SIV Escape from A Vaccine Targeting Cleavage Sites Is Associated with A Fitness Loss

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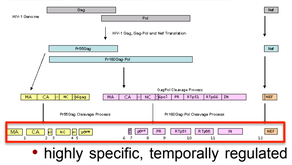
BACKGROUND

• Developing an effective vaccine for HIV-1 has proved be an enormous challenge and no vaccine exists for clinical use to prevent HIV-1 infection.

• Multiple approaches being undertaken, but these classical approaches have shown only limited efficacy in dealing with viral antigenic diversity.

• The identification of new targets that can potentially stimulate protective T and B cell immunity is urgently needed for the development of a safe and effective preventive HIV-1 vaccine.

Proteolytic Cleavage of HIV-1



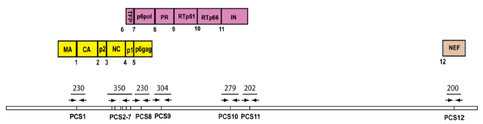
- mediated by HIV protease, a small 99-amino acid aspartic enzyme
- the cleavage of Gag, Gag-Pol and Nef precursor polyproteins
- highly specific, temporally regulated and essential for the production of infectious viral particles
- total twelve proteolytic reactions are required to generate a viable virion.
- each reaction occurs at a unique cleavage site that differs in amino acid

• A new vaccine candidate targeting protease cleavage sites (PCS) would have major advantages:
- destroy virus before its permanent establishment
- force the virus to accumulate mutations eliminating the function of protease
- avoid unwanted immune activation that often attract more targets for HIV-1 infection

• Luo et al. demonstrated the immunogenicity of sequences around HIV-1 protease cleavage sites in the previous study[1].

METHODS

• Seventeen Cynomolgus macaques were used in the study
- Vaccine group (n=12): immunized with a mixture of 12 rVSV each expressing a 20 amino acid peptides overlapping each of twelve unique cleavage sites of SIVmac239 PCS regions as shown below
- Control group (n=5):received no vaccinations



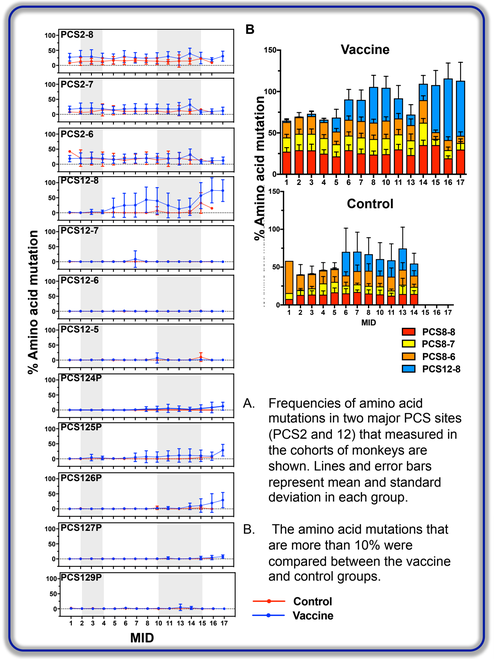
• All animals were then intrarectally (IR) challenged with SIVmac239

• Viral mutation was monitored by pyrosequencing

To determine the virologic correlates of protection of PCS vaccines we perform:
- Generation of SIV constructs with PCS mutation and preparation of transfection-derived viral stocks
- Characterization of transfection-derived viral stock
- Infectivity of viral stocks
- Analysis of proteolytic processing of $Pr55^{Gag}$ in both cell and virus lysates
- Replication and replicative fitness assay of PCS mutant viruses

RESULTS

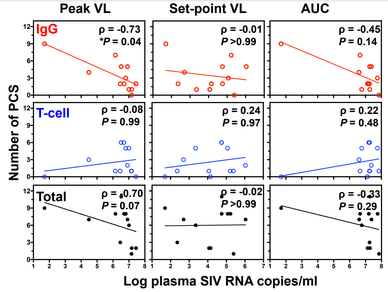
Figure 1. Comparison of the major PCS amino acid mutations between the vaccine and control groups



A. Frequencies of amino acid mutations in two major PCS sites (PCS2 and 12) that measured in the cohorts of monkeys are shown. Lines and error bars represent mean and standard deviation in each group.

B. The amino acid mutations that are more than 10% were compared between the vaccine and control groups.

Figure 2. Correlation between immune responses to the PCS and plasma SIV RNA copies in the vaccine group

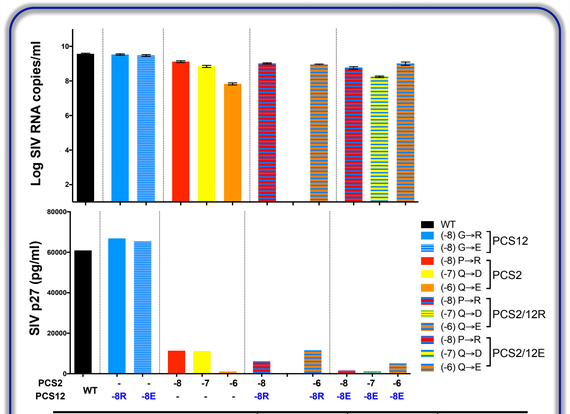


- Both antibody responses and T-cell mediated responses to the number of PCS peptides were correlated with Plasma virus RNA levels on weeks 2-4 (peak) and 10-15 (set-point) following SIV challenge separately and combined together.
- An area-under-the curve calculation was measured for the plasma SIV RNA levels between week 1 and week 14 following infection. Spearman's rank correlation was performed and Sidak adjusted *P* values were shown for multiple comparison.

Table 1. Locations of the PCS point mutations and the production of replication competent PCS mutant viruses

Site-directed PCS mutation	Amino acid sequence				Cells transfected
	PCS2 (CA(p27)/p2)			PCS12(Nef)	
	-8	-7	-6	-8	
SIVmac239 WT	P	G	Q	G	293FT
PCS12(-8)	P	G	Q	R	293FT
PCS12(-8)	P	G	Q	E	293FT
PCS2(-8)	R	G	Q	G	293FT
PCS2(-7)	P	D	Q	G	293FT
PCS2(-6)	P	G	E	G	293FT
PCS2(-8)/PCS12(-8R)	R	G	Q	R	293FT
PCS2(-7)/PCS12(-8R)	P	D	Q	R	293FT
PCS2(-6)/PCS12(-8R)	P	G	E	R	293FT
PCS2(-8)/PCS12(-8E)	R	G	Q	E	293FT
PCS2(-7)/PCS12(-8E)	P	D	Q	E	293FT
PCS2(-6)/PCS12(-8E)	P	G	E	E	293FT

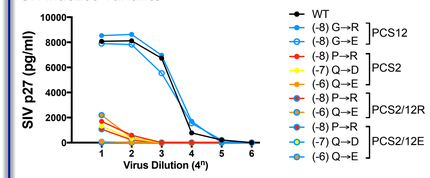
Figure 3. Virus content of transfection-derived PCS mutation SIVmac239 variants (vRNA and p27)



Viral stock	vRNA (Copies/ml)	p27 (pg/mL)	vRNA/p27 ratio
SIVmac239 WT	3.65E+09	6.15E+04	5.93E+04
PCS12(-8)	3.40E+09	6.19E+04	5.49E+04
PCS12(-8)	3.33E+09	6.08E+04	5.48E+04
PCS2(-8)	1.12E+09	1.31E+04	8.55E+04
PCS2(-7)	8.55E+08	1.23E+04	6.95E+04
PCS2(-6)	7.30E+07	9.59E+02	7.61E+04
PCS2(-8)/PCS12(-8R)	9.27E+08	5.41E+03	1.71E+05
PCS2(-6)/PCS12(-8R)	1.21E+09	1.09E+04	1.11E+05
PCS2(-8)/PCS12(-8E)	4.64E+08	1.37E+03	3.39E+05
PCS2(-7)/PCS12(-8E)	1.35E+08	1.08E+03	1.25E+05
PCS2(-6)/PCS12(-8E)	8.94E+08	5.27E+03	1.70E+05

Figure 4. Comparison of the infectivity of PCS mutant viruses

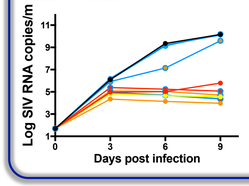
A. Quantitation of viable virions in PCS mutation SIVmac239 variants



Viral stock	$TCID_{50}$
SIVmac239 WT	3.23E+05
PCS12(-8)	2.05E+05
PCS12(-8)	3.26E+06
PCS2(-8)	8.00E+02
PCS2(-7)	1.17E+03
PCS2(-6)	3.27E+02
PCS2(-8)/PCS12(-8R)	3.17E+03
PCS2(-6)/PCS12(-8R)	3.17E+03
PCS2(-8)/PCS12(-8E)	1.29E+04
PCS2(-7)/PCS12(-8E)	3.17E+03
PCS2(-6)/PCS12(-8E)	5.17E+04

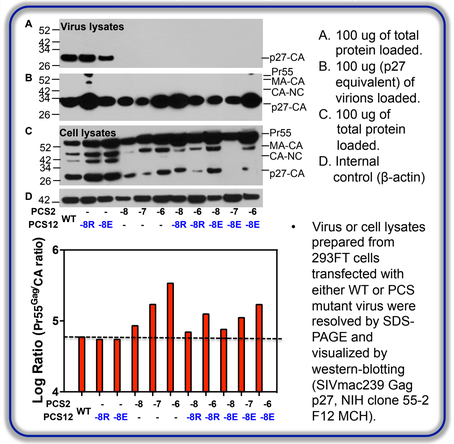
- Replication of virus in CEMx174 cells was assessed by measuring SIV p27 antigen in culture supernatants.
- $TCID_{50}$ of each virus stock was calculated using the Spearman-Karber method

B. Growth curves of PCS mutation SIVmac239 variants in CEMx174 cells



- Equivalent amounts of p27 (3ng) were used to infect $1x10^6$ CEMx174 cells.
- Viral replication was monitored by qPCR of culture supernatants.

Figure 5. Analysis of proteolytic processing of $Pr55^{Gag}$



A. 100 ug of total protein loaded.
B. 100 ug (p27 equivalent) of virions loaded.
C. 100 ug of total protein loaded.
D. Internal control (β-actin)

- Virus or cell lysates prepared from 293FT cells transfected with either WT or PCS mutant virus were resolved by SDS-PAGE and visualized by western-blotting (SIVmac239 Gag p27, NIH clone 55-2 F12 MCH).

CONCLUSIONS

- Immune responses to PCS peptides drive viral mutation.
- Vaccinees showed higher frequencies of mutations in both PCS2 (p27-p2) and PCS12 (Nef) regions that correlated with reduced viremia following challenge with SIVmac239
- Comparisons of eight PCS mutant viral stocks harboring either single PCS2 or combinations of two separate amino acid mutations one in the PCS2 site and the other within the PCS12 region to WT SIVmac239 indicate:
  - significantly lower viral RNA level and p27-CA content
  - impaired cleavage of $Pr55^{Gag}$ in virus lysates
  - 10-1000 fold differences in calculated $TCID_{50}$
  - impaired ability to replicate in CEmx174 cells
- The addition of the PCS mutation in PCS12 partially compensates the defects incurred by PCS2 and restores viral replication of PCS2 mutants.
- Virion production of PCS12 variants is nearly identical to those of WT SIVmac239 and replicate at comparable rates to WT SIVmac239.

REFERENCES

Luo M, Capina R, Daniuk C, Tuff J, Peters H, Kimani M, et al. Immunogenicity of sequences around HIV-1 protease cleavage sites: Potential targets and population coverage analysis for a HIV vaccine targeting protease cleavage sites. Vaccine. 2013 Jun 24;31(29):3000-8.

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