Allergy and Immunology

AIMS Allergy and Immunology, 2(3): 113–125.
DOI: 10.3934/Allergy.2018.3.113
Received: 30 May 2018
Accepted: 25 July 2018
Published: 31 July 2018

http://www.aimspress.com/journal/Allergy

Review

Unique natural and adaptive response mechanisms to control and eradicate HIV infection

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Abstract: During the more than 35 years since the discovery of HIV as the causative agent of AIDS a preventative and/or therapeutic vaccine has not been achieved in spite of innovative and unique break-through discoveries in the diagnosis, monitoring of disease progression and pharmaceutical advances. In part the inability to cure HIV either through immunologic and/or pharmacologic approaches is a result of distinctive features of HIV-1 pathogens—the capability to persist in cryptic tissue reservoirs. Recent immunologic discoveries may lead to eradication of this virus. The recognition of adaptive immunologic approaches has led to the development of unique genome editing tools in the form of artificial nucleases that can and have been applied to the editing of selected HIV-1 genomic sequences. This review addresses recent developments in immuno-pharmacologic approaches in the form of inducing reversal agents, latency activation and gene editing which exemplify ongoing unique and imaginative efforts of investigators to achieve a "cure" for HIV/AIDS. The several strategies for the control and treatment of HIV with the aim of eradication of the virus from all tissue reservoirs are discussed.

Keywords: treatment of HIV; HIV infection; HIV latency; gene editing; CRISPR/Cas 9

1. Overview and background

Since first recognized in 1981, considerable innovative research and clinical progress has been made in the diagnosis and treatment of HIV/AIDS [1]. During the ensuing 37 years major

breakthroughs and advances have been and are being made in the diagnosis, treatment, control with the goal, ultimately, of eradication of the disease. During the decades since the initial report in 1981, advances in molecular medicine coupled with nucleic acid technology and targeted antiviral therapy achieved great gains in arresting the spread of the disease and extending the life-span of individuals living with AIDS [2]. Today, available combination antiretroviral therapy (cART) allows those living with HIV, nearly 40 million people worldwide, to enjoy longer, healthier lives, an outcome that once seemed unattainable. As a result there have been ongoing declines in both the number of new HIV infections as well as the mortality rates of those infected [3,4].

cART encompasses several classes of anti-viral agents which have the capability to act at various points of viral maturation within T cells, from entry to budding (see Figure 1) [5]. Although cART has been proven to be effective keeping HIV at undetectable levels in the bloodstream which reduces the risk of transmission by treated individuals to HIV-negative persons, it does not eliminate the virus. Latent virus persists as provirus in latently infected resting memory $CD4^+$ T cells and can be found in many tissue compartments, namely, peripheral blood [6–9], brain [10–12], lymphoid tissue [13,14], gastrointestinal tract [14,15] and other reservoirs [16,17]. Thus the capability to truly cure an infected patient defined as elimination of virus from all body compartments remains a significant challenge. One clinical example of a cured individual known as the "Berlin Patient" was proven to be free of detectable virus in all tissue compartments [18]. The "cure" resulted from the patient receiving an allogenic hematopoietic stem cell transplant from a donor who harbored a homozygous deletion of the receptor CCR5, CCRΔ32, the receptor necessary for HIV-1 attachment and proliferation.

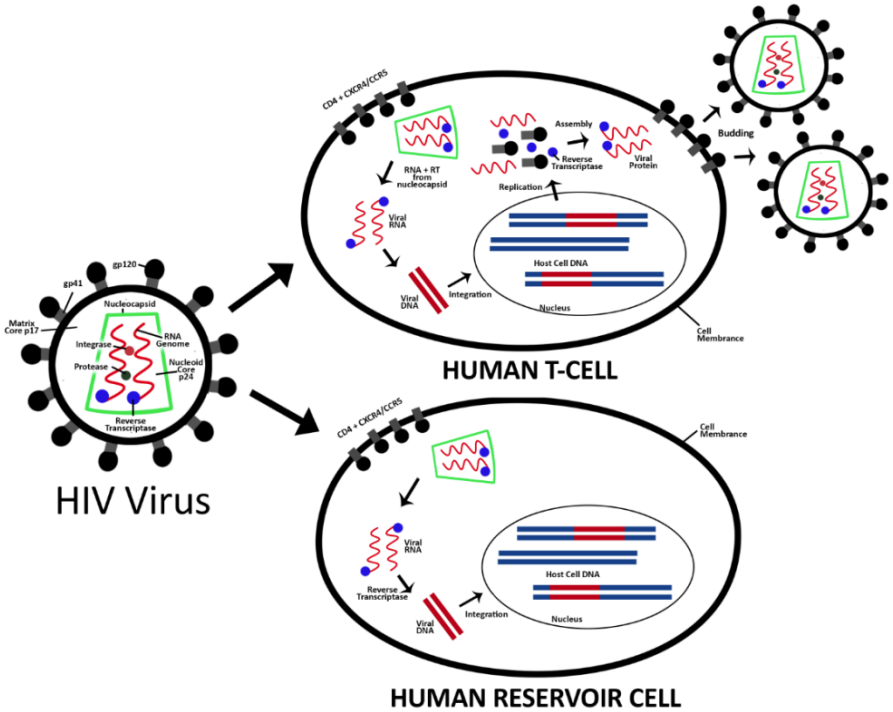

Figure 1. Represents the two courses that HIV infection will follow: Active infection in the human T cell; Latent infection (as a provirus) in the human reservoir cell [5].

Pharmacologic and other immunological approaches such as the use of antibodies and associated immunotherapy are addressed below with a focus on cutting edge genome editing innovative tactics that may represent the answer to this viral plague. In this critical evaluation, various strategies for the control and treatment of HIV with the aim of eradication of virus from all tissue reservoirs are reviewed.

2. Pharmacologic approaches

With the availability of antiretroviral agents in the 1990s, it was presumed these drugs could completely inhibit HIV replication as with any effective anti-infective agent against its microbial target. Specifically, based on the pharmacologic and pharmacokinetic attributes and dynamic properties of the antiretroviral agents (ARA) it was calculated they could clear HIV infection within approximately three years [19]. However, due to the incomplete understanding of mechanisms that allow HIV to persist during cART this goal was not realized. Moreover, the finding that certain tissues have ARA concentrations that were sub-inhibitory lends credence to the concept that the virus exhibits ongoing replication within selected tissue reservoirs during cART. However, investigations of viral sequences from patients displaying cART resistance have failed to provide evidence of evolving viral drug resistant variants [20,21]. Thus, evolving viral drug-resistant variants have not been shown to be the explanation for patients' cART non-responsiveness.

As researchers investigated new approaches to treat and potentially cure HIV/AIDS, decreasing contagion of this virus was also a crucial goal of therapy. Pre-exposure prophylaxis (PrEP) is a newer adjunctive treatment regimen introduced in the last decade as an approach to minimizing contagion of HIV. The application of PrEP was introduced to reduce the risk of infection among men who have sex with men. PrEP utilizes the drug tenofovir, a nucleotide reverse-transcriptase inhibitor that is delivered orally [22]. On occasion it has been combined with another nucleoside reverse transcriptase inhibitor oral agent, emtricitabine. In 2017 the CDC and WHO recommended the daily oral combination of tenofovir and emtricitabine for use as PrEP [23].

3. PD-1/ Checkpoint inhibitors

A strategy in the armamentarium of immune mechanism for limiting the progression of HIV-1 infection is the use of checkpoint molecules. Programmed cell death protein-1 (PD-1) and other checkpoint blocks have the capability to restore T cell function which ultimately may impact the establishment and maintenance of the HIV-1 viral reservoir [24]. It appears that the extent of the reservoir is positively correlated with the frequency of PD-1 expressing cells [25]. PD-1 is transcriptionally induced in activated T cells, B cells, natural killer T cells and monocytes. As the inhibitory receptor of activated T cells, the mechanism of $CD8^+$ CTL functional exhaustion was understood [26]. It has been shown that during chronic HIV-1 infection there is PD-1 overexpression in $CD8^+$ T cells [27]. A direct correlation between PD1 levels and viral load and an inverse correlation with the capacity of HIV-1 specific $CD8^+$ T cells to proliferate [28] has been identified. From this information it can be concluded that PD-1 expression predicts disease progression in that it correlates positively with increase in HIV specific $CD8^+$ T cells and plasma viral load while correlating negatively with $CD4^+$ T cell counts. Further work is needed to establish whether PD-1 block has a consistent effect on HIV.

4. Latency and latency reversal agents

It is clear that the utilization of cART throughout the world has seemingly blunted the spread of new cases of HIV infection and impacted the expansion of the disease [29]. Yet, despite effective cART, HIV infection can persist for decades, due to the persistence of the virus as provirus within long-lived resting memory $CD4^+$ T cells [13,30,31]. Latent infection is established within days of infection into a fraction of the cells that are infected and persists with a half-life of 40–44 months requiring extended cART to prevent re-infection [32]. Many options/strategies have been explored to reverse this barrier termed latency. Of the several approaches, a pharmaceutical option utilizing latency reversal agents (LRAs) such as vorinostat and disulfiram has been studied [33].

cART has been shown to significantly improve the clinical outcome for HIV infected patients. This highly effective treatment which drives viremia to undetectable levels in serum does not represent a cure. Latent virus persists as proviruses in latently infected resting memory $CD4^+$ T cells and can be found in many tissues as previously noted, namely blood, lymph nodes, the gut, and the central nervous system, notably the microglia and astrocytes. Eradication of the latent reservoir and the ability to truly cure an infected patient remains a significant challenge.

A major barrier to effectively curing HIV is the persistence of integrated provirus [34]. Multiple factors associated with the viral long terminal repeat (LTR) repress transcription [35,36]. Chromatin-modifying proteins and histone deacetylases alter the chromatin structure of integrated viral promoter to prevent access and binding by positive transcription factors. As these latently infected cells produce little to no viral proteins, they readily escape identification as harboring the virus. The absence of targets from these latently infected cells permits them to evade the actions of the immune system or antiviral drugs.

Several attempts have been taken to reactivate latent HIV provirus. Histone deacetylases (HDACs) maintain stable HIV infection. Drugs that block HDACs include trichostatin and valporic acid. The protein C activators (prostratin and bryostatin) and histone deacetylase inhibitors (vorinostat and panobinostat) have been shown to efficiently reactivate latent virus and have shown to be safe although limited in reducing the size of the latent reservoir. Another strategy termed “shock and kill” has been proposed in which reactivation is followed by a different treatment to kill the infected cell. In this approach, patients remain on cART to prevent the spread of infection to new cells [36]. It is anticipated that activation of virus will coincide with expression of marks that can be used to kill the newly infected cell either immunologically or through pharmaceutical intervention. Potential barriers to this approach include the inability to reach drug levels capable of reactivating all latent virus, increased susceptibility of T cells to infection following reactivating treatments and suppression of cytotoxic T lymphocytes following treatment with nonspecific reactivating agent. The limited capability of pharmaceutical or immunological approaches to reactivate latent virus and thus expose them to the activity of cARTs requires new and additional approaches that can elicit HIV reactivation with a high degree of specificity and low index of toxicity.

Latent infection is established early—within a day of viral entry in host cells and appears to be established in a fraction of the initial cells that becomes infected [37]. Infection occurs when HIV-1 provirus, the DNA form of the HIV genome becomes transcriptionally inactive leading to an accumulation—a reservoir of provirus. Latency decays slowly, requiring lifelong cART to inhibit re-establishment of infection. A detailed understanding of the dynamics that allow HIV infection to persist for years despite effective cART is needed to manage the treatment and course of a patient’s

disease. A central problem in this seesaw dilemma of proviral latency is the persistence of quiescent but replication-competent provirus resting in $CD4^+$ T lymphocytes and other cell populations.

5. Cellular and humoral adaptive immune responses

During natural human HIV type 1 infection, cellular and humoral adaptive immune responses to a limited extent, control virus replication [38,39]. $CD8^+$ T-cell responses act to reduce viral load after acute infection and polyfunctional $CD8^+$ T-cells select for escape viral mutants [40–42]. HIV-1 antibodies comprise two (2) major classes, neutralizing antibodies (NAbs) and non-neutralizing antibodies (non-Abs). The former, NAbs can prevent infection of target cells by binding to the virion envelope glycoprotein; the non-Abs are unable to bind to virion envelope and prevent infection but are capable of reorganizing HIV-1 Env on the surface of HIV-1-infected cells at the time of virus entry and assembly. They mediate antiviral activity through Fc effector functions [43,44]. HIV type 1 uses the CD4 marker as its principal target to infect T-cells. The HIV-1 genome integrates into the host cell, leading to persistent infection permitting it to remain transcriptionally silent in latently infected $CD4^+$ T-cells [13].

The characterization of antibodies from people infected with HIV has evidenced the neutralization of many HIV variants; they are referred to as broadly neutralizing antibodies (bnAbs) [45]. These antibodies have been shown to be detected in about 25% of persons with untreated HIV-1 infection, reflecting a host immune response to ongoing viral replication with evolving viral variants and shifting antigen exposure. The bnAbs may possess some selective pressure, but generally, they do not reduce the viral load, slow the progression of disease or improve health. Numerous bnAbs have been identified. The bnAbs that have been the focus of the greatest attention are those capable of neutralizing the largest member of HIV-1 strains, including those that are usually neutralization resistant [46,47]. The major goal of the initial search to identify and understand HIV bnAbs was for the purpose of elucidating HIV-1 envelope proteins that could be used in an effective HIV-1 vaccine [48].

The major barrier to eradicate HIV in lieu of a preventative or therapeutic vaccine is the latent proviral reservoir that persists despite long-term highly active cART [49]. Integration of reverse transcribed viral DNA into the host cell genome is an essential step during the HIV-1 infective life cycle. The integrated retroviral DNA is referred to as a provirus which serves as the primary source of viral protein production. Latent infection occurs when HIV-1 provirus becomes transcriptionally inactive resulting in a latent reservoir.

As noted earlier, nearly 40 million people worldwide are living with AIDS and considerable scientific effort is directed toward exposing and eliminating HIV reservoirs. In addition to various tactics using cART, other pharmaco-immunologic approaches to include the use of checkpoint inhibitors, and genome editing tools which employ artificial nucleases, i.e. endonucleases such as zinc-finger nucleases (ZFNs) and other transcription activator-like effector nucleases (TALENs) (see below). Each genome editing nuclease bears specific DNA-binding modules that recognize the HIV-DNA sequences in order to access and modify the nucleic acid sequence that is targeted.

6. Genome approaches: genetic editing technologies

6.1. RNAI & HIV

Within the past two decades, several technologies have emerged enabling modification of gene expression or genomic organization. Direct inhibition of gene expression by RNA interference (RNAi) has been achieved through the availability of genome wide RNAi libraries that have enabled screens to identify key elements in the life cycle of HIV [50]. RNAi technology has been applied to target all stages of HIV replication from viral entry to integration and transcription [51–53]. These seemingly promising results have only been obtained in HIV for in vitro assays and have yet to be efficiently transposed to the clinical setting. A limitation of the RNAi strategy has been associated with complications in the emergence of resistant strains [54]. Avoidance of this complication can be attained by bypassing target highly conserved regions of the HIV genome or utilization of multiple targets necessary for HIV infection and/or replication [55]. As an example, the co-receptors CCR5 and CXCR4 have been within the sights of this investigative direction [56–58]. Effective RNAi based antiviral, i.e. HIV, has the limitations associated with use of an effective delivery method, usually trialed in the form of lentiviral or adeno-associated virus [59]. Ongoing investigations using RNAi and genome editing tools (see below) as potential anti-HIV therapy aim to generate cells resistant to infection by simulating the effect achieved in the case of the "Berlin Patient"—an HIV-positive individual cured after stem cell transplant with CCRΔ32.

6.2. Genetic editing systems

Since the early 2,000s, advances in genome editing technologies based on programmable nucleases have markedly improved the capability to engineer precise changes in the DNA of eukaryotic cells [60]. Genetic engineering systems seemingly can direct this type of specific editing enabled by artificial nucleases as discussed below. Two recognized first generation genome editing technologies that include ZFNs and TALENs modifying a specific effector protein to edit a designated focus such as the promoter of the HIV provirus. Specifically, they were capable of disrupting CCR5 [61–63] or CXCR4 [64,65] co-receptors, therefore inhibiting HIV entry. ZFNs and TALENS have demonstrated significant applications in studies of HIV/AIDS prevention and therapy [66–69]. However, both of these methodologies exploit the principle of protein-DNA recognition but often involve a certain degree of ambiguity.

6.3. CRISPR/Cas 9

Given the limitations of the ZFNs and TALENs, newer genome editing technologies that could lead to better control of HIV have been explored. Over time bacteria and archaea evolved a unique antiviral defense system to combat invasion and infection by hostile viruses. The system is composed of clustered regularly interspaced palindromic repeats (CRISPRs) with associated genes—CRISPR associated genes (Cas 9). The CRISPR/Cas 9 system develops an adaptive immune resistance to foreign plasmids and viruses by establishing site-specific DNA double-stranded breaks (DSBs). Structurally, Cas 9 is a nuclease of 1368 amino acids comprising two nucleases activity domains: HNA and RUVC. Each domain can cleave a DNA strand directed by a complementary short

guide (sgRNA). The CRISPR/Cas 9 gene editing nuclease requires association with the sgRNA to hybridize to the target DNA site and recruit the Cas 9 endonuclease [70].

The CRISPR/Cas 9 system possess the capability to disrupt latently integrated viral genomes and has been shown to provide long term defense against viral infection, expression and replication in human cells. Unlike the CRISPR/Cas 9 system initially extant in bacteria, contemporary CRISPR/Cas 9 systems have been manipulated and adapted for genome editing. Gene repair is the ultimate goal of gene editing; other gene-editing tools have demonstrated the potential for therapeutic application. As previously noted, ZFNs and TALENs, surgical nucleases, fuse DNA nuclease and DNA binding domains to cut DNA at specific loci. In contrast, Cas 9 nucleases work with guide RNA-sgRNA to break/cut DNA at specific loci. Systems utilized currently include sgRNA and other defined RNAs which excise the target DNA and sgRNAs which direct Cas 9 to target sites [71,72]. The initial attempt to utilize CRISPR/Cas 9 for the eradication of HIV was reported from Japan in 2013 [73]. In other studies, investigators attempted to eliminate HIV provirus from T cells by targeting the LTR region of the provirus as HIV-1 gene expression is regulated by the LTR promoter [73,74].

HIV receptors have also been the target for HIV prevention by many investigators. CD4 is the primary receptor on human T cells and CCR5 and CXCR4 are necessary secondary constituents (see Figure 1) [5]. CRISPR/Cas 9 T cell genome engineering used by Hou et al. [74] satisfactorily disrupted CXCR4 in human primary $CD4^+$ T cells. Multiple sites within this gene locus were targeted and as a result expression was decreased approximately 30% [74]. The re-engineered T cells exhibited resistance to HIV infection and marked reduction of p24 antigen without damage to the cells' capability to divide and propagate normally. Similarly, Cas 9/sgRNA ribonucleoproteins (Cas 9 RNPs) were used to ablate CXCR4 in human T cells. The successful outcome realized by the case of the Berlin patient prompted Ye et al. [75] to produce CCRΔ32 pluripotent stem cells using the CRISPR/Cas 9 system. In these experiments, nearly 100% of the stem cells were successfully modified. The monocytes and macrophages derived from the bioengineered stem cells were found to be resistant to HIV infection [75]. In a coupled experiment, the CRISPR/Cas 9 technology was used to block CCR5 expression in human CD^+ cells.

In addition to the experiments demonstrating depletion or disruption of HIV receptors on human cells, many investigators have directed CRISPR/Cas 9 genome engineering to remove or disrupt HIV provirus in latently infected cells (see Figure 2) [5]. The CARES study group for the first time demonstrated the feasibility of the elimination of HIV provirus with CRISPR/Cas 9 in a single patient [76]. Ten sites of the HIV provirus were targeted by Zhu et al. [77] to eradicate HIV DNA from the Jukat human T cell line. These multiple sites were distributed among the LTR region and the *pol* and rev genes of the virus.

With the assistance of programmable sgRNAs directed by Cas 9, cleavage of specific sequences of the HIV provirus is possible. Repair of this broken DNA by the cells non-homologous end joining machinery leads to insertions and deletions (indels) that interfere with normal DNA function. Wang et al. 2016 demonstrated that many of the indels are in fact lethal for the virus, but others lead to the emergence of replication competent viruses that are resistant to the actions of Cas 9/sgRNA [78]. This unexpected finding that HIV-1 is able to escape from Cas 9/sgRNA may not be surprising given that HIV-1 has been shown to develop resistance to cART, immune responsiveness and other pressures.

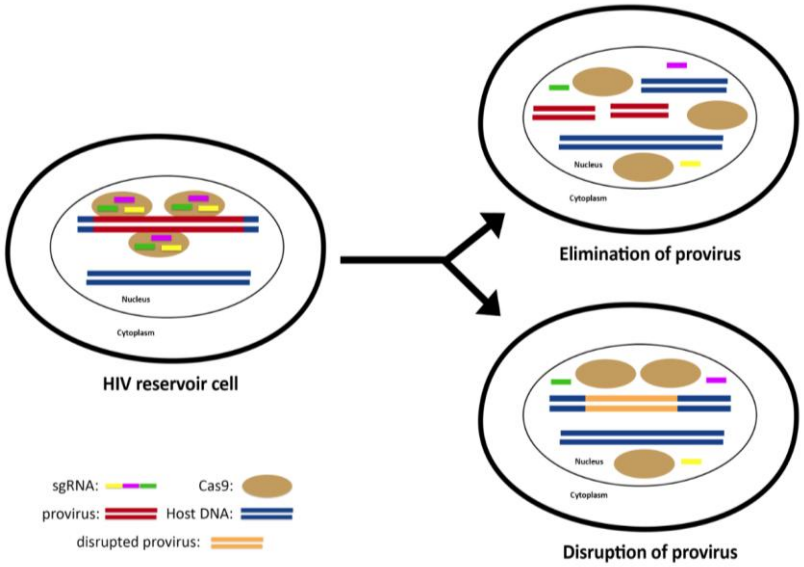

Figure 2. Eradication of HIV provirus from tissues reservoir cells utilizing CRISPR/Cas 9: Elimination of provirus; Disruption of provirus [5].

7. Conclusions and future directions

Although individuals currently infected with HIV appear to have clinical control of their disease and thrive as a result of effective cART, cART does not eradicate the infection. Pre-exposure prophylaxis limits the number of newly infected persons, however HIV infected individuals are never cured. Cure is the hallmark of ideal treatment of infectious diseases i.e. elimination of the infecting organism, in this case HIV. HIV persists as a result of the capability of the virus to become transcriptionally inactive resulting in latent reservoirs in several body compartments. Innovative approaches, including the recently introduced gene-editing tools, particularly CRISPR/Cas 9, hold great promise for the eradication of HIV from all the latent tissue reservoirs and/or the deletion of HIV receptors. A cautionary note is the observation that although some gene editing tools can inhibit HIV replication initially, HIV-1 variants can escape as a result of mutations around the Cas 9 cleavage sites. Hence, the goal for the cure of this widespread infectious viral agent remains elusive.

Acknowledgements

The author wishes to acknowledge the invaluable assistance of Dr. B. Ostrov of Penn State College of Medicine for her critical review and comments which added clarity and meaning to this work.

Conflict of interest

The author declares no conflicts of interest in this paper.

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