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Rapid development of an infant-derived HIV-1 broadly neutralizing antibody

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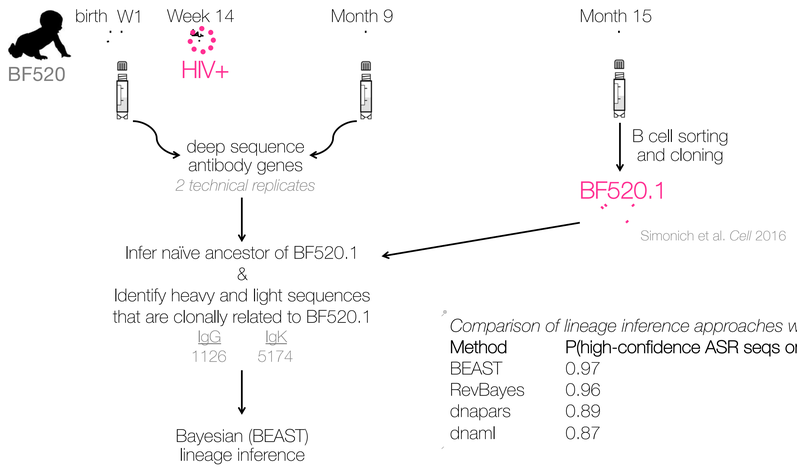
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Importance and Relevance

- Eliciting broadly neutralizing antibody (bnAb) responses against HIV-1 is a central goal of vaccine efforts, but the unusual nature and extensive mutation required to generate bnAbs has made this elusive
- Defining the developmental pathway of infant antibody BF520.1 could provide an attractive template for vaccine design because this antibody naturally developed rapidly (<1 year) and with low levels of SHM (<7%) Simonich *Cell* 2016
- Accuracy in lineage determination is critical for the efficient design of vaccines that mimic these natural processes of bnAb development in infection. We developed a Bayesian antibody lineage inference approach that displays multiple possible lineages and their relative confidences and is thus more robust than the current standard in the field (maximum-likelihood phylogenetic estimates).

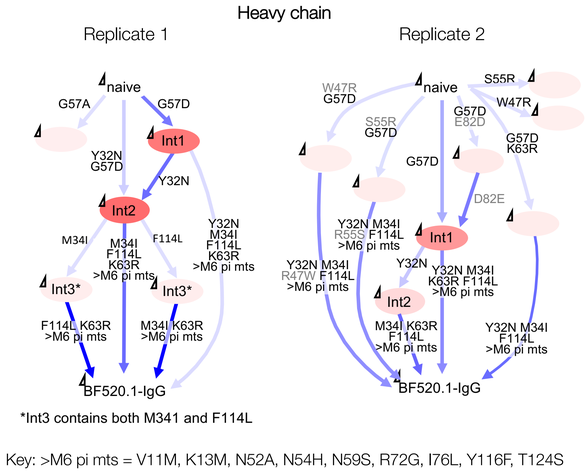
Study Goal: Determine how the BF520.1 infant antibody developed

Approach: Bayesian lineage inference using longitudinal antibody sequences



Comparison of lineage inference approaches with simulated data

Method	P(high-confidence ASR seqs on True Lineage)
BEAST	0.97
RevBayes	0.96
dnapars	0.89
dnaml	0.87



Key: >M6 pi mts = V11M, K13M, N52A, N54H, N59S, R72G, I76L, Y116F, T124S

BF520.1 antibody heavy and light chain lineage intermediates

M0-M9 Bayesian lineage inference by deep sequencing

M9-M15 Rational selection of additional mutations, progressively added:
1) CDR mutations
2) FR mutations in close proximity to CDRs
3) Additional non-conservative FR mutations

BF520.1 Heavy Chain

```
                FR1          CDR1        FR2        CDR2              FR3                    CDR3          FR4
Naïve QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGPFPNYYGPGSYWGGFDYWGQGTLVTVSS
Int1  ------------------------------------------------------------D-------------------------------------------------------------------
Int2  ------------------------------------------N-------------------D-------------------------------------------------------------------  M0-M9
Int3  ------------------------------------------N-I-----------------D-------------------------------------------------L-----------------
Int4  ------------------------------------------N-I-----------------A----D-------------------------------------------------L-----------------
Int5  ------------------------------------------N-I-----------------A-H--D-------------------------------------------------L-F---------  M9-M15
Int6  ------------------------------------------N-I-----------------A-H--D-----------G-------------------------------------L-F---------
VH    ----------M-M-------------------N-I-----------------A-H--D-S---R--------G---L-------------------------------------L-F-------S---
```

BF520.1 Light Chain

```
                FR1          CDR1        FR2        CDR2              FR3                    CDR3          FR4
Naïve EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYNNWPSPFTFGPGTKVDIK
Int1  ------------------------------------------------------------------------------------M-------------------------------------------
Int2  ------------------------------------------A-----------------------F-----------M-------------------------------------------
Int3  ------------------------------N-A-----------------------S-----------F-----------M-------------------------------  M0-M9
Int4  ------------------------T---N-A-----------------------S-----------F-----------M-------------------------------------------
Int5  ------------------------T---N-AY----------------E---S-----------F-----------M-------------------------------------------
Int6  ------------------------T---N-AY-F--------------E---S-----------F-----------M-------------------------------------------  M9-M15
Int7  ----------------L---------T---N-AY-F--------------E---S-----------F-----------M-----------------------------H--
VK    ----------------L---------T---N-AY-F--------------E---S----T------F-----------M-----------------------------H--
```

Early BF520.1 lineage mutations enable cross-clade HIV neutralization

BF520.1 heavy chain intermediates paired with mature kappa chain

	naïve	naive$_{VH}$	Int1$_{VH}$	Int2$_{VH}$	Int3$_{VH}$	Int4$_{VH}$	Int5$_{VH}$	Int6$_{VH}$	mature
			G57D	Y32N	M34I F114L	N52A	N54H Y116F	R72G	
		mature$_{VK}$							
Schematic of lineage pairing									
% VH SHM	0.0	0.0	0.5	0.8	1.3	1.8	2.4	2.6	6.5
% breadth relative to mature	0	54	46	77	92	100	100	100	100
geometric mean IC$_{50}$ (μg ml^{-1}) for Tier 2 viruses neutralized	>20	5.7	5.3	5.4	4.1	2.0	1.5	2.2	1.9

- Mature Kappa chain contributes to Env binding and cross-clade neutralization
- Y32N in CDRH1 (Int2$_{VH}$) expands breadth

BF520.1 kappa chain intermediates paired with mature heavy chain

	naïve	mature$_{VH}$								
		naive$_{VK}$	Int1$_{VK}$	Int2$_{VK}$	Int3$_{VK}$	Int4$_{VK}$	Int5$_{VK}$	Int6$_{VK}$	Int7$_{VK}$	mature
			L78M	S30A S67F	S28N T53S	A25T	S31Y G50E	L33F	P15L D107H	
Schematic of lineage pairing										
% VK SHM	0.0	0.0	0.3	1.2	1.8	2.1	3.4	3.7	4.3	4.6
% breadth relative to mature	0	8	8	62	69	85	100	100	100	100
geometric mean IC$_{50}$ (μg ml^{-1}) for Tier 2 viruses neutralized	>20	>20	>20	8.5	3.0	3.6	3.0	1.8	2.1	1.9

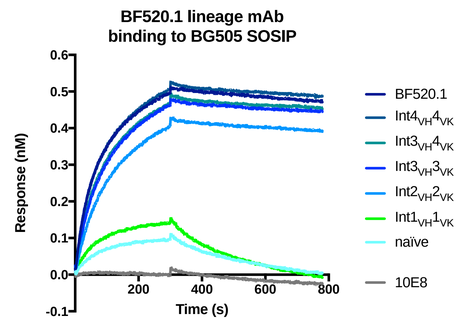
- S30A in CDRL1 and S67F in FR3 (Int2$_{VK}$) enable cross-clade HIV neutralization breadth

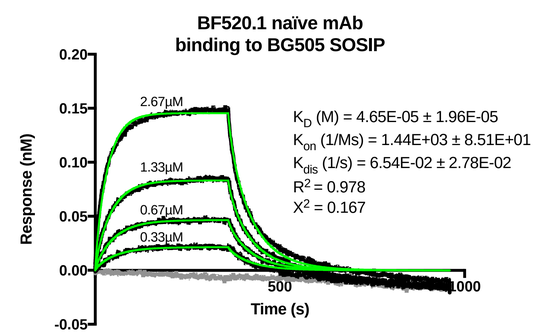
BF520.1 lineage paired intermediates

	naïve	Int1$_{VH}$	Int2$_{VH}$	Int3$_{VH}$	Int3$_{VH}$	Int4$_{VH}$	Int4$_{VH}$	Int4$_{VH}$	Int5$_{VH}$	Int6$_{VH}$	mature	Minimal$_{VH}$
		G57D	Y32N	M34I F114L		N52A			N54H Y116F	R72G		Y32N M34I N52A F114L
		Int1$_{VK}$	Int2$_{VK}$	Int3$_{VK}$	Int4$_{VK}$	Int4$_{VK}$	Int5$_{VK}$	Int6$_{VK}$	Int6$_{VK}$	Int7$_{VK}$		Minimal$_{VK}$
		L78M	S30A S67F	S28N T53S	A25T		S31Y G50E	L33F		P15L D107H		S28N S30A S31Y G50E T53S S67F
Schematic of lineage pairing												
% SHM	0.0	0.4	1.0	1.5	1.7	2.0	2.5	2.7	3.0	3.4	5.6	2.0
% breadth relative to mature	0	0	8	31	31	69	100	100	100	100	100	100
geometric mean IC$_{50}$ (μg ml^{-1}) for Tier 2 viruses neutralized	>20	>20	>20	5.7	3.1	3.3	3.2	1.9	1.6	2.3	1.9	3.4

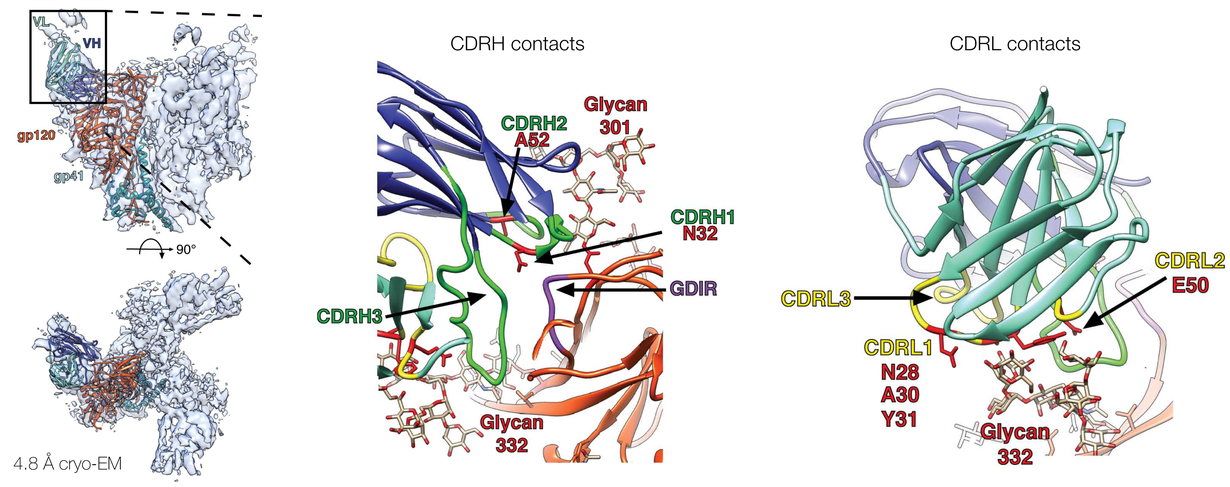
- Very limited SHM required for Tier 2 cross-clade neutralizing activity (Int3$_{VH}$ + Int3$_{VK}$) and full breadth (Minimal Mutant)
- N52A in CDRH2 (Int4$_{VH}$) augments neutralizing breadth

BF520.1 inferred naïve ancestor binds HIV Envelope





CDRL1 makes extensive contacts with N332 glycan of BG505.SOSIP.664 trimer



Conclusions

- BF520.1 naïve ancestor binds HIV Env
- Kappa chain maturation is critical for bnAb functionality
- Cryo-EM structure reveals contacts between CDRL1 and glycan 332
- CDR1 and CDR2 regions in heavy and light chains make more contacts with Env than CDR3 regions
- Very limited SHM is required for BF520.1 neutralization breadth