O Broad Anti-Influenza Antibody, Where Art Thou?

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Introduction

Influenza virus is a major public health threat that continues to be a looming concern. It is categorized as a Virus of Special Concern by the CDC[1]. Pathogenic avian influenza viruses cause sporadic outbreaks in humans with a high mortality rate, increasing the risk of another pandemic.

Influenza A infections account for most hospitalizations and are the only type to cause pandemics[2]. Influenza A is subtyped by its two major surface proteins, hemagglutinin (HA) and neuraminidase (NA)[3]. To date, there are 18 HA and 11 NA subtypes identified and characterized[4,2]. HA is the main target of neutralizing antibodies that are induced by infection or vaccination[5]. It has been hypothesized that broadly cross-HA subtype reactive antibodies have the potential as therapeutic agents and studies on their mechanism of action, epitope specificity, and ontogeny could inform the design of cross-protective influenza virus vaccines.

In this work, we develop a workflow to isolate antibody-secreting cells from influenza-vaccinated donor samples and screen antibodies using AbTheneum™, a single B cell antibody discovery method to rapidly screen and sequence antibody-secreting cells in parallel across multiple parameters. Antibodies from vaccinated subjects were profiled by screening their reactivity to four highly diverse HA proteins (A/California/7/2009 HA, A/Vietnam/1194/2004 HA, A/California/7/2004 HA, and A/Shanghai/1/2013 HA which are named in this work as H1N1, H3N2, H5N1, and H7N9, respectively) and their ability to compete with a benchmark neutralizing antibody (MEDI8852[6]). We discovered HA subtype-specific and cross HA subtype-reactive antibodies, many of which compete with the benchmark, including 6 mAbs that are broadly-reactive to 8 diverse HA proteins. AbTheneum screening is shown to be an excellent predictor of antibody activity.

AbTheneum Overview

AbTheneum is an antibody discovery engine that screens and sequences antibody-secreting cells. The workflow is shown in Figure 1. Cells are isolated and deposited onto a picoliter device with over 90,000 picowells. Multiple devices can be run simultaneously to increase throughput. Antibodies are captured onto capture slides and all independently and simultaneously profiled with fluorescent screening molecules. Off-line assays allow multiparametric analysis of antibodies including affinity ranking, cross-reactivity, specificity, competition, and blocking of receptors.

After capturing antibodies, all cells are lysed and mRNA for heavy and light chains are captured on a custom microarray. All IgGs are sequenced by NGS. The informatics process analyzes sequences and image analysis from antibody screen to cross-reference antibody sequence with screened properties.

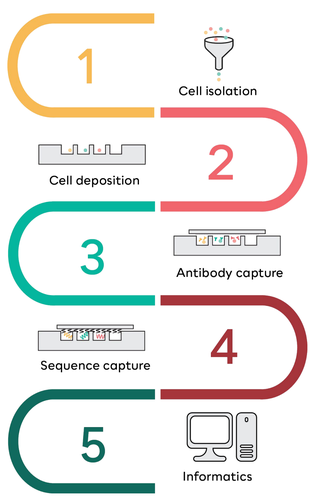

Figure 1. AbTheneum workflow from cells to cross-referenced dataset.

Cell Isolation

Peripheral blood mononuclear cells (PBMCs) were collected from 3 donors who had received their seasonal flu shot within 6 weeks.

PBMCs were thawed and Class-Switched Memory B Cells (CSMBCs) were isolated from PBMCs using a memory B cell isolation kit (Miltenyi). The CSMBCs were activated with a proprietary cytokine cocktail for 5 days (similar products by Miltenyi), with an antigen-specific sort using MACSQuant Tyto (Miltenyi Biotec) on Day 3 to enrich for antigen-specific cells.

On the day of the screen, activated CSMBCs were stained with anti-CD38 Brilliant Violet 421 (Biolegend), 7-AAD (ThermoFisher), and a mixture of 4 HA proteins labeled with AlexaFluor 647. MACSQuant Tyto was used to sort for 7-AAD-/CD38+/influenza+ cells. We observed over 4-fold increase in antigen-specific cells after sorting (Figure 2).

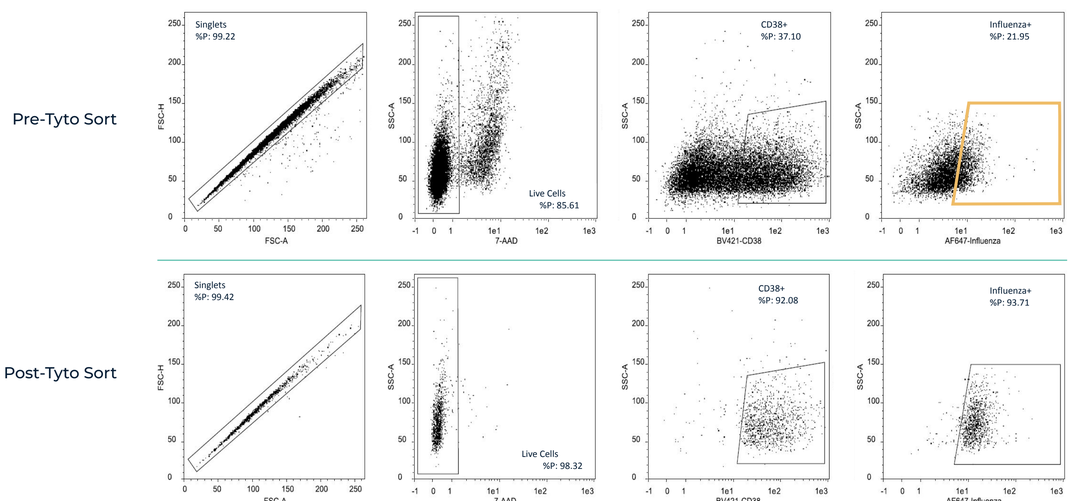

Figure 2. Cell analysis plot taken by MACSQuant 10 of cells pre and post sort by MACSQuant Tyto. Cells were stained with 7-AAD, Anti-CD38 Brilliant Violet 421, and 4 influenza HA proteins in AlexaFluor 647. The sort gate for Tyto sorting is highlighted in yellow.

Antibody Screening by AbTheneum™

Cells sorted from Tyto were deposited onto picoliter devices. Antibodies from the activated cells were captured onto 3 activated capture slides. The 3 slides were screened against 4 highly variable HA proteins: H1N1, H3N2, H5N1, H7N9. We also screened all antibodies for competing with a clinical benchmark antibody – MEDI8852. The screening plan for the 3 capture slides is outlined in Table 1.

Table 1. Screening plan for broadly-cross subtype reactive anti-influenza antibodies. Each slide is washed and scanned on a fluorescent slide scanner after each stain. Slide 1 screens all antibodies for competition with MEDI8852 antibody (negative hits are competing, positive hits are non-competing). Slide 2 screens all antibodies for binding to H1N1 and H5N1. Slide 3 screens all antibodies for binding to H3N2 and H7N9. All slides detect all IgGs on the last stain.

Slide	Stain 1	Stain 2	Stain 3
1	Unlabeled HA proteins	MEDI8852-555*	Anti-Human IgG-647*
2	H1N1-555*	H5N1-647*	Anti-Human IgG-647*
3	H3N2-555*	H7N9-647*	Anti-Human IgG-647*

Figure 3 shows a small region of all images after each stain to show the data output. After capturing antibodies, all cells were lysed and sequences captured en masse. All IgGs were sequenced by NGS and image analysis and bioinformatics mapped sequences back to their capture locations.

The data output of full-length sequences of all antigen-specific antibodies with each antibody's binding characteristics were recovered. The output from 2,593 antibodies profiled is displayed in Figure 4. The screening plan was designed to allow assigning antibodies to categories (e.g., highly specific to a single HA, or cross-reactive to 2, 3, or 4 HAs).

Slide	Stain 1	Stain 2	Stain 3
1	No image Unlabeled HA proteins	MEDI8852-555*	Anti-IgG-647*
2	H1N1-555*	H5N1-647*	Anti-IgG-647*
3	H3N2-555*	H7N9-647*	Anti-IgG-647*

Figure 3. Small cropped region from the same area on all 3 slides across 8 staining conditions. Slides were stained on a fluorescent slide scanner that has 2 lasers capable of detecting dyes exited at 532 and 635 nm. Images are aligned across all slides and images, analyzed for fluorescent intensity, and individual antibody binding properties are reported for each screen condition.

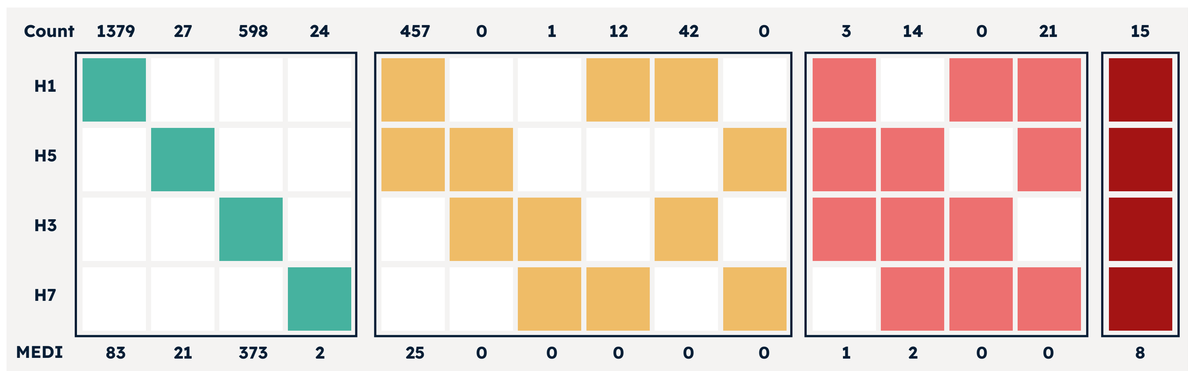

Figure 4. Data output of 2,593 antibodies discovered by AbTheneum. Each antibody was screened for binding against the H1N1, H5N1, H3N2, and H7N9 HA proteins. All 15 possible binding properties are shown using colored columns. For example, H1 and H5 colored yellow with H3 and H7 white means antibodies that bind to H1N1 and H5N1, with no binding to H3N2 and H7N9. The top x-axis lists the count of mAbs that exhibit the binding profile each colored column. The bottom x-axis lists the number of mAbs in the group that compete with MEDI8852.

AbTheneum Screening Validation

We selected 12 antibodies for reconstruction and expression and ran ELISA against recombinant 8 HA proteins. The 4 HA proteins used for screening in the discovery workflow were included and 4 additional HA proteins to add more diversity. Figure 5 displays the ELISA results for 12 antibodies and MEDI8852 against all 8 HA proteins. The ELISA results confirm the screening results from AbTheneum with high fidelity.

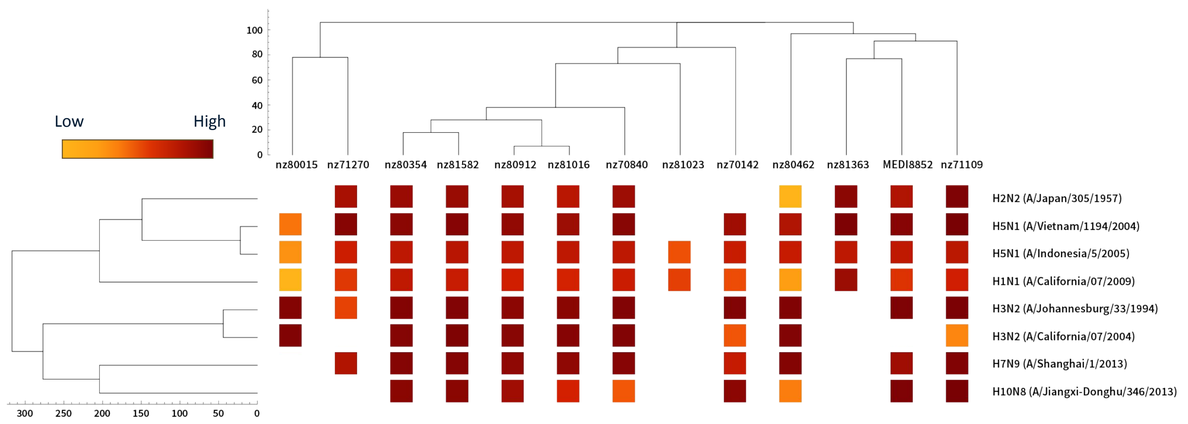

Figure 5. ELISA validation of 12 expressed antibodies, clustered on the x-axis by antibody sequence similarity and y-axis by HA protein sequence similarity. The heat map indicates the strength of the binding interaction of the antibody to each HA protein by ELISA at 1 nM of antibody concentration.

Conclusion

We demonstrate a strategy for enriching antigen-specific B cells that elevate the odds of identifying hits with rare binding profiles. AbTheneum screening technology was able to discover and confirm broadly-subtype reactive anti-influenza antibodies from human donors in less than 60 days. This timeline could be improved with higher speed antibody expression. These broadly subtype-reactive antibodies show promise as therapeutic agents worthy of further study.

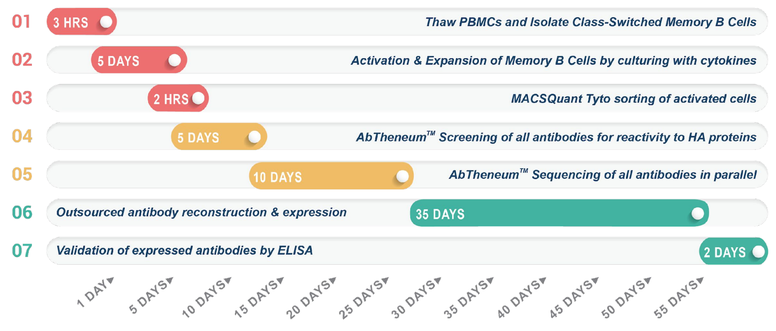

Figure 6. Timeline from donor cells to validated expressed antibodies screened using AbTheneum was less than 60 days.

Using AbTheneum allowed rapid filtering from thousands of antibodies to find rare events. It also offers the advantage of layering multiple screens, delivering layers of data on top of the natively paired full-length sequences. The ELISA data confirmed the binding data captured by AbTheneum screening, showing that AbTheneum screening is a good predictor of antibody activity.

AbTheneum discovery targets antibody-secreting cells and leverages that secretion to screen before massively parallel sequencing.

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