Human Rhinovirus Presenting 4E10 Epitope of HIV-1 MPER Elicits Broadly Neutralizing Antibodies in Human ICAM-1 Transgenic Mice

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Background:

Induction of bnAbs remains a critical goal towards developing a vaccine against HIV-1. We previously grafted 4E10 epitope of HIV-1 gp41 on the surface of human rhinovirus connected via linkers of varying lengths and sequences to construct combinatorial HRV display libraries. One chimeric HRV:HIV immunoselected from the libraries was able to elicit broad neutralization against 10 of 12 HIV-1 pseudoviruses in guinea pigs. However, guinea pigs do not get infected with HRV because they lack the viral receptor, human ICAM-1 and thus the antibody titers were rather modest. Since the recently developed hICAM-1 Tg mice support productive HRV infection, in this study we evaluated the efficacy of chimeric HRV:HIV vaccine in these mice.

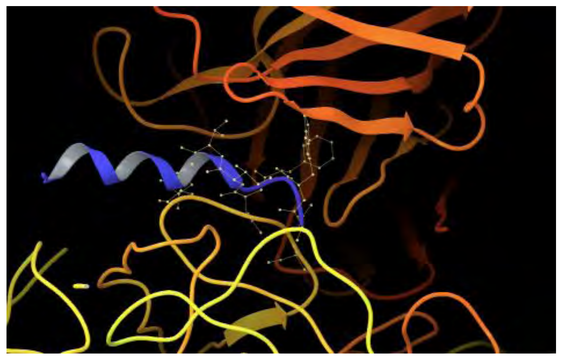


Figure 1. Docking of HRV14 (shown in yellow) with a crystallographic structure (1TZG from the Protein Data Bank) of a complex of the 4E10 Fab (orange) complexed with the cognate Ac-KGWNWFDITNWGK-NH_2 peptide (blue ribbon and gold stick-and-balls model).

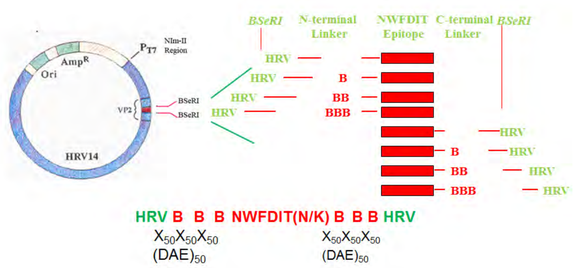


Figure 2. Design of the chimeric HRV:HIV-1 4E10 libraries. The libraries were made using eight overlapping oligonucleotides encoding the core NWFDIT and N- or C-terminal linkers of differing lengths and sequences.
X = any of the 20 amino acids; B = biased = 50% X, 50% DAE which used to promote helix conformation

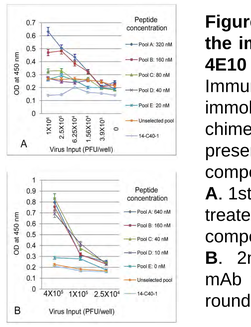


Figure 3. Direct ELISAs reflecting the impact of immunoselection of 4E10 library III, sub-library 13. Immunoselection was performed by immobilizing 4E10 and capturing chimeras that could bind in the presence of varying amounts of competing 4E10 peptide.
A. 1st round: Immobilized 4E10 mAb treated with library 13 plus competitive peptide.
B. 2nd round: Immobilized 4E10 mAb treated with Pool A from 1st round plus competitive peptide.

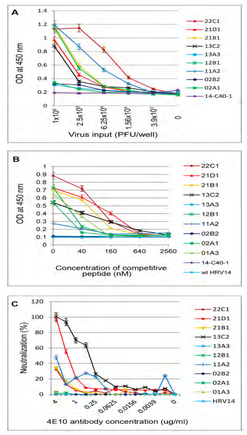


Figure 4. Antigenicity assays reflecting differences among individual chimeric viruses.
A. Direct ELISA using 4E10 to capture the chimeras.
B. Competitive ELISA using 4E10 to capture chimeras that could bind in the presence of competitive peptide, NK-15.
C. MTT cell-killing assay using 4E10 to bind to and neutralize the chimeric viruses, preventing HeLa cell death.

Methods:

hICAM Tg mice were intranasally immunized with three representative chimeric HRV:HIV viruses chosen from our previous study. The sera collected at different time points were tested for immunogenicity and HIV neutralization. ELISA was used to test the binding affinity against 4E10 peptide as well as HIV-1 gp140 trimer. The neutralizing ability against HIV-1 isolates was tested by single-round Tat-regulated luciferase assay in TZM-bl cells. Also, 11 HIV-1 internationally circulating isolates of diverse subtypes and coreceptor usages were used to test the breadth of neutralizing ability. HRV pre-immunized Tg mice were further immunized with chimeric HRV:HIV to test whether nasal administration can bypass HRV pre-immunity.

Results:

The sera from the immunized mice showed good binding affinity with 4E10 peptide as well as with HIV-1 gp140 trimer. All three chimeric HRV:HIV elicited neutralizing antibody responses in hICAM-1 Tg mice against diverse isolates of HIV-1, with one eliciting anti-HIV antibodies capable of neutralizing 9 of the 11 internationally circulating HIV-1 isolates at IC50 level (from six subtypes and two co-receptor usages of HIV-1). Furthermore, intranasal administration of chimeric HRV:HIV could bypass the preexisting immunity to HRV to elicit anti-HIV response.

Chimeric viruses	HRV	N linker	Core epitope	C linker		HRV
4E10 Library III Chimeras						
01A3	... DLSS		NWFDIT	N	E	VGG...
02A1	... DLSS		NWFDIT	K	EE	VGG...
02B2	... DLSS		NWFDIT	K	LE	VGG...
11A2	... DLSS	S	NWFDIT	N	E	VGG...
12B1	... DLSS	P	NWFDIT	K	EP	VGG...
13A3	... DLSS	E	NWFDIT	N	EAN	VGG...
13C2	... DLSS	E	NWFDIT	N	NLV	VGG...
21B1	... DLSS	DD	NWFDIT	N	T	VGG...
21D1	... DLSS	SD	NWFDIT	N	E	VGG...
22C1	... DLSS	DE	NWFDIT	K	N	VGG...

Table 1. Sequences of the most antigenic chimeras immunoselected and characterized from 4E10 Library. The red-highlighted chimeras were chosen to immunize hICAM-1 transgenic mice.

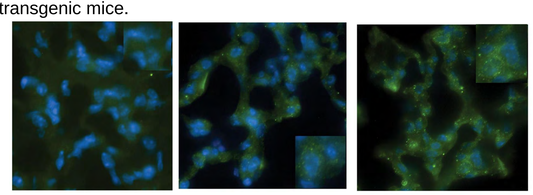


Figure 5. Expression of 4E10 epitope in the lungs of HRV-HIV infected mice. Mice were infected with WT HRV (a) or HRV-HIV chimeras 12B1 (b) or 22C1 (c) and lung sections obtained 3 days later stained with 4E10 mAb followed by Alexa Fluor® 488 labeled goat anti-human IgG. DAPI was used to stain the nuclei.

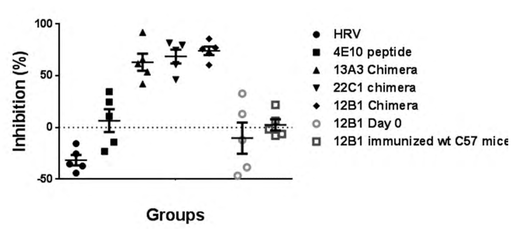


Figure 6. Neutralizing ability of immunized mice sera against HIV-BAL assessed by. **Tat-regulated luciferase assay.** HIV-BAL (200 pg of p24) was incubated with 1:50 dilution of sera for 2h at 37ºC before infecting TZM-bl cells and 24h later, cell lysates tested for luciferase activity. Pre-immune sera and 12B1-immunized WT C57 mice were used as negative controls. One-way ANOVA was used to test the significance between groups. *p<0.05.

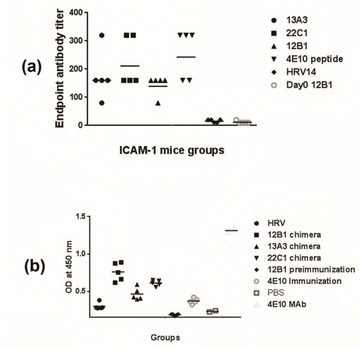
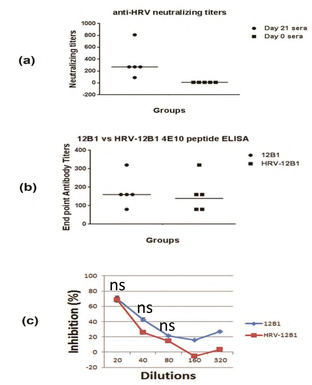


Figure 7. Sera from hICAM Tg mice immunized with HRV-HIV chimera bind 4E10 peptide as well as HIV-1 SF162 gp140 trimer. (a) Serial dilutions of sera were tested for binding 4E10 peptide (K7-D11 lactam-bridged Ac-NWFDITK 7WLWD 11KKK-NH2) by ELISA. Each serum sample was tested in triplicate and geometric mean titers are shown for each mouse. Positive binding was defined as > 3x mean of background (OD450 obtained with preimmune sera). (b) Immunized mouse sera were tested for binding HIV-1 SF162 gp140 trimer at a 1: 50 dilution by ELISA. One-way ANOVA was used to test the significance between groups. *p<0.05.

Figure 8. Pre-existing immunity to HRV does not compromise anti-HIV antibody response (a) hICAM-1Tg mice were intranasally immunized with 2.5x10^6 wild type HRV-14 and after three weeks, serial dilutions of sera, along with preimmune sera obtained before immunization (day 0) tested for neutralization of HRV-14 by plaque reduction assay. Each symbol represents an individual mouse. The neutralizing titers (PRNT 50) are shown as reciprocal of serum dilutions. (b) Prior HRV-14 immunized mice in (a) or naïve mice were intranasally immunized with HRV-HIV chimera and sera obtained after 3 weeks titrated for binding 4E10 peptide by ELISA. (c) Dilutions of sera in (b) were tested for HIV-BAL neutralization in TZM-bl cells. % inhibition of RLU obtained with preimmune serum is shown.



HIV-1 isolates	Immunogens (1/dilution)					mAbs (µg/ml)	
	12B1	13A3	22C1	4E10 peptide	HRV 14	VRC01	4E10
93RW(A, X4/R5)	74	63	64	<20	<20	4.7	2.5
92UG (A, X4)	30	31	37	<20	<20	5.1	1.2
Ker 2018 (A, R5)	<20	<20	<20	<20	<20	10.2	19.7
BK132 (B, X4)	21	<20	<20	<20	<20	2.3	4.7
BAL (B, R5)	38	<20	<20	<20	<20	2.2	N/A
TZBD (C, R5)	24	<20	<20	<20	<20	4.8	11.1
TZA246 (C, R5)	25	<20	<20	<20	<20	6.1	>20
93UG (D, X4)	28	23	<20	<20	<20	9.2	4.1
NI1052 (AE,X4)	58	37	155	<20	<20	5.2	3.8
55815 (AG, R5)	24	<20	<20	<20	<20	2.7	8.4
CAM0015(AG, R5)	<20	24	<20	<20	<20	11.7	19.6

Table 2. Neutralization of HIV-1 international isolates by sera from hICAM-1Tg mice immunized with chimeric viruses. Wild type HRV-14 and 4E10 peptide immunized hICAM-1Tg mouse sera were used as negative controls. Two bNAbs, VRC01 and 4E10 were used as positive controls. The neutralizing titers are shown as the reciprocal of serum dilution at which the luciferase expression is reduced by 50% (IC50) compared to preimmune serum.

Conclusion:

This work demonstrates that productive infection of HRV susceptible animals with chimeric HRV effectively elicits broadly neutralizing anti-HIV-1 antibodies. This strategy therefore appears to have potential for human vaccination.