New approaches in HIV eradication research

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Current Opinion in Infectious Diseases 2011, 24:593–598

Purpose of review

Despite the proven efficacy of highly active antiretroviral therapy in reducing mortality and morbidity of HIV infection, longer-term strategies are less well defined and there is renewed interest in HIV eradication. This review will describe the major obstacles that need to be overcome and the key new advances and strategies designed to achieve an HIV cure.

Recent findings

Characterization of the HIV viral reservoir over the past few years has led to a better understanding of which approaches might successfully lead to eradication. A number of approaches such as histone modification, immunotoxins, gene therapy and gene knockout strategies have resulted and have been explored initially *in vitro*. There has been progression from both laboratory and animal model studies, and clinical trials are now underway using new approaches such as histone deacetylase inhibitors and zinc finger nucleases.

Summary

Although there is currently no cure for HIV infection, there has been a resurgence of interest in the field with the development of a number of potential new approaches, some of which have entered clinical trials.

Keywords

eradication, HIV cure, reservoir, viral latency

Curr Opin Infect Dis 24:593–598

0951-7375

Introduction

To mark the 30th anniversary of the first reports of atypical opportunistic infections in Los Angeles which heralded the AIDS pandemic [1], The Economist published an editorial asserting that AIDS is – in principle – now beatable [2]. This was stimulated by data from HPTN052, which showed that not only does highly active antiretroviral therapy (HAART) reduce HIV-associated morbidity and mortality, but also transmission. As such, HAART – if given to all HIV-positive individuals – could bring the pandemic under control. In conjunction with behavioural change (i.e. safe sexual practices, condom use, etc.) and interventions such as male circumcision, the epidemic momentum could be stalled.

In reality, impacting behaviour has proved extremely difficult and provision of HAART to all who need it is logistically and financially untenable. The Centre for Global Development has estimated that to reach global targets for antiretroviral drug provision, the United States would be required to contribute 50% of its foreign aid budget by 2016, and all of it by 2024 [3]. This is at a time when richer nations are reviewing their contributions to the Global Fund – Holland and Spain have announced cuts, and Italy has withdrawn funding completely. This difficult economic scenario is juxtaposed with the possibility that life-long HAART may not fully restore life expectancy despite viral suppression [4,5], and that increasing numbers of presentations with non-AIDS morbidity are being recognized on HAART [6,7]. We do not fully understand the long-term effects of HAART – either good or bad (bearing in mind the cancer protection afforded by aspirin takes around five years to develop [8]) – and although we have learnt from 15 years of experience this is very different to prescribing therapy for life.

In view of these difficulties, there is increasing interest in devising new approaches to HIV eradication, or cure. Targeting therapies to 'latently' HIV-infected cells – those that are the source of the rebound virus when HAART is stopped – has opened a new chapter in HIV therapeutics. This review will highlight some of the key approaches being targeted at achieving this extremely challenging goal.

What are the key hurdles to HIV eradication?

The key barriers to HIV eradication are the persistence of viral DNA and RNA despite HAART [9–14], the

resulting ability of HIV to return to pretherapy levels of plasma viraemia on the cessation of drugs and the lack of any host characteristic – such as a robustly protective CD8-positive T-cell response – which could be induced or enhanced by an intervention.

Even after many years of full viral suppression on HAART, HIV DNA can be identified in a 'latent' form, integrated into the chromosomal DNA of resting CD4-positive T lymphocytes [15,16]. Although stable nonintegrated forms of HIV DNA exist – either as linear forms or as episomal 1-long terminal repeat (LTR) or 2-LTR circles – they have short half-lives and are unlikely to contribute to long-term HIV latency. Significantly, even when therapy has reduced the 'proviral load' to an estimated 1 HIV DNA copy in a billion CD4 T cells, the viral resurgence on stopping therapy is rapid [17•].

If there is on-going replication on HAART, the route to a cure may be the intensification of current antiretroviral regimes. However, most data indicate that residual viraemia on HAART is likely to represent sporadic release of latent virus rather than on-going replication – treatment intensification in chronic disease does not impact plasma viral load [18•], and phylogenetic analyses find no evidence for viral evolution, which would be expected if there was replication in the face of a selection pressure such as HAART [19–21]. However, we may have underestimated the degree of intensification required to show a difference at these low levels of viral replication, and most viral load assays do not reflect replication-competent viraemia, casting doubt on their clinical relevance. Studies that assess other parameters of viral replication suggest that there may be on-going replication – a transient increase in 2-LTR circles following treatment intensification with raltegravir may be evidence that viral replication continues [22•].

The cellular and anatomical source of this recrudescent virus following cessation of therapy remains unclear, although it seems highly likely that it arises from one or more latently infected cells. Phylogenetic studies have so far failed to link the resurgent virus with any particular anatomical sanctuary or cellular reservoir, although circumstantial data suggest that such a site exists [20,23]. In particular, one study associated the HIV sequences of episomal circles – rather than the integrated virus in blood CD4 T cells – with rebound virus, suggesting that a sanctuary site exists which may harbour replicating virus despite HAART [24•].

Strategies to achieving HIV eradication

The HIV reservoir can be approached with two different objectives. A 'sterilizing' cure would fit a more classical infectious diseases model in which an infecting pathogen is rendered either dead or nonreplicative and nonpathogenic, and the patient is considered cured. An alternative, possibly more realistic approach, would invoke a cancer model in which – through an intervention – a patient achieves a drug-free remission until therapy eventually needs to be restarted. Neither of these models is purely hypothetical. The well publicized case of Timothy Ray Brown, the HIV-positive 'Berlin Patient', who received total body irradiation, chemotherapy and a CCR5Δ32 stem cell transplant for acute myeloid leukaemia, shows that a sterilizing cure can be achieved, but with arguably unacceptable risks if there is no other indication for the intervention [25,26••]. The remission model is exemplified by 'elite controllers' – patients who maintain undetectable viral loads without therapy and whose disease progression and requirement for therapy is also negligible. Elite controllers have been well studied and although the CD8+ve cytotoxic T-cell response is a major component to this protection, no clear reproducible pathway has been elucidated [27–31].

Key points

- Current strategies for the management of the global HIV epidemic may be untenable in the long term.
- There is renewed interest in trying to find a 'cure' for HIV by either eradicating the virus from the body or producing a prolonged drug-free remission.
- Current approaches to a cure for HIV either 'exhaust', 'kill' or 'silence' the latent reservoir, or 'restore' the depleted population of CD4-positive T cells.
- A number of agents, such as histone deacetylase inhibitors, methylation inhibitors and NF-κB activators, can result in viral production from the latent reservoir *in vitro*.
- Clinical trials of antiretroviral drugs in combination with new antilatency agents are currently under way in patients with HIV infection.

From a strategic perspective, the latent HIV reservoir can be approached in four simple ways – 'exhaust' (activate the proviral reservoir in conjunction with HAART), 'kill' (target and kill latently infected cells), 'silence' (silence transcription from the proviral reservoir), or 'replace' (engineer a new population of HIV-resistant cells). We will explore each of these approaches in turn.

Exhaust

To date, the most widely explored approach has been to 'exhaust' the latent reservoir by activation in conjunction with HAART. Initially, it was predicted that around 3 years of fully suppressive HAART might lead to viral eradication [32], although further understanding of the decay characteristics of the viral reservoir resulted in revision of that figure to around 70 years [16].

Treatment in very early, or 'primary', HIV infection may result in a more rapid decline of the reservoir and some patients who received very early HAART show sustained control of HIV after treatment interruption [33]. The viral reservoir amongst these 'post-treatment controllers' was very low and stable, suggesting that early therapy could cause a reduction in the reservoir, perhaps to a point when the immune system might control HIV infection without HAART. Another study suggests that early therapy may impact the reservoir more effectively, resulting in a potential time to eradication of around seven years [34]. However, there are no reported cases in which HAART alone – even when given in acute infection [17•] – has resulted in clearance of the reservoir.

As a result, adjunctive agents have been sought which – in the presence of HAART – might activate resting $CD4^+$ T cells, induce HIV LTR transcription and viral expression. The rationale being that the reservoir can be depleted by activating infected cells with any resulting virions rendered incompetent by HAART. Approaches have so far been nonspecific, aiming to induce broad cellular activation. The problem with inducing cellular activation is the potential to increase the number of cellular targets for HIV infection, thereby replenishing rather than decreasing the reservoir. Approaches, therefore, need to be developed which result in viral transcription without widespread T-cell activation.

Nonspecific activation has been attempted in trials with agents such as IL-2 and OKT3 (an anti-T-cell receptor antibody). IL-2 alone (and HAART) had no significant clinical benefit [35], and when IL-2 and OKT3 were given together with HAART, there was evidence of marked immune activation, but no evidence of reduced viral burden [36]. Preclinical studies have shown that IL-7 can induce latent virus expression *ex vivo* from $CD4^+$ T cells from patients who are receiving HAART, although to differing extents, and IL-7 is currently being evaluated in clinical trials.

Another agent of interest is prostratin, a phorbol ester isolated from the Samoan medicinal plant 'Homolanthus nutans' [37,38], which can stimulate HIV transcription through nuclear factor kappa B activation, likely mediated by protein kinase C [39]. Prostratin reactivates HIV from primary blood lymphocytes and lymphoid tissue, but may also inhibit new infection by down-regulating the co-receptors CXCR4 and CD4, increasing the chance of HIV purging without the risk of new rounds of HIV infection [40,41]. Prostratin alone has also been shown to reactivate latent HIV from thymocytes and human peripheral blood lymphocytes in the SCID-hu (Thy/Liv) model, in the absence of cellular proliferation [42]. There are no in-vivo data of prostratin in patients, but this is awaited with great interest.

The histone deacetylase (HDAC) inhibitor class of drugs has received recent attention and potential promise for use in clinical trials. Valproic acid (VPA) – a weak HDAC inhibitor – increases HIV gene expression and virus production in cultured latently infected cells [43,44]. VPA alone, without ART intensification, was not sufficient for a significant reduction in HIV infection of $CD4^+$ T cells from HAART-treated patients [45], and even with HAART intensification there was no significant reduction in resting T-cell infection or plasma viral load [18•].

Suberoylanilide hydroxamic acid (SAHA; Vorinostat) is a more potent and selective class I HDAC inhibitor. SAHA induces HIV production from a latently infected Jurkat T-cell line and from resting CD4 cells from chronically HIV-infected patients [46], and is currently being evaluated in clinical trials of HIV-infected participants. As a class, the HDAC inhibitors may prove to impact the latent reservoir *in vivo* [47] and there are data showing that other HDAC inhibitors, such as oxamflatin [48], metacept-1, metacept-3 [49] and Scriptaid [50], also impact the proviral reservoir *in vitro*, and may prove to be interesting agents to assess in clinical trials.

An alternative epigenetic target is DNA methylation, which also helps maintain DNA latency [51,52]. Drug class investigations in this area are not as advanced as for the HDAC inhibitors, although in-vitro data show that the 5′LTR of HIV is CpG hypermethylated and the methylation inhibitor, 5-aza-2′-deoxycytidine (aza-CdR), may cause HIV reactivation [52]. Accordingly, this class of drug may prove useful, particularly in combination with other agents such as the HDAC inhibitors.

Kill

A second approach would be to identify those cells that are latently infected with HIV and kill them directly. This concept has been applied to tumour therapeutics, for example in the use of IL-2 bound to diptheria toxin in cutaneous T-cell lymphoma therapy [53] and anti-CD33 bound to calicheamicin (Gemtuzumab) in relapsing acute myeloid leukaemia [54,55]. For HIV, this strategy is hindered by the lack of any obvious characteristic to distinguish latently infected cells from others, especially in resting phases of the cell cycle. It is possible that some HIV proteins are present on the surface of latently infected cells to act as targets. Using HIV Envelope as a target, 3B3(Fv)-PE38 is a recombinant derivative of *Pseudomonas aeruginosa* exotoxin A combined with gp120 targeting moieties [56]. This immunotoxin selectively kills latently infected cells *in vitro* [57] and kills CD4 T cells *ex vivo* [58] with little evidence of toxicity in nonhuman primates [59]. There have been no clinical trials of HIV immunotoxins in combination

with HAART, although this is likely to be an area for exploration in the future.

Silence

An alternative approach to targeting the latent HIV reservoir would be to 'turn off' transcription. Specific control of gene expression at the transcriptional level has the potential to impact numerous diseases including cancers and HIV amongst others. Different approaches have been considered, including disrupting HIV-specific sequences with a third strand of DNA to make a replication incompetent 'triple helix' which, although possible *in vitro* [60], was not achievable *in vivo* due to physiological constraints. More recently small antisense RNA molecules have been developed with transcriptional silencing of numerous genes proposed; however, this has yet to translate into clinical practice. Specifically, for HIV, RNA interference (RNAi) has been reported *in vitro* [61], *in silico* [62] and in mouse models [63,64], particularly if multiple regions of the HIV genome are targeted simultaneously. Delivery of these agents has been problematic, but newer technologies – such as liposomal delivery vehicles [65] – may improve this. However, no human correlate yet exists with a dominant problem being HIV genetic variability and the need to inhibit conserved virological regions. Although an active research field, therapeutic options are still a long way from the clinic.

Restore

A number of research groups are reporting the use of zinc finger nucleases (ZFNs) to engineer populations of HIV-resistant cells, thereby restoring the CD4+ve T-cell population. The CCR5Δ32 mutation is naturally occurring and confers resistance to infection in homozygotes [66]. The phenotype of this naturally occurring variant can be reproduced using ZFNs to introduce mutations into the CCR5 gene rendering host cells uninfectable by HIV [67,68••]. ZFNs contain two domains – one a series of DNA triplet specific zinc finger peptides, the other a sequence specific endonuclease which cleaves the target DNA [69]. The DNA is subsequently repaired by nonhomologous end-joining which is highly error prone and leads to frame-shift and other nonviable mutations. The principle is that, should the affected cells remain viable and replication-competent, they can be expanded and transfused back into the host in which they provide a repository of uninfectable cells. Applied to CCR5, the safety of this approach has been evaluated in a phase I clinical trial [70] and there are preclinical data on applying the same technology to CXCR4 [71]. Although a technology in its infancy, these approaches show how the search for a cure to HIV is driving a new era in scientific innovation.

Conclusion

The cure to HIV, even if possible, is still a long way off. However, the growing focus of scientific enquiry and the application of previously unavailable technologies make this an extremely exciting field. There is a call for scientific researchers to work together under the umbrella of collaboration, and groups such as AmFar (The American Foundation for AIDS Research), the IAS global scientific 'Towards an HIV Cure' strategy and the UK CHERUB co-operative ('Collaborative HIV Eradication – a UK BRC Initiative') are examples of such an approach. The challenge of finding an HIV cure remains daunting, but the impact of success would be so profound at a global level to demand and justify on-going investment and collaboration.

Acknowledgements

The author thanks the Medical Research Council (UK), the Oxford NIHR Biomedical Research Centre for financial support, and Rodney Phillips and James Williams for helpful contributions to the manuscript. This work was supported by 'Collaborative HIV Eradication of viral Reservoirs: a UK BRC initiative' (UK CHERUB).

Conflicts of interest

There are no conflicts of interest.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 616).

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