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Public trimer-specific IgHV4-34 clonotype in bnAb inducers includes a novel V3-glycan bnAb subclass

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BACKGROUND

Several classes of HIV-1 broadly neutralizing antibodies (bnAbs) exhibit distinct immunoglobulin heavy chain variable (IGHV) gene usage, and several HIV-1 vaccine strategies aim to elicit public bnAb clonotypes. Here, we performed a systematic analysis of HIV-1 envelope (Env) trimer reactive B cell receptor (BCR) repertoires in people with HIV (PWH) with bnAb activity to define public features of IGHV and IGLV/KV gene usage, enabling the discovery of novel bnAbs.

METHODS

bnAb inducers (N=30) included in the XbnAb cohort (N=304; Pasin et al. biorXiv 2024) and PWH with no bnAb activity (nnAb inducers; n=10) were analyzed by single cell BCR cloning (10X genomics with Libra-Seq including 11 Env-trimer probes) to identify Env-trimer reactive BCRs. Data were processed using the Immcantation Framework (Gupta 2015). Clonal assignment was based on IGHV and IGHJ genes and a junction distance of 0.18 as determined by SHazaM. BCRs were classified as trimer-reactive when they scored in the top 0.5th quantile of the staining signal for at least 25% of analyzed trimers. Clonal families were defined as trimer-reacitve once a single member hit the trimer-reactivity threshold. Neutralization activity was measured against a 41-virus multi-clade Env pseudotype panel on TZM-bl cells. bnAb epitopes were defined by Env mutant screens (mutational scanning, binding, neutralization) and Cryo-EM structure analysis. BCR usage was investigated using logistic regressions with a random effect accounting for the donor and sample.

CONCLUSIONS

The high frequency of trimer reactive IGHV4-34 BCRs, and the identification of novel IGHV4-34 bnAbs suggests a high potential of targeting IGHV4-34 for HIV-1 bnAb vaccines, provided that IGHV4-34 autoreactivity can be controlled.

ADDITIONAL INFORMATION

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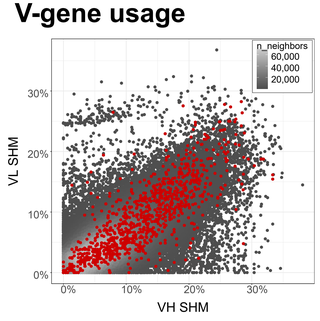
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Jia et al. Cell Host Microbe 2020
Pasin et al. biorXiv 2024
Van Schooten et al. PLOS Pathogens 2022

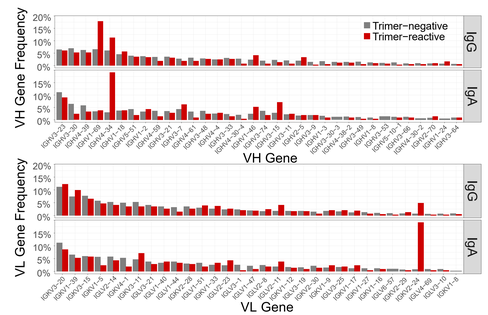
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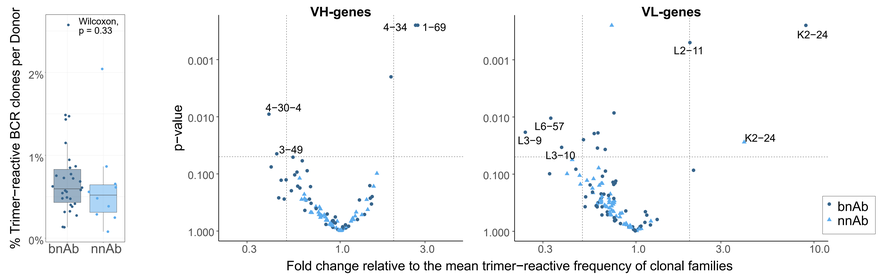
Trimer-reactive BCR lineages in bnAb inducers show differential V-gene usage



BCR repertoire analysis of 30 bnAb inducers retrieved 1008 individual IgG and IgA trimer-reactive BCRs/ 702 BCR lineages.

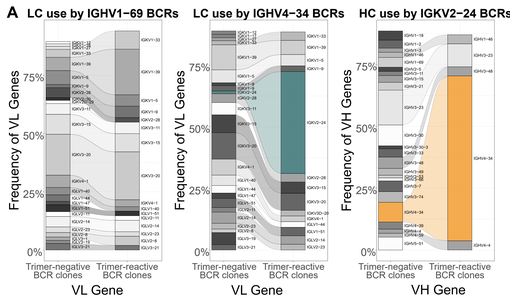


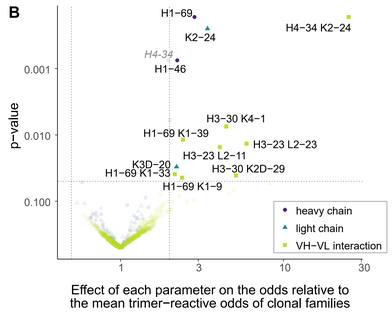
Trimer-reactive BCR repertoire showed highly differential VH/VL gene usage with large enrichment of IGHV4-34, IGHV1-69 and IGKV2-24. Genes with frequencies > 0.65% are displayed.

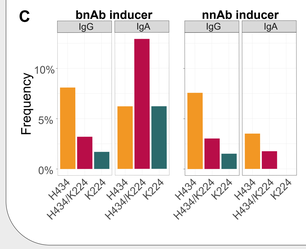


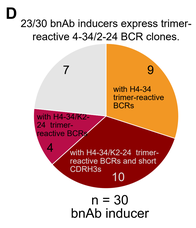
Frequencies of trimer-reactive clones in nnAbs (n=10) do not differ from bnAb inducers, but nnAb inducers show no enrichment of VH-genes among trimer-reactive clones. VH and VL genes with significant enrichment, i.e. p-value < 0.05 and with > 2- or < 2-fold change are indicated.

IGHV4-34/IGKV2-24 BCR lineages are highly enriched among trimer-reactive BCR clones in bnAb inducers



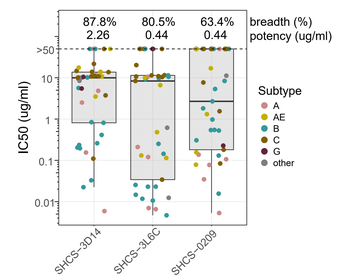




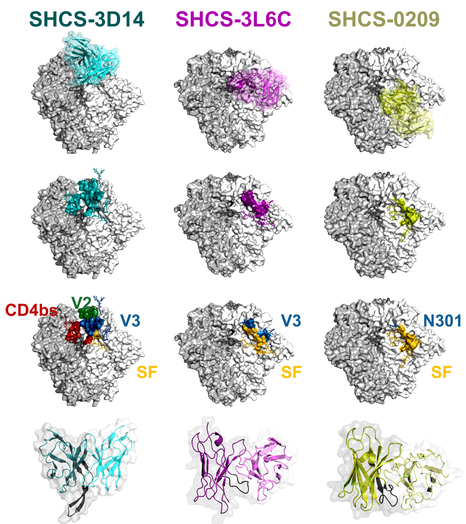


[A.] Paired VH/VL-genes of trimer-negative and trimer-reactive BCR clones using IGHV1-69, IGHV4-34 and IGKV2-24. Only genes with frequencies > 1% are displayed. **[B.]** Logistic regression analysis illustrating the individual effects of each IGHV gene, IGLV gene, and their interaction. The total odds ratio for trimer-reactivity in a given pair is the combined effect of these three components. **[C.]** Frequencies of IGHV4-34 (but not IGKV2-24), IGHV4-34/IGKV2-24 and IGKV2-24 (but not IGHV4-34) trimer-reactive BCR clones in bnAb inducers and nnAb inducers. IGHV4-34/IGKV2-24 IgA BCR clones are highly enriched in bnAb inducers. **[D.]** Prevalence of IGHV4-34 among bnAb inducers on the per donor level.

Three broad and potent IGHV4-34 bnAbs identified

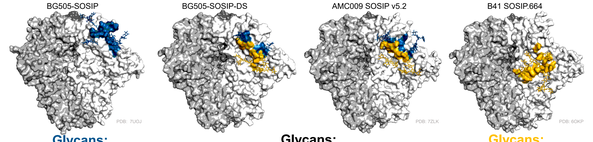


	SHCS-3D14	SHCS-3L6C	SHCS-0209
Donor	S51434	S51434	S32615
Donor subtype	B	B	AE
XbnAb Rank	2	2	58
IGHV/ IGLV	H4-34/ K3-15	H4-34/ K2-24	H4-34/ K2-24
CDRH3 length	24	10	14



IGHV4-34 are known to be autoreactive. Autoreactivity is determined through codon 26, and can be overcome through mutation of this codon. Codon 26 was retained in SHCS-3L6 and SHCS-3D14 but mutated in SHCS-0209.

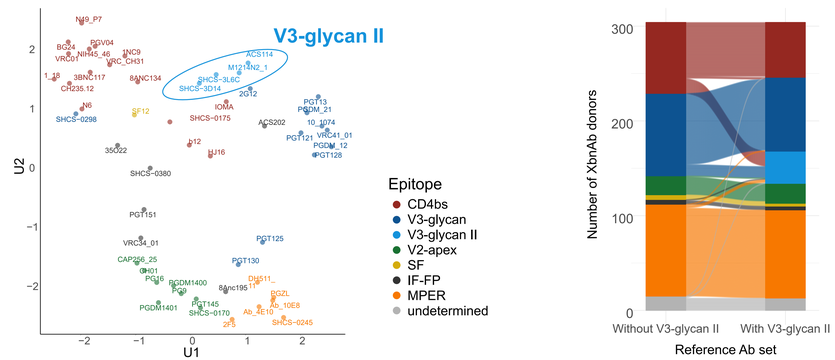
SHCS-3L6C - a new public bnAb clonotype



bnAbs M1214-N2 (Jia 2020) and ACS114 (Van Schooten 2022) are independently isolated representatives of this public clonotype.

IGHV4-34 V3 bnAbs define a novel bnAb subclass V3-glycan type II

Highly correlated, unique neutralization fingerprints of IGHV4-34 bnAbs define the V3-glycan II bnAb class



Neutralization fingerprinting defines 92/304 (30%) XbnAb donors with V3-glycan II activity (dominant specificity in 34 donors, sub-specificity in 58 donors).