Biomarker discovery to detect antibodies specific for *Wuchereria bancrofti*

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BACKGROUND

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The World Health Organization (WHO) neglected tropical diseases road map for 2021–2030 calls for better diagnostic tests that would allow greater precision in program delivery. Tests are needed both for diagnosing active infections and detecting recrudescence during the post-MDA surveillance phase. To address this, we sought to identify new antibody biomarkers that are sensitive and specific for bancroftian lymphatic filariasis (LF).

OBJECTIVES

➢To identify antibody biomarkers for bancroftian LF that are not cross-reacted to onchocerciasis-positive sera.

➢To develop an antibody detection assay for LF that could be used for post-MDA surveillance.

METHODS

1. SERA

SERA (serum epitope reservoir analysis) incorporates a large random peptide library, Next Generation Sequencing (NGS), and custom bioinformatics tools to map immunogenic antigen epitopes targeted for a given infection.

An antibody-containing patient sample (typically serum or plasma) is incubated with a bacterial library displaying random peptides at the cell surface. Antibodies bind to peptide library members that mimic the binding site on their natural protein target. Antibody (IgG total, IgG1, and IgG4 subclasses) bound bacteria are magnetically separated to isolate a unique set of antibody-binding peptides for each patient. NGS is used to sequence each patient's set of millions of antibody-binding peptides (i.e. each patient's antibody epitope repertoires). From these peptide datasets, custom computer algorithms identify amino acid motifs that occur in the diseased patient samples and are absent from controls. Motifs are aligned to proteome sequences of interest to identify likely antigen candidates eliciting the patient's antibody response. The most sensitive and specific motifs and antigens are identified for the disease cohort compared to controls and additional databased samples. A validation cohort of samples is then used to evaluate the performance of the selected motifs and antigens. The best-performing antigens are candidates for testing using a simple diagnostic platform such as ELISA or lateral flow (Kamath, K, et al. Sci Rep. 10, 5294 (2020); Reifert, J, et al. J Clin Microbiol. 59, e01836-20 (2021); Pantazes, RJ, et al. Sci Rep. 6, 30312 (2016); Haynes, WA, Kamath, K, Wait,z R, Daugherty, PS, Shon, JC. Front Immunol. 12, 625311 (2021)).

2. Peptide Array

The protein sequences of *W. bancrofti* and *O. volvulus* from SERA platform and from secretomes and glycosyl-phosphatidyl inositol (GPI)-anchored proteins were converted into a lymphatic filariasis peptide microarray. Peptides with a length of >15 amino acids were converted into 15 amino acid peptides with a peptide-peptide overlap of 13 amino acids. The resulting lymphatic filariasis peptide microarrays contained 4,879 different peptides printed in duplicate (9,758 peptide spots) and were framed by additional HA (YPYDVPDYAG, 108 spots) and polio (KEVPALTAVETGAT, 108 spots) control peptides. The slides were washed using PBS, pH 7.4 with 0.05% Tween 20 for 3 x 10 sec followed by a 30-minutes blocking step in Rockland blocking buffer MB-070. The slides were then exposed to *W. bancrofti* positive, Wuchereria bancrofti negative (from non-endemic countries), *O. volvulus* positive from *W. bancrofti* non-endemic area, and *Brugia malayi* positive sera diluted 1:200 (IgG total and IgG4) and 1:100 (IgG1) in 10% blocking buffer. Sera incubation was carried out for 16 h at 4°C and orbital shaking at 140 rpm. After washing as above, goat anti-human IgG (Fc) DyLight680 (0.1 µg/ml), mouse anti-human IgG4 DyLight800 (0.2 µg/ml), and mouse anti-human IgG1 Cy3 (0.2 µg/ml) were added for 45 minutes staining in the blocking buffer at RT. The fluorescence was read by a scanner (LI-COR Odyssey Imaging System) with scanning offset 0.65 mm, resolution 21 µm, scanning intensities of 7/7 (red = 680 nm/green = 800 nm) and Genepix Imaging System, resolution 10 µm, gain of 500 PMT(red = 632 nm / green = 532 nm)

3. Peptide ELISA

Peptides identified through the SERA platform and peptides that showed significant signal-to-noise ratios in the peptide array were synthesized using the service of a commercial company. After peptide resuspension to the respective diluent at 2 mg/mL, peptides were diluted to 10 ug/mL in sensitization buffer (50 mM Tris-HCl, ph 8.0 + 0.3 M KCl + 2 mM EDTA) and added to wells and then peptide sensitization was carried out at 4 C overnight.

The plate was washed with PBS/0.3% Tween-20 and then diluted serum (1:50 in PBS/0.3% Tween-20/5% milk) was added to the wells. After 30 minutes of incubation, the plate was washed and secondary antibodies (either IgG total, IgG1, or IgG4) – HRP conjugated was added. After a further 30 minutes of incubation, the plate was washed, and TMB substrate was added. The reaction was observed at A650 nm.

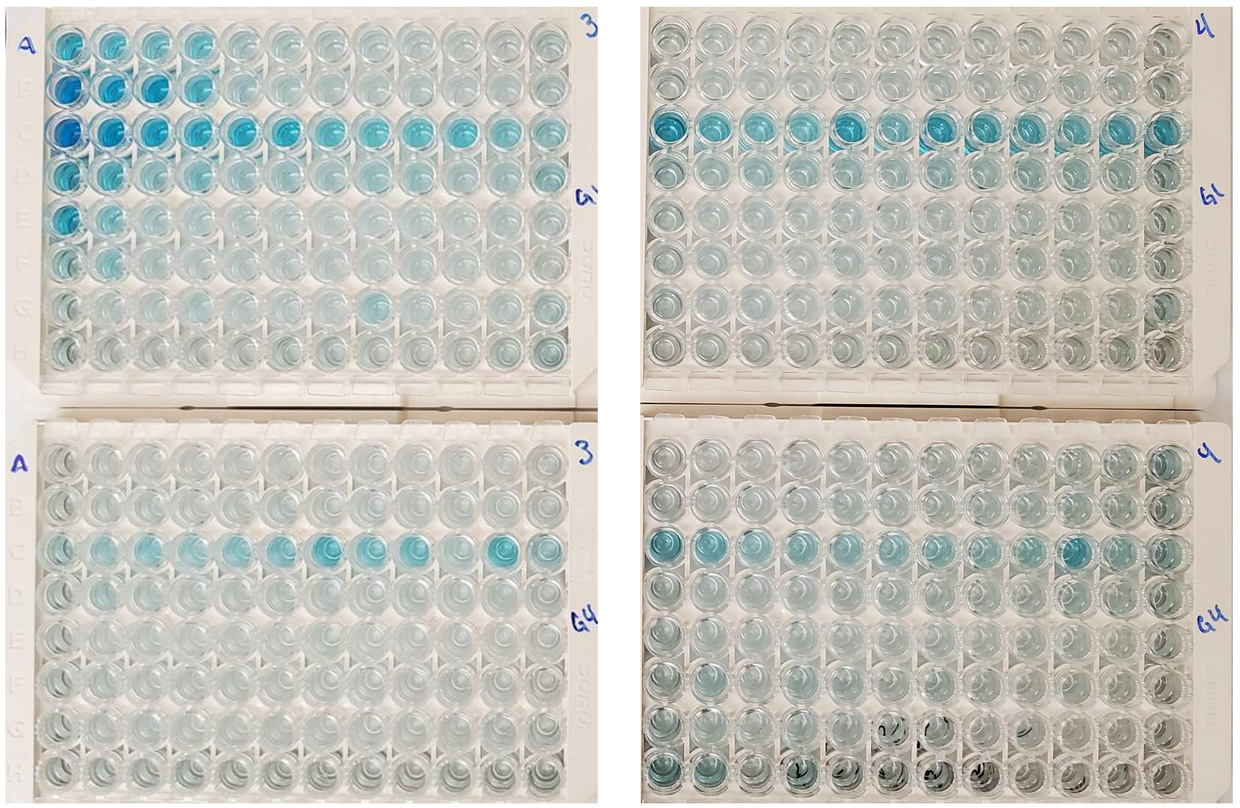

Figure 1: ELISA results of peptide array. Peptides were tested against IgG1 and IgG4.

IgG1	2	3	5	7	8	9	12	15	16	17	18	19
Negative	0.464933	0.1321	0.187933	0.0505	0.016067	0.009767	0.0082	0.0348	0.000333	0.026133	0.00185	0.000733
Wb	1.433933	0.7421	0.4563	0.359933	0.431	0.4375	0.268	0.239333	0.2261	0.3164	0.1618	0.0874
Ov	0.175933	0.1472	0.015	0.078733	0.0406	0.0586	0.0458	0.076233	0.0493	0.0599	0.0379	0.072
S/N Wb	3.1	5.6	2.4	7.1	26.8	44.8	32.7	6.9	678.3	12.1	87.5	119.2
S/N Ov	0.4	1.1	0.1	1.6	2.5	6.0	5.6	2.2	147.9	2.3	20.5	98.2
S/N Wb/O	8.2	5.0	30.4	4.6	10.6	7.5	5.9	3.1	4.6	5.3	4.3	1.2
IgG4	2	3	5	7	8	9	12	15	16	17	18	19
Negative	0.0026	0.0052	0.0127	0.0014	0.003033	0.005567	0.010933	0.003467	0.009567	0.00185	0.0075	0.0048
Wb	0.07545	0.1184	0.156333	0.1157	0.149067	0.203667	0.313733	0.203533	0.2234	0.05915	0.2964	0.114967
Ov	0.00415	0.0602	0.004533	0.0111	-0.00513	0.007767	0.006933	0.004433	0.0389	-0.00055	0.0065	0.010567
S/N Wb	29.0	22.8	12.3	82.6	49.1	36.6	28.7	58.7	23.4	32.0	39.5	24.0
S/N Ov	1.6	11.6	0.4	7.9	-1.7	1.4	0.6	1.3	4.1	-0.3	0.9	2.2
S/N Wb/O	18.2	2.0	34.5	10.4	-29.0	26.2	45.3	45.9	5.7	-107.5	45.6	10.9

IgG1	20	21	22	23	24	27	28	29	30	31	32	33
Negative	0.010867	0.001117	0.0151	0.0149	0.0018	0.016	0.0016	0.003683	0.008733	0.003	0.0053	0.0298
Wb	0.4724	0.25235	0.1989	0.1931	0.406467	0.164333	0.412033	0.32825	0.281167	0.193933	0.3053	0.395333
Ov	0.0386	0.02105	0.0563	0.0627	0.038467	0.033633	0.055933	0.03355	0.058967	0.052733	0.0417	0.039033
S/N Wb	43.5	226.0	13.2	13.0	225.8	10.3	257.5	89.1	32.2	64.6	57.6	13.3
S/N Ov	3.6	18.9	3.7	4.2	21.4	2.1	35.0	9.1	6.8	17.6	7.9	1.3
S/N Wb/O	12.2	12.0	3.5	3.1	10.6	4.9	7.4	9.8	4.8	3.7	7.3	10.1
IgG4	20	21	22	23	24	27	28	29	30	31	32	33
Negative	0.010133	0.0063	0.012067	0.018833	0.000633	0.006867	0.006667	0.021367	0.006567	0.000333	0.0026	0.018233
Wb	0.2178	0.1999	0.11	0.1371	0.1019	0.102733	0.1061	0.107233	0.104267	0.3164	0.0969	0.1058
Ov	0.0103	0.013	0.0127	0.0137	0.0021	0.008333	0.0037	0.026733	0.001867	0.0306	0.01	0.0252
S/N Wb	21.5	31.7	9.1	7.3	160.9	15.0	15.9	5.0	15.9	949.2	37.3	5.8
S/N Ov	1.0	2.1	1.1	0.7	3.3	1.2	0.6	1.3	0.3	91.8	3.8	1.4
S/N Wb/O	21.1	15.4	8.7	10.0	48.5	12.3	28.7	4.0	55.9	10.3	9.7	4.2

Figure 2a and 2b: Signal-to-noise (S/N) calculations for Plate 1-IgG1 and IgG4 (top) and Plate 2-IgG1 and IgG4 (bottom).

RESULTS

➢SERA Results

➢60 defined bancroftian LF (infected – antigen and microfilariae positive and exposed – negative for LF antigen and microfilariae) from Haiti, 60 defined onchocerciasis negative sera, and 60 defined onchocerciasis positive sera from Guatemala were subjected to the SERA platform.

➢There are 49 specific epitopes for IgG total, 93 epitopes for IgG1, and 28 epitopes for IgG4 for LF infected or exposed defined sera while there are 8 specific epitopes for IgG total, 5 epitopes for IgG1, and 96 epitopes for IgG4.

➢These epitopes and additional peptides identified through secretome, and GPI-anchored proteins were further screened using a peptide array. Based on significant signal-to-noise results (S/N > 10), we synthesized 99 peptide sequences for further screening in peptide ELISA.

➢Peptide Array Results

The ELISA results can be seen in Figure 1. Based on the criteria of S/N W. bancrofti to negative > 10 and S/N W. bancrofti to O. volvulus > 5, we identified 22 peptides for detection of IgG1 antibody responses and W. bancrofti infection and 21 peptides for detection of IgG4 antibody. It seems that IgG4 antibody responses yield a better S/N ratio than IgG1 antibody responses.

DISCUSSION

While the peptide ELISA gave several potential peptides for the detection of IgG1 and IgG4 antibody responses to *W. bancrofti* infection, there is a possibility that some peptides did not bind well to the well of the ELISA plate.

Before we conclude the study on antibody biomarkers for *W. bancrofti* infection, we need to use a potentially better binding of peptide to the well by biotinylating of peptides and adding them to the streptavidin plate.

CONCLUSION AND NEXT STEPS

SERA platform, peptide array, and peptide ELISA identified several potential peptides for the detection of IgG1 and IgG4 antibody responses to *W. bancrofti* infection in less than 5 months.

Conduct biotinylated peptide ELISA on a streptavidin plate.

Significant peptides will be used to produce a chimeric protein containing several peptides.

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