A Vaccine Targeting Protease Cleavage Sites Protects Cynomolgus Monkeys Against SIV

Ma Luo, David Tang, Rupert Capina, Xin-Yang Yuan, Jorge F Correia-Pinto, Cecilia Prego, So-Yon Lim, Christina Barry, Richard Pilon, Christina Daniuk, Mikaela Nykoluk, Stephane Pillet, David La, Tomasz Bielawny, Jeffrey Tuff, Chris Czarnecki, Philip Lacap, Gary Wong, Shaun Tyler, Binhua Liang, Zhe Yuan, Qingsheng Li, Terry B Ball, James Whitney, Maria J Alonso, Paul Sandstrom, Gary Kobinger, Francis A. Plummer

National Microbiology Laboratory, Winnipeg, Manitoba, Canada; University of Manitoba, Winnipeg, Manitoba, Canada; University of Santiago de Compostela, Spain; University of Nebraska-Lincoln, Nebraska, USA
Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA; Ragon Institute of MGH, MIT, and Harvard, Cambridge, MA 02139, USA

ABSTRACT

Background: With the sobering results of the STEP and HVTN 505 HIV vaccine clinical trials, novel approaches to HIV vaccine development must be explored. The HIV protease is a 99-amino acid aspartic enzyme that mediates the cleavage of Gag, Gag-Pol and Nef precursor polyproteins. This process is a highly specific, temporally regulated and essential for the production of infectious viral particles. A total of 12 proteolytic reactions are required to generate an infectious virion, and a single impaired cleavage reaction can render the virus noninfectious. Thus, HIV vaccine-elicited responses targeting the protease cleavage sites (PCS) could be highly efficacious.

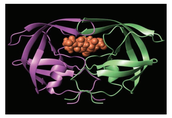
Methods: We assess vaccine immunogenicity elicited against PCS immunogens using a modifiedVesicular stomatitis virus (VSV) vector combined with a nanodelivery system. We then evaluate protective efficacy to disrupt SIV acquisition and disease progression using a Cynomolgus macaque (Macaca facicularis, Philippines) and SIVMAC239 intrarectal challenge model. To examine whether the immune driven viral mutations surrounding the PCS were detrimental to the virus, we amplified and sequenced the plasma viruses by 454-pyrosequencing and correlated the amino acid mutations surrounding PCS with alterations in viral load and CD4 count.

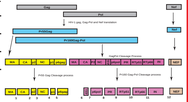
Results: PCS peptides expressed by rVSVs and packaged in nanoparticles are able to generate both antibody and T cell responses in macaques. The ability of macaques to withstand high dose SIVMAC239 challenge was significantly correlated with the antibody and antibody/T cell responses to the number of PCS peptides(p=0.0005, R=0.8005). This combination imparted resistance to all but the higher SIV doses that were required to infect vaccinnees (p=0.01). the vaccine group maintains significantly higher CD4 counts (p=0.0002). Amino acid mutations (SN or NS) surrounding PCS correlated significantly with reduced viral RNA levels (p<0.0001).

Conclusions: We show here in a Cynomolgus macaque/SIV model that a candidate HIV vaccine focusing immunologic responses to the regions surrounding the SIV protease cleavage sites can force viral mutation resulting in impaired SIV fitness. Focused immune response to the PCS region enables the macaques to withstand multiple high dose pathogenic SIVMAC239 intrarectal challenges. Virus recovered from vaccinnees harbored mutations surrounding the protease cleavage region that correlated significantly with reduced viral load, and the maintenance of CD4+ T cells in vivo.

INTRODUCTION

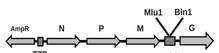
Developing an effective preventative vaccine for HIV has proved to be an enormous scientific challenge. The classical approach to HIV vaccine development has shown at best, only modest efficacy. An effective HIV vaccine should not only generate potent immune responses capable of destroying infected cells, but also limit aberrant immune activation that increases the susceptibility of the HIV target cell population to infection. Vaccines that generate focused immune responses to highly conserved regions of HIV protease cleavage regions (PCS) might be able to strike such a balance. The HIV protease is a 99-amino acid aspartic enzyme that mediates the cleavage of Gag, Gag-Pol and Nef precursor polyproteins. This process is highly specific, temporally regulated and essential for the production of infectious viral particles (1-4). Twelve proteolytic reactions are required to generate an infectious virion, and a single impaired cleavage reaction can render the virus noninfectious (5). Thus, an HIV vaccine eliciting immune responses targeting the sequences surrounding these PCS could be highly efficacious.



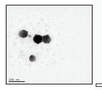


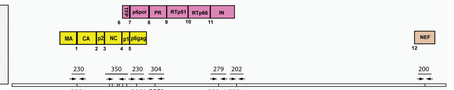
METHODS

we assessed vaccine immunogenicity elicited against PCS immunogens using a modified Vesicular stomatitis virus (VSV) vector combined with a nanodelivery system. We then evaluated protective efficacy to disrupt SIV acquisition and disease progression using a Cynomolgus macaque (Macaca facicularis) and SIVMAC239 intrarectal challenge model. To examine whether the immune driven viral mutations surrounding the PCS were detrimental to the virus, we amplified and sequenced the plasma viruses by 454-pyrosequencing and correlated the amino acid mutations surrounding the PCS regions with alterations in viral load and maintenance of CD 4 T cell counts.



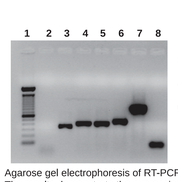
The nucleotide sequences encoding 20 amino acids overlapping each of the 12 protease cleavage sites, Gag or Env were cloned into pATX VSV-G vector at Mlu1-Bln1 site.





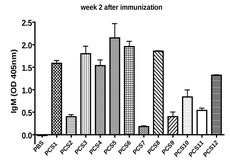
RESULTS

A vaccine expressing PCS peptides generates antibody and T cell responses

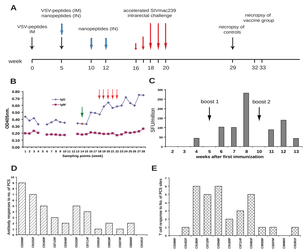


1: 100bp ladder
2: negative control
3: VSV-L, 357bp
4: VSV-M, 419bp
5: VSV-N, 423bp
6: VSV-P, 476bp
7: VSV-G, 820bp
8: PSC site 2

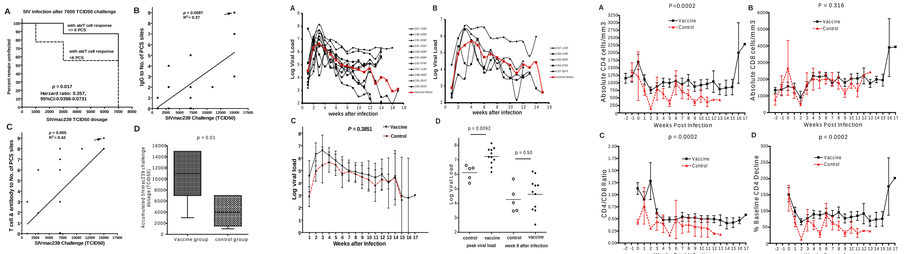
Agarose gel electrophoresis of RT-PCR products. The results demonstrate the expression of RNA of peptides overlapping the p27/p2 site of protease cleavage site of SIVmac239.



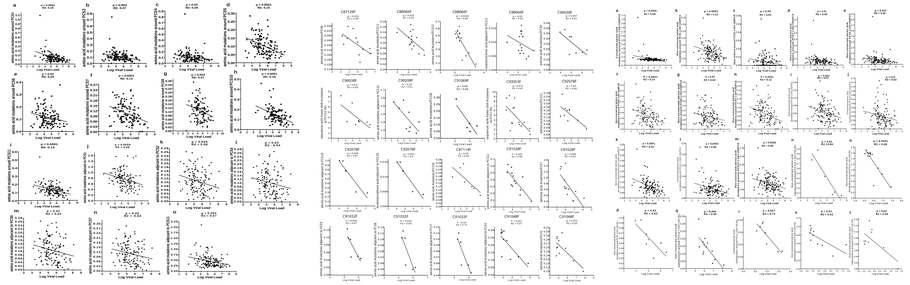
IgM antibodies to the peptides overlapping the 12 protease cleavage sites were detected in BALB/c mice 2 weeks after immunization with recombinant vesicular stomatitis virus.



PCS specific immune responses protect cynomolgus monkeys against pathogenic SIVMAC239 infection and disease progression

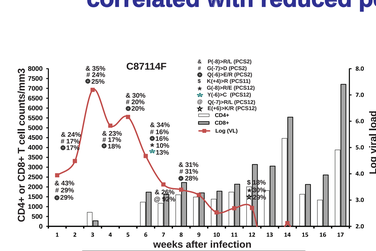


Immune responses to PCS peptides drive viral mutation that is detrimental to the challenge virus



RESULTS

Amino acid mutations surrounding PCS at the peak and set point correlated with reduced peak and set point viremia

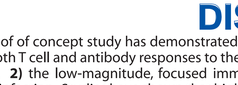




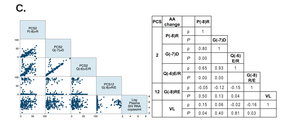
	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10
G	G	P	I	Q	K	A	I	R	L	M	A	E	A	L	K	E	A	L	A	P



	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10
L	V	S	Q	G	I	R	Q	Y	L	F	L	R	K	I	E	P	A	Q	E	



	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10
N	Q	G	Q	Y	M	N	T	P	W	R	N	P	A	E	E	R	E	K	L	



DISCUSSION

Our proof of concept study has demonstrated the feasibility of this vaccine approach in several ways. **1)** it is possible to generate both T cell and antibody responses to the 12 20-amino acid peptides using a modified VSV vector and a Nano delivery system. **2)** the low-magnitude, focused immune responses to multiple PCS regions can provide protection against SIVmac239 infection. Studies have shown that higher viral load increases the risk of sexual HIV transmission, and mother-child HIV transmission(6). The increased dose of SIVmac239 intrarectal challenge in our study simulates the high viral load condition in sexual HIV transmission. The resistance of macaques to the higher SIVmac239 challenge dose is significantly correlated with their ability to generate immune responses to the number of PCS.

The second measurement of the effectiveness of the vaccine is whether a vaccine targeting PCS can offer protection from disease progression after infection. Our study showed that a vaccine targeting PCS can also protect macaques against disease progression. HIV disease progression is measured by rapid CD4 decline; this is also shown in monkey models. We showed that **1)** PCS vaccinated monkeys can maintain significantly higher and healthy CD4 counts. **2)** there is a significant inverse correlation between mutations surrounding the PCS and the viral load. **3)** both conserved and non-conserved amino acid substitutions around the PCS correlated significantly with lower viral load. The data demonstrated that protease cleavage of the pathogenic SIVmac239 is extremely vulnerable to any amino acid alternations around PCS, thus the sequences surrounding PCS are good targets for an effective HIV vaccine. Our proof of concept study demonstrated that disrupting HIV maturation process by vaccination against sequences surrounding the PCS is feasible and effective.

References

1. Jacks T, Power MD, Masiarz FR, Luciw PA, Barr PJ, Varmus HE. Characterization of ribosomal frameshifting in HIV-1 gag-pol expression. Nature. 331:280. **2.** Kaplan AH, Zack JA, Knigge M, Paul DA, Kempf DJ, Norbeck DW, et al. Partial inhibition of the human immunodeficiency virus type 1 protease results in aberrant virus assembly and the formation of noninfectious particles. J Virol. 67:4050. **3.** Krausslich HG, Ingraham RH, Skoog MT, Wimmer E, Pallai PV, Carter CA. Activity of purified biosynthetic proteinase of human immunodeficiency virus on natural substrates and synthetic peptides. PNAS U S A. 86:807. **4.** Louis JM, Ishima R, Torchia DA, Weber IT. HIV-1 protease: structure, dynamics, and inhibition. Adv Pharmacol. 55:261. **5.** Billich S, Knoop MT, Hansen J, Strop P, Sedlacek J, Mertz R, et al. Synthetic peptides as substrates and inhibitors of human immune deficiency virus-1 protease. J Biol Chem. 263:17905; **6.** Chappell CA, Cohn SE. Prevention of perinatal transmission of human immunodeficiency virus. Infect Dis Clin North Am. 28:529

Acknowledgement

We thank the staff of VTS of National Microbiology laboratory and Canadian Food Inspection Agency (CFIA) for their dedication in this study; Ms. Michelle Janes for her technical support in viral load analysis; and the professional support from the staff of Genomic Core and Bioinformatics Core of National Microbiology Laboratory for SIV pyrosequencing and data analysis, as well as the project support from Dr. Mike Drebot and Dr. Graham Tipples. The study is supported by a CIHR/CHVI catalyst grant, the National Microbiology Laboratory, Public Health Agency of Canada and a NIH grant R01AI111805.