* 2013; **3**(7): pii: e002835.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Pyle DM, Yang VS, Gruchalla RS, *et al.*: **IgE cross-linking critically impairs human monocyte function by blocking phagocytosis.** *J Allergy Clin Immunol.* 2013; **131**(2): 491–500.e491–495.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Open Peer Review

Current Peer Review Status:

Version 1

Reviewer Report 08 February 2016

<https://doi.org/10.5256/f1000research.7637.r11338>

© 2016 Luty A. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Adrian J. F. Luty

IRD MERIT UMR 216, French Research Institute for Development (IRD), Paris, France

Over the last 3 decades or so the development and testing of candidate vaccines for malaria has seen numerous advances but an almost equal number of setbacks. RTS,S (aka Mosquirix) stands apart, for now, as the only such vaccine to have advanced as far as registration. Whilst that is clearly an achievement to be applauded, there nevertheless remain substantial gaps in our basic understanding of the immunological mechanisms involved in the protection conferred by the vaccine. The study reported here by Darawan Rinchai and colleagues represents an attempt to fill some of the gap in that knowledge.

The source data that Rinchai and colleagues were able to access for their analyses were derived from an original study authored by Kester and colleagues¹ that comprised a comparison of outcomes, including efficacy, of two formulations of RTS,S in different adjuvants administered to adult volunteers. Notably, that study documented higher titres of CSP-specific IgG as well as higher frequencies of 'activated' CSP-specific CD4⁺ T cells and IFN- γ -producing T cells in individuals who were protected from an experimental challenge infection with sporozoites compared to the study individuals who were not protected. The results of an adjunct to Kester and colleagues' study were published subsequently by Vahey and colleagues². That study documented gene expression analyses of peripheral blood mononuclear cells isolated from the volunteers at different time-points and identified - through the use of high-resolution geneset analysis - immunoproteasome processing of MHC peptides as predictively distinguishing protected from unprotected individuals.

Rinchai and colleagues' analytical approach employed a 'modular' method that they had previously developed 'in-house' and had used in other studies of vaccine-induced gene profiles. Here they used the same approach to assess gene expression profiles in the dataset generated by Vahey and colleagues. On that basis it would have been instructive for them to demonstrate the validity of their approach by assessing the immunoproteasome geneset identified by Vahey and colleagues in the context of its predictive capacity. Also, they need to justify the rationale for the module filtering procedure that discarded any displaying changes <15%. On what basis was that threshold chosen? In passing, for accuracy they also need to modify their repeated incorrect

reference to 'blood' proteome to the correct 'peripheral blood mononuclear cell' proteome.

Rinchai and colleagues' principal finding concerning the association between upregulated expression of an 'interferon module' response and protection conferred by RTS,S appears to be consistent with the enhanced T cell responses associated with protection reported by Kester and colleagues in the same individuals. It would also be consistent with the known role for IFN- α in enhancement of naive B cell differentiation³. My main concern in the context of their discussion and conclusions relates to their speculative interpretation for the under-expression/down-regulation of the interferon module response they recorded both 3 & 14 days after the third RTS,S immunization in non-protected individuals. They contend that that particular profile is most likely to be linked to an aberrant IgE rather than IgG response to CSP. They conclude that this would result in IgE-CSP immune complex-mediated cross-linking of IgE receptors on myeloid cells that would in turn mediate the type of down-regulation of pDC activity they observed in the non-protected individuals. Whilst this may indeed be a plausible explanation for their observations, it is far from being the only one, and the authors need to recognize this. Firstly, it is of possibly central relevance here that RTS,S is a hybrid molecule comprising the CSP sequence conjugated to a 226 amino acid stretch of hepatitis B surface antigen (HBsAg). The same portion of HBsAg is co-expressed in unconjugated form along with the conjugate such that the purified vaccine spontaneously forms virus-like particles. Importantly, HBsAg has been shown to inhibit IFN- α production by pDC⁴, implying that the 'non-malaria' component of RTS,S could thus potentially be exerting some influence on the outcome of immunization in terms of the anti-malarial protection generated. Secondly, a role for IgG-CSP immune complex-mediated inhibition of IFN- α production could also be envisaged through their interaction with the inhibitory Fc γ RIIB receptor expressed on pDC⁵. Thus, I feel, the authors need to modify their article to incorporate discussion of these alternative, but not necessarily mutually exclusive explanations for their findings. In the context of their preferred conclusion, I wonder if might it not also be a possibility that they re-interrogate their dataset to try to identify possible changes in relevant 'modules' related to IgE/FCERI expression levels?

Finally, I also suggest strongly that they remove references to and discussion of publications that have proposed a role for IgE in malarial morbidity. Disease manifestations due to infection with *Plasmodium falciparum* result from pathophysiological events associated with the asexual blood stage multiplication phase, reference to which in the context of putative antibody responses to a component of a vaccine that targets the pre-erythrocytic sporozoite stage are entirely erroneous in my view.

In conclusion I would say that Rinchai and colleagues' findings certainly provide some pointers to elucidating the immunological mechanisms governing the capacity of RTS,S to induce protective responses in some individuals but not others. I would nevertheless add the rider that the vaccine's capacity for induction of immunological responses in malaria non-exposed adults in North America may be quite far removed from its capacity to induce responses in sub-Saharan African newborns or infants, many of whom could have had some exposure to the pathogen or its products already either *in utero* or in early life. Such exposures could plausibly modulate their capacity to respond to vaccines in general and to a malaria vaccine in particular.

References

1. Kester KE, Cummings JF, Ofori-Anyinam O, Ockenhouse CF, et al.: Randomized, double-blind, phase 2a trial of falciparum malaria vaccines RTS,S/AS01B and RTS,S/AS02A in malaria-naïve

- adults: safety, efficacy, and immunologic associates of protection. *J Infect Dis.* 2009; **200** (3): 337-46 [PubMed Abstract](#) | [Publisher Full Text](#)
2. Vahey MT, Wang Z, Kester KE, Cummings J, et al.: Expression of genes associated with immunoproteasome processing of major histocompatibility complex peptides is indicative of protection with adjuvanted RTS,S malaria vaccine. *J Infect Dis.* 2010; **201** (4): 580-9 [PubMed Abstract](#) | [Publisher Full Text](#)
3. Gujer C, Sandgren KJ, Douagi I, Adams WC, et al.: IFN- α produced by human plasmacytoid dendritic cells enhances T cell-dependent naïve B cell differentiation. *J Leukoc Biol.* 2011; **89** (6): 811-21 [PubMed Abstract](#) | [Publisher Full Text](#)
4. Shi B, Ren G, Hu Y, Wang S, et al.: HBsAg inhibits IFN- α production in plasmacytoid dendritic cells through TNF- α and IL-10 induction in monocytes. *PLoS One.* 2012; **7** (9): e44900 [PubMed Abstract](#) | [Publisher Full Text](#)
5. Flores M, Chew C, Tyan K, Huang WQ, et al.: Fc γ RIIB prevents inflammatory type I IFN production from plasmacytoid dendritic cells during a viral memory response. *J Immunol.* 2015; **194** (9): 4240-50 [PubMed Abstract](#) | [Publisher Full Text](#)

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 22 Jun 2017

Damien Chaussabel, The Jackson Laboratory for Genomic Medicine, USA

We would like to thank the reviewers for their time and constructive feedback. In this revised version, we have added a new result section and figure showing FCER1 transcript profiles in vaccinated individuals, as well as newly generated IgE profiling data measured in plasma samples of children from malaria endemic areas who received the RTS,S vaccine or a comparator vaccine. We have included below our response to the points that were raised in the review.

Approved with Reservations

...

Rinchai and colleagues' analytical approach employed a 'modular' method that they had previously developed 'in-house' and had used in other studies of vaccine-induced gene profiles. Here they used the same approach to assess gene expression profiles in the dataset generated by Vahey and colleagues. On that basis it would have been instructive for them to demonstrate the validity of their approach by assessing the immunoproteasome geneset identified by Vahey and colleagues in the context of its predictive capacity.

Authors: *Indeed, this is an important point. We assessed the overlap of the proteasome signature reported by Vahey et al. with our modular genesets. Six genes overlapped with module M5.13 and three with module M5.4 (Supplementary Figure 1). However, neither M5.13 nor M5.4 were identified as being associated with protection, or lack of protection following RTS,S vaccination.*

We added the paragraph below in the result section “Decreased interferon module response at

days 3 and 14 correlate with lack of protection” and show results in supplementary figure 1

“Notably, we found that the genes constitutive of M1.2, M3.4 and M5.12 do not overlap with the day 14 immunoproteasome signature (32 genes) described by Vahey et al (Supplementary figure 1). All but three of the 32 genes mapped to the modules constituting our repertoire. Six genes mapped to module M5.13 and three to module M5.4, the remaining 23 genes mapped to 18 other modules. However, none of the genes mapped to either of the three interferon modules identified as associated with protection by RTS,S vaccine at Day 1, 3 or 14 after the third vaccination. Notably, IFNG, which was part of the 32 gene signature is among the genes constituting M3.6, which is associated with NK cells/Cytotoxic activity.”

Also, they need to justify the rationale for the module filtering procedure that discarded any displaying changes <15%. On what basis was that threshold chosen?

Authors: *We have added the sentence below in the manuscript to clarify this point;*

“This arbitrary cutoff is set at three times the false discovery rate used for multiple testing correction (5%), which allows effective filtering of false positive results.”

In passing, for accuracy they also need to modify their repeated incorrect reference to 'blood' proteome to the correct 'peripheral blood mononuclear cell' proteome.

Authors: *The manuscript was edited accordingly and reference now being made to “Peripheral blood mononuclear cell transcriptome....” Throughout.*

Rinchai and colleagues' principal finding concerning the association between upregulated expression of an 'interferon module' response and protection conferred by RTS,S appears to be consistent with the enhanced T cell responses associated with protection reported by Kester and colleagues in the same individuals. It would also be consistent with the known role for IFN- α in enhancement of naive B cell differentiation³. My main concern in the context of their discussion and conclusions relates to their speculative interpretation for the under-expression/down-regulation of the interferon module response they recorded both 3 & 14 days after the third RTS,S immunization in non-protected individuals. They contend that that particular profile is most likely to be linked to an aberrant IgE rather than IgG response to CSP. They conclude that this would result in IgE-CSP immune complex-mediated cross-linking of IgE receptors on myeloid cells that would in turn mediate the type of down-regulation of pDC activity they observed in the non-protected individuals. Whilst this may indeed be a plausible explanation for their observations, it is far from being the only one, and the authors need to recognize this. Firstly, it is of possibly central relevance here that RTS,S is a hybrid molecule comprising the CSP sequence conjugated to a 226 amino acid stretch of hepatitis B surface antigen (HBsAg). The same portion of HBsAg is co-expressed in unconjugated form along with the conjugate such that the purified vaccine spontaneously forms virus-like particles. Importantly, HBsAg has been shown to inhibit IFN- α production by pDC⁴, implying that the 'non-malaria' component of RTS,S could thus potentially be exerting some influence on the outcome of immunization in terms of the

anti-malarial protection generated. Secondly, a role for IgG-CSP immune complex-mediated inhibition of IFN- α production could also be envisaged through their interaction with the inhibitory Fc γ RIIB receptor expressed on pDC⁵. Thus, I feel, the authors need to modify their article to incorporate discussion of these alternative, but not necessarily mutually exclusive explanations for their findings. In the context of their preferred conclusion, I wonder if might it not also be a possibility that they re-interrogate their dataset to try to identify possible changes in relevant 'modules' related to IgE/FCER1 expression levels?

Authors: *These comments have contributed significantly to the discussion and interpretation of the IgE profiling data presented in this revised manuscript:*

With regards to the first point made in the comment above:

"An alternative explanation is advanced by Dr Luty in his comments, who mentions the potential relevance of the use of a 226 amino acid stretch of HBsAg as a conjugate for the CSP antigen in the RTS,S vaccine. Indeed this molecule has also been shown to inhibit Toll-like Receptor (TLR)9-mediated IFN- α production by pDC [Shi B., et al., PLoS One. 2012], and "could thus potentially be exerting some influence on the outcome of immunization in term of the anti-malarial protection generated" [Luty AJF., Referee report., F1000Research 2015, 4:919]. This is indeed another possibility, which warrants testing of the inhibitory capacity of the protein fragment used in RTS,S vaccine formulation. Furthermore the cell populations and signaling pathways involved in elicitation and modulation of interferon responses by RTS,S vaccine would also need to be investigated. It was found for instance that, rather unexpectedly, the induction of interferon response by the trivalent influenza vaccine is mediated by immune-complexes rather than TLR engagement [O'Gorman WE., et al., Vaccine. 2014]."

With regards to the second point made in the comment above:

*"At Dr Luty's suggestion we also examined levels of abundance of FCER1 subunits transcripts in the RTS,S vaccine dataset used in our analysis (**Figure 4**). Consistently with what is observed during acute malaria infection (GSE34404) [22949651], FCER1A and FCER1G levels were respectively increased and decreased one day post-RTS,S vaccination. However, notably, FCER1G levels remained significantly elevated at day 3 post-vaccination in non-protected individuals, while they decreased to baseline levels in protected individuals (**Figure 4D**). Abundance of FCER1G transcript was also significantly elevated in a third dataset where changes were measured in patients during episodes of asthma exacerbation (GSE24745) [22316092]. Moreover, we also checked expression level of FCGR2A and FCGR2B in the same dataset and found that abundance of FCGR2A and FCGR2B were not different in subjects who were protected and in those who were not protected."*

Finally, I also suggest strongly that they remove references to and discussion of publications that have proposed a role for IgE in malarial morbidity. Disease manifestations due to infection with *Plasmodium falciparum* result from pathophysiological events associated with the asexual blood stage multiplication phase, reference to which in the context of putative antibody responses to a component of a vaccine that targets the pre-erythrocytic sporozoite stage are entirely erroneous in my view.

Authors: *The IgE profiling results that have been newly generated illustrate the point made by Dr Luty. It shows a weak association with elevated baseline levels of CSP-specific IgEs and subsequent malaria morbidity, but a much stronger association with MSP-specific IgEs. These findings make the point raised and citations in question all the more relevant. The discussion points have been modified accordingly:*

*"Further evidence pointing to the influence of IgE responses comes from the literature and from results of our preliminary investigation reported below showing a dependence on both stages and antigen specificity of IgE on outcomes of malaria infection in children from endemic areas. Perlmann et al. identified IgE as a pathogenic factor in malaria, with immune complexes contributing to excess TNF induction in PBMC in vitro ²⁶. Mice deficient for the high affinity IgE receptor have shown increased resistance to blood-stage of parasite infection, specifically implicating FCER1 expressing neutrophils as pathogenic mediators ²⁷. A more recent study has established a link between asthma and atopic dermatitis and delayed development of clinical immunity to *P. falciparum* ²⁸. Notably, in addition to shifting cytokine balance by promoting IL-10 and TNF production, engagement of high affinity IgE receptors has been reported to critically impair phagocytic function of monocytes, a mechanism that is essential for the control of malaria infection ²⁹. The antigen specificity of IgE responses that have been associated with enhanced malaria morbidity remains to be determined. It is of relevance to RTS,S vaccination, at least in naïve subjects, since the vaccine targets the liver stage rather than the blood stage of the parasite."*

Competing Interests: No competing interests were disclosed.

Reviewer Report 21 December 2015

<https://doi.org/10.5256/f1000research.7637.r11692>

© 2015 Milon G. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Geneviève Milon

Immunophysiology and Intracellular Parasitism Unit, Institut Pasteur, Paris, France

It is a pleasure to provide my comments and highlight some issues in your well-conceived and well-conducted re-analysis of dataset generated and assembled by Vahey *et al*; this latter study was conducted to investigate changes in transcript abundance in Peripheral Blood Mononuclear Cells/PBMCs prepared from blood sampled from human volunteers who were recipients of three doses of the recombinant RTS,S vaccine containing a protein named Circum Sporozoite Protein/ CSP. Of note the CSP is displayed at the surface of by the human- invasive 3D7 *Plasmodium falciparum* developmental stage population named the invasive sporozoite population.

The invasive properties of sporozoite population

- reflect maturation within the salivary glands of the blood-feeding *Anopheles stephensi*/A.s
- and is transmitted when these blood-feeding anopheline mosquitos are
 1. either probing the skin.
 2. or sampling their blood meals,
- whether it is one or the other process it is coupled to delivery of both saliva *A. stephensi* **saliva** molecules and sporozoites.

Your re-analysis did allow generating interesting data sets

- with the identification of an interferon transcriptional signature at day 1 post-vaccination, correlating with protection,
- as well as the identification of distinct interferon transcriptional signatures at days 3 and 14 post-vaccination correlating, at these time points, with reduced or lack of protection of study subjects from subsequent challenges with sporozoites delivered by insectarium reared-blood-feeding *Anopheles* hosting mature sporozoites in their salivary glands

When concluding your re-analysis and the putative model accounting for the contrasted interferon signatures monitored at day 1 and days 3 and 14 post RTS,S vaccination, please could you also consider another biologically sound variable, namely the potentially contrasted ratio profile of the *Anopheles* saliva molecules-binding IgE/IgG: indeed you are aware that infants and adults who share durably the habitats of blood feeding *Anopheles* spp are more frequently exposed to *Anopheles* females that do not act as *Plasmodium* sporozoite vectors but that deliver saliva in the dermis a site where many saliva derived agonists are rapidly sensed by immune sensors the T cell receptors and the membrane Ig receptors included; such features are reflected by the ability to detect saliva-reactive T cells and saliva-reactive Ig molecules in the blood at very early times points post either skin probing or blood sampling by blood feeding insects.

Please note that blood-feeding *Anopheles* does not transmit malaria – the latter term is expected to depict only the symptoms that occur over the asexual *P. falciparum* intra-erythrocytic developmental program: the blood feeding *Anopheles* delivers saliva while transmitting sporozoites that will further reach the hepatocytes where proceeds the generation of *P. falciparum* merozoites that are invasive for erythrocytes.

Please could you review the Fig 3 legend:

"Figure 3. Proposed immunological mechanism determining protection – or lack thereof – in adult human individuals to whom was inoculated the RTS,S vaccine.

Our model infers that the interferon signatures observed on days 1, 3 and 14 post-vaccination correlating with outcome of the processes that deploy post the co-delivery of Anopheles saliva and P. falciparum sporozoites, are the result of engagement of Fc receptors by immune complexes.

*According to our model no interferon signatures should be observed following administration of the first vaccine dose of the CSP- containing RTS,S vaccine, in absence of pre-existing immune effectors/regulators reactive to *P. falciparum* Circum Sporozoite Protein/CSP.*

The injection of the first two doses of vaccines should elicit a humoral response, which in non-protected individuals is dominated by CSP-binding IgE rather than IgG. CSP-Ig immune complexes should form when the third vaccine dose is administered.

The transient interferon response elicited in individuals who develop a protective response and that we observed at Day 1 could be mediated by engagement of the FcγR by IgG-CSP immune complexes as has been described earlier in the context of influenza vaccination)²².

Our model predicts that IgE-CSP complexes form in non-protected individuals and cross-link the high affinity IgE receptor at the surface of cells of the myeloid lineage. FCER1 engagement would in turn mediate reduction in levels of IFN-inducible (IFI) transcripts that is observed on days 3 and 14.

This reduction of constitutive levels of IFN inducible transcripts in the non-protected group would be countered at least partially on Day 1 by residual IgG-IC response in subjects displaying mixed IgE/IgG humoral responses.

If this is indeed the case the levels of M1.2 reduction on Day 1 in individuals displaying CSP-binding IgE should be inversely correlated with levels of CSP-specific IgG."

Many thanks for your attention

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 22 Jun 2017

Damien Chaussabel, The Jackson Laboratory for Genomic Medicine, USA

We thank you very much for your time and valuable comments.

All your edits have been incorporated in the legend of Figure 3.

At the end of the conclusion we added the following sentence to reflect the suggestion that you have made, which is made all the more relevant by the data that we have generated and are now presenting in the second version of this manuscript.

"Furthermore, as pointed out by Dr Milon in her comments, extending the investigation to include profiling of IgE which are specific for antigen present in the saliva of the vector is also warranted [REF]."

REF: Milon G. Referee Report For: Blood Interferon Signatures Putatively Link Lack of Protection Conferred by the RTS,S Recombinant Malaria Vaccine to an Antigen-specific IgE Response [version 1; referees: 1 approved, 1 approved with reservations]. F1000Research 2015, 4:919

Competing Interests: No competing interests were disclosed.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research