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Engineering hematopoietic stem cells toward a functional cure of human immunodeficiency virus infection

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Abstract

The battle with human immunodeficiency virus (HIV) has been ongoing for more than 30 years, and although progress has been made, there are still significant challenges remaining. A few unique features render HIV to be one of the toughest viruses to conquer in the modern medicine era, such as the ability to target the host immune system, persist by integrating into the host genome and adapt to a hostile environment such as a single anti-HIV medication by continuously evolving. The finding of combination anti-retroviral therapy (cART) about 2 decades ago has transformed the treatment options for HIV-infected patients and significantly improved patient outcomes. However, finding an HIV cure has proven to be extremely challenging with the only known exception being the so-called "Berlin patient," whose immune system was replaced by stem cell transplants from a donor missing one of HIV's key co-receptors (CCR5). The broad application of this approach is limited by the requirement of an HLA-matched donor who is also homozygous for the rare CCR5 delta32 deletion. On the other hand, the Berlin patient provided the proof of concept of a potential cure for HIV using HIV-resistant hematopoietic stem cells (HSCs), revitalizing the hope to find an HIV cure that is broadly applicable. Here we will review strategies and recent attempts to engineer HIV-resistant HSCs as a path to an HIV cure.

Key Words: *gene therapy, human immunodeficiency virus–resistant, stem cells, transplantation*

Introduction

Human immunodeficiency virus (HIV) is the causative agent of acquired immune deficiency syndrome (AIDS). With 36.9 million people infected worldwide, HIV infection stands as a huge health and economic burden, especially in sub-saharan Africa. The development of highly active anti-retroviral therapy (HAART) or combination antiretroviral therapy (cART) initiated about 2 decades ago has transformed the treatment options for HIV-infected patients and significantly improved patient outcomes [1,2]. However, cART can neither eliminate latent reservoirs of virus nor fully restore the health of patients [3–5]. Successful control of HIV infection requires a lifetime of excellent patient adherence to the treatment protocol, and the medications themselves can be quite expensive. Furthermore, the pharmacological toxicities associated with the treatment regimen can be intolerable for some patients. Hence, searching for an HIV cure is the ultimate goal of many researchers. With the recent report of the first cured Berlin patient [6,7], the field is revitalized with the hope of finding a broadly applicable cure.

Viral reservoirs and a functional cure

HIV is an enveloped RNA virus. HIV infection is initiated through the binding of viral envelop protein gp120 to cellular CD4 receptor, followed by conformational changes in gp120 and subsequent binding to the CCR5 or CXCR4 co-receptor, which then triggers structural changes in gp41 and ultimately leads to membrane fusion and virus entry. Viral genomic RNA (gRNA) is released into the cytoplasm upon uncoating and is reverse transcribed into viral genomic DNA (gDNA). Viral gDNA is translocated into the nucleus of the infected cell in the form of a pre-integration complex, and then integrated into host chromosomes (provirus). After transcription and translation, viral proteins and gRNA are packaged (assembled) into a new virion and released from the cell membrane by budding to spread the infection [8]. Various processes in the HIV life cycle are targeted for anti-HIV therapy [9]. Most components of the current cART regimen mainly target the post-entry process and are highly efficient in inhibiting HIV replication. However, once the virus gets integrated into the host cell genome and becomes dormant, it is mechanistically difficult to remove the integrated viral DNA.

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The infected cells, especially those long-lived cells such as memory T cells, with dormant virus form a major component of the viral reservoirs [10,11].

Based on the definition by amfAR, the Foundation for AIDS Research, to be considered cured, an infected person would need to meet three criteria: (1) be able to live a normal, healthy lifespan; (2) be off antiretroviral therapy or any other HIV-related medications; and (3) be incapable of transmitting the virus to others. A "sterilizing" cure, comprising a complete eradication of all replication-competent HIV from the body, is desirable but would be extremely difficult to achieve and nearly impossible to prove [12,13]. Thus, a functional cure, where the virus may not have been completely eliminated but would be effectively controlled and prevented from causing disease progression may be the most realistic goal in the near term [13]. A functional cure generally means people can live a reasonably normal life, perhaps a completely normal life, without the need for cART. Remission or long-term remission, the latest definition to enter the HIV cure dialog, refers to control of HIV viral load at low levels (such as <50 copies/mL) in the absence of cART treatment for a relatively long period of time (unspecified so far), which could have the potential to rebound due to low levels of HIV replication [14].

T.B., the Berlin patient, represents a functional HIV cure, and possibly a sterilizing cure [6]. He had both HIV and leukemia and underwent intensive radiation and chemotherapy, which can potentially wipe out a large portion of viral reservoirs, and received hematopoietic stem cell transplantation (HSCT) twice from an HIV-resistant donor with the "delta 32" mutation in the CCR5 gene (CCR5 Δ32/Δ32). The 32-base pair (bp) deletion (Δ32) in the CCR5 gene causes a frame shift mutation at position 185 and in turn leads to dysfunction and diminished surface expression of the CCR5 receptor. Individuals with a homozygous Δ32 deletion are consequently highly protected from infection with HIV-1. The naturally occurring mutation is most common in white populations where about 1% of the population are homozygous for the mutation [15,16]. The most ultrasensitive tests have detected no replication-competent HIV in the patient's tissues collected about 5 years after his radical treatment. In addition, anti-HIV antibody and cytotoxic T lymphocyte (CTL) responses decreased or disappeared [7]. The Berlin patient has remained off of cART for about 9 years now. Of note, the stem cell donor was both an appropriate tissue match and homozygous for the CCR5Δ32 mutation, which is extremely rare. In addition, a graft-versus-host disease (GVHD) developed after the treatment, where donor-derived immune cells likely attacked/eradicated residual leukemia cells as well as HIV-infected donor immune cells including viral reservoirs. More recent studies of the two "Boston patients" [17,18], who also received cytoablative conditioning and allogenic HSCT, but from wild-type CCR5 donors, initially reported markedly reduced viral reservoirs and undetectable HIV viremia for months. However, the virus later returned in both patients after ceasing cART, highlighting the potential necessity of using HIV-resistance HSCs as donor cells for transplantation to attain a cure.

The "Mississippi baby" and the 14 post-treatment controllers from the ANRS VISCONTI study (Viro-Immunological Sustained CONtrol after Treatment Interruption study funded by the French National Agency for Research on AIDS and Viral Hepatitis) [19–21], who had anti-HIV treatment initiated very early and had remission without viremia for much longer than the typical 2–4 weeks after therapeutic interruption, provided evidence for the effective control of viral reservoirs by starting cART early after infection. Early treatment is thought to potentially reduce the size of viral reservoirs, preserve patients' immune responses and protect them from chronic immune activation. However, despite the early hope of a cure for this child, detectable HIV has now been measured in the Mississippi baby's blood. Thus, these studies highlight how stealthy and pernicious HIV is and how challenging it can be to eradicate viral reservoirs with cART alone.

Multiple strategies have been pursued to eradicate the viral reservoirs. One of the most discussed and tested strategies is the so called "shock and kill" or "kick and kill." The dormant reservoirs are activated by various latency reversing agents (LRAs) and subsequently eliminated by the viral cytopathic effect or by the immune system in the presence of cART to prevent new infections [22,23]. Several epigenetic and non-epigenetic LRAs have been tested and appear promising, including inhibitors of histone deacetylases (HDAC), histone methyltransferase (HMT), and DNA methyltransferases (DNMT), protein kinase C (PKC) agonists and others [22,23]. However, portions of the HIV reservoir (the "deep reservoir") appear to be refractory to LRA-mediated activation [24]. Alternatively, the LRAs tested have poor *in vivo* pharmacokinetic activity to reach and activate the target cells, or possibly not all infected cells were eliminated by the viral cytopathic effect or the immune system following LRA-mediated activation [25]. Hence, several immunologic strategies (immunotherapy) are being explored to augment the capacity of the host immune system to eliminate viral reservoirs, including therapeutic vaccines, broadly neutralizing monoclonal antibodies, HIV-specific CTLs and various immune modulating agents including those that target the immune checkpoints PD-1 and CTLA-4 [22,26,27]. However, the ability of HIV-1 to destroy activated CD4 T cells and the defect in normal CD4 T-cell function in HIV-infected patients have probably played an important role in the largely unsuccessful attempts so far.