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Review

Engineered DNA modifying enzymes: Components of a future strategy to cure HIV/AIDS

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ABSTRACT

Despite phenomenal advances in AIDS therapy transforming the disease into a chronic illness for most patients, a routine cure for HIV infections remains a distant goal. However, a recent example of HIV eradication in a patient who had received CCR5-negative bone marrow cells after full-body irradiation has fuelled new hopes for a cure for AIDS. Here, we review new HIV treatment strategies that use sophisticated genome engineering to target HIV infections. These approaches offer new ways to tackle the infection, and alone or in conjunction with already established treatments, promise to transform HIV into a curable disease.

Contents

1. Introduction

Combination antiretroviral therapy (ART), introduced into clinical practice in the mid-1990s, has profoundly reduced HIV-associated morbidity and mortality, changing a lethal disease into a chronic illness (Palella et al., 1998; Thompson et al., 2010). Although ART can suppress viral loads below the detection limit of standard clinical assays (<50 HIV-1 RNA copies/mL), it cannot eliminate HIV. This is due on the fact that ART targets virus entry or the viral enzymes, but not the integrated provirus. Therefore, ART requires lifelong treatment, potentially leading to problems of cost (Chen et al., 2006; Schackman et al., 2006), adherence (Mannheimer et al., 2002; Paterson et al., 2000), drug resistance (Little et al., 2002; Richman, 2006) and toxicity (Dybul et al., 2002). Particularly, long-term treatment frequently results in secondary complications, such as diabetes, hyperlipidemia, cardiovascular disease, osteoporosis, and chronic kidney disease (Calmy et al., 2009; Deeks and Phillips, 2009).

More importantly, patients successfully treated with ART for several years still do not fully recover their immune responses, and show increased levels of immune activation along with its harmful effects (Ostrowski, 2010; Plana et al., 1998). Consequently, low-level viral replication may persist along with an established pool of latently infected cells (Finzi et al., 1997, 1999; Palmer

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et al., 2008). When ART treatment is interrupted viral load can quickly rebound, even in patients who have suppressed plasma viremia to levels below detection limits for many years (Davey et al., 1999). Therefore, developing novel therapeutic strategies aiming to cure HIV infection must address the pool of cells that harbor the latent HIV reservoir (Chun and Fauci, 2012; Deeks et al., 2012; Richman et al., 2009).

In principle, two qualitatively different types of cure have been defined (Dieffenbach and Fauci, 2011; Lafeuillade, 2011; Lewin et al., 2011). In a "functional cure" the patient's immune defense fully controls HIV in the absence of ART. However, proviral DNA can still be found in the body. In contrast, a "sterilizing cure" eradicates HIV and no viral genes remain in the infected host. Clearly, a functional cure may be easier to achieve, but a sterilizing cure is considered to be the holy grail of HIV therapy.

2. Purging the reservoir

The long-lived resting cells that contain HIV reservoirs reside primarily in tissues, in other words sanctuary sites that may not be easily accessible (Lafeuillade, 2012; Palmer et al., 2011; Smith et al., 2012). The proviral DNA (i.e. the integrated replication-competent HIV genome) in these cells is transcriptionally silenced, mainly due to epigenetic modifications of the viral long terminal repeat (LTR) promoter region (Coiras et al., 2009; Geeraert et al., 2008; Richman et al., 2009). Hence the viral antigens are not expressed, and in consequence, these HIV-infected host cells evade immune surveillance. Importantly, the existence of these viral reservoirs is believed to be the main hurdle to quantitatively clearing the virus from an infected organism. So far, the main and also best characterized reservoir comprises latently infected resting memory $CD4^+$ T cells containing replication-competent but dormant proviral DNA (for recent reviews on HIV reservoirs see Chun and Fauci, 2012; Lafeuillade, 2012; Margolis, 2011a; Palmer et al., 2011; Smith et al., 2012).

An important mechanism for maintaining transcriptional quiescence of the provirus, and hence viral latency, relies on cellular chromatin remodeling enzymes, in particular histone deacetylases (HDACs) (Hakre et al., 2011; Margolis, 2011b). Therefore, a main strategy currently being investigated for eliminating HIV reservoirs is based on pharmacologically inhibiting HDACs, thereby specifically activating latent proviral genomes in resting $CD4^+$ T cells. Upon HIV antigen expression, it is expected that these cells will be eliminated through either direct cytophatic viral effects or immune responses of the host (e.g. cytotoxic T cells; CTL).

Indeed, the HDAC inhibitor (HDACi) suberoylanilide hydroxamic acid (SAHA; Vorinostat), an FDA-approved drug for treating cutaneous T cell lymphoma, did specifically reactivate HIV from latency in chronically infected cell lines and primary cells (Archin et al., 2009; Contreras et al., 2009; Edelstein et al., 2009). More recently, SAHA has been administered to ART-treated HIV-positive patients with fully suppressed viremia (Archin et al., 2012). In a majority of these patients, SAHA not only affected cellular acetylation but also upregulated HIV-specific RNA expression in their resting $CD4^+$ T cells. Clearly, this increase in cell-associated HIV RNA does not necessarily imply that the respective cells could produce viral progenies. Nevertheless, reactivation of latent HIV expression by applying chromatin remodeling drugs, such as HDAC inhibitors, may be an essential mechanism to trigger HIV eradication *in vivo* (Durand et al., 2012). Doubtless, such a strategy will be applied in combination with ART to avoid *de novo* infection during activation of the latent virus reservoir.

As mentioned above, HDACi-induced (i.e. SAHA-induced) activation of latent HIV was generally expected to result in cell death due to either cytopathic viral effects or CTL action. Unfortunately, in another recent study it was shown that neither is the case, even when autologous CTLs from ART-treated patients were present (Shan et al., 2012). Instead, after virus reactivation $CD4^+$ T cells were only killed by CTLs when the cytotoxic T cells were pre-stimulated with HIV-1 Gag peptides.

These data demonstrate that HDAC inhibitor-induced activation of latent HIV will presumably not suffice to eradicate the long-term viral reservoirs by clearing the pool of latently infected cells. It has therefore been suggested that some form of therapeutic vaccination and/or additional interventions may be required for successful purging/eradication attempts (Archin et al., 2012; Shan et al., 2012). These may include gene therapy strategies (Kiem et al., 2012; van Lunzen et al., 2011). This notion is also supported by a more recent study in which various HDAC inhibitors (HDACis), including SAHA, were analyzed with respect to HIV production (Blazkova et al., 2012). It was demonstrated that, in aviremic individuals, HDACis only stimulate HIV expression in a small fraction of latently infected resting $CD4^+$ T cells and may therefore not be able at eliminating these viral reservoirs. The authors therefore suggested that alternative therapeutic strategies that incorporate HIV-specific targeting and/or immune activation approaches will be necessary to clear latent HIV (Blazkova et al., 2012).

3. A singular case of an HIV cure

In 2009, publication of the so-called "Berlin patient" case report revived the notion that a cure for HIV infection might be feasible (Hütter et al., 2009). This HIV-infected patient suffered from acute myeloid leukemia (AML). After failure of chemotherapy, the patient received hematopoietic stem cells (HSCs) from an HLA-identical donor selected for CCR5Δ32 homozygosity. This very rare mutation in Caucasians (~1% occurrence) inactivates the *CCR5* gene which encodes a critical HIV co-receptor (Liu et al., 1996). The patient received fully ablative and potentially lethal conditioning regimes in combination with two successive HSC transplantations. This procedure led to a complete remission of the AML (Hütter et al., 2009). Importantly, however, prior to transplantation the patient discontinued ART and for more than five years now shows no signs of HIV infection (Allers et al., 2011; Hütter and Thiel, 2011).

This is of particular interest, since before treatment a minor population (2.9%) of CCR5-independent virus variants (i.e. CXCR4-tropic or dual-tropic viruses) was also detected in the patient. Why these viruses did not rebound after ceasing ART, particularly in light of the fact that a high proportion of potential target cells (e.g. activated memory $CD4^+$ T cells) were recovered after transplantation, is unclear at the moment (Hütter and Ganepola, 2011). Nonetheless, it is conceivable that the harsh myeoablative conditioning of the patient or other immune reactions may have been responsible for this fortunate outcome.

Obviously, this approach cannot be applied to larger HIV patient cohorts for various reasons. For example, HLA-matched CCR5Δ32 homozygous donors are extremely rare, which in fact has so far prevented the treatment of another patient (Hütter and Thiel, 2011). Also equally prohibiting is the relatively high rate of mortality (~26%) connected with the procedure of allogeneic HSC transplantation (Gooley et al., 2010). Nevertheless, this unique case of the "Berlin patient" obviously jump-started the field of HIV eradication and latency research by demonstrating that an HIV cure is possible under certain, although extremely rare conditions. This case may also suggest that the genetic alteration of host cells, rendering them resistant to HIV, may be an important component of future eradication strategies.