AID-induced insertions and deletions complement point mutations to massively expand the diversity created by somatic hypermutation of antibodies

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Abstract

During somatic hypermutation (SHM), deamination of cytidine by activation-induced cytidine deaminase (AID) and subsequent DNA repair generates mutations within immunoglobulin V-regions. Nucleotide insertion and deletions (indels) have recently been shown to be critical for the evolution of antibody binding. We analyzed the affinity maturation of 53 antibodies using *in vitro* SHM in a non-B cell context and compared mutation patterns with SHM *in vivo*. The origin, frequency and location of indels observed during in vitro affinity maturation is similar to that observed *in vivo.* Indels are localized to CDRs and AID hotspots and secondary mutations within insertions further optimize antigen binding. Structural determination and analysis of an antibody matured *in vitro* and comparison with human derived antibodies containing insertions reveals conserved patterns of antibody maturation. These findings indicate that AID acting on V region sequences is sufficient to initiate authentic formation of indels *in vitro* and *in vivo*, and that point mutations, indel formation and clonal selection form a robust, tripartite system for antibody evolution.

Samples

In vitro SHM coupled with mammalian cell display of full-length IgGs was used to affinity mature 39 human germline antibodies, and 14 CDR-grafted antibodies directed against 21 unique antigens. Sanger sequencing was performed during each round of maturation

Antibodies were sequenced from cell populations without AID as a negative control for potential sequencing and technology related errors

In order to characterize the unbiased spectrum of indels created by AID In vitro, samples from cells co-expressing AID with a HC/LC pair in the absence of selection for improved antigen binding were sequenced using Illumina

To characterize the indel repertoire of antibodies *in vivo*, normal human PBMC samples composed of both immature and mature B cells were sequenced using NGS and high quality reads were mapped to the closest human germline V-region sequence

Table 1: Sequence analysis of SHM in vivo and in vitro

		In vitro SHM, no selection	In vitro, No AID	In vitro SHM, with selection	In vivo NGS
HC	Samples	18	3	132	68
	Reads	1,672,921	363,535	143,329	285,331
	Indels	113	0	54	1,173
LC	Samples	16	NA	132	1
	Reads	1,419,998	NA	118,762	197,374
	Indels	91	NA	51	658

Statistics shown for Indels ≥3bp in length;; signal peptide excluded from in vivo and in vitro analysis for both indels and mutations; * indicates the number of sites that show a statistically significant number of mutations relative to that predicted by site specific sequence quality metrics and accompanying error model; # indicates the number of individual mutations observed when comparing reads with their germline V gene. Indel analysis not performed for in vivo human IgG dataset from NCBI

References

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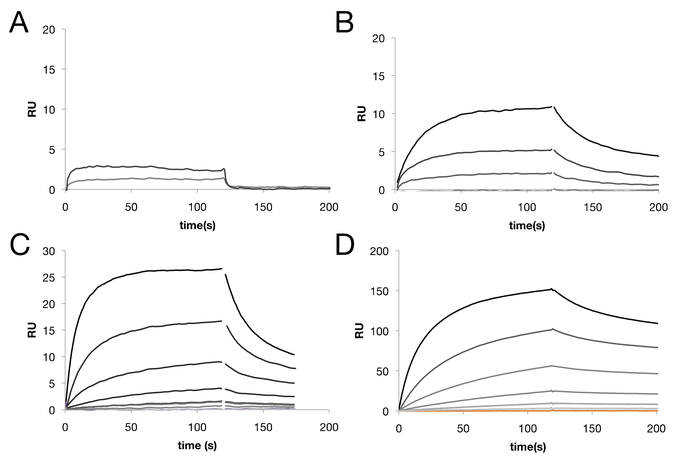
Figure 1 A spectrum of related indels are generated during in vitro SHM affinity maturation of an antibody to anti-hβNGF

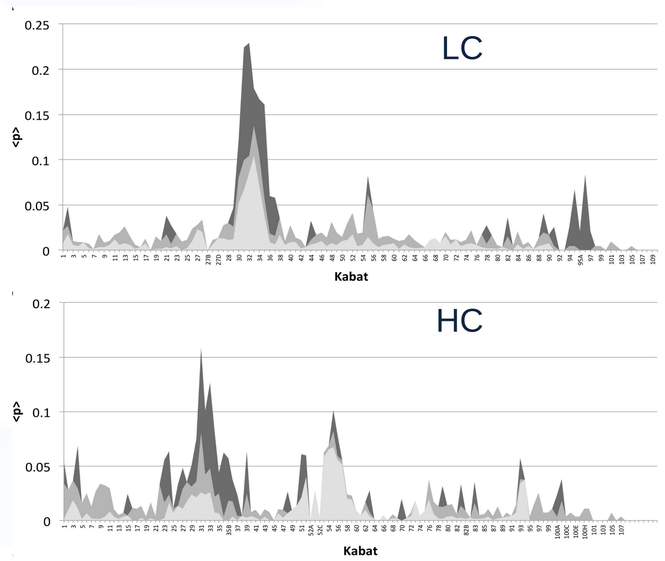
V sequences are shown containing unique insertions recovered during *in vitro* affinity maturation for the HC of an anti-hβNGF antibody. Amino acid sequences shown are on top and the respective DNA sequence below. CDRH1 regions are highlighted with a box, originating sequence is shown in light gray, inserted sequence in black, and point mutations from the parental sequence shown in dark grey.

Figure 2 Multiple, related indels identified by in vitro AIM result in significant improvement in antigen binding



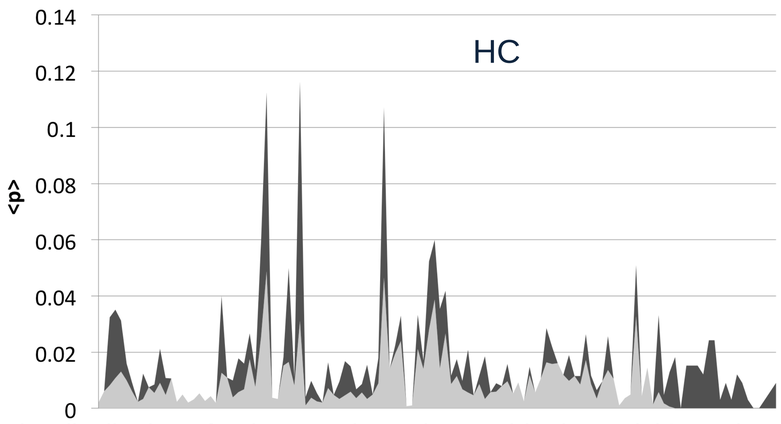
Improvement of antigen binding affinity for anti-hβNGF antibodies containing CDRH1 insertions, respectively. SPR sensorgrams for (A) an anti-hβNGF human antibody containing two point mutations, S31N and L45F; (B, C, D) the same antibody with incorporated insertions derived from *in vitro* SHM (corresponding to Figure 1B HC4-HC6). No non-specific binding was observed for these antibodies.

Figure 3 Distribution of indels in in vivo and in vitro Abs



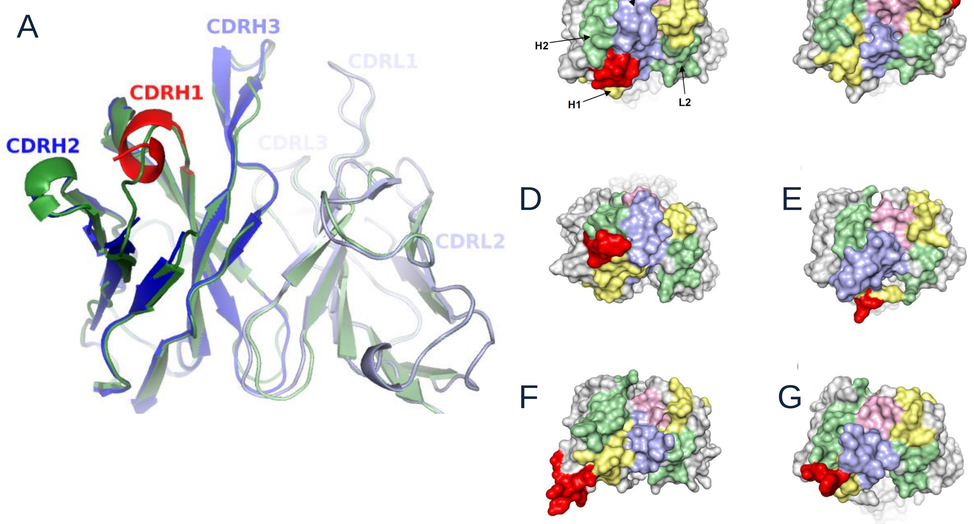
Indel distribution in variable regions of antibodies subjected to *in vitro* AID without maturation (dark gray), *in vitro* AID with maturation in 53 antibodies derived from human and mouse sources (black), compared to *in vivo* data for immature and mature B cells (light gray).

Figure 4 Distribution of point mutaitons in in vivo and in vitro Abs



The distribution of point mutations observed in the variable regions of antibodies subjected to *in vitro* AID with antigen binding selection for 53 antibodies derived from human and mouse sources (black; n=330 non-synonymous mutations), compared to *in vivo* data for immature and mature B cells (light gray; n=63549 non-synonymous mutations).

Figure 5 Fab crystal structures of an anti-human βNGF antibody with and without a 9 amino acid insertion in CDRH1. Comparison with PDB structures of antibodies featuring insertions.



(A) Cartoon representation of the superimposed structures of the variable domains with and without CDRH1 insertion. (B-G) Molecular surface representation of the Fab fragment crystal structures of human antibodies with distinct specificities featuring insertions ranging from 4 to 9 amino acids. (B) APE1551, an anti-hβNGF antibody with a 9 residue insert in; (C) bH1, a dual specific antibody to HER2 and VEGF, 4 residue insert in CDRL1 (3BDY, [Bostrom 2009]); (D) PGT127, an anti-gp120 antibody, 6 residue insert in CDRH1, 4 residue deletion in CDRL1 (3TWC, [Pejchal 2011]); (E) C05, an anti-haemagglutinin antibody, 5 residue insert in CDRH1 (4FP8, [Ekiert 2012]); (F) VRC06, an anti-gp120 antibody, 7 residue insert in heavy chain FR3, 1 residue deletion in CDRL1 (4JB9, [Georgiev 2013]); (G) PGT135, an anti-gp120 antibody, 5 residue insert in CDRH1 (4JM2, [Kong 2013]).

Conclusions

Indels are associated with SHM in vivo and in vitro, but were not observed in the absence of AID

Multiple, sequence related indels are often generated during maturation to an antigen in vitro

Indel formation and point mutations are biased toward the complementarity-determining regions and AID hotspots at positions expected to result in changes in binding affinity

Indels occur with a similar frequency and length distribution in vivo and in vitro

Structure determination of an antibody with and without an in vitro SHM-derived in vitro showed no large scale structural perturbations, and comparison of with PDB structures containing indels shows conserved patterns of augmentation to extend the the antigen recognition surface

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