Proteomic Characterization of the Evolution of the Circulating Antibody Response to Hepatitis B Virus Vaccination

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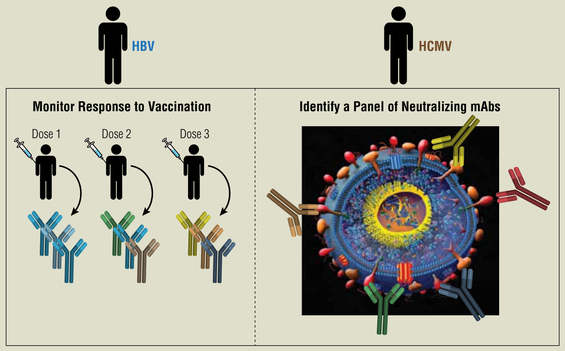
Abstract

A potent polyclonal antibody response is essential for host protection against pathogens. Methods to elucidate the antibody composition of the human serological polyclonal response have so far been elusive. For development of vaccines, the ability to monitor the individual monoclonal components of circulating antibodies elicited against the immunogen would be highly desirable. Previously, we described a novel approach using mass spectrometry based proteomics and next-generation sequencing to identify and isolate antigen-specific antibodies from circulation in immunized animals[1], and more recently, we demonstrated that this approach can also be applied to clone vaccine-specific monoclonal antibodies from a donor immunized against HBV, and neutralizing monoclonal antibodies against CMV from a naturally infected donor[2]. To further investigate the evolution of the circulating antibody response during HBV vaccination, we conducted proteomic analyses on longitudinal samples from the same donor that was vaccinated against HBV. The majority of vaccine-specific monoclonal antibodies observed in circulation one week after the second immunization were still present one week and six weeks after the third immunization. Furthermore, we observed from the later time-points the emergence of variants of the highest affinity (517pM) antibody that was cloned initially from the earliest time-point. We also observed persistent presence of two antibodies specific to neutralizing epitopes within the antigenic loop of the hepatitis B virus surface antigen. As such, we present a rapid proteomic method to accurately monitor the circulating antibody response elicited against vaccines.



NG-XMT™ identifies the monoclonal components of circulating antibodies elicited in response to a specific antigen. This platform can be used to monitor the humoral response to vaccines (**left**) and/or to isolate and clone endogenous monoclonal antibodies with a specific functional activity (**right**).

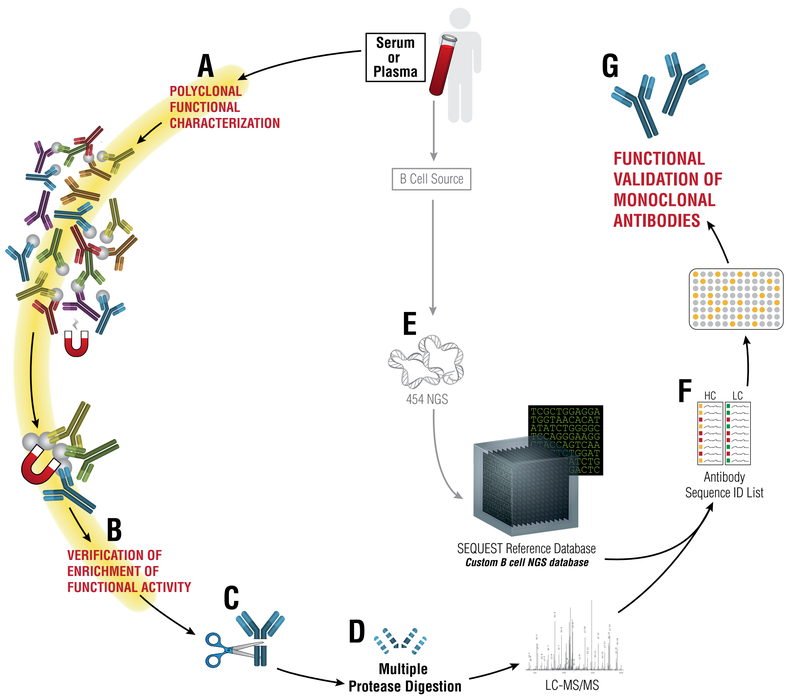


Figure 1. NG-XMT™ is a proteomic approach to identify antigen-specific human monoclonal antibodies in circulation. (**A**) Identification of desired activity in serum or plasma. (**B**) Specific and stringent, magnetic bead-based affinity purification to isolate antigen-specific antibodies enriched in the same functional activity. (**C**) Elimination of Fc with a site-specific endopeptidase. (**D**) Digestion of F(ab')$_2$ with multiple proteases and analysis by LC-MS/MS. (**E**) Generation of custom sequence reference database from B cells isolated from the same donor. (**F**) Identification and cloning of heavy and light chain variable region sequences and expression of monoclonal antibodies. (**G**) Validation and characterization of each monoclonal antibody for the desired functional activity.

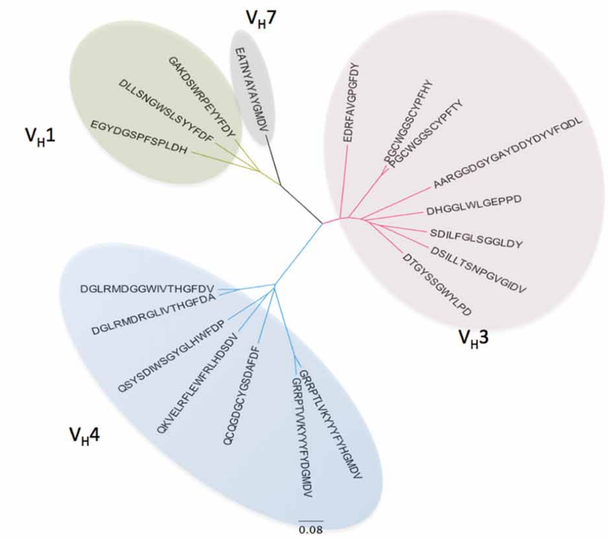


Figure 2. Diverse, high affinity antibodies against HBV identified from a vaccinated donor. Phylogenetic tree (generated using neighbor joining method for multiple sequence alignment by CLC Bio's genomic workbench software) of all 19 heavy chain variable sequences identified from affinity-purified IgG from donor C037. Sequences that utilize VH1, 3, 4, and 7 gene families are shown in green, pink, blue, and grey, respectively. The scale represents the number of substitutions per 100 residues (display was generated using Fig Tree [http://tree.bio.ed.ac.uk/software/figtree/]).

Clone	H-CDR3	L-CDR3	K_a ($M^{-1}s^{-1}$)	K_d (s^{-1})	K_D (M)
C037(1+49)	DLLSNGWSLSYYFDF	QQYHTWPFT	1.69×10^{5}	4.27×10^{-4}	2.53×10^{-9}
C037(3+53)	AARGGDGYGAYDDYDYVFQDL	QQYSAYPIT	4.03×10^{4}	2.09×10^{-5}	5.17×10^{-10}
C037(5+55)	GRRPTVVKYYYFYDGMDV	QQSASSPRT	3.06×10^{4}	5.65×10^{-4}	1.85×10^{-8}
C037(7+63)	DGLRMDGGWIVTHGFDV	QHRSNWPPLFT	9.80×10^{4}	1.99×10^{-3}	2.03×10^{-8}
C037(9+61)	QCQGDGCYGSDAFDF	QQRSNWPMYT	n.d.	n.d.	n.d.
C037(11+63)	DGLRMDRGLIVTHGFDA	QHRSNWPPLFT	n.d.	n.d.	n.d.
C037(14+75)	DTGYSSGWYLPD	QQYYGAPIT	1.98×10^{5}	4.32×10^{-4}	2.18×10^{-9}
C037(15+91)	EDRFAVGPGFDY	GTWDTTLSAGV	8.95×10^{4}	4.10×10^{-4}	4.58×10^{-9}
C037(17+55)	GRRPTLVKYYYFYHGMDV	QQSASSPRT	4.20×10^{4}	4.99×10^{-4}	1.18×10^{-8}
C037(19+89)	QKVELRFLEWFRLHDSDV	ASWDDSLKGVL	7.80×10^{4}	4.71×10^{-4}	6.04×10^{-9}
C037(21+57)	GAKDSWRPEYYFDY	QQYNSFAFN	3.21×10^{5}	1.62×10^{-3}	5.04×10^{-9}
C037(25+105)	PGCWGGSCYPFTY	QSYDNSLSGHNYV	3.63×10^{5}	3.63×10^{-4}	2.08×10^{-9}
C037(27+103)	SDILFGLSGGLDY	QVWDGISNRVV	4.23×10^{5}	5.08×10^{-4}	1.20×10^{-9}
C037(29+107)	DHGGLWLGEPPD	ASFTPRYTWV	n.d.	n.d.	n.d.
C037(31+105)	PGCWGGSCYPFHY	QSYDNSLSGHNYV	5.63×10^{5}	7.45×10^{-4}	1.32×10^{-9}
C037(34+87)	QSYSDIWSGYGLHWFDP	HNYGTIPGT	1.77×10^{4}	4.50×10^{-4}	2.54×10^{-8}
C037(35+97)	DSILLTSNPGVGIDV	QVWDTTTDHMI	4.61×10^{5}	1.19×10^{-3}	2.57×10^{-9}
C037(37+95)	EATNYAYAYGMDV	QSYDGSLSRSTV	n.d.	n.d.	n.d.
C037(44+87)	EGYDGSPFSPLDH	HNYGTIPGT	n.d.	n.d.	n.d.

Table 1. Anti-HBsAg monoclonal antibodies isolated from HBV vaccinated donor, C037.

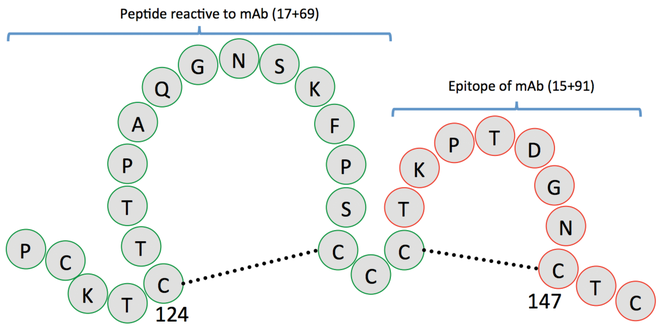


Figure 3. Two HBsAg-specific monoclonal antibodies recognize neutralization-sensitive epitopes within the antigenic loop of HBsAg. Linear epitopes were mapped by ELISA using overlapping HBsAg peptides.

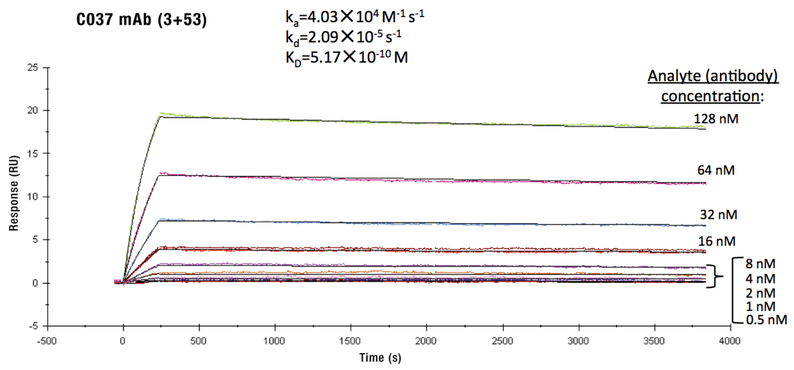


Figure 4. Kinetics measurement of a picomolar affinity anti-HBV antibody. Binding kinetics curves of C037 monoclonal antibody (3+53) were generated by Biacore T200 with the antibody as the analyte and HBsAg as the ligand immobilized at low density on the chip surface to minimize effects of avidity.

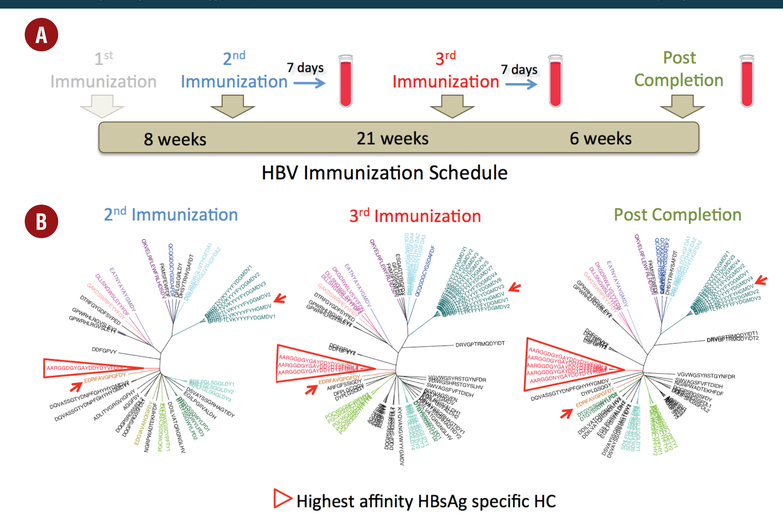


Figure 5. (**A**) **Immunization schedule and blood draw of HBV vaccine recipient C037.** Donor C037 received the three HBV vaccine series over a course of 29 weeks. Blood samples were collected 7 days after the 2nd and 3rd immunizations, and 6 weeks after the 3rd immunization (post completion sample). Blood sample following the first immunization was not available because the first collection was after the second immunization. (**B**) **Phylogenetic tree of heavy chain (HC) sequences identified by NG-XMT™ in each time-point.** Antigen-specific antibodies were affinity purified from each time-point and Ig variable region sequences were identified by LC-MS/MS using sequence databases created from NGS of Ig variable regions of memory B cell libraries from each corresponding time-point. Heavy chain variable region sequences of HBV-specific antibodies were aligned using neighbor joining method for multiple sequence alignment by CLC Bio and represented as a dendrogram. Each unique sequence is indicated by its CDR3 amino acid sequence. Heavy chains with colored sequences generated HBV-specific antibodies when paired with its corresponding light chain (see **Table 1**). The sequences shown in black have not been characterized.

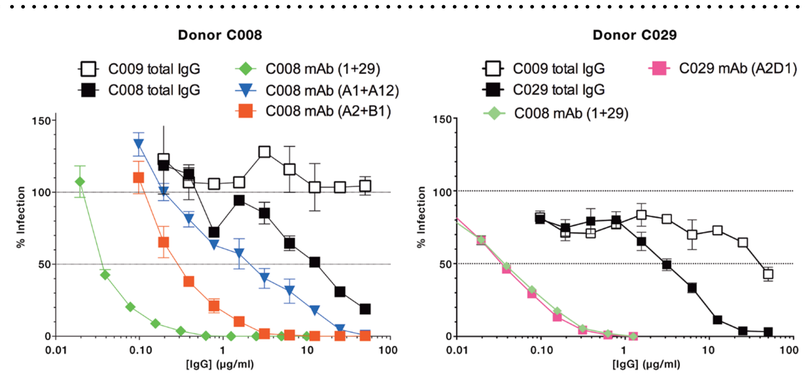


Figure 6. Potent CMV-neutralizing antibodies identified from two naturally infected donors. Antibodies specific to cytomegalovirus (CMV) glycoprotein B were cloned from two donors naturally exposed to CMV. *In vitro* neutralizing activity was measured in MRC5 cells (fibroblast cell line) with AD169 CMV strain as previously described.

Conclusion

We employed a mass spectrometry-based proteomic approach to identify and clone human antiviral antibodies from donors: anti-HBV antibodies from an HBV vaccine recipient; CMV-neutralizing antibodies from two naturally infected donors. We monitored the evolution of the circulating antibody response to the HBV vaccine by analyzing longitudinal samples from the same HBV vaccine recipient.

:: The anti-HBV antibody possessing the highest affinity (517 pM), which was cloned 7 days following the second immunization, had expanded into a clonal family in the latter time-points, as observed by a number of closely related but distinct sequences.

:: The majority of anti-HBV clones observed in the first time point, including those that specifically bind to the neutralization-sensitive epitopes in the antigenic loop of HBsAg, persisted throughout the immunization.

References

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