A monoclonal antibody-based immunoassay to measure the antibody response against the repeat region of the circumsporozoite protein of *Plasmodium falciparum*

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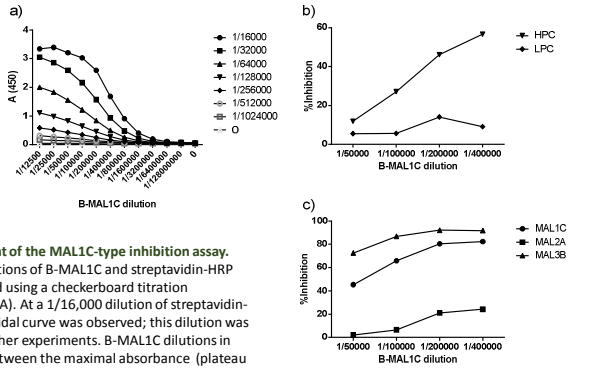
Abstract

The malaria vaccine candidate RTS,S/AS01 (GSK Vaccines) induces high IgG titers against the circumsporozoite protein (CSP) of *P. falciparum*. In human vaccine recipients circulating anti-CSP antibody titres are associated with protection against infection but appear not to be the ultimate correlate of protection. However, in a humanized mouse model of malaria infection prophylactic administration of a human monoclonal antibody (MAL1C) directed against the CSP repeat region conveyed full protection in a dose-dependent manner [1] suggesting that antibodies alone are able to prevent *P. falciparum* infection when present in high enough concentrations. A competition ELISA was developed to measure the presence of MAL1C-like antibodies in polyclonal sera from RTS,S vaccine recipients and study their possible contribution to protection against infection. Serum samples were taken at different time points from participants of 2 RTS,S/AS01 vaccine studies. Vaccine-induced protection status of the study participants was determined based on the outcome of experimental challenge with infectious mosquito bites after vaccination.

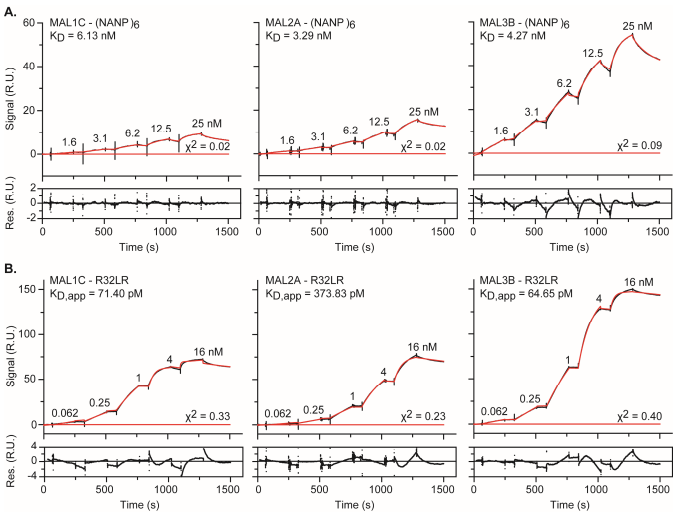
Methods

A competition ELISA was developed to measure the presence of MAL1C-like antibodies in polyclonal sera from RTS,S/AS01 vaccine recipients and study their possible contribution to protection against infection. The assay relies on the competition of MAL1C-like antibodies present in polyclonal vaccine-induced sera with biotinylated monoclonal antibody MAL1C for binding sites on the capture antigen consisting of the recombinant protein encompassing 32 NANP repeats of CSP (R32LR). Serum samples were taken at different time points from participants of 2 RTS,S/AS01 vaccine studies. Vaccine-induced protection status of the study participants was determined based on the outcome of experimental challenge with infectious mosquito bites after vaccination.

Results



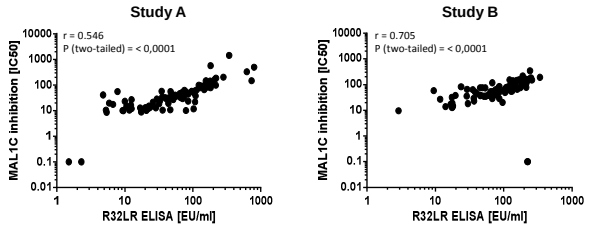
Development of the MAL1C-type inhibition assay. Optimal dilutions of B-MAL1C and streptavidin-HRP were defined using a checkerboard titration experiment (A). At a 1/16,000 dilution of streptavidin-HRP a sigmoidal curve was observed; this dilution was used for further experiments. B-MAL1C dilutions in the range between the maximal absorbance (plateau observed at 1/50,000) and half max (max/2 observed at 1/400,000) were explored in an inhibition set up using defined sera with high and low antibody content in R32LR ELISA assay (B) and the three monoclonal antibodies, MAL1C, MAL2B and MAL3A (C). The data shown in panels B and C were obtained with the highest serum concentrations (starting dilution 1/5) and with mAb concentrations of 5 µg/mL.



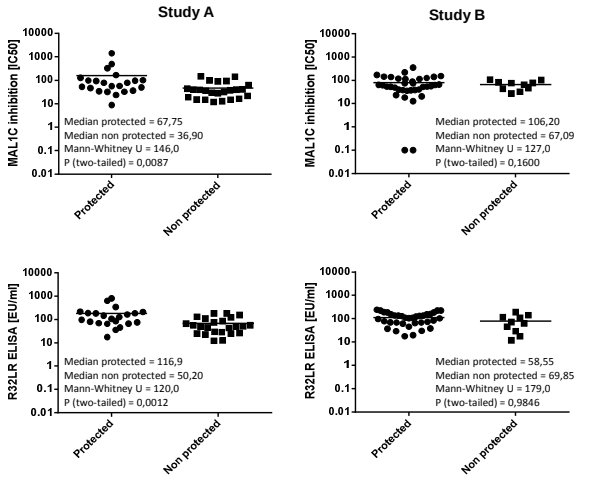
Interactions between the three mAbs (MAL1C, MAL2A, and MAL3B) and the CSP-derived peptides as measured by SPR. The data for the mAb:CSP-peptide interaction were measured in the format of a kinetic titration [2], with mAb as the ligand (i.e., coated onto the sensor surface) and either $(NANP)_6$ (A.) and R32LR (B.) as the analyte. In both panels, the top graphs display the sensorgrams (black traces) and the fit to the data with a 1:1 Langmuir binding model (red traces). The residuals of the fit are shown in the bottom graph. The (apparent) affinity constants for the interactions and the chi2 of the fit are also shown for convenience.

References

1. Foquet L. 2014, *Vaccine-induced monoclonal antibodies targeting circumsporozoite protein prevent Plasmodium falciparum infection.* The Journal of Clinical Investigation 124(1): 140-144.
2. Karlsson R. 2006, *Analyzing a kinetic titration series using affinity biosensors.* Analytical Biochemistry 349(1): 136-147.
3. Clement F. 2012, *Validation of an enzyme-linked immunosorbent assay for the quantification of human IgG directed against the repeat region of the circumsporozoite protein of the parasite Plasmodium falciparum.* Malaria Journal, 11.



Correlation between R32LR ELISA and MAL1C-type inhibition assay. Sera from participants from Study A and Study B taken after the second and third vaccine doses (immediately prior to mosquito bite challenge) were analyzed with the standard R32LR ELISA [3] and the MAL1C-type inhibition ELISA. A Pearson product-moment correlation coefficient was computed to assess the relationship between the antibody concentrations measured in both assays. In both studies there was a strong, positive correlation between the results obtained in the two assays.



Correlation between protection from infection and antibody content, measured with both R32LR ELISA and MAL1C-type inhibition assay. Antibody concentrations in the sera obtained immediately before challenge with infected mosquito's from participants at the A (n=46) and B (n=46) studies were measured with the R32LR ELISA and MAL1C-type inhibition assay. We ran a Mann-Whitney's U test to evaluate the difference in the antibody concentrations in protected and non-protected vaccine recipients. In study B no significant differences were observed irrespective of the assay used, whereas in study A the difference was significant.

Conclusions

A competition ELISA, developed to measure polyclonal MAL1C-like antibodies in sera from RTS,S vaccine recipients, was robust and specific, but did not show any new correlates of protection. Further work is needed to evaluate the role of other factors in protection against malaria.

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