REVIEW ARTICLE

The Elusive Nature Toward a Cure for HIV

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Abstract

Extensive efforts have been engaged over the past two decades to characterize and attempt to eradicate a pool of long-lived, virus-infected cells in patients living with human immunodeficiency virus (HIV). This pool of cells, which persists in HIV-infected patients, consists largely of infected CD4+ T lymphocytes that enter and maintain a resting or quiescent memory state in which viral expression is repressed. The longevity of this pool of cells, referred to as the latent reservoir, precludes the possibility of patient withdrawal from potent anti-retroviral therapy (ART) without a predictable rebound of blood born virus despite long-term administration of ART. This circumstance mandates that the vast majority of HIV-infected individuals remain on ART indefinitely. There are compelling reasons to pursue ablation of the latent reservoir since it serves as a persistent source from which viremia ensues upon withdrawal of ART. A means to eradicate this reservoir would be regarded as tantamount to a cure of the infection and relieve patients from life-long administration of ART. This review outlines the mechanisms of latent reservoir formation and its sustenance. It presents circumstances of individuals who are naturally refractory to viremia without the necessity of ART or have been treated to become so. Both of these circumstances may serve as mechanistic models toward the design and implementation of a sterilizing cure. Finally, a number of molecular-based strategies are discussed which are directed toward the complete and sustained suppression of virus expression and/or eradication of the latent reservoir which still remains an elusive goal.

Keywords: HIV latent reservoir, HIV-infected resting CD4+ cells, HIV Anti-Retroviral Therapy

1. Introduction

Almost four decades have passed since the ongoing era of HIV/AIDS began with the recognition of a cluster homosexual men presenting with *Pneumocystis carinii*, cytomegalovirus (CMV), candida mucosal infections and other opportunistic infections of unknown etiology. A few years later, the etiologic agent responsible for the depletion of patient immune systems was identified as a novel retrovirus ultimately referred to as HIV (Human Immunodeficiency Virus) and its genome sequenced.[1,2] By 1985, the first generation of anti-retroviral therapeutics was developed by rational drug design and their administration to HIV-infected patients brought about a rapid inflection in circumstance from AIDS as a lethal disorder to one that could be managed as a chronic, non-lethal disease though not a means toward a cure.[3] The mechanism underlying the chronic nature of the infection became evident with the detection of a persistent pool of latently HIV-1 infected cells in patients despite potent anti-retroviral therapy (ART).[4,5,6] Moreover, this reservoir of long-lived cells may contribute to the on-going, low-level release of virus along with metabolically active non-reservoir cells both of which are refractory to complete viral suppression and clearance from within patients despite years of ART administration.[7,8,9]

2. Early Establishment of the HIV Latent Reservoir

The HIV persistent latent reservoir is established rapidly and within a very limited window of time after exposure to the virus.[4] This narrow window for latent reservoir formation was observed to occur within as little as three days post-infection as evidenced in a number of Simian Immunodeficiency Virus (SIV) studies.[10] Select human case studies have suggested even more rapid establishment of the latent HIV reservoir post-infection. The Mississippi baby is a case of particular interest. The specifics of this case indicated that the latent reservoir is likely established in as little as 30 hours post-infection given the rapid administration of ART in this case. The child, born prematurely to an HIV-infected mother, was administered liquid ART 30 hours postpartum and continued therapy for 18 months but was then briefly lost to the health care system and the child's ART treatment discontinued. Some months later, re-engagement and testing of the child revealed undetectable viremia and this status continued for more than two years without reapplication of ART.[11] The child eventually incurred viral rebound just short of 4 years of age.[12] While rebound was an unfortunate development, the case of the Mississippi baby, indicates that latent reservoir formation can be established very early after exposure to virus, and further,

that periods of non-viremia for relatively lengthy periods of time could be possible for some individuals after withdrawal from ART. Given that reservoir seeding is now recognized to occur early after infection, it has become common practice to engage ART as a prophylaxis for high-risk populations or as close after the time of known exposure to virus which may potentially decrease the likelihood of latent reservoir establishment.[13,14,15]

3. Establishment of the Latent HIV Reservoir and Its Sustenance

HIV-1 is a monotropic and T-cell tropic virus primarily infecting monocytes and macrophage as well as T-lymphocytes. The persistent pool of HIV-1 latently infected cells is comprised largely of resting memory CD4+ T cells bearing HIV-1 proviral genomic DNA.[16,17] The mechanistic bases that underpin the silencing of viral DNA within resting CD4+ T-cells as well as non-resting cells are varied and occur at several levels. The primary mechanism of latent reservoir formation seems most closely linked to reversion of actively replicating HIV-infected CD4+ T cells into a resting status referred to as the quiescent or latent state.[17] Such cells exhibit a memory phenotype identified via the presence of specific cell surface markers indicative of a resting state. Apart from cellular quiescence triggering HIV latency, additional molecular mechanisms may also contribute to the silencing of HIV proviral DNA which can occur in the in actively dividing infected cells as well.[18] Among these are mutations within proviral DNA that silence viral gene expression. Such mutations may revert to replication competent status and re-engage virion production particularly in actively dividing cells. Integration of the HIV provirus into heterochromatin as well as methylation events that reconfigure chromatin structure that results in epigenetic silencing of proviral DNA transcription have also been observed.[19,20] This chromatin-based silencing is reversible however.[21]

The metabolic dynamics of quiescent cells appear not to support viral expression owing to the down-regulation of select transcription host cell factors necessary for viral gene expression.[22,23] The low-level of metabolic activity in latently infected cells affect host cell transcriptional and post-transcriptional regulation, cell cycle progression and cell division that collectively result in multiple metabolic blocks of viral gene expression.[18] It is of interest that the blockade to viral expression can be overcome upon cellular activation of the HIV-infected resting T cell population. This has been demonstrated by exposure of latently infected cells to a wide variety of agents that trigger cellular activation thereby re-engaging robust virion production.[24,25,26] The induction of virus gene expression by sporadic activation of latently infected reservoir cells in patients can

lead to localized virion spread. Unfortunately, ART also appears insufficiently robust toward completely seizing on-going low-level viral expression. This can also result in virion release from infected cells to neighboring uninfected cells.[27,28,29] These dynamics present considerable challenges toward the complete eradication of HIV infection with the currently available armamentarium of anti-retroviral agents.[30]

4. Long-Term Non-Progressors and Elite Controllers

There is a wide spectrum of individuals that exhibit substantially different responses with regard to viremia and disease progression following HIV infection. This spectrum consists of reasonably defined categories including: rapid disease progressors, slow disease progressors, long-term non-progressors (LTNP) and elite controllers (EC).[31]

The frequency of the individuals in the latter two groups, referred to as long-term non-progressors or elite controllers are quite rare in the overall population of individuals infected by HIV yet are of considerable interest owing to their ability to maintain very low or undetectable levels of viremia for extensive periods of time in the absence of ART.[31] Consequently, the mechanisms that allow these individuals to constrain viral expression without ART, if clearly defined, could facilitate the development of a "functional cure" for HIV infection. The term functional cure can be defined as the ability to persistently and effectively control HIV infection in the absence of ART despite the ongoing presence of a HIV infected cells.

With regard to most ECs, viremic and disease progression control seems related to their vigorous and durable anti-HIV immune response.[32] Soon after HIV infection, ECs rapidly down-regulate viral replication in tissue compartments to a very low level especially in lymphoid tissue and very few circulating HIV-infected CD4 cells are present which is linked to a very slow manifestation of HIV-related disease progression. Unfortunately, it remains unknown as to whether LTNPs and ECs can maintain their constraint of viremia and disease-free progression status indefinitely. Nonetheless, a precise understanding of the underlying cellular, physiological and immune-based mechanisms involved in the control of viremia by LTNPs and ECs may point to useful strategies to suppress viral expression in the larger population of individuals that are unable to do so without ART.

5. The Berlin Patient: Over A Decade Free of HIV

Over a decade following intensive chemotherapy followed by allogeneic hematopoietic progenitor stem transplantation to treat leukemia, Timothy Ray Brown referred to as the Berlin Patient, continues free of HIV.

Timothy Brown underwent transplantation stem cell therapy (SCT) in Berlin performed by Gero Hutter and his medical team. Mr. Brown was infused with donor cells which bore a homozygous mutation in the CCR5 cell surface receptor. CCR5 is a necessary co-receptor for virion binding and entry into monocytes/ macrophage. As a consequence of SCT, Mr. Brown has remained free of viremia in the absence of ART. Mr. Brown is presumed "cured" of HIV infection since virus has remained undetectable in the absence of ART as confirmed by several biopsies of various tissue compartments including peripheral blood cells, liver, gut and brain.[33,34] There have been additional attempts in the use of SCT to "cure" HIV infection, whether that be directed at absolute eradication of virally-infected cells or a re-population of immune cells resistant to infection toward a functional cure. Another attempt of using SCT, similar to that of the Berlin patient, pointed to a drawback in the overall utility of this approach as the patient incurred viral rebound of a non-CCR5 viral variant.[35] Furthermore, SCT, as a broad-based approach to cure HIV infection, has many complexities and potential complications including, but not limited to, risks associated with the procedure and scarcity of a compatible donors that bear the homozygous CCR5 mutation.[36]

6. Eliminating HIV-1 Infection Via Gene Delivery or Editing Therapies

Recent gene delivery or editing systems offer intriguing methodologies toward potential resistance to, or eradication of, HIV infection. These DNA-based delivery and editing systems have been employed in a variety of ways to either protect HIV susceptible cells from *de novo* infection, inhibit viral gene expression of existing infected cells, prevent viral budding or precisely "snip out" infectious proviral DNA from infected cells. The methodologies employed vary in the technologies used and their conceptual objective toward a "cure" but all share the common goal toward durable protection from infection or latent reservoir eradication for individuals currently infected with the virus requiring ART.

A brief survey of approaches include the expression of trans-dominant viral proteins to block virion production. An initial example for this type of approach was the use of a lentiviral vector to express transdominant-negative mutant of Rev (TdRev) in cells which prevented the nuclear export of unspliced HIV RNA transcripts.[37] The use of HIV-1 or lentiviral-based vectors to express gene products has the advantage of vector delivery into non-dividing hematopoietic cells. CD34+ cells, transduced with the TdRev expressing vector, were transplanted into patients and prolonged stable expression of TdRev was

noted but patients were not withdrawn from ART so the efficacy of this approach and well as those using other trans-dominant target proteins toward a durable cure remains inconclusive.[37]

Intracellular RNA expression-based virus inhibitory therapies are currently being evaluated which represent a completely different approach to halt viral expression relative to trans-dominant viral protein inhibition. Directed destruction of viral or cellular RNAs whose synthesis, protein translation and/or stability are necessary for viral entry, expression or virion budding are all feasible targets for inclusion as gene therapeutic approaches toward a functional or even complete cure of infection. The number of such studies using this approach is impressive.[38,39] As well, there are many intracellular delivery modes of expressing inhibitory RNAs or ribozymes that specifically direct cleavage of either viral or host cell RNAs which are critical for HIV expression.[40] These delivery modes include a variety of viral RNA expression vectors, liposomes, protein or chemical moiety RNA conjugates and synthetic bead or nanoparticles carrying antiviral agents of varying composition.[40,41] However, it could be expected that these RNA-based gene therapy approaches may encounter the same problem as conventional ART – specifically the emergence of mutation-based viral resistance to the inhibitory RNA sequences.

Perhaps the final frontier toward eliminating the persistent latent reservoir is direct DNA-based ablation of proviral genomes in quiescently-infected cells. The proof of concept of this approach has been demonstrated recently using the CRISPR/Cas9 gene guide RNA editing system. This system can introduce, edit or delete specific DNA sequences with great precision. HIV-1 replication can be completely shut down and the virus eliminated from infected cells in animals with a powerful gene editing technology known as CRISPR/Cas9. The use of the CRISPR/Cas9 system has the ability to inactivate expression of integrated HIV DNA genomes in select animal model systems that parallel the human infection recapitulating both acute and latent human infections.[42]

Recently, a model of latent HIV-1 infection was generated in mice engrafted with human immune cells and those cells infected with HIV *in vivo*. Importantly, these animals bore latent HIV in the genomes within the mice-engrafted human T cells. Treatment with saCas9 and multiplex guide RNAs, in this proof of concept experiment, successfully excised proviral DNA with great efficiency from the latently infected human cells embedded in mouse tissues and organs thereby providing a new molecular approach toward reservoir eradication.[43]

7. Conclusions

The possibility of a cure for HIV infection remains hopeful. Understanding mechanisms whereby elite controllers constrain viremia without therapy could play a significant role in developing immunotherapeutic strategies toward a "functional" cure for HIV-infected individuals currently requiring ART. Delivery of molecular based inhibitors into cells which comprise the latent reservoir, directed toward targeting complete down-regulation of viral expression or the removal of these cells is technically feasible but not yet demonstrated to be capable of complete silencing or ablation of the latent reservoir in patients. Finally, the seek and destroy approach to cripple or excise latent proviral genomes with extreme precision via gene editing systems presents yet another exciting advancement toward the goal of latent reservoir eradication.

Almost 25 years ago, efforts to save lives from the ravages of HIV by rational anti-retroviral drug design lead to the development and evolution of ART which eliminated the lethal face of this infection. Although the challenge of a cure for this contagion remains elusive, recent efforts which demonstrate unprecedented efficiency and specificity toward reservoir eradication offers guarded hope and optimism toward this goal.

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