Monitoring the evolution of antigen-specific antibodies in circulation and their corresponding B cell clones in longitudinal immunization case studies through the use of NG-XMT™

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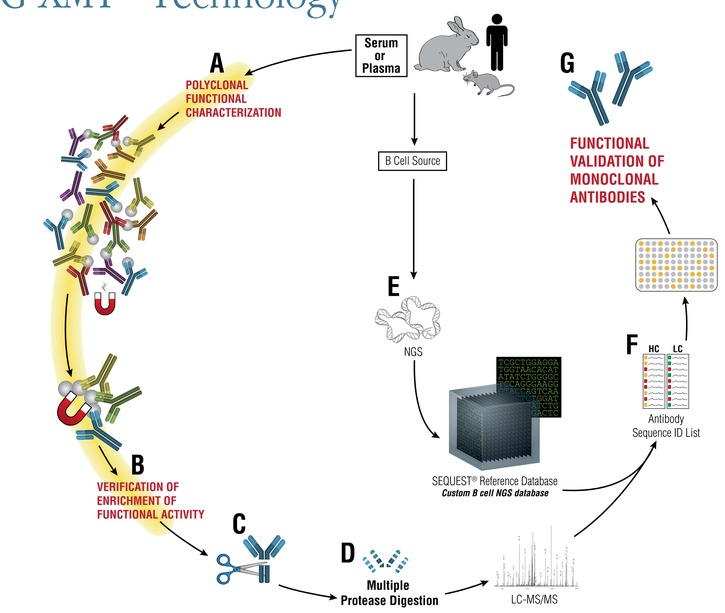
Abstract

Previous studies aimed at determining the affinity maturation pathways of monoclonal antibodies during an immunization have relied on comparing germ-line with mature forms of antibodies present in either transgenic B cell receptor mouse models or by cloning circulating human B cells present in peripheral blood (PB) of pre and post-immunized individuals. These approaches have been insightful, but they do not identify in confidence the true B cell clone(s) producing the antigen-specific antibodies found in the sera of immunized animals or humans. We recently developed a technology, NG-XMT™, encompassing tandem mass spectrometry and next generation sequencing (NGS)[1,2], which for the first time enables the **1)** identification of the anatomical location of the B cell clone(s) corresponding to the antigen-specific serum antibodies **2)** investigation of clonal diversity of antigen-specific B cell clones residing in primary and secondary lymphoid organs and circulating in PB and **3)** analysis of the evolving affinity mature serum antibodies, in two longitudinal immunization studies of rabbits and humans. These results not only advance our understanding of the affinity maturation process and the dynamics of memory B cells and plasma cell development that occurs during an immunization response, they also allow us to use this information to generate useful recombinant antibodies that can be utilized in passive immunization strategies.

NG-XMT™ Technology



NG-XMT™ Technology. NG-XMT™ is a proteomic approach to identify antigen-specific human, rabbit and mouse 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 for 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.

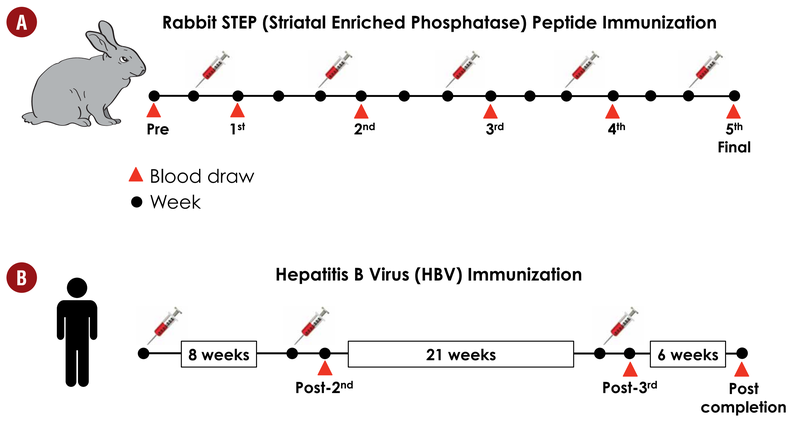
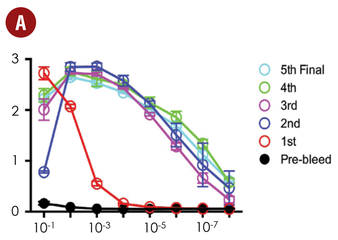
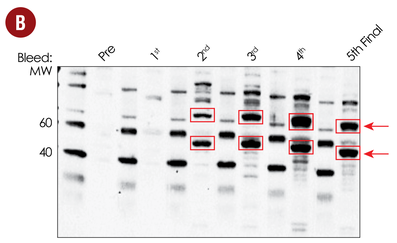


Figure 1. (A) Rabbit Immunization Strategy. Rabbits were immunized in 3 week intervals with plasma and peripheral blood mononuclear cells (PBMCs) collected pre-immunization and 1 week after each boost. PBMCs were immediately processed and prepared for NGS using the Illumina® platform. At terminal bleed, spleen and bone marrow were harvested and cells were processed for sequencing. **(B) Immunization schedule and blood draw of HBV vaccine recipient C037.** Donor C037 received the three HBV vaccine series over a course of 29 weeks. Serum and PBMCs were collected 7 days after the 2nd and 3rd immunizations and 6 weeks after the 3rd immunization (post-completion). PBMCs were immediately processed and prepared for NGS.

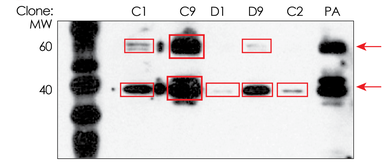
Rabbit Immunization



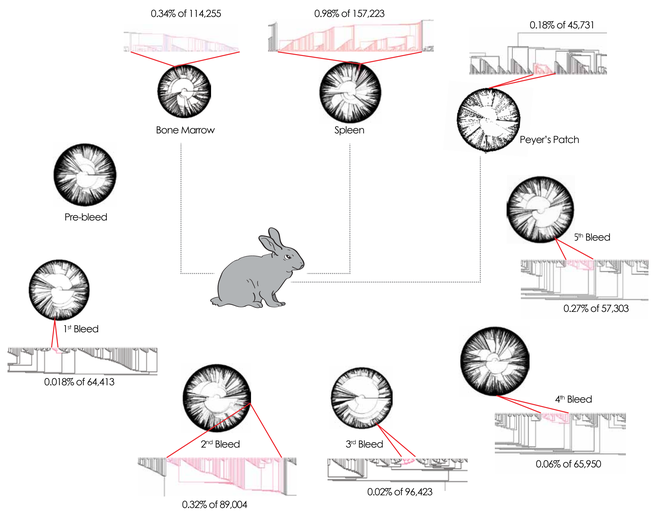


C

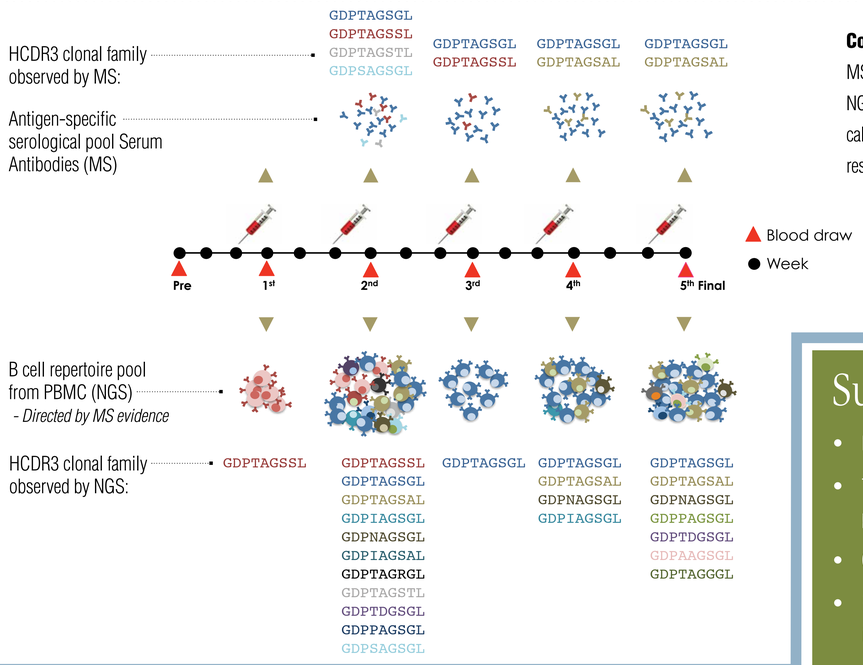
Clone	H-CDR3	L-CDR3	ELISA (OD 450)
C1	GDPTAGSGL	AGGYRSNSDSG	3.66
C9	GDPTAGSGL	AAGYSRNSDSG	3.42
D1	GDPTAGSAL	AGGYRSNSDSG	3.16
D9	GDPTAGSAL	AAGYSRNSDSG	3.49
C2	GDPTAGSGL	AGGYSSNSDNA	2.15



Antigen specific activity of sequential bleeds and subsequent purifications. Anti-STEP specific activity of crude plasma collected at different time-points was determined via **(A)** STEP peptide ELISA and **(B)** Western Blot using rat brain lysates. Plasma activity to both cytosolic STEP (46 Kd) and membrane-associated STEP (61 Kd) is noted. **(C)** NG-XMT™ technology revealed heavy and light chain variable region sequences, which were cloned and expressed as recombinant monoclonal antibodies. Validation and characterization of each monoclonal antibody was determined by STEP specific ELISA **(C)** and Western blot **(D)**. PA- Protein A material. MW- Molecular weight.



The antigen specific heavy chain sequence family GDPxxGSxL represents a small subset of heavy chain variable sequences present in systemic immune tissues and sequential bleeds. Phylograms of Ig heavy chain variable region sequences present in spleen, bone marrow, Peyer's patches, and PBMCs collected a week post antigen boost at sequential time intervals. GDPxxGSxL sequence family is highlighted red in each circular phylogram. Percentage of familial sequences from total heavy chain sequences is annotated. Sequences were aligned using neighbor joining method for multiple sequence alignment by MAFFT and circular phylograms were generated using Fig Tree software (http://tree.bio.ed.ac.uk/software/figtree/).

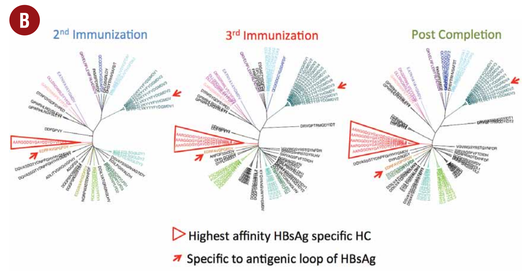


Correlation of antigen-specific serological and cellular response during immunization. MS sequence data of antigen specific purified IgGs of plasma collected after each immunization was paired with NGS data of PBMCs from corresponding time point bleeds to determine presence of both antigen specific serological and cellular response. Heavy chain CDR3 sequences of GDPxxGSxL family members are noted. Antibodies corresponding to cellular clones are color matched.

Human Immunization

A

C037(1+49)	DLLSNGWSLSYYFDF
C037(3+53)	AARGGDGYGAYDYDYVFQDL
C037(5+55)	GRRPTVVKYYYFYDGMDV
C037(7+63)	DGLRMDGGWIVTHGFDV
C037(9+61)	QCQGDGCYGSDAFDF
C037(11+63)	DGLRMDRGLIVTHGFDA
C037(14+75)	DTGYSSGWYLPD
C037(15+91)	EDRFAVGPGFDFDY
C037(17+55)	GRRPTLVKYYYFYHGMDV
C037(19+89)	QKVELRFLEWFRLHDSDV
C037(21+57)	GAKDSWRPEYYFDY
C037(25+105)	PGCWGGSCYPFTY
C037(27+103)	SDILFGLSGGLDY
C037(29+107)	DHGGLWLGEPPD
C037(31+105)	PGCWGGSCYPFHY
C037(34+87)	QSYSDIWSGYGLHWFDP
C037(35+97)	DSLLTSNPGVGIDV
C037(37+95)	EATNYAYAYGMDV
C037(44+87)	EGYDGSPFSPLDH
C037(1+49)	QCQYHTWPPT
C037(3+53)	QQYSAYPIT
C037(5+55)	QCQSASSPRT
C037(7+63)	QHRSNWPPLFT
C037(9+61)	QQRSNWPMYT
C037(11+63)	QHRSNWPPLFT
C037(14+75)	QQIYYGAPIT
C037(15+91)	GTWDTTLSAGV
C037(17+55)	QQSASSPRT
C037(19+89)	ASWDDSLKGVL
C037(21+57)	QQYNSFAFN
C037(25+105)	QSYDNSLSGHNYV
C037(27+103)	QVWDGISNRVV
C037(29+107)	ASFTPRYTWV
C037(31+105)	QSYDNSLSGHNYV
C037(34+87)	HNYGTIPGT
C037(35+97)	QVWDTTTDHMI
C037(37+95)	QSYDGSLSRSTV
C037(44+87)	HNYGTIPGT



Identification of human monoclonal antibodies specific to Hepatitis B surface antigen (HBsAg) during the course of an HBV vaccine series. (A) Table depicting heavy and light chain CDR3 sequences of anti-HBsAg monoclonal antibodies isolated from HBV vaccinated donor, C037. **(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's Genomic Workbench software and represented as a circular 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 in **(A)**). The sequences shown in black have not been yet characterized.

Summary of Data

- NG-XMT™ Technology identified heavy and light chain antibody families with specificity to STEP peptide antigen in rabbits.
- The heavy chain GDPxxGSxL family identified was present in all lymphoid tissues analyzed, albeit expansion of this clonal population was much less evident in gut associated lymphoid tissues (i.e. Peyer's patches).
- GDPxxGSxL represented only a small HC subset of the large heavy chain sequences obtained from lymphoid tissues.
- PB B cell clones expressing clonal IgG heavy chain CDR3 sequence, GDPxxGSxL, emerged immediately after the first immunization and remained through out.
- Clonal diversity of PB B cells grew after subsequent immunizations. In contrast, serum antibody diversity diminished with subsequent immunizations, with only a few dominant antibody clones persisting in circulation.
- NG-XMT™ Technology was used to identify several human anti-HBV antibodies from the serum of a vaccine recipient. As demonstrated in our rabbit studies, we were able to monitor the evolution of these circulating antibodies in response to the HBV vaccine, and we were able to reveal persistent clones.
- Herein, using our NG-XMT™ technology, we reveal that antigen specific activity present in the serum of an immunized rabbit or human corresponds to only a few dominant and persistent circulating B cell clones.
- NG-XMT™ enables the identification of antigen specific antibodies that are directly involved in the serological response driven by an active immunization or other immune insult.

References

1. ***Nat. Biotechnol.*** **2012,5:447**
2. ***Nat. Biotechnol.*** **2012,11:1039**

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