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VH gene polymorphism associated with potent anti-SIV neutralizing antibody induction

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BACKGROUND

Induction of broadly neutralizing antibodies (bnAbs) is considered crucial for an effective HIV-1 vaccine. To clarify the mechanism to induce bnAbs, induction of B404-class antibodies, anti-SIV bnAbs, induction was analyzed using SIV-infected macaques.

METHODS

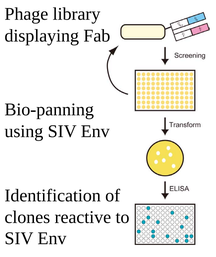
- Six rhesus macaques were infected with SIVsmH635FC, and blood and lymph nodes (LNs) were collected.
- Monoclonal anti-SIV Fab clones were isolated from LNs by bio-panning using phage display.
- B cell receptor (BCR) VH sequences were analyzed by next generation sequencing (NGS).

RESULTS

Isolation of anti-Env monoclonal Fab clones

Anti-Env Fab clones with neutralizing activity were obtained from SIV-infected macaques by phage display method. B404-class antibodies were isolated only from MM621.

Isolation of Fab clones



Number of anti-Env Fab clones obtained from LNs by Bio-panning

	51 wpi		55 wpi	
animal	total	nAb	total	nAb
MM617	22	5	ND	ND
MM618	18	5	20	6
MM620	14	0	ND	ND
MM621	9	9	10	10
MM622	13	2	15	1

IC_{50} (μg/ml) of neutralizing Fab clones

animal	clone	V gene	IC_{50} (μg/ml)				
			SIVsmH635FC	SIVsmE543-3	SIVmac316	SIVsmH805	SIVmac239
MM617	617-12wL02	VH4.28	2.1	>8	>8	>8	>8
	617-51wK01	VH1.53	0.026	0.030	1.4	>8	>8
	617-51wK03	VH1.53	0.014	0.051	0.077	>8	>8
	617-51wK12	VH1.53	0.047	0.092	>8	>8	>8
	617-51wK15	VH1.53	0.013	0.012	0.11	>8	>8
	617-51wK18	VH1.53	0.059	0.051	2.5	>8	>8
MM618	618-12wL03	VH4.38	0.012	0.073	0.84	>8	>8
	618-12wL07	VH4.38	0.023	0.10	0.19	>8	>8
	618-12wL08	VH4.38	0.013	0.018	0.12	2.7	>8
	618-24wK07	VH1.59	0.25	0.13	>8	5.7	>8
	618-24wL02	VH3.9	0.021	0.22	0.091	6.6	>8
	618-24wL06	VH4.38	0.032	0.074	0.13	5.1	>8
	618-51wK04	VH1.59	0.0019	0.0048	0.16	>8	>8
	618-51wL07	VH4.38	0.0019	0.0073	>8	>8	>8
	618-51wL09	VH4.22	0.011	0.030	4.1	>8	>8
	618-51wL10	VH4.38	0.0026	0.013	0.074	>8	>8
	618-51wL16	VH4.38	0.0041	0.066	0.11	>8	>8
	618-55wK05	VH1.59	0.013	0.024	0.73	>8	>8
	618-55wK06	VH4.40	0.0034	0.0037	0.0014	0.15	>8*
	618-55wL01	VH4.38	0.0038	0.017	0.033	1.3	>8
	618-55wL03	VH4.57	0.0027	0.0082	0.84	>8	>8
	618-55wL05	VH4.38	0.0067	0.034	0.28	>8	>8
	618-55wL06	VH4.38	0.0044	0.016	0.071	>8	>8
MM621	621-51wL02	VH3.33	0.013	0.0062	0.0022	>8	>8
	621-51wL03	VH3.33	0.18	0.015	0.29	>8	>8
	621-51wL07	VH3.33	0.12	0.016	0.011	>8	>8
	621-51wL08	VH3.33	0.048	0.015	0.0094	>8	>8
	621-55wL01	VH3.33	0.0095	0.012	0.0030	>8	>8
	621-55wL04	VH3.33	0.0093	0.018	0.0021	>8	>8
	621-55wL06	VH3.33	0.0083	0.011	0.0013	>8	>8
	621-55wL07	VH3.33	0.0044	0.020	0.0017	>8	>8
MM622	622-24wL05	VH5.7	>8	>8	2.8	>8	>8
	622-51wK02	VH5.7	0.31	>8	4.0	>8	>8
	622-51wL06	VH4.11	0.093	0.010	0.015	>8	>8
	622-55wL02	VH5.7	>8	>8	7.5	>8	>8

< 0.01 μg/ml
0.01 - 0.1 μg/ml
0.1 - 1.0 μg/ml
1.0 - 8 μg/ml
> 8 μg/ml

- **Polymorphism of immunoglobulin germline gene significantly restricts induction of bnAbs with preferential gene usage.**
- **The 38th amino acid of VH3.33 germline gene was critical for induction of B404-class bnAb induction in macaques.**

bnAb induction



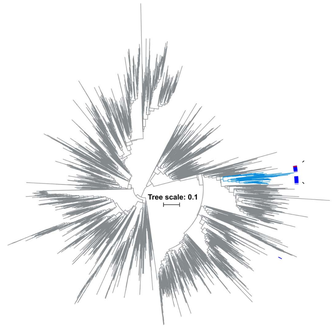
No bnAb induction



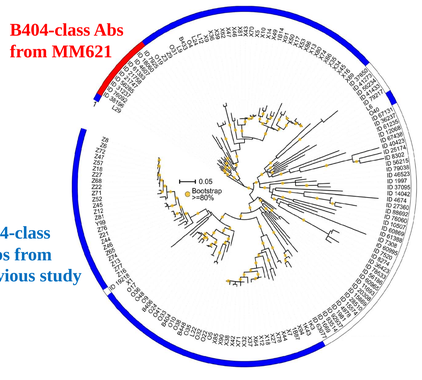
Sequences close to the B404-class antibody clones were only from MM621

NGS analysis revealed that B404-class antibodies clustered with sequences from MM621, but not from other macaques.

Phylogenetic tree of all the obtained VH sequences and B404-class Abs



Cluster containing B404-class Abs



Sequences detected in the B404-class Ab cluster

Sequence ID	618 W51	618 W55	619 W21	620 W51	617 W51	621 W51	621 W55	621 W55*	622 W51	622 W55	CDR3 aa sequence	length	residue 38	63	65
ID_38196											TRGLSFYNFWNGDNEREQEYFEF	23	E	D	T
ID_76092											TRGLSFYNFWNGDNEREQEYFEF	23	E	D	T
ID_31237											TRGLSFYNFWNGDNEREQEYFEF	23	E	D	T
ID_56285											TRGLSFYNFWNGDNEREQEYFEF	23	E	D	T
ID_21747											VRGLSFDNFWNGDNEREQEYFDF	23	E	D	T
ID_21758											TRGLSFDNFWNGDNEREQEYFEF	23	E	D	T
ID_61383											TRGLSFDNFWNGDNEREQEYFEF	23	E	E	T
ID_4607											TRGLSFDNFWNGDNEREQEYFEF	23	E	D	T
ID_18060											TRGLSFDNFWNGDNEREQEYFEF	23	E	D	T
ID_7925											TRGLSFDNFWNGDNEREQEYFEF	23	E	D	T
ID_37850											TRRVWTGTIHFDY	13	E	D	T
ID_56234											SRTTGVLLRFDS	12	E	D	T
ID_61131											ARAPAFDFSFDY	12	E	D	T
ID_36237											ARGMGVLPFLESLFDFYFDY	20	E	G	T
ID_61235											TRGMGVQQFLEWFLDFYFDY	20	E	D	T
ID_12068											IRGKPVTGPQLGDY	14	E	G	T
ID_40423											VRDAWVAHFDS	11	D	T	T
ID_25174											VRDLWVAHLDS	11	A	T	T
ID_8302											VRDLWVAHFDP	11	D	S	T
ID_56215											TKGSDIVPYYFDY	13	V	G	T
ID_40432											VRDAWVAHFDS	11	V	T	T
ID_25174											VRDLWVAHLDS	11	A	T	T
ID_8302											VRDLWVAHFDP	11	D	S	T
ID_56215											TKGSDIVPYYFDY	13	V	G	T
ID_1997											CRGGYSGFRRFEY	13	E	Y	T
ID_4674											ASDKGARTFDS	12	E	G	T
ID_77360											CRDMGARTLDY	11	E	Y	T
ID_88692											CRDMGARTFDL	11	E	Y	T
ID_76060											CRDMGARTFDY	11	E	Y	T
ID_60869											ARDDSNGWDFDY	12	E	S	T
ID_61388											TRAGSGRFPDFDY	13	E	D	T
ID_7308											VREDLHSDSIFDF	13	V	G	K
ID_60995											VREEEIFELVPEFWAFDY	18	G	G	T
ID_56423											ARGDTDEDEYGYWILDN	17	D	V	T
ID_56186											SRGLQFLEWSHPYYFDS	17	E	D	T
ID_60965											TRGLDFWSPYYFDY	14	E	D	T
ID_61693											TRGLEISEWSFYFDY	15	E	D	T
ID_20308											TRGLEISEWLLYFDY	15	E	D	T
ID_15869											SRGLQFLESLFYFDS	15	E	D	T
ID_28510											TRGLQFLESLFYFDS	15	E	D	T
ID_15974											TRGADIVMVSAAYFDY	17	E	K	K
ID_4978											TRVGDTAGTDFDS	13	E	D	T
ID_1981											TGAEAGTFDY	10	E	N	T
ID_1659											TRMTAAAAHFDY	12	E	D	V
ID_19218											TRGLQFLEWFSGSDECFDF	19	E	G	T

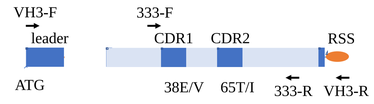
Abundance ratio(%)
>0.1
0.05-0.1
0.025-0.05
0.0125-0.025
0.0063-0.0125
0.0031-0.0063
0.0012-0.0031
<0.0012
N.D

CDR3 Length (aa)
11-15
16-20
20<

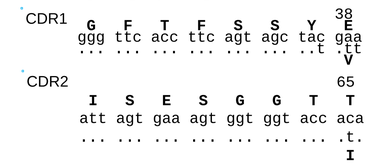
VH3.33 allele was associated with B404-class Ab induction

VH3.33 gene, which is used by B404-class Abs, has polymorphic sites in CDR1 and CDR2. Animals with VH3.33_ET allele showed B404-class Ab induction.

VH3.33 gene structure



VH3.33 gene polymorphism



VH3-33 allele and B404-class Ab induction

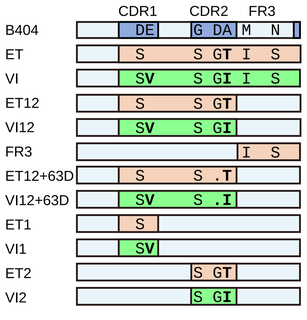
animal	VH3-33 allele		B404-class Ab induction
H704	ET	ET	Yes
H709	ET	ET	Yes
H714	ET	VT	Yes
H723	ET	VI	Yes
MM617	VI	VI	No
MM618	VI	VI	No
MM619	VI	VI	No
MM620	VI	VI	No
MM621	ET	VI	Yes
MM622	VI	VI	No

ET; 38E and 65T
VI; 38V and 65I
VT; 38V and 65T

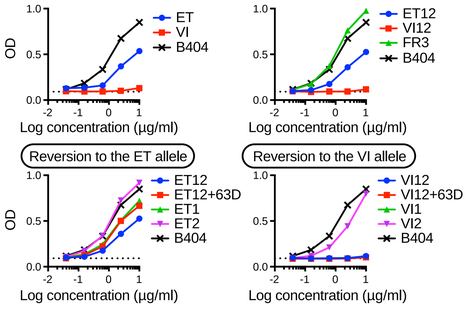
Reactivity to Env was maintained by reversion to the ET allele, but not by the reversion to the VI allele.

Germline-reverted B404 mutants were constructed, and their reactivity to Env was examined by ELISA. All the ET-reverted mutants bound to Env, but the reversion to the VI allele, especially 38V mutation, abolished the reactivity to Env.

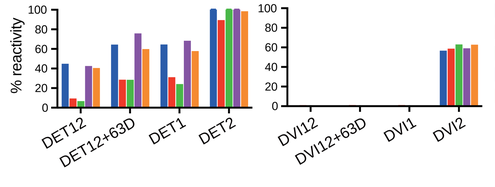
B404 germline-reverted mutants



Binding activity to Env



Reactivity of B404 mutants to various SIV strains



Neutralizing activity of B404 mutants

