

Pathogenesis of HIV: Pathway to eradication

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ABSTRACT

The introduction of combined therapy has been an important breakthrough in the treatment of HIV-1 infection, however for proper eradication, HIV pathogenesis has to be understood. Despite treatment with combinatorial therapies, a functional cure approach would be very effective in suppressing viraemic load in the host to such low levels that the immune system would be able to control the infection without the need for antiretroviral therapy, neither inflicting harm upon the host nor transmission to other individuals. Scientific focus should be on proper approach, that is direct functional cure, which provides present and future possible treatment strategies to completely eradicate HIV persistence in the reservoirs within the host through full understanding of the mechanism behind HIV pathogenesis. However, for proper eradication of HIV in infected individuals, there should be a better understanding of the immunologic and virologic mechanisms that contribute to viral persistence in infected individuals. The full understanding of HIV pathogenesis is the only scientific way by which its eradication will be fully accomplished.

Keywords: Human Immunodeficiency Virus, Pathogenesis, Antiretroviral Therapy, Functional cure, Eradication.

INTRODUCTION

The human immunodeficiency virus (HIV) is a retrovirus belonging to the family of lentiviruses. Retroviruses generally contain ribonucleic acid (RNA) and not deoxyribonucleic acid (DNA). They are unable to replicate outside of living host cells but are able to use their RNA and host DNA to make viral DNA through reverse transcription [1]. Once the virus is within the host cell, the viral particle uncoats from its spherical envelope to release its RNA where it continuously uses new host cells to replicate itself.

The virus is diploid and contains two plus-stranded RNA copies of its genome. The approximately 9kb RNA genome encodes at least 9 proteins, Gag, Pol, Env, Tat, Ref, Nef, Vif, Vpr and Vpr. The three principal genes are gag, pol and env. The gag encodes core proteins, the pol gene encodes the enzymes reverse transcriptase, protease and integrase while the env gene encodes the HIV structural components known as glycoproteins. The other RNA genome are important for viral replication and enhancing HIV's infectivity rate [2,1,3].

HIV consists of a cylindrical centre surrounded by a sphere-shaped lipid bilayer envelope. There are two major viral glycoproteins in this lipid bilayer, gp120 and gp41. The major function of these proteins is to mediate the recognition of CD4+ cells and chemokine receptors mainly CCR5 and CXCR4, thereby enabling the virus to attach to and invade CD4+ cells [4]. Other cells bearing CD4 and chemokine receptors are also affected, including resting CD4 T cells, monocytes and macrophages, and dendritic cells. HIV exists in two different types, HIV-1 and HIV-2, both believed to have been transmitted to humans from African non-human primates by blood [5,6,3].

The use of highly active antiretroviral therapy (HAART) has significantly reduced the mortality rate and has increased the lifespan of HIV- infected patients by maintaining viral loads below detection levels, thus preventing the onset of AIDS [7]. HAART drug cocktails primarily targets the reverse transcriptase (RT) and protease (PR)

enzymes potentially suppressing viral loads and transmission rates, but complications can arise from compound toxicity and the emergence of resistant strains [4].

HIV Lifecycle

HIV replication cycle is complex and not completely understood. HIV-1 life cycle can be categorized into two phases. The early stage occurs between entry into host cells and integration into its genome. The late phase occurs from the state of integrated provirus to full viral replication [8,9]. The HIV lifecycle characterizes multiple interactions between viral and host-cell proteins. These interactions aid viral replication or involve host defences and their counteraction by the virus [10].

One of the HIV proteins proven to be essential for the replication of the virus and progression of AIDS is Nef. Nef is a regulatory protein present only in the primate lentiviruses HIV-1, HIV-2 and SIV. The *nef* gene is situated at the 3'-end of the viral genome. Originally it was believed that the *nef* gene inhibits transcription from where it got its name the negative factor. The Nef protein functions abundantly in the early stages of viral replication during the interaction of HIV infected cells and CD4+ cells [11,12,13].

Over time, virus replication leads to a slow and progressive destruction of the immune system. One important issue is that HIV-1 makes use of the replication machinery of the host cell, which minimizes the number of potential viral targets. On the other hand, the close host-virus relationship limits the evolutionary freedom for the viral components that interact with the host molecules [2].

The HIV lifecycle involves six stages: binding and fusion, reverse transcription, integration, transcription, translation or assembly and budding. All these stages are involved in the replication of the virus in the host cell.

• Binding and Fusion

HIV binds to the CD4+ receptor on the outside of CD4 cells using gp120 found on the surface of the virus and macrophages, which in turn allows the entry of HIV into host cell. Once HIV binds to the receptor, other proteins such as CCR5 and CXCR4 are activated in order to complete its fusion with the cell. There are essentially four steps of viral entry, attachment, CD4 binding, co-receptor binding and membrane fusion [14].

The binding of gp120 to CD4 and the viral coreceptors results in conformational changes and triggers the establishment of a "fusion complex" composed of gp41 trimers [15]. The trimers play important roles in membrane fusion and cell entry. After fusion, the viruses release RNA, its genetic material, into the host cell.

It is interesting to know that individuals without CCR5 expression are highly resistant to HIV infection [10]. Binding and fusion can be blocked by entry inhibitors and fusion inhibitors respectively.

• Reverse Transcription

This stage involves the conversion of single-stranded HIV RNA to double-stranded HIV DNA by an enzyme called reverse transcriptase. Before HIV RNA can be incorporated into the DNA of the CD4+ cell, it must be converted to DNA. Reverse transcription can be blocked by Nucleoside Reverse Transcriptase Inhibitors (NRTIs) and Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) [7].

• Integration

Once reverse transcription has occurred, the viral DNA can enter the nucleus of the CD4+ cell. The viral enzyme 'integrase' then incorporates the viral DNA into the CD4+ cell's DNA. HIV-1 integrase is a viral protein with several roles. After reverse transcription of the viral RNA genome into DNA, it appears in part to be responsible for transporting and inserting the viral DNA into the nucleus of the cell, where it has the ability to closely associate with one of the chromosomes of the host cell [16,17,18]. Integration can be blocked by integrase inhibitors.

• Replication

The new DNA formed by integration causes the production of messenger DNA that initiates the synthesis of HIV proteins. Vpr is a virus-associated and multifunctional protein, localized in cytoplasm as well as nucleus of infected cells, which functions in the regulation of viral replication, cellular events like NF- κ B-mediated transcription, apoptosis and cytokine production. It is essential for viral replication in T cells but indispensable in macrophages [19,20,21,22].

• Transcription and Translation

Transcription involves the formation of a new strand of viral RNA, sometimes called messenger RNA (mRNA), from the division of the two strands of DNA.

- **Budding**

Viral budding is blocked by Interferons.

- **Assembly and Maturation**

Virus assembly and maturation is blocked by Protease inhibitors.

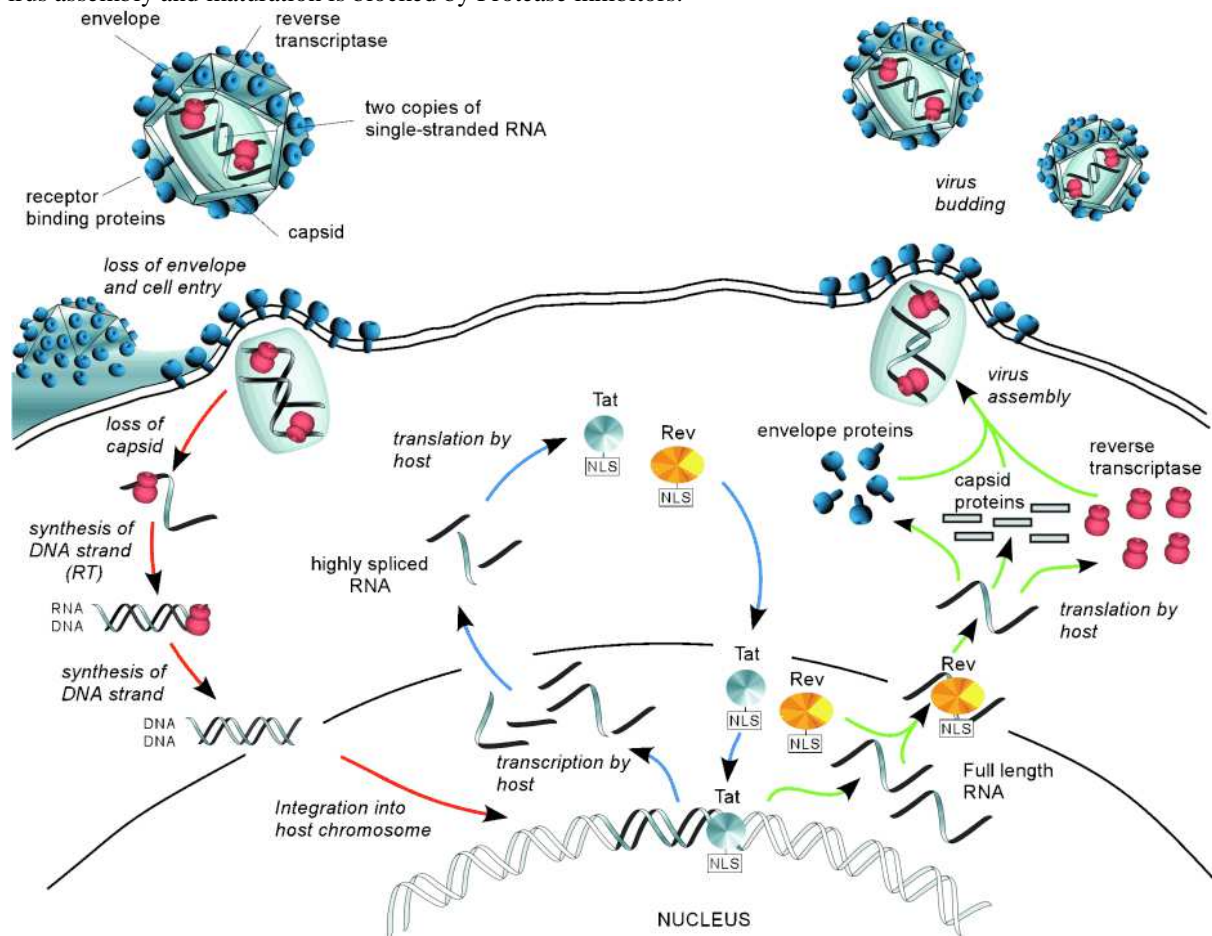


Figure 1: Diagram showing the replication cycle of HIV-1

Source: Weizman and Tor [23].

Mechanism of Pathogenesis of HIV Infection

The pathogenesis of HIV infection is a function of the virus life cycle, host cellular environment, and quantity of viruses in the infected individual [24]. HIV pathogenesis is basically a competition between HIV replication and the immune responses of the subject or patient via the cell-mediated and immune-mediated reactions [25].

Having established that the main target of HIV is activated CD4 T lymphocytes [3] through the depletion of the total host cell CD4⁺ T-cell pool, via interactions with CD4 and the chemokine co-receptors, CCR5 or CXCR4, the mechanism of pathogenesis still has to be understood. Pathogenesis results in host immunodeficiency [26].

Pathogenesis of HIV-1 is complex and is characterized by the interaction of both viral and host factors [7]. In HIV infection, disease progression correlates both with increased viral load [27] and elevated levels of apoptosis [28]. Usually, T-lymphocyte cells inversely correlate with levels of apoptosis [29]. T-cell apoptosis, a complex process, involves both HIV-infected and uninfected cells [30].

HIV-1 uses different strategies to survive within infected individuals. Importantly, the HIV-1 Nef protein aids in viral infection with three main functions. It changes the signalling pathways of the cell, increases viral infectivity and down regulates the surface antigens of infected cells [31,32]. Nef is a small HIV protein that is essential for the replication of the virus, progression of acquired immune deficiency syndrome (AIDS), and is expressed abundantly during the early stages of infection in a host cell [33]. It is composed of a flexible N-terminal membrane bound loop and a well reserved C-terminal core region.

According to Weber [6], Garcia *et al.*, [34] and Sundquist and Kräusslich [1], the mechanism of HIV-1 infection can be explained with respect to the level of infection in the host; primary HIV-1 infection, chronic phase of asymptomatic HIV infection and late HIV disease. The probability of infection is a function of both the number of infective HIV virions in the body fluid which contacts the host as well as the number of cells available at the site of contact that have appropriate CD4 receptors.

Primary HIV-1 infection is manifest by a viraemic peak associated with a temporally related decline in CD4⁺ T-cells; the viraemia is probably curtailed by an HIV-specific CD8⁺ CTL (cytotoxic T lymphocyte) response [6,34]. Chronic asymptomatic HIV infection is associated with highly dynamic, persistent viral replication, with the production of approximately 10⁸ virions per day. Viral replication leads to loss of CD4⁺ T-cells, which could be due either to increased cell death, or to reduced production, or both. The increased turnover of both CD4⁺ and CD8⁺ T-cells in HIV-1 infected subjects compared to controls supports the killing of virally infected cells by HIV-specific CTL as a leading hypothesis for CD4⁺ T-cell decline in HIV infection. However, the direct relationship between plasma viral load and rate of CD4 decline suggests that viral replication also contributes, directly or indirectly, to CD4 loss [6].

Late stage HIV-1 infection manifests an increased rate of CD4 loss through expansion or broadening of the viral tropism mediated by a switch in co-receptor usage from CCR5 to CXCR4. Thus, the loss of cellular immune regulation results in AIDS which in turn suppresses the regulation of the SI/CXCR4 variants [6,34].

As reported by Battistini and Sgarbanti [35], the major obstacle towards HIV-1 eradication is the life-long continuity of the virus in reservoirs of latently infected cells. Also, cell-to-cell transmission of HIV represents an obstacle as it allows the infection of cell types which are not easily infected by HIV, leading to the establishment of long-lived viral reservoirs. This phenomenon allows the escape of HIV elimination by the immune system [36]. In these cells, the proviral DNA remains bound to the host's genome but does not actively replicate, becoming invisible and unaffected by existing antiviral drugs. Latently infected cells do not express viral proteins, hence, they remain hidden to the immune system. In reservoirs of latently infected cells integrated provirus remains quiescent due to transcriptional silencing which makes them unaffected by c-ART that normally targets actively replicating virus. Interestingly, these cells can be activated thereby promoting new rounds of viral replication with systemic infection. Study suggests that at least two reasons explain the reason behind the persistence of infected cells for extended periods. First, the latently infected, resting CD4⁺ T cells exhibited primarily a memory phenotype CD45RO⁺, implying that such infected cells could be long-lived. Second, resting CD4⁺ T cells carrying infectious HIV could do so without expressing viral antigens on their cell surface, thereby enabling them to escape recognition by the host's immune system as well as resist virus-induced Cytopathic effects [37].

Cell-to-cell transmission aids in viral pathogenesis with the following characteristics: HIV escape from suppressive effects of antiretroviral drugs, promotion of viral dissemination including various types of cells of the immune system, with important role in sexual and mother-to-child transmission of HIV and spread within the central nervous system and gut-associated lymphoid tissues [36].

Interferons, importantly interferon- α has been identified as one of the key cytokine markers of HIV pathogenesis. While IFN expression in the initial stages of HIV infection can restrict viral replication, the HIV-mediated overstimulation of IFN responses during chronic infection fuels pathogenesis and disease progression [38].

According to Lane [26], immune activation in HIV infection includes two components; the homeostatic response to CD4⁺ cell depletion and an inflammatory response that includes both an HIV-specific immune response and "bystander" immune activation as a result of the HIV-specific immune response thereby leading to substantial endorgan damage outside of the immune system. This explains the difference between CD4⁺ and CD8⁺ immune activation. Lane [26] reported from a study in which T-cell proliferation was measured in HIV-infected patients with respect to viral load and CD4⁺ count that the level of CD8⁺ cell activation correlated almost exclusively with viral load while the level of CD4⁺ cell activation associated with both viral load and CD4⁺ cell depletion. CD8⁺ cell activation can likely suggest ongoing viral replication.

According to Fraser *et al.* [39], HIV pathogenesis should not only include host and its immune responses but greater emphasis should be on the virus and its genotype, how viral variation interacts with the host response, and how certain heritable viral characteristics influence the course of disease.

HIV Eradication

According to Tae-Wook and Anthony [37], plasma HIV viraemia can be suppressed and maintained below the limits of detection for prolonged periods of time in the vast majority of HIV-infected individuals who receive antiretroviral

therapy (ART). However, ART alone cannot eradicate HIV in infected individuals due to the persistence of viral reservoirs in the peripheral blood and lymphoid tissues of infected individuals despite the suppression of plasma viraemia.

According to Battistini and Sgarbanti [35], as a result of the present failure in the eradication of HIV-1 infection, novel therapeutic approaches have been adopted to overcome viral resistance to drugs, however the immune factors responsible for maintaining natural suppressive response have to be taken into consideration before developing safe and effective immune interventions [40]. The two major approaches to cure HIV-1 infection include curative strategies (or sterilizing cure) and reducing the reservoir strategy (that is, reaching a drug-free control of infection-functional cure), however, a functional cure is more feasible than a sterilizing cure [36]. According to Sivro *et al.* [38], functional cure cannot be achieved due to the establishment of viral reservoirs.

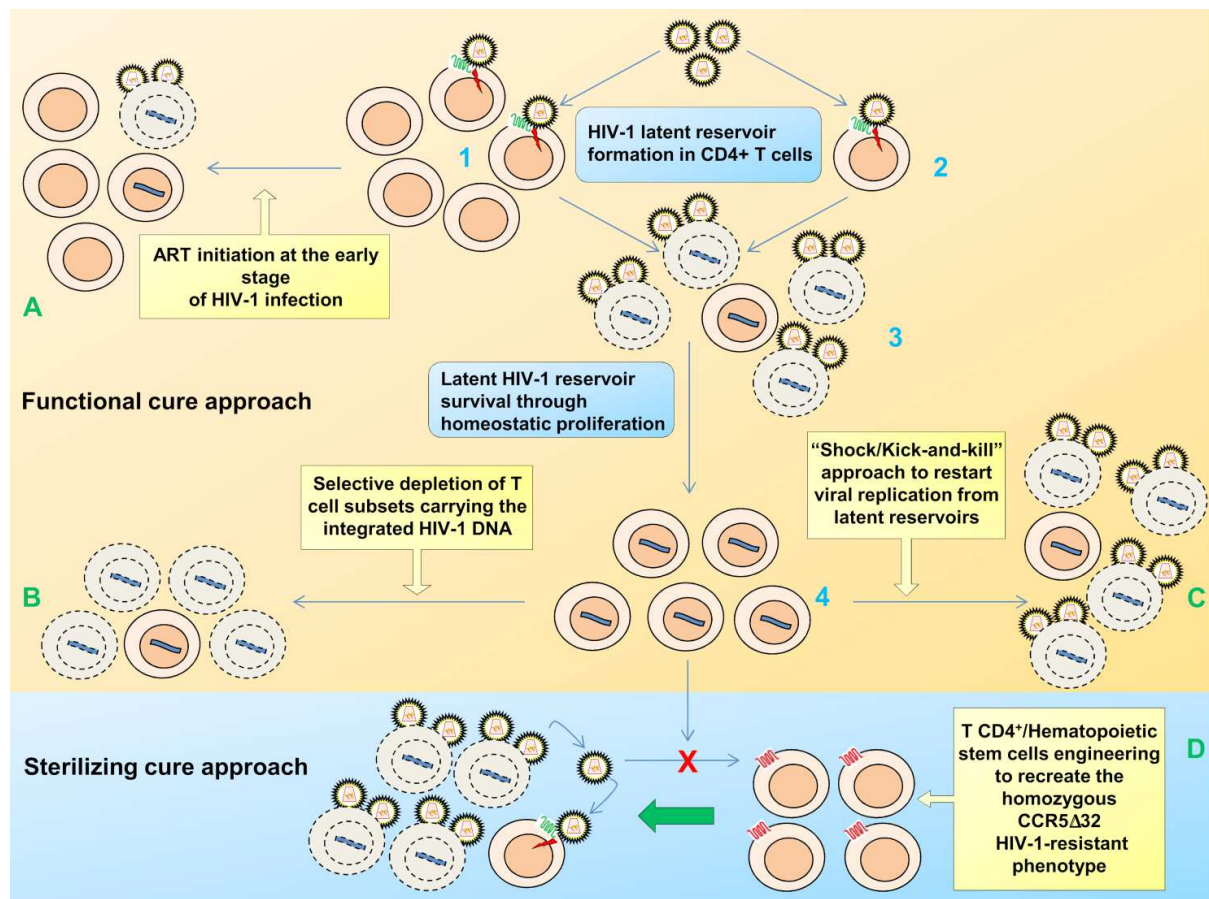


Figure 2: Diagram showing the novel therapeutic approach to HIV-1 infection

Source: Battistini and Sgarbanti [35].

Interestingly, some HIV-infected patients tend to exhibit control of viral replication in the absence of treatment. This control appears to be mediated by a unique CD8+ cell response that identifies an important target for potential HIV vaccines and HIV-specific, immune-based therapies [26].

- Therapeutic vaccines

Therapeutic vaccines are immunotherapies aimed at restoring a cellular immunity importantly through the control of HIV-1 reservoir size. For a therapeutic vaccine to be effective, it should induce HIV-1 specific immune responses that have been previously absent [40]. This approach according to studies could re-stimulate CTL responses, reactivate virus and induce HIV-1 replication [41,42,43,44,45,46,47,48,49,50].

Based on the observation that several vaccination regimens and pathogen infections have been shown to gradually increase viral RNA in plasma of HIV-1 infected patients undergoing ART, recently, the use of Toll-like receptors (TRLs) agonist has been recommended to both reactivate HIV-1 from latently infected cells and boost HIV-specific cytotoxic CD8+ T cell immunity [49].

- Immune-based therapies

Immune-based therapies in combination with reactivating compounds enhance the clearance of reactivated latently-infected cells as well as improving host immune responses. Cytokines and agents that antagonize negative regulators of immune activation have been considered among immune-based therapies in their ability to reversing virus silencing and restoring immune functions [49,35]. Cytokines, also, functions to stimulate HIV-1 replication and interfere with mechanisms responsible for HIV-1 latency [50].

A recent study found the use of pegylated interferon alpha-2A (clinical trial NCT005948) as another immune-mediated approach, which controlled HIV replication and decreased HIV-1 integration in patients after interruption of ART [51,38].

CONCLUSION

Although ART has suppressed viral load in infected patients, complete eradication is not possible without clearance of HIV-1 from latent reservoirs. Therefore, the efficacies of the current antiretroviral agents and treatment strategies cannot be simply assessed by quantitation of CD4+ T-lymphocytes but on their ability to reconstitute the immune system on a wider scale. In the future, combinatorial therapies equipped with precise delivery tools can fulfil the scientific dream of the complete eradication of HIV-1 from infected patients.

However, for proper eradication of HIV in infected individuals, the followings need to be put in mind. First, there should be a better understanding of the immunologic and virologic factors/mechanisms that contribute to viral persistence in infected individuals receiving ART taking note of the precise mechanism by which the latent viral reservoir is established and maintained, the role of tissue-derived productively infected cells in the viral persistence, the origin of rebounding plasma viraemia upon discontinuation of therapy and the effect of immune activation on viral burden in infected individuals receiving ART. Second, the impact of the use of aggressive ART regimens administered following the course of HIV infection should be addressed. Third, clinically relevant and reproducible high-throughput virologic assays need to be developed in order to measure the efficacy of agents that are designed to target persistent HIV reservoirs. Fourth, strategies aimed at targeting both latent and non-latent viral reservoirs may need to be pursued simultaneously in order to eradicate HIV. Finally, eradication strategies may need to include immune-based therapies such as therapeutic vaccination that are capable of boosting the host's immune responses. Given the many strategies HIV employs to evade immune recognition and susceptibility to antivirals, a functional cure would be very effective in suppressing it to such low levels that the host's immune system would be able to control the infection without the need for antiretroviral therapy, harm would not be inflicted upon the host and HIV would not be transmitted to other individuals.

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