



Decelerating HIV-1 Viral Escape by Inhibiting Entry into CD4 T Cells

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Abstract

Decelerating the viral escape of HIV-1 could serve to provide more time for antibodies and vaccines to neutralize the virus. This review attempts to advocate for an inclusion of antiviral methods in a vaccine design that can prolong the viral escape of HIV-1. Delaying viral escape can enhance the effectiveness of vaccination. The gene editing of T cells through CCR5 disruption can lower the frequency of infection. A lesser rate of infection due to CCR5 disruption can reduce the rate of viral escape because according to Yang et al., the rate of viral escape is directly proportional to the T cell response. If HIV-1 is prevented from entering and infecting resistant CD4 T cells, viral escape can be delayed. A CD4 T cell entry inhibitor called eCD4-Ig can trap the HIV virus by mimicking the CD4 T cell receptor and then lock in the virus. HIV cannot completely escape from eCD4-Ig as it lowers the viral escape of the virus. This delay may offer more time for antivirals, vaccines, and other innate immune responses to recognize and neutralize the virus. The time of the initial HIV-1 infection plays a major role in the efficacy of antivirals and determines the kinetics of HIV-1 viral escape. Combining gene disruption of CCR5 and a eCD4-Ig HIV-1 entry inhibitor with vaccines may help to delay viral escape and control HIV-1 replication.

Keywords

HIV-1; CCR5; T Cells; Viral Escape; Entry Inhibitors; Glycoproteins

Introduction

HIV was first discovered and made aware of 30 years ago. One million deaths were reported in 2015 [1]. Only two individuals have been cured. An HIV-positive patient was engrafted with a bone marrow transplant as treatment for his leukemia. The bone marrow transplant had a mutation in the C-C chemokine receptor 5, CCR5 gene loci. This mutation was a 32 base pair deletion in the CCR5 loci [1]. This disrupted CCR5 suppresses the initial stages for entrance of the virus into CD4 T cells that can lessen the infection. Ten years after his transplant, he remains without HIV infection or its complications. This has spun numerous methods to target and block the expression of CCR5. The life cycle of HIV-1 includes, fusion with the host cell membrane, a release of its genome into the host cell, reverse transcription of its RNA into DNA, integration of HIV-DNA into the host genome, synthesis of viral proteins, assembly, budding, and its exit from the host cell.

HIV Life Cycle HIV-1 binds to the CD4 receptor via the CCR5 co-receptor. After initial attachment, the HIV virus fuses with the plasma membrane of the T cell, and then the virus secretes its viral RNA into the cytoplasm of the host cell. The viral RNA is reverse transcribed into viral DNA that then integrates into the host cell genome. The viral DNA is transcribed and translated into the proviral proteins needed for the outer coat, capsid core, and the glycoproteins. After each proviral DNA is translated, the newly formed proteins enter into a process of assembly. HIV-1 buds from the T cell and then is released from the host cell (Figure 1).

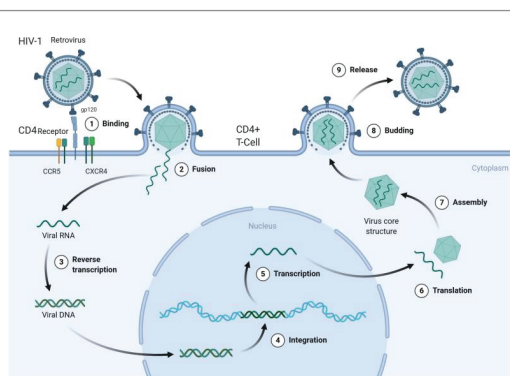


Figure 1: HIV Life Cycle

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The C-C chemokine receptor 5, CCR5, is a coreceptor from CD4 T cells that allows HIV viruses to enter these cells. The HIV glycoproteins bind to CCR5; thus, CCR5 is a favorable target for many forms of therapies specifically for gene disruption. Mega nuclease transcription activator-like (megaTAL) were used to target CCR5 and cleave this site. About 70% to 90% of CD4+ T cells showed CCR5 disruption [2]. To only select CCR5-edited T cells a drug-resistant gene was added and coupled with CCR5-disruption. The drug methotrexate or MTX was used during the chemoselection process for screening mutant human dihydrofolate reductase or mDHFR to determine the cells resistant to MTX. CD4+ T cells resistant to MTX were also found to lack CCR5 expression.

HIV-1 has a glycoprotein positioned on its outer envelope that mediates with the fusion of the virus to host cell membranes. The virus can then enter the host cells. The envelope glycoprotein or Env of gp160 is cut into two fragments that interact called gp120 that binds the host cell receptor and gp41 needed for fusion. When each fragment is replicated three times, a viral spike including gp120 and gp41 is generated. A gp120 binds to the CD4 receptor and a co-receptor CCR5 or CXCR4 that cause many changes in conformation. These changes in conformation affect and initiate gp120 to disconnect and cause folding of gp41. The gp41 develops into reassortments that link the viral membrane with the host cell membrane, causing fusion. HIV-1 gp120 binds to the CD4 binding site and the gp41 binds with its membrane proximal external region (MPER) that has glycan independent epitopes. However, the gp120 has glycan dependent epitopes on its V1/V2 and V3 sub-domain sites that are vulnerable to glycan-like binding antibodies [3].

CCR5 and CXCR4 are G protein-coupled receptors or GPCRs. Each co-receptor has seven transmembrane sections. Viruses can choose CCR5 or CXCR4. Viruses that select CCR5 cause viral transmission. Viruses that choose CXCR4 or both co-receptors occur further into the progression of HIV-1. CCR5 and CXCR4 have an N-terminal domain, three extracellular loops (ECL), three internal loops (ICL), and a C-terminal ending in the cytoplasm [4]. According to crystal structures, CXCR4 has mutations in its C-terminals with a T4 lysozyme for fusion with different ligands [4]. CCR5 has a similar structure with CXCR4. CCR5 can perform rubredoxin fusion with anti-HIV drugs called maraviroc or with an edited chemokine [4]. The N-terminal part of CXCR4 and CCR5 develops chemokine recognition site 1 or CRS1 for interaction with chemokines as their seven transmembrane segments mold a binding pocket called the chemokine recognition site 2 to bind chemokines at the N-terminus of each co-receptor.

After the HIV virus binds to the coreceptors C-C chemokine Receptor Type 5 or to CXC Chemokine Receptor Type 4, many changes in shape develop to cause fusion between the viral and host cell membranes. After fusion, the viral core that contains RNA enters the host cell. A virion that can attach to either coreceptor leads to different patterns of pathogenesis of HIV, affecting the types of treatments needed. All transmitting HIV viruses are mainly CCR5-tropic; however, 50% of HIV-1B type patients will produce CXCR4-tropic X4 HIV viruses [5]. Individuals who exhibit X4 viral infection have an unfavorable clinical outcome and prognosis.

The bottleneck effect of HIV-1 transmission mirrors a favorable selection of previously activated memory T cells from the past phase of HIV viral infection. This source of natural selection creates a pressure for HIV-1 to infect naïve T cells even past the threshold and the initial progression of the disease when most T cells have been reduced in numbers [5]. With the progression of the HIV/AIDs disease, drugs that target CCR5 can become ineffective due to the coreceptor switch to CXCR4. A screen must be applied to confirm the coreceptor type, R5 or X4 currently utilized by the virus before any drug treatments are given [5].

HIV strains that use CXCR4 and CXCR6 instead of CCR5 cannot prevent CCR5 disrupted cells from HIV-1 infection. Therefore, individuals homozygous for the 32 base pair deletion of CCR5, can still become infected with non-CCR5 selecting viruses. For this reason, alternative gene editing methods are required to develop HIV-resistant cells such as retroviral or lentiviral gene therapy developed by vector mediation [6]. Vector mediation could include CCR5 disruption combined with other site-specific gene therapies.

To disrupt CCR5, site-specific anti-HIV factors can be added to the CCR5 locus. For example, anti-HIV chimeric antigen receptors can be integrated into a disrupted CCR5 site, which produces HIV-targeting CAR T cells. These CAR T cells can prevent R5-tropic infection by HIV [6]. Lessening the expression of CCR5 prevents HIV infection of CD4 T cells. A low infection rate lowers the mutation rate of HIV. HIV mutates its DNA inside of the host cell's genome. Blocking the virus' entry into the host cell eliminates its reverse transcription stage that is necessary for HIV proviral DNA to integrate into the host cell's genome. Less HIV-DNA mutations cause less frequent events of viral escape that can lower the virus' ability to evade immune responses.

A major issue for developing vaccines for HIV-1 is viral escape. HIV-1 mutates its proviral DNA and replicates viruses with different epitopes on its surface that immune cells cannot recognize, neutralize, or attack. For developing an effective vaccine for HIV-1, the HIV-1 epitopes and targets of broadly neutralizing antibodies need identification. An increased understanding of the process for broadly neutralizing antibodies development is also required [3]. The envelope glycoprotein called Env, helps with the fusion of the viral membrane with the host cell membrane via coreceptors CCR5 and/or CXCR4. This fusion is extremely difficult for most cells of the human immune system to target and neutralize the virus. The Env glycoprotein has many different and varied compositions of amino acids with diverse types of glycan additions. Only broadly neutralizing antibodies or bnAbs have been found to identify and then neutralize multiple types of HIV strains while HIV evades many immune responses [7]. After the progress of infection overtime, the bnAbs are produced. The bnAbs usually have long edited loops after translation modification. These loops develop after exposure to conserved regions on the Env surfaces. eCD4-Ig can inhibit the entry of HIV, lower its viral escape, and attract antibodies to the virus. The subunits of eCD4-Ig have a CD4 site that is similar to the host cell CD4 co-receptor and acts as a decoy for HIV to bind. The IgG hinge can attract and attach to antibodies from B cells. The E51 fragment of eCD4-Ig is a peptide that binds to the gp120 glycoprotein and then locks in the virus where it cannot escape or evade immune responses. The purpose for this review includes advocating for an addition of antivirals to the HIV-1 vaccine design such as CCR5 gene silencing and the use of eCD4-Ig because each can provide a delayed HIV escape response. "Cornering" HIV-1 in with denying entry through lowering the expression of CCR5 on the surface of cells and trapping it with eCD4-Ig can provide more time for vaccines to induce an effective and timely immune response.

Survey Methodology

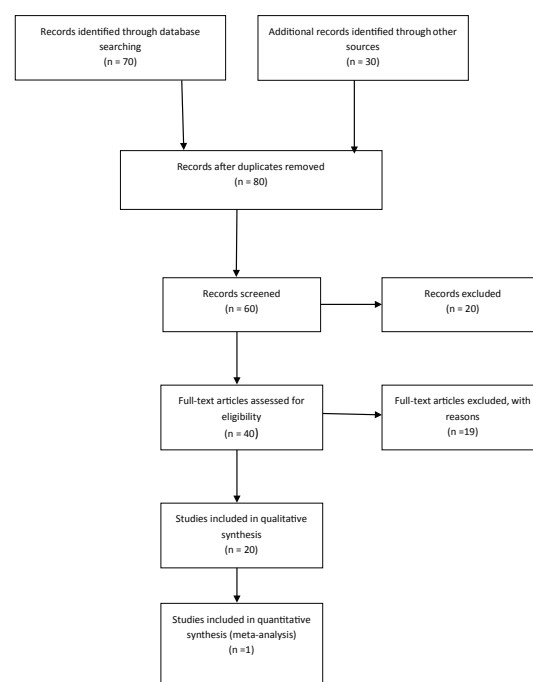


Chart 1: Survey Methodology

Broadly Neutralizing Antibodies and Vaccines

The bnAbs have five types, categories, or epitopes that are present on its surface. Point mutations that arise in Env of HIV-1 envelope glycoproteins affect the efficiency for bnAbs neutralization of HIV viruses [7]. Therefore, studying bnAbs are a prominent source of research and vaccine design. One of the causes for vaccine failures can be attributed to HIV-DNA viral mutants escaping from immune cells. The mutations block T cells from identifying these escape mutants of HIV viruses. To design more effective vaccines, viral escape relative to CD8+ T cell actions of response may need further elucidation. Humoral and cellular immunity cannot recognize HIV viruses that have an ability to perform a viral escape. However, the potency of immune responses that is required for inducing a viral escape is still not completely measured or quantified [8].

General Vaccination Process Antigens are produced through the process of vaccine design. Antigens enter the host through the process of vaccination. The antigen induces an immune response. The B cells bind to the antigen and begin to generate memory B cells and antibodies that match with and can bind to the epitopes of the disease causing protein or antigen. This is the first exposure. After a second exposure to a virus, B cells recognize and attach to the familiar antigenic epitopes of the virus. The B cells recruit memory B cells, and the memory B cells release antibodies specific for the virus. The antibodies neutralize the virus (Figure 2).

Researchers can analyze the viral escape and evasion of bnAb as an alternative tool. Studying monoclonal antibody therapy in trials can help us better understand viral escape through the provision of data gathered from viral escape predictions of variants. By understanding the innate process of HIV virus' ability to escape bnABs, we can research alternative ways to delay viral escape [7]. To produce bnAbs the viral and subsequent immune responses will need to be mimicked in the process of designing vaccines [9]. The envelope proteins of the HIV virus stimulate the most bnAbs to neutralize the virus. The HIV replicates with V1 loops that were longer by seven amino acids were resistant to neutralization [10]. Therefore, the time between HIV-1 transmission and the development of bnAbs needs further research and study. Inducing passive immunization through broadly neutralizing antibodies can decrease HIV-1 infections.

Passive immunity can regulate the infection and remove latent reservoir cells. However, antibodies that quickly neutralize the viruses in the early stages of the disease, initiate viral escape. The cost of fitness can be lowered if the virus begins to produce more mutations. The fast escape of the virus from neutralization is a result of a high mutation rate, the strong ability for the envelope glycoprotein

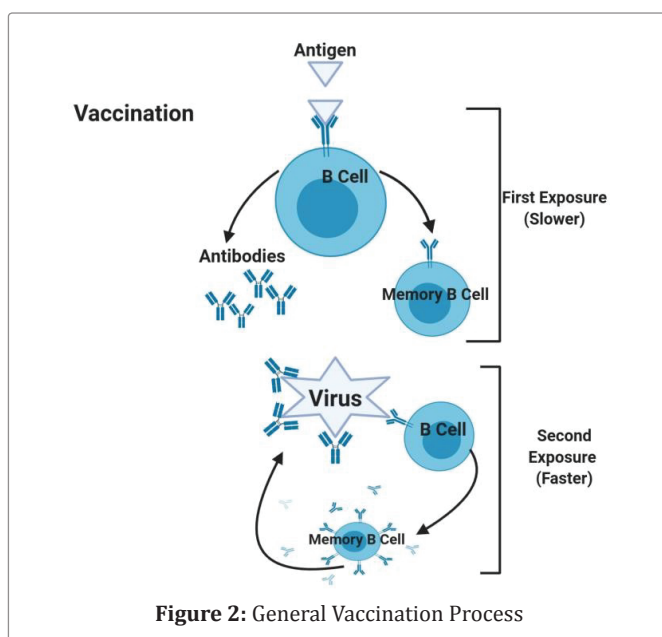


Figure 2: General Vaccination Process

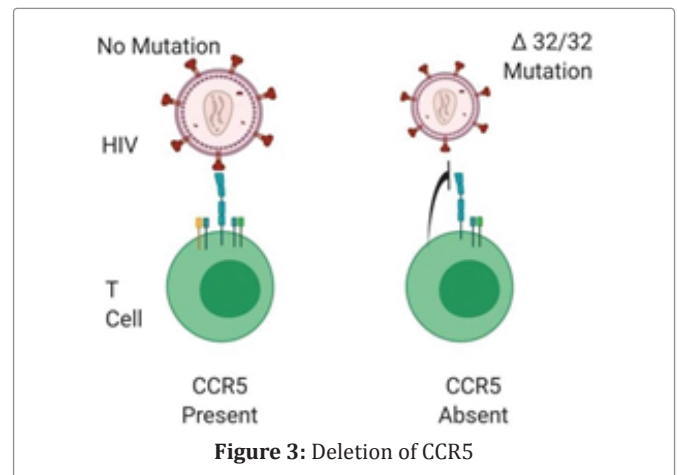


Figure 3: Deletion of CCR5

to change its structure and conformation, and the antibody size of its epitopes that are larger than the binding receptor sites of Env. An antibody that is too large and is rare will fail to bind with many Env epitopes. To develop an effective vaccine for HIV-1, we need to understand the ways in which vaccines alter and affect viral escape. During early and acute infection before the 20-30 days, the rate of escape was 0.14 per day as the virus escaped cytotoxic T lymphocytes (CTL) [11]. The chronic infection of HIV-1 was lower with an escape rate of 0.04 per day, which shows that the CTL-attacking of HIV-1 viruses is more strenuous during the acute infection than the chronic phase [11]. Viral escape is characterized by many frequent epitope changes, diversity, and epitope disorder (entropy).

Disrupting CCR5

Genetic variations in the CCR5 gene can create HIV resistant immune cells such as T cells and macrophages. Homozygous individuals have a delta32 allele deletion of the CCR5 gene that does not express CCR5 on the host cell surface. The lack of CCR5 expression on the cell surface leads to HIV-1 resistance [12]. Two individuals with HIV infection named the Berlin and the London patient received bone marrow transplants for their leukemia and lymphoma, respectively. The stem cells in the bone marrow transplants had a mutation that deleted 32 base pairs of the CCR5 gene sequence. After both men received their transplants, they were HIV negative, and they no longer needed their antiretroviral drugs. Each patient sustained their HIV negative status long term. CCR5 is also a target for many drugs acting against the transmission of HIV/AIDS.

The CCR5 co-receptor, after its deletion, HIV-1 is limited and inhibited from binding to the CD4 receptor of the CD4 T cell through the CCR5 co-receptor. The HIV-1 CCR5 disruption can prevent HIV 1 from infecting T cells and many other lymphocytes (Figure 3). C-C chemokine receptor 5 or CCR5 and C-X-C chemokine receptor 4 (CXCR4) allow HIV-1 to enter T cells. Because many new drugs for HIV-1 lack high-thorough point screening, the development of drugs has decelerated. A novel drug screening called a luciferase assay can image CCR5 and CXCR4 with promoter-dependent firefly and Renilla luciferase expressing vectors (pGL4.10-RLUC-CCR5/CXCR4) [13]. The drugs were imaged to observe and determine the drugs' efficient regulation of CCR5 and CXCR4. The promoters for CCR5 and CXCR5 were inserted into a recombinant plasmid, and then through transfection the vectors were delivered into T white blood cells and leukemia cell lines called H9 [13]. The transfected cells, after treatment with additional Chinese medicinal compounds, showed increased suppression of CXCR4 and reduced CCR5 activities at their subsequent promoter sites. This dual-luciferase reporter assay can reveal the efficacy of anti-AIDs/HIV drugs.

After CRISPR reduced the expression of CCR5, the efficacy of silencing CCR5 was 5.20% to 8.28% from samples of bone marrow after a 19-month phase [14]. In many hematopoietic diverging cell types (HSPC), there was observed CCR5 reduction and ablation. The

transplantation of CCR5 produced HSPCs from patients showed long-term CCR5 silenced expression. CD4⁺ T cells that showed increased CCR5 indels were produced more with increased exiting into blood circulation. The CD4⁺ T cell counts were restored gradually over a 6-month period after the engraftment of CRISPR-CCR5 edited HSPCs.

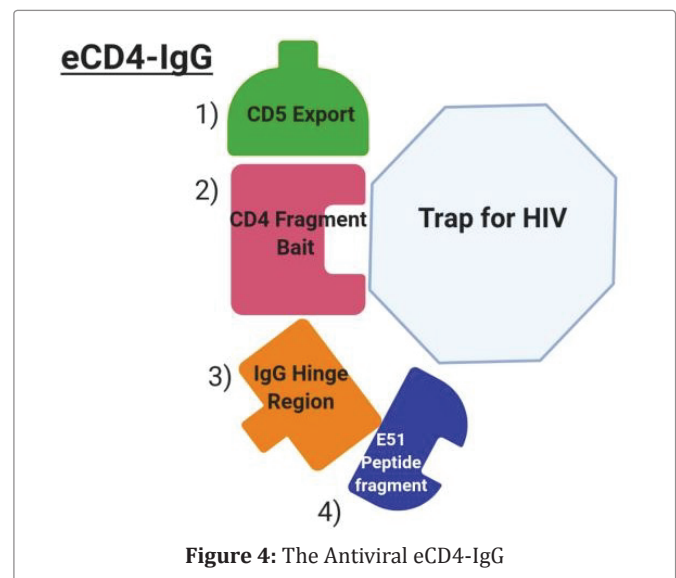
Because exogenous DNA was incorporated into the T cell genome, the previous HSPC gene therapies became less efficient, and induced an acute neoplasia with other signs of innate immune response. However, researchers used non-viral transfections to deliver Cas9 ribonucleoproteins, therefore, preventing the inclusion of exogenous DNA, which allowed Cas9 to remain in target cells for a longer term. The Cas9 editing may have still resulted in the occurrence of off target indels [14]. Researchers used a high-throughput whole-genome assay to observe the pre-transplantation and post-transplantation of cells after 15 weeks to 19 months [14]. Their whole-genome assay did not discover any point mutations, larger deletions, or any chromosomal translocations after CRISPR gene editing. There were no off-target changes, which gives much support for the safety of CRISPR gene editing of CCR5.

Four drug type analogs were studied to confirm the activation, the expression of T-cells, and the secretion of anti-HIV compounds at a drug potency of 25 pM [15]. An anti-HIV drug analog with a high concentration and potency is a result of the drugs' capability to attach and bind to receptors that are not available for innate chemokines. Researchers used dual-color fluorescence cross-correlation spectroscopy assays to label fluorescent analogs and detect monomeric CCR5 labeled with fluorescence. Researchers detected low nanomolar binding affinities for two drug analogs 5P12-RANTES (regulated on activation normal T cell expressed and secreted) and 5P14-RANTES [15]. They concluded that drug analogs all bind at the same binding site because innate host chemokines could not remove the attached fluorescent drug analogs.

Insertion to edit the CCR5 locus with transgenes can control and suppress HIV viral infection. Researchers created a hybrid of the Methotrexate (MTX) drug with nucleases to edit the CCR5 locus of the CD4⁺ T cells. Approximately 82% of the nuclease-induced HDR activities at CCR5 remained long-term in vivo without any off-target mutations and inserts [16]. This approach can be repeated and reproduced with zinc-finger nucleases (ZFNs) and CRISPR. However, challenges remain for both gene editing strategies to produce additional loads of gene-edited cells. An adeno-associated virus (AAV) delivered meganuclease transcription activator-like (megaTAL) treatments into a DNA donor template where the mDHFR components were inserted into the CCR5 locus. After inserting dihydrofolate reductase (mDHFR) into the CCR5 locus, the CD4⁺ T cells were resistant to the MTX drug and lacked CCR5 expression on its outer surface. By inserting drug-resistant genes and then selecting only the enhanced gene-edited CCR5 CD4⁺ T cells, can improve the exclusive selection of viable gene-disrupted human CD4⁺ T cells [2]. By lessening the co-receptor presence on host cells or through decreasing the co-receptor affinity for gp120, the rate of membrane fusion will reduce [17]. Although editing co-receptor CCR5 can lessen the entrance of the HIV-1 virus into T cells, we should also modify CCR5 and CXCR4 co-receptors. Modifying CXCR4 co-receptors does not express any negative effects in cells. The gene editing of co-receptors should be combined with the CRISPR-Cas9 eradication of the HIV-1 genome from host cells [18].

Delaying HIV-1 viral escape by inhibiting entry into T cells

The eradication of T cells determines and calculates the timing and speed of HIV viral escape [19]. The rate of viral escape is proportionally relative to the effectiveness of T cell immune responses. The speed and rate of viral escape is determined by the early frequency of viral mutation, viral mutation rate, and by the effective decay of T cells. For example, Yang et al. found four T cell responses that produced three viral escapes to these responses after 4 years of HIV viral infection [19]. The T cells responded to the Gag



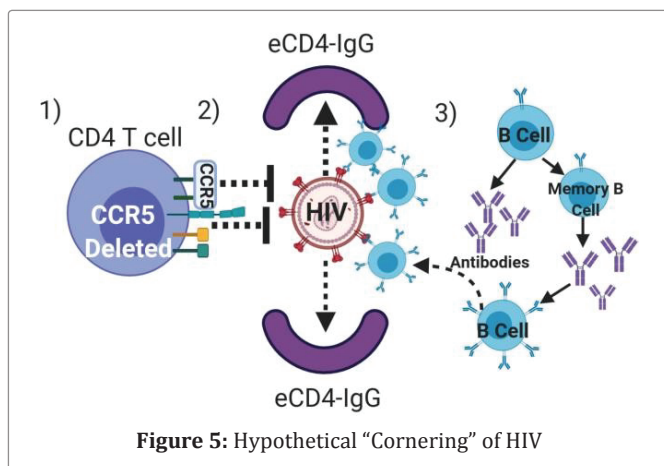
HIV-DNA translation into TPQDLNTML that was then changed into an escape mutant of GagTPQDLNTMLNTGGHQAA. This escape mutant appeared after 1,132 days from the early inception of the disease [19]. The Yang et al. [19] mathematical model for viral escape confirmed the chances of cytotoxic T cells to kill and eradicate HIV viruses were dependent on the escape rate. The escape rate is proportional to the level of cytotoxic T lymphocyte (CTL) elimination of HIV viruses.

An antiviral called eCD4-Ig can reduce and lessen the frequency of viral escape. A tremendously effective entry inhibitor molecule called an eCD4-Ig is a combination of an inhibitor, CD4-Ig, and a sulfated tyrosine amino acid type of peptide coreceptor. eCD4-Ig has four parts 1) an ED5 for export of the antibody from the cell 2) a CD4 decoy that mimics the CD4 T cell receptor, 3) an IgG hinge region, 4) an E51 small peptide fragment that binds to the gp120 of HIV, and locks it in a trap. eCD4-Ig readily neutralized 270 subtypes of HIV-1, HIV-2, and the simian immunodeficiency virus [20]. The eCD4-Ig had an 80% inhibition of HIV for concentrations less than 10 grams per milliliters. eCD4-Ig vectored into *Rhesus macaques* were prevented from developing SHV-AD8 and SIVmac239 for 1 year [20]. The C-terminus of the coreceptor peptide increases the effects of eCD4-Ig. The peptide present on the C-terminus of eCD4-Ig can more effectively bind to HIV-1 Env, preventing Env to bind its coreceptor. This delays the viral escape from immune cells (i.e. CD4-Ig). HIV-1 Env slowly escapes from eCD4-Ig due to the viruses' lack of ways to escape because HIV-Env mutations against eCD4-Ig were produced at a high fitness cost [20]. Therefore, eCD4-Ig is broad, challenging to escape, any resistance induces a high cost of fitness, and it can combine efforts with normally active and innate antibodies to help eradicate HIV viruses.

The antiviral eCD4-IgG

There four subunits of eCD4-IgG that can trap HIV and lower viral escape. 1) The CD5 export system allows the exit of eCD4-IgG externally from a cell. 2) CD4 is similar to CD4 co-receptor and acts as a decoy for HIV to bind. 3) The IgG hinge can attract and attach to the antibodies of B cells. 4) The E51 fragment is a peptide that binds to the gp120 glycoprotein and then locks in HIV where the virus cannot escape or evade immune responses (Figure 4).

However, to produce viral elimination in the latent reservoir, a gene editing method is needed to increase HIV-1 coreceptor disruptive cells. Researchers used zinc finger nucleases to edit the CCR5 loci by inserting stop codons into the coreceptor. Samples of bone marrow from viremic HIV individuals were used for zinc finger nuclease gene editing. After performing PCR, to measure the number of insertions into CD32⁺ T cells, approximately 0.5% of CD34⁺ cells contained the CCR5 loci stop codons [21]. Using this method allows



the facile return of the T cells into the bone marrow. Moreover, there are three positions external to V3 that influence the coreceptor shift between R5, the CD4 binding site, and the surface connection between gp120 to gp41 in the segments of gp41 [5]. The residues that were found of the R5 and the CD4 contain high coreceptor specificity for entering viruses as new mutations for gp120 to gp41 may cause a shift in coreceptor viral entry. The structural changes via gp120-gp41 binding to coreceptors for CD4⁺ T cells could be further studied. Inhibiting these highly specific residues between R5 and CD4 through CCR5 gene disruptive insertions, may prolong viral entry, lower the rate of viral escape, which may provide more time for an efficacious vaccine to bind the Env proteins, neutralizing the HIV virus. Because CCR5 disrupted T cells can block viral entry, HIV-1 viruses may more frequently contact the eCD4-Ig CD4 receptor decoy and become trapped. Denying viral entry can push the virus into an eCD4-Ig trap to provide additional time for antibodies and CTLs to capture the virus at its Env proteins. Inhibiting viral entry into lymphocytes through CCR5 disruption and eCD4-Ig, may decelerate HIV viral escape and provide sufficient time for vaccines to induce an innate immune response. Gene editing of CCR5 and eCD4-Ig can optimize the effectiveness of vaccines.

Hypothetical "Cornering" of HIV By surrounding HIV with multiple antiviral responses, HIV may become "cornered" with not many places to escape immune attacks. Three phases of "cornering" include: 1) CCR5 disruption inhibits HIV entry, which then lowers the frequency of infection, reduces the mutation rate, and decreases the mutations required for an increased rate of viral escape; 2) eCD4-IgG further lowers the rate of viral escape by trapping the virus by mimicking a CD4 receptor to lure in HIV, and then the virus is locked in via the E51 fragment binding to the viral gp120; and 3) The IgG hinge fragment of eCD4-IgG recruits antibodies and immune responses from B cells (Figure 5).

Conclusion

The rationale of this review includes the process of blocking HIV entrance through disrupting CCR5 and using eCD4-Ig as traps for rendering T cells resistant to HIV infection. The malfunctioning of CCR5 can interrupt the virus' entrance and delay the rate of infection. HIV-1 mutates its proviral DNA and the mutation allows the virus to synthesize proteins with epitopes that lymphocytes and antibodies cannot recognize. Thus, the virus evades many immune responses, vaccines, and antibodies through a rapid viral escape. Preventing an entrance of HIV can decrease its mutation rate by preventing its fusion, release of its genome, its reverse transcription, and its integration of proviral DNA into the host cell genome where HIV-DNA mutations occur. The HIV virus could lower the HIV-DNA mutation rate, reducing the virus' ability to evade immune responses, and reduce the frequency of viral escape. CRISPR gene editing has been proven to be an effective and safe source for gene editing. CRISPR insertions into the CCR5 gene sequences successfully inhibit CCR5 expression without many off target mutations.

The eCD4-Ig can prevent the entrance of HIV viruses into CD4 T cells. eCD4-Ig carries a CD4 like receptor protein that can act as a decoy for HIV-1 to bind. The CD4 binding site of eCD4-Ig mimics the CD4 receptor of T cells. The HIV-1 virus binds to this eCD4-Ig site, and then the E51 fragment binds to the gp120 Env protein of HIV-1, trapping the virus. The eCD4-Ig trap of HIV-1 lowers its viral escape where the virus needs much energy to produce mutants that can evade eCD4-Ig. Also, eCD4-Ig can attract antibodies to trapped viruses. Combining multiple methods such as CRISPR gene editing of CCR5 and entry inhibitors as eCD4-Ig with a vaccine design can possibly offer a functional cure for HIV infected individuals. Therefore, the effective delivery of an HIV-1 vaccine depends on the initial period of the acute infection where the kinetics of HIV-1 viral escape is extremely rapid. Inhibiting viral entry through CRISPR-CCR5 disrupted T cells and by trapping viruses with eCD4-Ig may delay viral escape to provide sufficient time for vaccines to allocate a successful cell and humoral immune response. However additional sequencing of HIV env DNA sites is needed to select antigens specific for any current viral escape Env mutants, inducing a stronger binding affinity between HIV-1 Env glycoproteins and antibodies.

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