2015

Comparative fitness analysis of proteolytic cleavage site vaccine variants in simian immunodeficiency virus

https://hdl.handle.net/2144/16186

Boston University
COMPARATIVE FITNESS ANALYSIS OF PROTEOLYTIC CLEAVAGE SITE VACCINE VARIANTS IN SIMIAN IMMUNODECIFIENCY VIRUS

by

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B.S., Georgia Institute of Technology, 2012

Submitted in partial fulfillment of the requirements for the degree of Master of Science

2015
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ACKNOWLEDGMENTS

I would like to express my sincere gratitude towards Drs. James Whitney and So-Yon Lim for their continued and tireless support over the past year. Dr. Whitney, thank you for giving me the opportunity to learn and to grow as a scientist in your lab, and for the help and encouragement throughout the thesis writing process. So-Yon, thank you so much for your dedication, guidance and patience towards me during all of the experiments, mistakes and successes we went through. I will never again forget where and how to store bacterial cultures and will slowly get over my fear of aspirating. Completing my thesis would not have been possible without the both of you. To Oscar, thank you for being my partner in crime this past year and more specifically, for all the inoculation help. To the other members in the lab, Dr. Christa Osuna, Sri Sanisetty, and Jess, thank you for always offering your help when I needed it, and for the daily conversations and laughter. To Dr Rachel Fears, thank you for your suggestions, help and time with editing this thesis. Finally, I want to thank my family for their never-ending support for me and for the pursuit of my studies.
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FRANCES Y CHIANG

ABSTRACT

Over the past few decades, the human immunodeficiency virus and its progression to acquired immunodeficiency syndrome has become one of the most prominent global health issues. As the number of infected persons continues to grow, it is increasingly important to develop a protective vaccine to stop HIV transmission, and a cure for those already infected. Although current combination antiretroviral therapy can help patients maintain undetectable levels of the virus throughout their bodies, once the treatment is stopped, the virus will rebound. In this project, the effects of a vaccine therapy that targets the protease cleavage sites (PCS) of the HIV protease were evaluated in 16 Cynomolgus macaques. Preliminary results of the study show that in the vaccine group (n=11), a disruption to one or more of the HIV protease cleavage sites leads to a better maintenance of CD4+ T cells versus that in the control group (n=5). Furthermore, a correlation between the percentage of PCS mutations and viral load was also observed. Upon closer analysis, it was determined that the most common sites of mutation occur at PCS2 and PCS12. To assess the impact of these PCS mutations on viral fitness, we used site directed mutagenesis to introduce single amino acid mutations into a fully infectious SIV clone (SIVmac239). Ongoing studies include producing virus stocks of the SIVmac239 mutants (with multiple PCS mutations) and evaluating the viral fitness of the SIVmac239 clones in cell lines using growth competition assays. The data from this
study and future studies will help provide information in the areas of vaccine and therapy development for HIV.
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LIST OF ABBREVIATIONS

AIDS acquired immunodeficiency syndrome
ART antiretroviral therapy
bNABs broadly neutralizing antibodies
CA capsid
cART combination antiretroviral therapy
cDNA complementary DNA
CMV cytomegalovirus
CTL cytotoxic T lymphocyte
CTLA-4 cytotoxic T-lymphocyte-associated protein 4
CypA cyclophilin A
dNTP deoxynucleotide triphosphate
dTT dithiothreitol
EDTA ethylenediaminetetraacetic acid
Env envelope
FBS fetal bovine serum
Gag group specific antigen
p27-CA 27 KDa SIV capsid antigen
gp glycoprotein
HIV-1/HIV-2 human immunodeficiency virus type 1/ type 2
HLA human leukocyte antigen (several)
IL-2 Interleukin-2
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>kb</td>
<td>kilobase</td>
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<tr>
<td>kDa</td>
<td>kilodalton</td>
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<td>LAG-3</td>
<td>lymphocyte-activation gene 3</td>
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<td>MA</td>
<td>matrix</td>
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<td>MHC</td>
<td>major histocompatibility complex</td>
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<td>MOI</td>
<td>multiplicity of infection</td>
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<td>mRNA</td>
<td>messenger ribonucleic acid</td>
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<td>MVA</td>
<td>modified vaccinia Ankara</td>
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<td>NC</td>
<td>nucleocapsid</td>
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<tr>
<td>Nef</td>
<td>negative regulatory factor</td>
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<td>NHP</td>
<td>non human primate</td>
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<td>nt</td>
<td>nucleotide</td>
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<td>PBMC</td>
<td>peripheral blood mononuclear cells</td>
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<td>PBS</td>
<td>phosphate buffered saline</td>
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<td>PCR</td>
<td>polymerase chain reaction</td>
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<td>PCS</td>
<td>protease cleavage site</td>
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<td>PD-1</td>
<td>programmed cell death 1</td>
</tr>
<tr>
<td>Pol</td>
<td>polymerase</td>
</tr>
<tr>
<td>RPMI</td>
<td>Roswell Park Memorial Institute</td>
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<td>RTase</td>
<td>reverse transcriptase</td>
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<tr>
<td>RT-PCR</td>
<td>reverse transcription - time polymerase chain reaction</td>
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<td>RTC</td>
<td>reverse transcription complex</td>
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<tr>
<td>Term</td>
<td>Description</td>
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<td>--------------------------------------------------------------</td>
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<tr>
<td>SIVmac251</td>
<td>simian immunodeficiency virus 251 (biological quasispecies)</td>
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<td>SIVmac239</td>
<td>simian immunodeficiency virus 239 (clone derived from SIV251)</td>
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<tr>
<td>STLV-III</td>
<td>simian T-cell lymphotropic virus type III (STLV-III)</td>
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<tr>
<td>Tat</td>
<td>transactivator</td>
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<tr>
<td>TCID$_{50}$</td>
<td>50% tissue culture infectious dose</td>
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<tr>
<td>TIM-3</td>
<td>T-cell immunoglobulin mucin 3</td>
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INTRODUCTION

*Human Immunodeficiency Virus (HIV)*

Human immunodeficiency virus (HIV) is a retrovirus that infects and results in the destruction of CD4+ T lymphocytes. Left untreated, this CD4+ destruction ultimately results in Acquired Immunodeficiency Syndrome (AIDS), due to multiple opportunistic infections. In 2013, there were more than 35 million people in the world living with HIV, with the majority of the infected persons from underdeveloped regions in Africa and Southeast Asia. Two genetically distinct forms of HIV have been found to be present in individuals with AIDS: HIV-1 and HIV-2. HIV-1 is the more common form found in most patients living with AIDS, while HIV-2 is much less prevalent, as well as of reduced pathogenicity. HIV-2 is found predominantly in West Africa. The global HIV-1 disease burden, incidence of new infection, and its death toll are shown in Table 1. Notably, the number of fatalities due to HIV-1/AIDS has been decreasing due to HIV education and antiretroviral treatment.

<table>
<thead>
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<th>Global Summary of the AIDS Epidemic (2012)</th>
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<td>Number of people living with HIV</td>
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<tr>
<td>Total</td>
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<tr>
<td>Adults</td>
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<tr>
<td>Women</td>
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<tr>
<td>Children (&lt;15 years)</td>
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<td>People newly infected with HIV in 2012</td>
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<tr>
<td>Total</td>
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<tr>
<td>Adults</td>
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<td>Children (&lt;15 years)</td>
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<td>AIDS deaths in 2012</td>
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<tr>
<td>Total</td>
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<tr>
<td>Adults</td>
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<td>Children (&lt;15 years)</td>
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Table 1. Summary of the number of people living with HIV, the number of newly infected people, and the number of deaths due to AIDS in 2012.
However, current treatment methods targeting HIV-1 do not fully eradicate the virus. The clinically asymptomatic period between HIV-1 acquisition and the first clinical symptoms of AIDS can be on the magnitude of years. Once infected, the clinical hallmark of HIV is the depletion of CD4+ T cells. The course of HIV-1 viremia (as measured in the blood plasma) begins with a sharp increase in viral RNA levels, followed by a decrease and fairly constant level of viremia, or “set point” during the asymptomatic chronic phase. This set point, is a steady state that can be maintained for years and its magnitude correlates with both the prognosis and time of progression to AIDS. During the asymptomatic phase, the total numbers of CD8+ and CD4+ T lymphocytes continues to decrease, eventually dropping to a level at which viral replication can no longer be controlled. This leads to increased viral loads and progression to AIDS. The course of infection is depicted in **Figure 1**.

![Figure 1](image.png)

**Figure 1.** Diagram showing the course of HIV infection with changes in CD8+ T lymphocytes (blue), CD4+ T lymphocytes (green), and plasma virus load (red)
Current Treatment Methods

The current treatment for individuals with HIV-1 is combination antiretroviral therapy (cART). cART is currently effective at maintaining an undetectable level of viral load of less than 50 copies/mL in infected persons. Depending on the class and subtype of HIV that a person has been infected with, specific drugs with different mechanisms of inhibition can be used: nucleoside reverse transcriptase inhibitors, integrase inhibitors and protease inhibitors. Additionally, a drug, nevirapine, a non-nucleoside reverse transcriptase inhibitor, is being used to prevent the transmission of all subtypes of HIV from mother to child, although its effect is highly variable depending on the subtype of the virus. Furthermore, integrase inhibitors such as raltegravir or dolutegravir are preferred in combination treatment due to their efficacy and lack of severe side effects.

While current therapies may help to manage and control HIV, they are not a cure. After prolonged exposure to the antiretroviral drugs or patient non-compliance, the virus can quickly mutate and develop resistance to the treatment. Furthermore, patient adherence to the drug is critical for infected persons to maintain undetectable levels of viremia. While treatments can help infected persons manage the disease, there are negative side effects of the long-term use of cART, ranging from fatigue, nausea and vomiting, to more severe symptoms of insulin resistance, pancreatitis and dyslipidemia. Until a cure is developed for HIV-1, it is important to find a balance between the effectiveness of cART and the long-term drug toxicity and physiological side effects on the patient. With the many factors that affect the efficacy and safety of using cART, it is increasingly important to develop curative therapies.
Experimental Treatment Methods

Currently, there are ongoing experimental treatments being tested to combat the HIV-1. In addition to cART, HIV-specific monoclonal antibodies have been used in an attempt to eradicate the disease. Structurally, the Env epitope is very exposed on the virus, thus making it a potential target for therapeutic treatments. Cocktails of antibodies targeting different areas including the CD4 binding site and the N332 glycan region on the Env epitope, were used in an attempt to create a broad effect. However, due to viral mutations, the effects of these treatments were found to be thwarted after an extended period of time. However, in a study performed by Diskin et al., broadly neutralizing antibodies (bNAbs) were developed that were found to block potential pathways for the virus to mutate against the antibodies, which may be a potential route for the discovery of a more long term and effective treatment.

HIV Dynamics and Viral Load

To determine the efficacy of treatment methods on eliminating HIV from the body, viral dynamics are used to study the relationship between treatment and viral load. The viral load of HIV is known as the amount of HIV RNA that is detectable within the plasma. A major obstacle that challenges scientists in this area is the viral latency period of HIV. Once an individual is exposed to the virus, the virus establishes a latent reservoir almost immediately after infection. While there are current drugs that can lower the viral load of HIV to undetectable levels, those values represent the amount of active HIV RNA that is found within the plasma and do not measure the latent reservoir pool.
Evidence of an early seeding of the viral reservoir before day three of infection was shown in rhesus monkeys infected with SIV. The discovery of this early establishment of the viral reservoir presents a huge challenge in the pursuit of finding a cure for HIV. The viral reservoir of SIV was found to be established during the eclipse phase between the time of infection and the production of new virions. Although there was no detectable viremia at the start of ART on day 3, after ART discontinuation, viral rebound was observed in all monkeys. With the help of Bayesian statistics, viral dynamic models can be created to not only show current effects of a treatment, but also to predict future viral loads of HIV over an extended period of time.

**HIV Pathogenesis**

When determining how the course of HIV can progress in an infected person, it is important to take into consideration each person’s individual genetic makeup. Humans can show a combination of six different HLA class I alleles from the three most common MHC class I loci HLA-A, HLA-B and HLA-C. The rate of infection and progression to AIDS is dependent on which alleles are displayed, representing the different ways the host immune system can recognize the virus and destroy it. For example, persons displaying alleles HLA-B57, HLA-B27 and HLA-B51 are found to be more likely to have long term control of the virus that does not progress to AIDS, while persons with HLA-B35 progress rapidly.
The HIV Replication Cycle

The first step in the life cycle of HIV begins with the attachment of HIV-1 to the host cell. This is carried out by the viral envelope glycoprotein, which begins as a gp160 molecule and is subsequently cleaved into gp120 and gp41, two necessary molecules for proper membrane fusion. A specific amino acid sequence on the glycoprotein molecule gp120 found on the surface of the viral envelope creates a binding site for the CD4 surface protein found on various host cells including T lymphocytes, monocytes and macrophages. The gp120 molecule recognizes one or both of the chemokine receptors, CCR5 and CXCR4, which may be expressed on the host cell to attach to the membrane. These receptors are responsible for determining which type of cell HIV-1 will infect: The CCR5 receptor is found mostly on macrophages, while the CXCR4 receptor is found mainly on T cells. Any changes to this steps in the mechanism may cause the virus to lose the ability to bind to the host cell. During the final step of attachment, the viral envelope fuses with the host cell membrane with the help of the gp41 molecule.

Similar to other retroviruses, the HIV genome enters the cytoplasm of the host cell and becomes incorporated into the host genome with the help of various factors and host proteins. Once in the cytoplasm, the reverse transcription complex (RTC), which is comprised of the reverse transcriptase, integrase, protease and other accessory proteins, becomes activated and produces a strand of complementary DNA (cDNA). The cDNA then enters the nucleus and becomes incorporated into the genome, where it may remain latent for years until it is activated by antigens or cytokines to form infectious viral particles.
The HIV RNA genome is transcribed by cellular RNA polymerase II. New particles of HIV are synthesized as three polyproteins that are eventually cleaved to give rise to the Gag, Pol and Env genes. The Gag domain consists of the viral core, the Pol domain is comprised of the viral enzymes, and the Env domain contains the glycoproteins that form the viral envelope. Before the cleavage of the polyproteins into mature particles, the virus first buds from the host cell. The accessory protein, Vpu, has been found to be a necessary factor in helping to promote the budding and release of new viral particles. Once the immature viral particle has been released from the host cell, both the Gag and Pol polyproteins must be cleaved in a tightly regulated manner by the HIV protease to produce mature, infectious HIV particles. Without the proper function of the HIV protease, which could be due to mutations within the gene or on the protease cleavage sites, the new viral particles formed will not be infectious.

The HIV protease cleaves the Gag polyprotein into the p17 matrix (MA), p24 capsid (CA), p7 nucleocapsid (NC) and other smaller glycoproteins. The production of the capsid, a protein made up of two domains, stimulates the maturation process, during which a shell is formed around the viral RNA/NC complex and rearranges to form a conical core, a core characteristic of an infectious virion. The two domains of the capsid are the N-terminal region and the C-terminal domain. The N-terminal domain has been found to play a role in viral maturation and the incorporation of cyclophilin A (CypA) while the C-terminal domain helps with the Gag-gag interactions. This Gag-Gag interaction plays a role in the communication of cholesterol raft binding that is required at the plasma membrane during viral budding.
Throughout the HIV replication cycle, there are many steps that can be targeted by treatment and therapy to halt the replication of and to destroy the virus. These different steps are depicted in Figure 2 and include therapy that targets the entry, reverse transcription, integration and activity of the HIV protease.

Figure 2. Potential steps in the HIV replication cycle that can be targeted for treatment

CTL Immune Response and Escape

One of the most critical components of the host immune response to HIV infection is CD8+ cytotoxic T lymphocyte (CTL) activity. In a study performed by Borrow et al., the CTL response towards the HIV envelope protein, gp160, was inversely correlated with viral load. Furthermore, the inhibition of HIV-1 replication in peripheral blood lymphocytes in vitro has been found to be dependent on the presence of CD8+ lymphocytes. This immune response and control of viral replication is important in the development of vaccines and treatments and may play a role in the maintenance of suppressed viremia.
However, as the virus replicates and persists within the host, it is able to mutate and ultimately evade the immune system. As a result, CTLs alone are unable to prevent infected patients from succumbing to AIDS. It has been found that the escape variants occur by amino-acid replacements within CTL epitopes. In SIV infected rhesus macaques, it was shown that changes in the CTL epitopes of Env and Nef genes lead to changes in the MHC class I binding and disease progression. In a study on rhesus macaques performed by Evans et al., different variants in amino acid replacements were found, including on the Mamu-A*11 and Mamu-B*03 Env epitope, as well as on the Mamu-B*03, Mamu-B*04 and Mamu-B*17 Nef epitopes. All these changes resulted in a lowered CTL response to SIV infection and pose a major barrier to the development of a vaccine for HIV.

The impact of CTL escape mutations on a population depends on the transmission ability of the virus and its stability after transmission. Reversion of an HIV-1 CTL epitope mutation after transmission was first observed on the TW10 epitope, which is found in a large percentage of the HLA-B57 or HLA-5801 positive population and is the immunodominant CTL response in acute infection. The escape mutation, T242N, found on the TW10 epitope, has been successfully transmitted from persons to persons. After transmission of the T242N mutation, the mutation persisted in persons who are HLA-B57/HLA-5801 positive (4 of 19 persons during the acute phase of infection), but not in persons without the particular alleles (0 of 187 persons), suggesting that the virus reverted back to the wild type in the absence of HLA-B57/HLA-5801. Reversion of a mutation back to its wild type could be due to the associated fitness cost.
Determining Viral Fitness

The fitness of a virus is defined as its ability to adapt to the environment and continue to replicate and produce infectious copies of itself\(^4\). Mutations within the viral genome will inevitably occur due to natural selection as the virus undergoes various changes towards selective pressures such as the development of drug resistances to reverse transcriptase and protease inhibitors, fusion inhibitors and CCR5 inhibitors. In a study performed by Anastassopoulou et al., fitness was defined as the ability of the HIV strain to replicate in tissue cultures. The viral fitness of HIV after developing resistance to a CCR5 inhibitor drug, AD101, was examined by using a comparative fitness analysis. Using a dual-infection growth competition assay, a resistance to AD101 which occurs by a change in four amino acids within the V3 region of gp120, showed no changes viral fitness and did not lead to a fitness loss \(^4\). Understanding viral behavior towards treatment drugs and how its fitness changes in response can play a vital role in the development of a cure for HIV.

Using the Simian Immunodeficiency Virus as a Model

The best model to date for the study of HIV and the development of a vaccine has been the non-human primate (NHP) and the simian immunodeficiency virus (SIV) system. There are a number of key features that the model must display\(^4\). The model has many features that recapitulate HIV/AIDS in humans:

1. Demonstrate persistent, progressive systemic infection after inoculation
2. Use the CCR5 co-receptor for infection
3. Undergo acute depletion of memory CD4+ T cells, specifically from mucosal sites

4. Establish an initially disease-free plateau phase

5. Show progression to AIDS over a period of several months to years

The simian immunodeficiency virus from macaques, previously known as simian T-cell lymphotropic virus type III (STLV-III), is related to HIV in humans in many ways, including by its antigenicity, morphologic and growth properties. These similarities make it the best model for studying HIV.

Furthermore, after SIV infection, the progression of the virus is very similar to that of HIV in humans. There is an extremely high rate of infection and a rapid destruction of memory CD4+ T cells. At the peak level of SIV infection of rhesus macaques, approximately 30-60% of all memory CD4+ T cells become infected and at four days post-peak infection, about 80% of the infected cells are destroyed.

Current Investigation and Specific Aims

As HIV and AIDS continue to infect and kill people worldwide, it is increasingly important to find and develop a full eradication strategy targeted against the virus. Targeting the HIV protease as a mechanism for stopping HIV replication is a potential avenue in vaccine development. Altering the function of HIV protease leads to the production of immature, noninfectious viral particles, thus halting the replication of HIV. The HIV protease is required by the virus to process the polyproteins Gag and Gag/Pol, which are necessary for complete replication within a host cell. The HIV protease
functions by identifying cleavage sites on the proteolytic precursors Pr55 for Gag and Pr160 for Gag/Pol. Using an *in vitro* HIV protease assay, Kohl *et al.* determined that a single mutation that caused an amino acid change at position 25 on HIV protease from aspartate to asparagine rendered the HIV protease to become nonfunctional. This alteration stopped the ability of HIV protease to cleave HIV gag p55 to gag p24 and gag p17. Without proper identification, the tightly regulated normal processing function and rate of cleavage by the protease can be lost. Factors that may alter the processing and cleavage include the sequence, structure and availability of the cleavage site to the HIV protease.

Previous studies have been carried out to determine the effect of protease inhibitors on HIV. A study was performed and analyzed the infectivity of viral particles produced in the presence of protease inhibitor and viral particles produced from proviral plasmids with mutations to its cleavage sites. It was found that even low concentrations of a protease inhibitor caused a small, but effective enough change on the polyprotein processing of Gag that it led to a decreased production of infectious viral particles.

The aim of this project is to investigate the use of HIV’s protease cleavage sites (PCS) in the development of a vaccine that can be used as a cure for infected individuals, as well as to prevent future infections. The results that have been shown in this thesis were generated in Dr. Ma Luo’s laboratory in the University of Manitoba, as part of an ongoing collaboration. The data were analyzed and discussed in collaboration with Dr. Whitney’s laboratory at the Beth Israel Deaconess Medical Center as a part of an HIV vaccine development consortium. Using the previously obtained data (unpublished data
from Luo et al.), we were able to determine immune correlates, single mutations and interacting mutations, which allowed us to better develop the basis for beginning this specific phase of the project of creating SIV clones. The protease cleavage sites of interest for this project are sites 2 and 12, which are located on the p2 Gag spacer and Nef protein, respectively. Viruses with the mutants will be synthesized using site directed mutagenesis, and their fitness will be evaluated by growth competition assays. These experiments will be carried out using the SIV model.
METHODS

Animals, SIV Challenge and Vaccination Protocol

This study enrolled and monitored 17 adult Cynomolgus macaques (*Macaca fascicularis*). All animals were genotyped for their MHC haplotypes by both PCR and MHC-amplicon sequencing. The monkeys were housed under biosafety level-2+ conditions, and were routinely monitored by physical examination and T cell subset analysis before and after SIV infection.

Specimen Collection and Processing

Peripheral blood was collected weekly from each monkey. The PBMC’s were separated on Ficoll-Hypaque cushions following standard protocols. All PBMC cultures were first purified by CD8 depletion using a commercial MACS magnetic bead depletion assay (Miltenyibiotec Inc.) and then cyropreserved and frozen at –180 °C for later use. After thawing, all PBMC were stimulated for 48 hours with Concanavalin A, prior to use in experiments. The PBMC’s were maintained in a lymphocyte medium (RPMI 1640 with 10% FBS and 40 U/mL IL-2). Blood plasma was immediately frozen and maintained at –80 °C until use.

Viral RNA Extraction and qRT-PCR Detection

Viral RNA was routinely isolated from 140 µL of blood plasma using the QIAamp Viral RNA Mini Kit (Qiagen) following the manufacturer’s protocol. RNA recovered from spin columns was eluted into a final volume of 60 µL.
**cDNA Synthesis**

A 10 µL volume of extracted RNA was reverse transcribed to single-stranded cDNA as described by the manufacturer in the Superscript III protocol (Invitrogen). The RNA, deoxynucleotides (0.5 mM each), and 0.24 µM primer OR9608 (5’ – CTCATCTGATACATTTACGGGG – 3’) were then incubated for 5 minutes at 65 °C. The sample was then chilled on ice for 1 minute, followed by a brief centrifugation. The first strand of the cDNA synthesis was completed by the addition of 10 µL of 5x reaction buffer, 2.5 µL of 0.1 M DTT, 2.5 µL of RNase Out, and 2.5 µL of Superscript III RT to the sample. The mixture was incubated as follows: 60 minutes at 50 °C, 60 minutes at 55 °C and 15 minutes at 70 °C. Finally, 5 units of RNase H were added and the mixture was incubated for 20 minutes at 37 °C.

**QPCR assay**

The SIV RNA standard was transcribed from the pSP72 vector containing the first 731 base pairs of the SIVmac239-Gag gene using the Megascript T7 kit (Ambion Inc., Austin, TX). RNA was isolated by phenol-chloroform purification followed by ethanol precipitation. All purified RNA preparations were quantified by optical density and the RNA quality was determined by the Agilent bioanalyzer RNA chip (Agilent Santa, Clara CA).

Quantitative RT-PCR was conducted in a 2-step process. First, RNA was reverse transcribed in parallel with an SIV-gag RNA standard using the gene-specific primer s-Gag-R: 5’CACTAGGTGTCTCTGCACATCTCTTTG-3’). Each 50 µL reaction
contained 1X buffer (250 mM Tris-HCL pH 8.3, 375 mM KCl, 15 mM MgCl₂), 0.25 µM primer, 0.5 mM dNTPs (Roche), 5 mM dTT, 500 U Superscript III RTase (Invitrogen, Carlsbad, CA), 100 U RnaseOUT (Invitrogen, Carlsbad, CA), and 10 µL of sample. The PCR conditions used were as follows: 1 hour at 50 °C, 1 hour at 55 °C and 15 minutes at 70 °C. All samples were then treated with RNAse H (Stratagene, Cedar Creek, TX) for 20 minutes at 37 °C. All real-time PCR reactions used EZ RT PCR Core Reagents (Applied Biosystems, Foster City, CA) following the manufacturer’s suggested instructions under the following conditions: the 50µL reactions contained 1X buffer (250 mM Bicine, 575 mM potassium acetate, 0.05 mM EDTA, 300 nM Passive Reference 1, 40% (w/v) glycerol, pH 8.2, 0.3 mM each of dATP, dCTP, dGTP, 0.6 mM dUTP, 3 mM Mn (OAc)₂, 0.5 U uracil N-glycosylase, 5 U rTth DNA Polymerase, 0.4 uM of each primer, and 10µL of sample template. PCR reagents were assembled at room temperature (RT) and spun briefly to eliminate air bubbles. Following 2 minutes at 50 °C, the polymerase was activated for 10 minutes at 95 °C, and then cycling proceeded at 15 seconds at 95 °C and 1 minute at 60 °C for fifty cycles. Primer sequences were adapted from those described by 55, forward primer s-Gag-F: 5’-GTCTGCGTCATCTGTTGTGCATTC-3’, reverse primer s-Gag-R: 5’-CACTAGGTGTCTCAGCTCATCATGTTTTG-3’, and the probe s-Gag-P: 5’-CTTCCTCAGTGTGTTCACACTTTCTCTTTCTCTGCG-3’, linked to Fam and BHQ (Invitrogen, Carlsbad, CA). All reactions were carried out on a 7300 ABI Real-Time PCR system (Applied Biosystems) in triplicate according to the manufacturer’s protocols.
Construction of PCS mutant SIV clones

Two mutated SIV clones were created: SIV-PCS2-mtracking vector and SIV-PCS12-mtracking vector. The first step in the construction of the mutated SIV-PCS2-mtracking vector, SIV-PCS2-m, involved subcloning of the region located between restriction sites BamHI (position 2104) and SphI (position 6701) from the full-length infectious clone of SIVmac239, derived from a biological isolate of SIVmac251, as described by Whitney et al.\textsuperscript{56} into pSP73 (Promega). The first step in the construction of the mutated SIV-PCS12-m-tracking vector, SIV-PCS12-m, involved subcloning of the region located between restriction sites SphI (position 6701) and XhoI (position 10535) from the full-length infectious clone of SIVmac239, derived from a biological isolate of SIVmac251, as described by Whitney et al., into pSP73 (Promega). The resulting plasmid was subjected to site-directed mutagenesis to insert 3 and 2 nonsynonymous mutations into the PCS2 or PCS12 region, respectively, using the Stratagene Quickchange I kit, as specified by the manufacturer. The mutagenized BamHI-SphI fragment and the SphI-XhoI fragment were then inserted into the parental clone to produce the PCS2-mutant (PCS2-M) and PCS12-mutant (PCS12-M) clones, respectively. Infectious virus stocks were prepared by transient transfection of 293T cells with SIVmac239 wild type or PCS2/12-M plasmids using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions.
**Virus stocks**

Viruses used in the fitness studies are both biological isolates and chimeric virus derived from the vaccine and control arms of a primate-based HIV-candidate vaccine study. Stocks of each isolates were prepared. All infectious stocks were stored in aliquots at -80 °C. The titers 50% tissue culture infectious dose (TCID$_{50}$) of all stocks were determined in PBMC culture by standard methods and data was analyzed using the method described by Spearman and Karber.

**Growth-Competition Assays**

Growth competition assays will be performed in 12-well plates seeded with $2 \times 10^6$ PHA-activated rhesus PBMC’s in 2 mL of total volume. The two viruses under evaluation are added to the target cells at individual MOI of 0.001 or 0.005, which are generally accepted to be low enough to prevent recombination. To limit the inherent variability of PBMC replication assays, each data point is derived from duplicate cultures on the same plate, and all experiments will be performed twice. Competition cultures involving viruses will be maintained for 14 days and supernatants are taken at days 3, 5, 7, 9 and 12. PBMC’s will be harvested, washed twice with phosphate-buffered saline (PBS), and pelleted for DNA extraction using the QIAamp DNA Blood Mini Kit, according to the manufacturer's protocol. Virus production in culture fluids are monitored by both RT-PCR and SIV p27 antigen capture assay (Coulter Immunotech Inc., Westbrook, ME, U.S.A.).
Single Genome Amplification of SIV Envelope

Viral cDNA is diluted in 96-well plates to yield fewer than 30% of positive wells for amplification to ensure that positive amplifications are a result of a single cDNA. The first round of PCR is carried out in a reaction mixture containing the following: 1X buffer (Platinum Taq HF Kit, Invitrogen), 0.2 mM dNTP mix, 2 mM Mg$_2$SO$_4$, 0.2 μM primer OF6207 (5’ – GGGTAGTGGAGGTTCTGGAAG – 3’), 0.2 μM primer OR9608 (5’ – CTCATCTGATACATTTACG – 3’), and 0.025 units of Platinum Taq High Fidelity polymerase to give a total volume of 20 μL for each reaction. The PCR mixtures are then loaded into MicroAmp Optical 96-Well Reaction Plates (Applied Biosciences) and the conditions for the first round of PCR are programmed as follows: 5 minutes at 94 ºC, 35 cycles of 15 seconds at 94 ºC, 30 seconds at 52 ºC, and 4 minutes and 15 seconds at 68 ºC, followed by a final extension time of 10 minutes at 68 ºC. For the second round of PCR, 2 μL of the first round PCR product was mixed with 1X buffer (Platinum Taq HF Kit, Invitrogen), 0.2 mM dNTP mix, 2 mM Mg$_2$SO$_4$, 0.3 μM primer IF6428 (5’ – CGTGCTATAACACATGCTATTG – 3’), 0.3 μM primer IR9351 (5’ – CCCTACCAAGTCATCATCTTC – 3’), and 0.025 units of Platinum Taq High Fidelity polymerase to give a total volume of 20 μL for each reaction. The PCR conditions for the second round are programmed as follows: 5 minutes at 94 ºC, 45 cycles of 15 seconds at 94 ºC, 30 seconds at 51 ºC, and 3 minutes and 30 seconds at 68 ºC, followed by a final extension time of 10 minutes at 68 ºC. The amplicons from the cDNA dilutions that resulted in less than 30% positive reactions will be sequenced at the Dana-Farber/Harvard Cancer Center DNA Resource Core. Eight sequencing primers spanning the length of the
SIV envelope are used for complete envelope sequencing. For each monkey, between 15-30 SIV envelope sequences are analyzed per time point.

**SIV Envelope Sequence Analysis**

Raw sequencing data will be analyzed utilizing the GeneCodes Sequencher 4.8 DNA sequencing software. Groups of primer sequence fragments are assembled into overlapping contigs utilizing Sequencher’s automatic assembly algorithm. Chromatograms of assembled contigs will be reviewed and manually corrected for all ambiguous bases.

**Bioinformatic Analysis: Alignments and Phylogenetic Analysis**

Edited contig consensus sequences will be exported to the Los Alamos National Laboratory (LANL) for translation. The HIV sequence database translate tool (http://www.hiv.lanl.gov) is also used for further bioinformatic analysis. Nucleotide and translated contig consensus sequences are aligned with the online database EBI Tools ClustalW \(^{60}\) under default conditions. When necessary, nucleotide alignments are manually aligned using the BioEdit Sequence Alignment Editor program to prepare them for phylogenetic analysis.

Edited sequences will also be sent to Dr. Bette Korber of the Los Alamos National Laboratory for phylogenetic analysis. All phylogenetic analysis of nucleotide sequences will be performed using the MEGA 4 evolutionary analysis software package. Evolutionary trees are inferred using the maximum parsimony and neighbor-joining
methods. The bootstrap consensus trees are inferred from 500 replicates and are taken to represent the evolutionary history of all taxa analyzed. Maximum parsimony trees will be obtained using the Close-Neighbor-Interchange method in which the initial trees are obtained with the random addition of sequences (10 replicates). All positions containing gaps and missing data will be eliminated from the datasets.
RESULTS

_Vaccine targeting of PCS controls SIV in Cynomolgus macaques following experimental challenge with SIV._

As classical vaccine approaches to elicit either T cell or antibody responses against HIV-1 have so far produced no or modest effects, new approaches are necessitated. The immunogenicity of the sequences around the protease cleavage sites (PCS) and the population coverage for a vaccine targeting HIV-1 PCS has been investigated\(^1\). In the pilot study, sequences around PCS were assessed as targets for the development of a new vaccine strategy using *Cynomolgus macaques* from the Philippines and SIVmac239 as an experimental model. The regimens have included vaccine immunogens that are cocktails of 20mer peptides overlapping the 12-protease cleavage sites (PCS).

Both plasma SIV RNA levels and CD4+ T cell counts were monitored in this cohort of cynomolgus monkeys through 20 weeks after SIVmac239 challenge. Plasma virus RNA levels looked similar between the vaccine and control group, and this observation is mostly due to higher dose of SIVmac239 used to infect the vaccine group (**Figure 3A**). Despite there being no obvious differences in the plasma virus RNA level between the vaccine and control groups, a comparison of the total numbers of CD4+ T cells between two groups showed that the vaccinated group maintained higher CD4+ counts. In the vaccine group, 6 out of 11 animals were able to maintain their CD4+ T cell levels until the end point of the study, while 4 of the 5 monkeys in the control group showed a decline in CD4+ T cell count during the progression of the study (**Figure 3B**,
Table 2). This maintenance of CD4+ T cells indicates that the virus was not successful in continuing to destroy the immune system via killing the CD4+ T cells. It shows that the vaccine might have produced a specific immune pressure to generate a mutation that makes the virus less fit and renders it unable to reproduce infectious particles.

Figure 3. The kinetics of plasma SIV RNA and total CD4+ T cell counts following SIVmac239 infection. (A) Plasma virus RNA levels were assessed in cynomolgus monkeys between weeks 1 to 20 after challenging with SIVmac239. Log_{10} SIV RNA copies/mL for each monkey in both the vaccine (red) and control (blue) groups are shown. Median viral loads in both the vaccine (black solid) and control (black dashed) groups are also shown. (B) Changes in CD4+ T cell counts for each monkey in both the vaccine (red) and control (blue) groups over time were plotted. Changes in the number of CD4+ T cell counts (ΔCD4+ T cells) were calculated by subtracting the absolute CD4+ T cell counts of the following week from those of the previous week.
Table 2. Change in total CD4+ T cell counts in *Cynomolgus macaques* following infection. Monkeys in the cohort are shown in 2 groups: one group of animals that showed a decline in total CD4+ T cell counts and the other group of animals that maintained CD4+ T cell counts.

This vaccine modality was also found to generate antibody and T cell responses in the macaques. The antibody response to the peptides overlapping the PCS is correlated with a reduction in viral load during the acute phase of SIV infection, while long-term durable control during the chronic stages, for example the set-point, is less pronounced (Figure 4A, 4B). Furthermore, vaccine-elicited control of viral load was maintained over the course of 20 weeks post-challenge, which is shown by significant associations between the vaccine elicited antibody response and an area-under-the curve calculation for the plasma SIV RNA levels between week 1 and 20 following infection (Figure 4C, Table 3).
**Figure 4.** Correlation between vaccine-induced immune responses and plasma SIV RNA levels in the vaccine group. Correlation matrices were generated using the Spearman nonparametric correlation to compute the correlation coefficients for each pair of variables including plasma antibody and Elispot responses to the number of PCS peptides and plasma SIV RNA levels in the vaccine group. The plasma SIV RNA levels were assessed on 2-4 weeks and 9-14 weeks following challenge, representing peak (A) and set point (B) viral load, respectively. Area-under-the-curve calculations (C) for the plasma SIV RNA levels were also assessed in these monkeys.
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**Table 3.** Correlation between vaccine-induced immune responses and plasma viral load in the vaccine group. 1) The Spearman correlation coefficients ($\rho$) are shown. 2) Exact P values for nonparametric Spearman correlation are also shown.

*Amino acid mutations in sequences around the PCS sites and correlations with plasma virus RNA levels*

*Figure 5* shows the locations of the twelve protease cleavage sites located at specific locations across the HIV-1 genome. Five cleavage sites are found on the Pr55Gag protein, six cleavage sites are found on the GagPol protein, and one cleavage site is found on the Nef protein.
Figure 5. A schematic diagram illustrating landmarks of the HIV-1 genome and its 12 proteolytic cleavage sites. Twelve unique cleavage sites between Gag, Gag-Pol and Nef precursor polyproteins are designated.

Viral sequence analysis revealed extensive mutations around PCS regions in both vaccinated and control monkeys over 20 weeks after infection (Figure 6). Comparisons of the PCS mutation frequencies were conducted at each time point between the vaccine and control groups. Evaluations of the percentage of PCS mutation were carried out using data during the MID periods. Two out of the twelve cleavage sites, PCS2 and PCS12, were found to have amino acid mutation frequencies of greater than 10% in response to the vaccine treatment. Figure 7 depicts the percentage of each mutation found on PCS2 and PCS12. Specifically, virus recovered from the vaccine group showed significantly higher frequencies of mutation in PCS12 region ($P=0.009$), which corresponds to a
change from glycine to either arginine or glutamic acid compared to the mutation frequencies in the control group. Amino acid mutation in PCS2 (-8) was associated with moderate yet significant increase of viral replication. However, amino acid mutation in PCS12 (-8) displayed a negative correlation with the plasma viral load (Figure 8). At the time-points when the frequency of the PCS mutation got greater, the plasma viral load was lower compared to the plasma viral load at the time-points when the PCS mutation frequencies are lower.
**Figure 6.** The major PCS amino acid mutations of plasma SIVmac239 in a cohort of monkeys. The frequencies of each PCS amino acid mutation following infection with SIVmac239 in the vaccine (**A**) and control (**B**) groups are shown in various colored solid lines and corresponding plasma SIV RNA copies are shown in the black solid line.
**Figure 7.** Comparison of the major PCS amino acid mutation frequencies between the vaccine and control groups. The total sum of frequencies found in the major PCS were compared. The amino acid encoded by wild type SIVmac239 (black) precedes the dash, which is followed by the amino acid encoded by the mutation (red). The comparison of the values from the groups of animals was determined using a non-parametric one-way ANOVA Kruskal-Wallis test with Dunn's multiple comparison test or the Mann-Whitney test. The amino acid mutations less than 10% in other PCS sites were not included in the analysis.
Figure 8. Correlation between the major PCS amino acid mutation and plasma viral load in the vaccine group. Correlation matrices were generated using Spearman nonparametric correlation to compute the correlation coefficient for each pair of variables including PCS amino acid mutations (PCS2: -8, -7, and -6 and PCS12: -8) and plasma SIV RNA levels in the vaccine group.

Construction of major PCS amino acid mutations

To introduce point mutations into each PCS region, the fragment between the BamHI and SphI sites, and the SphI and XhoI was subcloned into the pSP73 vector to generate a clone termed pSP73-PCS2, and the fragment between the SphI and XhoI sites was subcloned into the pSP73 vector to generate the clone termed pSP73-PCS12 (Figure 9A). The Quick Change site-directed mutagenesis kit (Stratagene, La Jolla, Calif.) was used to introduce PCS2 (-8, -7 and -6) and PCS12 (-8) point mutations procedures described by manufacturer (Figure 9B). The presence of all point mutations was confirmed by direct sequencing (Figure 9C). The BamHI-SphI fragment was cloned
back into SIVmac239 to generate the SIVmac239-PCS2 mutant clones; the SphI-XhoI fragment was cloned into the SIVmac239 to generate both SIVmac239-PCS12 (-8R) and SIVmac239-PCS12 (-8E) clones (Figure 9D). All SIVmac239 PCS mutants generated in the study are listed in Table 4.

Infectious virus stocks were prepared by transient transfection of 293T cells with SIVmac239 wild type or each PCS mutant plasmid as described above using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions.

A

SIVmac239 pSP73 Ligation

B

1. Mutant Strand Synthesis
   Perform thermal cycling to:
   - Denature DNA template
   - Anneal mutagenic primers containing desired mutation
   - Extend and incorporate primers with high-fidelity DNA polymerase

2. DpnI Digestion of Template
   Digest parental methylated and hemmethylated DNA with DpnI

3. Transformation
   Transform mutated molecule into competent cells for nick repair
Figure 9. Construction of major PCS amino acid mutation. (A) Digestion of both SIVmac239 and pSP73 vector with BamHI and SpH1 or SpH1 and XhoI followed by ligation using T4 DNA ligase to create pSP73-PCS2 and pSP73-PCS12, respectively. (B) Site-directed mutagenesis strategy to create the point mutations. (C) Sequencing alignments to confirm the successful introduction of each individual mutant into the 5' SIVmac239. A) PCS2-8, B) PCS2-7, C) PCS2-6, D) PCS12-8E, E) PCS12-8R. (D) Full length SIVmac239 PCS mutant clones.
Table 4. SIVmac239 PCS region mutant clones generated in the current study and the major amino acid mutation in PCS region. The amino acids encoded by wild type SIVmac239 are shown in black and the amino acid mutations generated as described in materials and methods are shown in red.

Future Work - Competitive Fitness Assay

To continue the evaluation of the PCS vaccine on SIVmac239 fitness, competitive fitness assays should be carried out as shown in Figure 10 and Figure 11. The details of how the experiments will be conducted are also described under the Methods section. Due to the highly frequent mutations that occur in SIV, it is important to understand how a mutation at a critical site can affect a virus’s ability to replicate and survive. To design a viral fitness assay, many factors must be taken into account (Figure 10).
A typical set up for a growth competition experiment is displayed in Figure 11. The experiment involves dual infections with a control virus, commonly the wild-type virus, and an experimental virus, for example, a mutated virus. The infections are carried out with different multiplicities of infection and are compared to the positive controls (wells I, V) and the negative control (well VI)\(^6\). Depending on the fitness of the two viruses in the study, one may be selected over the other indicating a greater fitness and likelihood of survival.

**Figure 10.** Practical considerations in the design of a competitive viral fitness assay. Schematic representation of each of 5 aspect to be considered in the selection of them most appropriate assay conditions including Culture environment 1, Virus isolate 2, Inoculum titration 3, Assay specifics 4, and Experimental endpoints 5. (Adapted from Quinones-Mateau and Arts 2006)\(^6\)
Figure 11. General setup of a virus competition assay. Fitness (W) can be calculated by the proportion of virus in a dual infection ($f_o$) divided by its initial level in the inoculum ($i_o$). The relative fitness difference ($W_{\text{diff}}$) can be determined by the ratio of the more fit ($W_M$) and less fit ($W_L$) virus. (Figure adapted from Quinones-Mateau and Arts, 2000)\textsuperscript{62}.
DISCUSSION

The advent of potent combination antiretroviral therapy (cART) has dramatically improved the quality of life and life expectancy of millions of HIV-infected individuals. HIV-1 infected patients could maintain near undetectable levels of viremia, if they have access to these medications and are motivated to take them daily for life. However, as antiretroviral drugs could not eradicate a stable latent reservoir in resting CD4+ memory cells that harbors virus that are refractory to antiretroviral drugs and HIV specific immune responses, providing a long-term archive of replication-competent virus. Moreover, even for every individual who gains access antiretroviral drugs, two to three individuals become newly infected. Thus, efforts to reduce or eradicate HIV reservoir are now centered on a new strategy aimed perturbation of the virus reservoir, followed by rapid removal of infected cells \(^6\). To augment the capacity of the host to eliminate latently infected cells after activation by latency-reversing agents, several immunologic strategies have been explored including 1) therapeutic vaccines, 2) monoclonal antibodies, and 3) inhibitors of immune check point molecules such as PD-1, CTLA-4, LAG-3 and TIM-3.

With the goal of eliciting virus-specific immune responses, a safe, effective and affordable vaccine is highly needed to counter the tide of new infections (i.e. prophylactic), accelerate the decay of the reservoir and improve the control of viral rebound after the interruption of cART (i.e. therapeutic). Unfortunately, the classical vaccine approach to elicit either T cell or antibody responses against HIV-1 has failed so far or produced a modest effect. Several novel vaccines have currently been explored or
are being evaluated in clinical trials within the next several years. Among such strategies including CMV vectors, AD26 prime and modified vaccinia Ankara (MVA) boost regimens, CMV vectors led to induce broad cellular immune responses and apparent clearance of SIV in about 50% of vaccinated monkeys\textsuperscript{65,66}.

The recent study showed that the sequences around the PCS are very immunogenic and could be viable vaccine targets\textsuperscript{61}. HIV-1 protease has been the major therapeutic target against AIDS due to its essential role in the production of infectious virions. The process of protease cleavage requires a tightly controlled, ordered sequence of proteolytic processing events mediated by different rates of cleavage at the different processing sites. Even the subtle disturbances may be sufficient to interrupt this delicately balanced process and drive it toward producing non-productive virions. Therefore, vaccine targets the 12 protease cleavage sites would yield major advantages as these cleavage sites are highly conserved among major subtypes of HIV-1. To test this hypothesis, preclinical animal studies has been conducted to test whether a vaccine generating focused immune responses to the 12 20-amino acid peptides overlapping the 12 protease cleavage sites can offer protection against HIV infection.

The preliminary data from the preclinical animal studies showed that two cleavage sites among 12 PCS, PCS2 and PCS12, have shown amino acid mutation frequencies greater than 10 % in the vaccine group. Mutations at protease cleavage sites might occur due to immunologic pressures induced by the vaccine as described above. Specifically, higher frequencies of mutation in PCS12 region found in the vaccine group were significantly correlated with the lower plasma viral RNA levels. Amino acid
mutation in PCS2 (-8) was associated with moderate yet significant increase of viral replication. However, to correlate these virologic measurements with the protection of 12-PCS vaccine, whether these mutations change viral fitness and its infectivity is yet to be determined. Experiments were designed and conducted to determine the virologic correlates of such protection of a PCS based vaccine using viral fitness analysis of major PCS mutations in SIVmac239 in vitro. We were able to construct SIVmac239 clones with five single amino acid mutation found in PCS2 (-8, -7 and -6) and PCS12 (-8) regions.

When determining how to construct the SIVmac239 clones, it was important to choose restriction enzymes that only had a single cut location. These enzymes should cleave both the plasmid vector and the full-length virus at specific locations to isolate the PCS2 and PCS12 areas. During the initial construction, the restriction enzymes were not correctly chosen and as a result, transformation of the SIVmac239 clones into competent cells was unsuccessful. The first set of enzymes used to construct the PCS2 and PCS12 mutants consisted of EcoRI + SspI and ClaI + XhoI, respectively. However, it was discovered that both EcoRI and ClaI recognize multiple cut site on the SIVmac239 backbone and within the insert. We re-analyzed the restriction map for both SIVmac239 and the plasmid vector pSP73 and determined that the combination of restriction enzymes that should be used were BamHI + SphI and SphI + XhoI for PCS2 and PCS12, respectively.

Further studies will include SIVmac239 clones with multiple permutations to further investigate the effects of how altering the HIV protease cleaving mechanism can aid in destroying the virus’s ability to survive. This information will provide important
information to aid the development of an effective preventative HIV-1 vaccine targeting PCS regions.
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CURRICULUM VITAE

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Year of Birth: 1990

EDUCATION

Boston University School of Medicine, Boston, MA | 2013 – present
Master of Science in Medical Sciences

Georgia Institute of Technology, Atlanta, GA | 2008 – 2012
Bachelor of Science in Biochemistry
Spanish Language Minor
Georgia Tech Honors Program

SKILLS

Languages
Native English speaker
Fluent in written and spoken Spanish
Conversationally fluent in Mandarin Chinese and Taiwanese

PUBLICATIONS


RESEARCH EXPERIENCE

Beth Israel Deaconess Medical Center, Boston, MA | August 2014 – present
Graduate Research Student, Center for Virology and Vaccine Research
• Conducted research for my Masters thesis with Dr. James B. Whitney
• Evaluated the use of HIV’s protease cleavage sites for the development of a vaccine treatment

Defense Forensic Science Center (DFSC), Atlanta, GA | September 2012 – May 2013
Forensic Science Intern
• Completed an independent research project investigating the effects of latent print development techniques on the recovery of touch DNA
• Submitted manuscript for DFSC internal review and possible journal submission
Children’s Healthcare of Atlanta, Atlanta GA | November 2012 – July 2013
Research Intern
- Worked on a project evaluating the viability of stem cells for bone marrow transplants after long term cryostorage
- Assisted in sample processing and organization of sample repositories for clinical trials from a laboratory standpoint

Children’s Healthcare of Atlanta, Atlanta, GA | June 2009 – August 2009
Pathology Department Summer Intern
- Published paper after researching and reviewing journal articles

Center for Advanced Brain Imaging, Atlanta, GA | August 2010 – September 2011
Undergraduate Research Assistant
- Helped run functional magnetic resonance imagining tests on participants to evaluate the responses to tactile stimuli of vibration intensity and timing

OTHER WORK EXPERIENCE

Beantown Physio, Inc., Boston, MA | August 2014 – present
Front Desk Coordinator
- Serve as the first contact point to meet and greet patients in the office
- Perform administrative duties including scheduling appointments, contacting insurance companies and balancing payments

LEADERSHIP EXPERIENCE

Georgia Tech Women’s Ultimate Frisbee Club Team | August 2009 – May 2012
President
- Communicated with the Georgia Tech Sports Club director and the Student Government Association to ensure details of practice, travel, and funding were kept up with in an organized manner
- Discussed with the coaches and other college ultimate teams in the Southeast to decide and choose which tournaments to attend during the season

Georgia Tech Trailblazers
Secretary and Treasurer | May 2009 – May 2010
- Organized and took meeting minutes
- Was responsible for the financial duties of the organization
Vice President of Alternative Breaks and Trip Leader | May 2010 – May 2012
• In charge of overseeing the planning of all fall and spring alternative break trips
• Worked closely with not only other members on the alternative breaks committee, but also members from the National Park and Forest Services to plan environmental awareness and community service trips
• Planned and led two alternative break trips to Virginia and South Carolina

Vice President of Local Projects | May 2011 – May 2012
• Managed a team of four local project committee members and planned monthly trips to help give back to the environment and community in and around the Atlanta/North Georgia area

Georgia Tech Honors Program Planning Committee | May 2009 – October 2009
Fall Retreat Planning Committee
• Helped to organize the annual GT Honors Program fall retreat for incoming freshman at a local state park in Georgia.

VOLUNTEER EXPERIENCE

Rosie’s Place, Boston, MA | January 2013 – present
• Serve as a volunteer in the dining room/kitchen or in the food pantry to assist and provide help to homeless women and children in the Boston area

Georgia Tech Alumni Association, Boston, MA | October 2014 – present
Boston Network, Philanthropy Chair
• Organized community service events for the Georgia Tech Alumni Association, including the 2014 TEAM Buzz event with The Food Project, a local urban farm, and the Annual Earth Day Charles River Clean Up

Camp Braveheart, Camp Twin Lakes, Atlanta, GA | May 25-31, 2013
Cabin Counselor
• Volunteered as a camp counselor at Camp Braveheart, a camp for children who have had a heart transplant or have a congenital heart disease
• Responsibilities included not only the traditional camp duties, but serving as a role model for the girls in my cabin

Children’s Healthcare of Atlanta, Atlanta, GA | Fall 2009 – Spring 2011
Hospital College Volunteer
• Volunteered in the operating room restocking supplies and preparing operating rooms
• Volunteered in the emergency department by interacting with patients and families to help pass their time spend in the hospital