

HIV gp120 and gp41 Mutation-Array in Measles Chimera, an HIV Vaccine Study

Background and Introduction

The HIV-1 lentivirus (family: retroviridae) is one of the deadliest viruses known to mankind. This pathogen is generally spread via sexual intercourse, however it can also be transmitted intravenously (i.e. drug use or transfusion), and vertically. It is believed that the virus crossed the species barrier from chimpanzees into humans in Africa sometime in the first half of the 20th century. Although the exact cause of the jump is unknown, scientists have many hypothesis: some believe that SIV was able to infect humans from sexual intercourse with chimpanzees; some scientists believe that the consumption of primate flesh, which is practiced in tribes in Africa, is the cause of the crossing of species. The HIV pandemic began in the 1980's, when the virus began to disseminate around the globe; it was not until this time that scientists began to realize the gravity of this immuno-compromising pathogen. By the year 2000, it was estimated that approximately 36.1 million people had been living with HIV/AIDS; there were approximately 5.3 million new infections every year; and this virus had claimed the lives of approximately 21.8 million people (CDC). Sub-Saharan Africa, an epicenter for HIV has felt the devastation of HIV more than any other continent. – nearly two-thirds of all HIV infected people live in this region of the world (CDC). Currently, there is no known effective vaccination against HIV. However, by analyzing HIV virulence factors, it is plausible that research may elicit the development of a vaccination against HIV, which may utilize a recombinant measles virus (rMV) in order to develop an adaptive immune response in uninfected (HIV-) hosts.

The HIV genome is approximately 9.1kb in size (Zheng, et.al). There are four main genes, in addition to a number of accessory genes. The *gag* and *env* genes are translated into structural and cell surface proteins, respectively; the *pro* and *pol* genes are necessary for infection and replication. Cumulatively, the main and accessory genes are a topic for antiviral drug research. Some prerequisite information regarding viral replication and virulence factors must be acknowledged in order to synthesize an effective vaccination.

The *gag* gene in the HIV genome encodes for viral structural proteins; these proteins are required for the assembly and morphogenesis of mature virions. The translated Gag proteins undergo proteolytic cleavage via viral protease, which manifests the creation of: matrix proteins (p17), capsid proteins (p24), nucleocapsid proteins (p7) and an additional protein (p6). In mature virions, p24 molecules bind to form a cone-shaped core that invaginates viral gRNA, p7, integrase and other proteins; this core is typically comprised of 1,000-1,500 p24 molecules, and some virions may actually have more than one core (Briggs, et.al). The p17 proteins form the viral matrix, which is ultimately encompassed by a lipid bilayer (envelope) upon budding.

The *pro* gene is translated into viral protease. This enzyme is responsible for cleaving monocistronic gene products into functional peptides. For example, viral protease cleaves the Gag protein into the aforementioned structural proteins, as well as the Env protein into gp41 and gp120. Although it would not be feasible to incorporate the *pro* gene into a chimera genome in order to elicit an adaptive immune response, HIV protease is a target for many antiviral drugs. Mutations in *pro* can occur via the selective pressure of protease inhibitors (Hickman, et.al.). Typically, HIV *pro* is highly conserved relative to other viral genes, however synthetically lethal mutations may be a means of controlling the progress of infection.

The *pol* gene encodes for three viral enzymes: reverse transcriptase, integrase and RNase-H. In HIV, the *gag*, *pro* and *pol* genes overlap, thus forming a genomic complex that has evolved in order to ensure a higher ratio of Gag proteins to protease and Pol proteins. Transcription of the *pol* gene requires a -1 ribosomal frameshift at a UUA codon in *gag* that is slightly upstream of a pseudoknot, or a stop codon read through at the adjoining end of *gag* (Vickers and Ecker). It is plausible that antiviral drugs that have an effect on the structure of the pseudoknot may inhibit viral enzyme translation. Reverse transcriptase is responsible for synthesizing dsDNA from ssRNA inside the host cell. Integrase is the viral enzyme required to lysogenically insert the dsDNA into the host genome randomly; RNase H is an enzyme that aids in this process.

The *env* gene in the HIV-1 genome is perhaps the most researched, in that it encodes for glycoproteins necessary for fusion to leukocyte membranes, which are potential targets for antiviral drugs, as well as vaccinations. The Env proteins, specifically gp120 and gp41, are viral surface glycoproteins that are derived from the proteolytic cleavage of the gp160 moiety. HIV-1 genes have high mutation frequencies due to rapid replication and poor fidelity of reverse transcriptase and host RNA Pol II due to a lack of proof-reading capability (Hickman, et.al.). Whereas other regions of the HIV genome are comparatively conserved (i.e. the *pro* and *pol* loci), the *env* locus has a high annual rate of change. This gene undergoes a mutative rate of approximately 2.5% per year; mutations most commonly occur within the hypervariable regions, and are driven by immune selection (Strauss). Unfortunately, this presents a problem for vaccine development, in that developing epitope-specific antibodies is difficult due to the said antigenic shift.

The trimeric envelope complex, also known as the Env protein, is primarily responsible for binding to leukocytes: CD4⁺ macrophages and T-cells are the cellular targets for HIV-1. The Env receptor is comprised of three gp120 molecules that are attached to three gp41 molecules, which are anchored into the membrane; the gp120 antigen elicits infection by binding to CD4 cellular antigens. After binding, gp120 undergoes a conformational change, which exposes the V3 loop of the glycoprotein; the V3 loop (that is typically shielded) successively binds a chemokine receptor (typically CCR5, a tyrosine-rich protein), which is necessary for viral entry into T-cells (Choe,

et.al.). The gp120 antigen is a typical target for vaccine developers, because neutralizing its epitopes would prevent infection.

However, antibodies typically target the immunodominant V3 co-receptor on gp120, and neutralizing antibodies that bind to this site can not control infection (Collado, et.al). Empirical evidence illustrates two means of circumventing this problem when developing a vaccination: a.) develop antibodies that have sulfated tyrosines in the CDR3 heavy chain, which are more potent at neutralizing this antigen (Choe, et.al); or b.) when using a recombinant chimera virus to elicit an adaptive immune response in a host, flank the *env* gene with proteomic genes under the same promoter that will mask the immunodominant epitope upon translation, so synthesized antibodies will respond to other epitopes (such as V1 or V2) on gp120, which would be more highly neutralizing (Collado, et.al.). Since the gp120 glycoprotein is possibly the most important surface protein for HIV-1 infectivity, developing a means to subvert this protein from effective binding, despite the fact that it undergoes regular antigenic shift, may be the key to preventing infection.

The gp41 glycoprotein can be referred to as the transmembrane glycoprotein, due to its proximal location within the HIV-1 virion. As mentioned before the HIV receptor protein (Env) is comprised of three anchored gp41 moities bound to three surface gp120 moities. Gp41 antigens are coiled-coil trimers comprised of an N51 protein binding domain, to which three C43 helices are bound (Chan, et. al). After initial docking to a cellular CD4 antigen, gp120 antigens fall off, thus exposing the gp41 antigens. These exposed antigens promote membrane fusion between HIV and the target cell, thus allowing for the intrusion of the viral capsids into the host cytoplasm. It is possible for neutralizing antibodies to bind the exposed gp41 region after disassociation of gp120 antigens, which can only be weakly, and ineffectively neutralized (Chan and Kim). However, this method of immunization is not highly effective due to the short length of time these antigens are bindable, due to gp120 interference.

After injection into the host cell cytoplasm, the enzyme reverse transcriptase begins copying the ssRNA genome into dsDNA. This forms the pre-integration complex, which is composed of dsDNA, integrase, p17, Vpr, reverse transcriptase and a DNA binding protein known as HMGI; this complex enters the host cell nucleus via nuclear localization signals in the complex proteins (Zheng, et.al). Afterwards, integrase lysogenically incorporates the dsDNA into the host cell genome. Host cell transcription machinery transcribes viral mRNA, which is eventually translated into the aforementioned HIV proteins (Env, Pol, Gag, etc.), or attached by p24 capsid proteins; spliced mRNA is translated into accessory proteins beforehand (i.e. Rev), which regulate transcription. The Gag proteins manifest viral assembly within the host cell. Env proteins are expressed on the host cell surface after traversing the golgi apparatus. Immature assembled virions acquire these receptors after exocytotic budding from the host cell, which is how the HIV envelope is formed.

Vaccine Description

The chimeric vaccine will be comprised of an attenuated measles virus, specifically containing a functional hemagglutinin surface protein to associate with CD46, CD150, and the C-type lectin DC-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN), recently discovered to be important in measles adherence and stabilization during entry into immature dendritic cells (de Witte, et al.) Included into the measles virus will be mutational variants of the HIV gp120 and gp41 genes, located downstream of measles virion packaging signals and within insertional sequences used to insert the gene into the host cell chromosome.

The hypervariable regions of the gp120 and gp41 proteins are the best at eliciting an immune response and are available for antibody-mediated immunity. The problem comes in that being the hypervariable region, escape variants emerge that are unrecognizable to the immune response mounted by the host. It takes time for an appropriate T cell to recognize this variant, and therefore the infection continues. By administering a vaccine comprised of a wide array of mutationally varied gp120 and gp41, it is hoped that a large fraction of this variability will be addressed, allowing the immune system to recognize these antigens during vaccination, rather than during actual HIV infection.

To create the array of gp120 and gp41 proteins, these genes are ligated into a plasmid. PCR is done using a primer with flanking sequences complimentary to the HIV gene, but containing non-matching bases in the middle to introduce a mutation at the nucleotide level. The PCR-amplified plasmid is sequenced to confirm introduction of the mutation, after which a co-transfection/infection into dendritic cells *in vitro*, of the plasmid with the attenuated measles virus is allowed to occur. After infection, measles virions are isolated and screened for addition of the chimeric gp120 and gp41 genes. Virions containing both chimeric genes are grown in culture into a heterogeneous virus population. These chimeric virions make up the array used in the vaccine.

Adjuvants:

Along with the antigen in the vaccine, adjuvants are added to enhance the immune response produced by the antigen. The main purpose of adjuvants is to attract and activate dendritic cells. However, other purposes are to increase immunological half-life of the antigen, improve antigen delivery to antigen presenting cells (APC) as well as their processivity and delivery by the APC, and increase cytokine production are also crucial to improve the immune response. Molecular adjuvants are used as a part of this vaccine. To produce a more effective response in an enveloped virus vaccine, a triple combination of adjuvants, GM-CSF, IL-12, and NFS (CD40L), were used. The combinations of these three will activate CD8⁺T cells, and they will increase activation of APCs and their ability to process antigens.

Risks:

Some of the risks involved with the vaccine are possible local reaction at the injection site such as inflammation and/or abscesses. However, some of the risks involve reaction to the adjuvants such as fever, malaria, adjuvant arthritis, or anterior uveitis. These reactions may be due to the interaction between the antigen and the adjuvants.

Immunity Assessment

T cell response

Upon inoculation, the dendritic cell-specific chimera virus will associate with CD46, CD150, and DC-SIGN molecules on the surface of the DC, promoting adsorption and fusion of the viral membrane with that of the host cell. After insertion of the viral genes into the host cell genome, including the HIV gp120 and gp40 chimeric genes, transcription and translation of these viral genes will lead to intracellular degradation of viral proteins via antigenic processing, and expression on the MHC-I complex. This gp120 and gp40-antigen presenting DC will migrate to the lymph nodes, where gp120 and gp40-specific naïve, mature T cell with the correct specific TCR, the CD3/CD8/TCR/MHCI complex will interact while the CD28/B7.1or2 complex will induce costimulation and activation of the naïve T cell. The activated Tc cell will make IL-2 and IL-2R, causing it to divide.

These activated Cytotoxic T cells will migrate into the periphery, finding cells infected with HIV, expressing gp120 and gp40 antigens on MHC-I complexes. Recognition of the specific TCR-Ag-MHC complex, along with other nonspecific cell adhesion molecules, will stimulate apoptosis of the infected cell.

A fraction of the chimera viruses will not fuse to the DC membrane, but instead be taken up by endocytosis and therefore placed in the MHCII pathway. These chimeric gp120 and gp40 surface protein peptide antigens will be presented on MHCII molecules, where naïve, mature Th cells will bind them on their TCR and be stabilized by the CD3/CD4/TCR/MHCII complex while CD28/B7.1or2 complex will activate the naïve T cell. The activated Th cell will make IL-2 and IL-2R, causing it to divide. Specifically of interest is the Th2 cell, for its stimulatory role in humoral immunity.

B cell response

B cells expressing BCRs specific for the HIV gp120 and gp40 envelope proteins will come into contact with extracellular whole virus, or from viral particles released during T-cell mediated death of cells infected by HIV or the chimera virus vaccine. Recognition of the BCR with the chimeric surface proteins will stimulate endocytosis of the BCR-Ag complex, and eventually presentation on MHC-II molecules on the B cell's surface. Activated, mature Th2 cells (as described earlier) will recognize the presentation

of this Ag on the MHC-II and CD40 with the CD40-L on the Th2 cell, stimulate both somatic hypermutation and expansion of the specific B cell and its BCR to make antibody-secreting plasma cells, and IL-4 will be secreted by the Th2 cell, inducing antibody isotype switching and secretion of HIV gp120 or gp40-specific IgG antibodies. These serum antibodies will serve to neutralize extracellular virus so it cannot fuse to susceptible host cells. Opsonization induced by antibody coating can induce phagocytosis and destruction of the virus by macrophages.

Testing

The testing for the efficacy of the vaccine will be carried out in a randomized double blind study. The test participants will be 100 HIV negative (HIV-) volunteers. The participants are then split into 2 groups, 50 injected with the vaccine/adjuvant cocktail, and 50 are injected with a saline solution not containing only the adjuvants.

Timing

Participants are exposed to the vaccine and placebo in the following manner: Initial baseline sample collection and administering of the vaccine is termed “day 0”. The immunological response tests are then carried out 14 days following initial vaccination. Participants are screened every 14 days until day 140, then at day 365 (1 year) and 730 (2 years). If an immune response is detected within the first 140 days, but is diminished at 365, the participant is revaccinated and begins the screening again at day 0.

Problems

The current test of HIV involves taking a serum sample from the individual being tested and then analyze it in one of a few ways.

The primary way of testing is to analyze the blood sample for antibodies against HIV antigen. The primary antigens in which the antibodies tested for react to are the gp120 and the gp41 antibodies. The serum sample is put through an Enzyme-Linked Immunosorbent Assay (ELISA), which in turn detects and measures the levels of antibodies to specific antigen.

The second method is to test for the HIV antigens themselves. The primary target for this test is the protein p24. This antigen provokes a very strong immune response in a new infection. The presence of the p24 antigen is measured by performing flow cytometry.

The third method of HIV testing is the most accurate; the blood sample is analyzed for the presence of HIV genetic material, DNA/RNA.

Due to the nature of this vaccine, the host immune system being exposed to the coat proteins of HIV (gp120 and gp41), and therefore the primary method of testing can

no longer be used to detect actual infection by HIV. This means one of the tests must be used in its place, each of which has its drawbacks.

If p24 detection becomes the mainstream test, it must be taken into account that even though it is quick and inexpensive, the levels of p24 can fall to undetectable levels after the HIV becomes fully established in the body. This can lead to an infected individual being tested after the infection is established, testing negative, and then accidentally transmitting the virus.

The drawback to the genetic material testing is that it is not only time consuming, but it is also extremely expensive when compared to the cost of the other two tests.

Testing Methods:

Flow Cytometry

Flow cytometry, among its many uses, can be used to detect cell-surface proteins specific to certain cell types to measure quantitatively the proliferative effect due to inoculation. Fluorochromes covalently linked to antibodies specific for each of these surface proteins are incubated with a serum sample and passed past a light beam in a single-cell width stream, which will induce fluorescence if the antibody is bound to the cell surface. Multi-color assays can compare multiple surface molecules to determine more specific types of cells or cell actions occurring due to treatment.

Molecules of interest:

CD3 – T cells

CD8 – Tc cells

CD49e – Memory T cells

CD25 – Activated T cells

MHC: Peptide Tetramers (PT) specific to gp41 or gp120

Control – p24 specific MHC: Peptide tetramers

Testing Parameters:

We will be testing by flow cytometry to measure differences in cell counts after inoculation with the vaccine. Baseline measurements of patient cell counts will be done before vaccination to help determine the immune response.

Tests:

CD3+/CD8+ → does the amount of Tc cells increase after inoculation

CD3+/CD4+ → does the amount of Th cells increase after inoculation

CD8+/CD25+/MHC:PT (41 and 120) → is there an increase in activated Tc cells specific for the gp41 and 120 antigens

CD4+/CD25+/MHC:PT (41 and 120) → is there an increase in activated Th cells specific

for the gp41 and 120 antigens
CD8+/CD49e+ → is there an increase in memory Tc cell production after inoculation
CD4+/CD49e+ → is there an increase in memory Th cell production after inoculation
CD49e+/MHC:P (41 and 120) → is there an increase in memory T cell
production after inoculation, specific to gp41 and 120 antigens
CD8+/MHC:PT (24) → does an HIV protein not included in the chimera virus induce
immune response after inoculation? (should be no if a functional control)
CD4+/MHC:PT (24) → does an HIV protein not included in the chimera virus induce
immune response after inoculation? (should be no if a functional control)

ELISA

Enzyme-Linked Immunosorbent Assay can be used to detect increase in cytokines, interleukins, or specific antibodies that would suggest immune recognition and stimulation to the vaccine. For the cytokines and interleukins, the microtiter plate will be treated with an antibody specific to these molecules and blocked with nonspecific proteins. Patient serum samples will be added to the plate, allowed to bind, and finally a second antibody specific to these molecules linked to a chromogenic enzyme will be added complete the antibody sandwich.

Antibody detection by ELISA involves coating the microtiter plate with purified gp120 and gp40 proteins, including many mutational variations to account for the sequence variation in our population of chimeric viruses. Then, patient serum samples will be added and incubated to allow for specific antibodies to bind, if present. Lastly, a chromogenic enzyme-linked anti-Ig antibody, and its chromogen-producing substrate, will be added for detection.

gp120 and gp40-specific IgG antibodies
Interferon gamma
Interferon alpha
Interferon beta
IL-2 (T cell proliferation)
IL-4 (B cell activator)
IL-6 (T and B cell proliferation)
IL-23 (memory T cell development)
IL-27 (IL-12R, T cell inducer)

References

Briggs, John A G, Martha N Simon, et.al. "The stoichiometry of Gag protein in HIV-1."
Nature Structural and Molecular Biology 11(2004): 672-675

- Chan, David C, Deborah Fass, et.al. "Core Structure of gp41 from the HIV Envelope Glycoprotein." *Cell*. 89(1997): 263-273.
- Chan , David C , and Peter S Kim. "HIV Entry and Its Inhibition." *Cell*. 93(1998): 681-684.
- Choe, Hyeryun, Wenhui Li, et.al. "Tyrosine Sulfation of Human Antibodies Contributes to Recognition of the CCR5 Binding Region of HIV-1 gp120." *Cell*. 114(2003): 161-170.
- Collado, Manuel, Dolores Rodriguez, et.al. "Chimeras between the human immunodeficiency virus (HIV-1) Env and vaccinia virus immunogenic proteins p14 and p39 generate in mice broadly reactive antibodies and specific activation of CD8+ T cell responses to Env ." *Vaccine*. 18(2000): 3123-3133.
- De Witte, Lot, et al. *Measles Virus Targets DC-SIGN To Enhance Dendritic Cell Infection*. *J Virol*. 80(Apr 2006): 3477-3486.
- Hickman, Peter J, Janet E Leigh, et.al. "Oropharyngeal candidiasis in HIV+ patients may influence the selection of HIV-1 protease variants." *Virus Research*. 87(2002): 97-106.
- HIV/Aids Surveillance Report." Center for Disease Control. June 2000. 4 Apr 2007 <<http://www.cdc.gov/hiv/topics/surveillance/resources/reports/pdf/hasr1201.pdf>>.
- Vickers, T A, and DJ Ecker. "Enhancement of Ribosomal Frameshifting by Oligonucleotides Targeted the HIV gag-pol Region." *Nucleic Acids Research*. 20(1992): 3945-3953.
- Strauss, Ellen G, and James H Strauss. *Viruses and Human Disease*. 1st ed . New York: Academic Press, 2001.
- Zheng, Yong-Hui, Nika Lovstin, and B. Matija Peterlin. "Newly identified host factors modulate HIV replication." *Immunology Letters*. 97(2005): 225-234 .