Protective Antibody Responses in Congenital Zika Virus Infection

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BACKGROUND

ZIKV infection during pregnancy is associated severe birth defects and mild neurodevelopmental deficits. However, it is unclear why some infants are severely affected, and others are asymptomatic. Some evidence indicates that the protection of infants from Zika virus is strongly associated with the maternal antibody response. Therefore, ZIKV immunotherapy is an attractive target to treat maternal ZIKV infection and limit vertical transmission to the fetus. We need to define antibody immune correlates of protection, specifically related to the breadth of the antibody response, in order to develop effective immunotherapy. Our goal is to define the magnitude and breadth of the maternal antibody response that correlates with fetal protection.

METHODS

We used a high-density peptide micro array to define the breadth, or number of reactive epitopes, of the antibody response. We measured the reactivity of ZIKV-immune serum (from 14 days post infection) to overlapping linear epitopes spanning the entire viral polyprotein. This pilot experiment explored the optimized experimental conditions by testing multiple serum and secondary antibody (anti-IgG, -IgM, -IgA) dilutions.

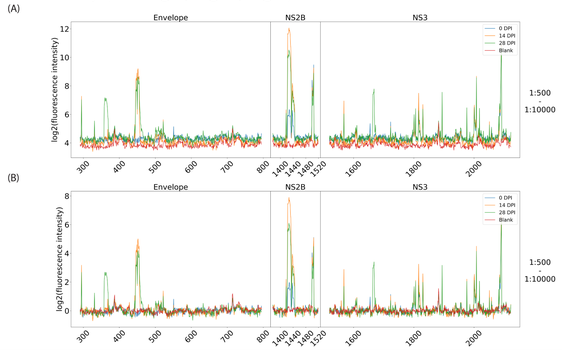


Fig.1 Z-scale normalization. (A) Signal plot of IgG at 14 days post infection before normalization. (B) Signal plot of IgG at 14 days post infection after normalization.

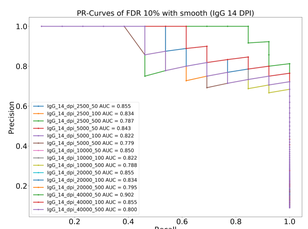
Fifteen experimental conditions of different serum and secondary antibody dilutions were tested in a checkerboard fashion. We corrected for systematic bias using Z-scale normalization[1] (Figure 1A and 1B). We defined the best experimental conditions with precision-recall curves and measuring the area under the curve (AUC). We defined reactive epitopes based on a false discovery rate approach.

- Establishment of a high-density peptide micro array analysis ***pipeline***.
- Defined the ***optimal experimental conditions*** for multiple secondary antibodies (anti-IgG, -IgM, -IgA).
- The number of reactive ZIKV epitopes was highest at 14 days post-infection for IgM and IgA antibodies, and highest at 28 days post infection for IgG antibodies.

RESULTS

We defined the most optimal experimental condition as one that has the precision-recall curve achieving the highest area under the curve (AUC). We demonstrate that 1:50 is the optimal serum dilution for all of the secondary antibodies (Figure 2B, 2C and 2D), and the optimal secondary antibody dilution differs among antibody classes, with a dilution of 1:20,000 optimal for anti-IgA and anti-IgM (Figure 2C and 2D), and a dilution of 1:40,000 optimal for anti-IgG (Figure 2D).

(A)



(B) IgG 14dpi PRC AUC

2nd Antibody / Serum	1:2500	1:5000	1:10000	1:20000	1:40000
1:50	0.855	0.843	0.850	0.855	0.902
1:100	0.834	0.822	0.822	0.834	0.855
1:500	0.787	0.779	0.788	0.795	0.800

(C) IgA 14dpi PRC AUC

2nd Antibody / Serum	1:2500	1:5000	1:10000	1:20000	1:40000
1:50	0.613	0.644	0.740	0.862	0.326
1:100	0.675	0.675	0.686	0.736	0.468
1:500	0.678	0.668	0.775	0.793	0.444

(D) IgM 14dpi PRC AUC

2nd Antibody / Serum	1:2500	1:5000	1:10000	1:20000	1:40000
1:50	0.728	0.586	0.591	0.742	0.566
1:100	0.697	0.698	0.692	0.709	0.702
1:500	0.612	0.493	0.489	0.498	0.575

Fig.2 Precision-recall curves of IgG. (A) Precision-recall curves (PRC) of IgG at 14 days post infection. (B) Area under curve (AUC) table of PRC of IgG at 14 days post infection. (C) Area under curve (AUC) table of PRC of IgA at 14 days post infection. (D) Area under curve table of PRC of IgM at 14 days post infection.

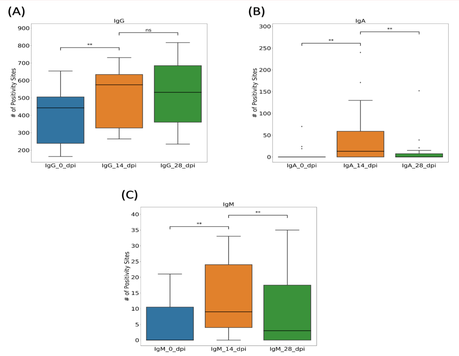


Fig.3 Number of positive epitopes bound by Zika virus-specific IgA, IgM and IgG antibodies at different days post infection. (A) IgG. (B) IgA. (C) IgM

We used the data generating under optimal experimental condition to compare the number of reactive epitopes at 0-, 14- and 28-days post-infection. The number of viral-specific epitopes, or positive epitopes, increased from 14 days post infection (dpi) to 28 dpi for Zika virus-specific IgG antibodies. In comparison, the number of epitopes bound by Zika virus-specific IgM and IgA antibodies peaked at 14 dpi (Figure 3).

CONCLUSIONS

We developed a standard high-density peptide micro array analysis pipeline, including data normalization and reactive epitopes detection, to ensure the reproducibility of data analysis results. We defined the optimal experimental conditions that will be used in for future high-density peptide micro array experiments. We also detected the reactive epitopes using data generating under optimal experimental conditions and found that the breadth, or number of positive epitopes, varies by time since infection and antibody class, with the number of IgM and IgA epitopes peaking earlier than the IgG epitopes. Future peptide microarrays will build on these optimal experimental conditions to define the breadth of the maternal Zika virus-specific antibody response.

ADDITIONAL KEY INFORMATION

Reference:

1. Imholte, Greg C., et al. "A computational framework for the analysis of peptide microarray antibody binding data with application to HIV vaccine profiling." Journal of immunological methods 395.1-2 (2013): 1-13.

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Acknowledgements:
This research was supported by Department of Pediatrics and the National Institutes of Health R01 AI116382-01A1S1 (David O'Connor), K08 AI139341 (Emma Mohr).