Research Highlight

HIV Cure and HIV Reservoirs*

YIN Qian Qian, Shao Yi Ming, and MA Li Ying#

The success of combined antiretroviral therapy (cART) has dramatically improved the clinical outcomes of HIV infection and made HIV infection a chronic disease. Nonetheless, cART alone cannot eliminate viral reservoirs and fully recover patients' health, so the patients have to receive this treatment for lifetime. Therefore, researchers have focused their efforts on the cure for HIV infection. In recent years, some intriguing and inspiring cases indicated that the cure of HIV infection (HIV cure) or durable alleviation of HIV infection may not be a dream any more. 'Berlin Patient' 'Mississippi Baby', and probably 'Long Beach Baby' have brought great hope to the HIV cure. Many scientists believe that the HIV cure is just a question of time.

Now, novel approaches to cure HIV infection which can bypass the limitations of current therapies and recover patients' health are being explored:

Hematopoietic Stem Cell (HSC) Transplantation

Using both HSC transplantation and antiretroviral therapy is a strategy for the eradication of HIV in HIV-infected patients with diagnosed leukemia and/or lymphomas. In 2007, 'Berlin patient', who had been diagnosed with acute myeloid leukemia and infected with HIV-1 CCR5-tropic variants for more than 10 years, received stem cells transplantation from a donor who was homozygous for CCR5 delta32. No viral rebound was detected in the patient after the transplantation and HAART interruption. It is wildly believed that this patient had been cured of HIV-1 infection[1]. The case demonstrated that HIV-1 reservoirs can be reduced or eradicated and ART can be stopped without subsequent viral rebound. Recently, two HIV infected patients with lymphoma received HSC transplantation and HIV seemed to be eradicated, they are known as 'Boston patients'. They stopped taking AIDS drugs in early 2013. However, it was reported that they experienced a strong viral rebound several months after treatment interruption in the 6th International Workshop on HIV Persistence, Reservoirs and Eradication Strategies.

Viral reservoirs still remain a major obstacle to the HIV cure. Through the failure of the 'Boston patients', we have discovered that HIV reservoir is deeper and more persistent than what we had known previously and current HIV detection techniques may not be sufficient. HIV persistence is thought to stem primarily from the presence of integrated proviral genome within long-lived cells which are likely to harbor latent provirus. Resting memory $CD4^+$ T cells are the best-characterized reservoirs of latent HIV[2]. Josefsson et al.[3] found that persistent HIV-1 in peripheral blood and gut-associated lymphoid tissue (GALT) is mainly in memory $CD4^+$ T cells and indicated that persistence of a remarkably stable population of infected memory cells will be the primary barrier to the cure of HIV. In a recent study[4], Buzon et al. showed that during suppressive antiretroviral therapy, $CD4^+$ T memory stem cells (T_{SCM} cells) harbor high per-cell levels of HIV-1 DNA and make increasing contributions to the total viral $CD4^+$ T cell reservoir. Although T_{SCM} cells had a small contribution to the cellular pool, reservoirs within T_{SCM} cells were stable. HIV-1 may exploit the T_{SCM} cells characteristics of that they can survive for long periods of time and potentially spread provirus vertically with self-renewal and differentiation to facilitate long-term viral persistence. Due to different T cell subsets different eradication strategies may be needed, there is great interest in identifying and characterizing the cell types that harbor latent provirus[5]. While Ya-Chi Ho et al. identified replication-competent noninduced proviruses which indicated the size of the latent reservoir might be up to 60-fold greater than previously estimated[6]. These proviruses pose a severe threat because they are likely to be activated despite the treatment with most effective drugs. This result undoubtedly indicated the difficulties in viral reservoirs activation

doi: 10.3967/ bes2014.078

State Key Laboratory for Infection Disease Prevention and Control, National Center for AIDS/STD Control and Prevention (NCAIDS), Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseas, Chinese Center for Disease Control and Prevention (China-CDC), Beijing 102206, China

research.

So far, the 'Berlin patient' is the only successful case of HSC transplantation to eradicate HIV and other attempts using this approach have ended in failure. Despite the effectiveness of the treatment on reducing HIV reservoirs, it is not a suitable option for the majority of HIV infected patients, because it is a risky and expensive procedure and recommended only for those who develop cancer. Owing to the impossibility of applying this strategy in large-scale clinical practice, many researchers have focused on other eradication strategies.

Gene Therapy The case of the 'Berlin patient' has stimulated researchers' great interest in gene therapy to delete the virus from infected cells or to produce HIV-resistant cells. The artificially modified nucleic acid enzymes-zinc finger nuclease (ZFN) is a common genome editing tool. Using ZFNs, Cannon et al.[7] disrupted CCR5 in human CD34+ hematopoietic stem/progenitor cells (HSPCs). The results supported the use of ZFN-modified autologous HSCs as a clinical approach to treat HIV-1infection. In another study by June et al. from University of Pennsylvania[8], 12 patients with chronic aviremic HIV infection and receiving HAART were enrolled. These patients were infused with SB-728-T, consisting of autologous CD4-enriched T cells in which the CCR5 gene was rendered permanently dysfunctional by a zinc-finger nuclease (ZFN). After treatment interruption 4 weeks after the infusion, HIV RNA became undetectable in one of four patients and the blood level of HIV DNA decreased in most patients, indicating that T cells modification has important value to avoid the lifetime use of antiretroviral therapy and provides fundamental to the 'functional cure' of HIV infection. The ZFN technology used in the study was from Sangamo BioSciences. According to the results published by this company at the 2011 Conference on Retroviruses and Opportunistic Infections (CROI), we know that CCR5-modified autologous T cells (SB-728-T) produced based on the ZFN have good safety on treating HIV/AIDS. Clinical trials are ongoing to evaluate the efficacy of SB-728-T. Moreover, ZFNs have been designed to target CCR5 and CXCR4 to produce primary $CD4^+$ T cells resistant to R5 and X4 viruses in humanized mouse models[9]. This strategy would tackle the risk of tropism switching. Nonetheless, the safety and efficacy of gene delivery and access to the treatments may be the major challenges to the gene therapy strategy.

Baby Steps Several recent studies have highlighted the potential impact of early ART on viral reservoirs and persistence. It is reported that an US baby girl treated very early is cured now-the case of 'Mississippi Baby' and 'functional cure'[10]. The infant, whose mother had HIV-1 infection, began to receive ART 30 h after birth for high-risk HIV-1 exposure. Due to the detection of HIV-1 DNA in peripheral blood mononuclear cells (PBMCs) and RNA in a separate blood sample, which met the standard diagnostic criteria for HIV infection, cART was continued. cART was discontinued when the child was 18 months of age, and the clinical assays indicated that the levels of plasma HIV-1 RNA, proviral DNA in PBMCs remained undetectable when the infant was aged 30 months, as were HIV-1 antibodies. Similarly, an HIV-infected baby from Long Beach, California started antiviral treatment 4 hours after birth and now has no detectable virus. Although this baby continues to receive antiretroviral therapy, it is hopeful that HIV infection has been cured. These cases indicated that very early ART in infants might alter the establishment and long-term persistence of reservoirs of replication-competent virus. Initiation of cART immediately after infection might dramatically change treatment strategies of the newborns whose mothers were HIV infected worldwide.

Visconti Cohort French researchers conducted a study of 14 patients who were infected with HIV in 1990s and 2000s, a group known as Visconti cohort[11], they were treated within 10 weeks after infection and began to stop taking drugs 3 years later on average. The researchers reported that even at seven years after the treatment, they still showed no signs of the virus rebound. Results showed that viral load was under control after stopping drug treatment. Drugs only keep the virus in control and cannot eradicate the virus from its hiding places inside the immune system. Generally, most patients who receive the same treatment will experience virus bounce back when the drug treatment stop, but some people will be the exception. French researchers reported that rapid treatment after HIV infection might be effective to achieve 'functional cure' in about 10% of early diagnosed HIV infected patients. This case shows that early initiation of therapy is effective. Josefssons' study also indicated that early treatment could result in lower HIV-1 reservoir size in peripheral blood and GALT. Early treatment might limit HIV reservoir formation and reduce the number of long-term reservoirs that become infected. Researchers believe that giving HIV-infected patients drugs as soon as possible after

they are diagnosed could be the future of treatment. However, identifying the people who are newly infected remains to be one of the biggest problems, since some people infected with HIV are thought to be completely unaware of their infection status.

Prospect Considering the limitations of current treatment and the possibility of the HIV cure, promising strategies to tackle HIV are being explored[12-13]. Such strategies will aim to limit the size of the viral reservoirs. Except HSC transplantation, gene therapy and early treatment, other strategies are being studied, but they are still in the early stages of development. For example, there is a 'kick and kill' strategy to treat patients with a latent antagonist[14] combined with cART to prevent new infections and latent reservoir reestablishment. The bi-functional antagonists of HIV-1 can be developed to purge viral reservoirs in the future drug therapies[15]. Moreover, enhancing susceptibility to apoptosis of HIV infected cells or impairing these cells homoeostatic proliferation and persistence are also the potential strategies to eliminate HIV reservoirs. However, the side effects of such approaches need to be evaluated carefully. Although there is still a long way to go for HIV cure at global level through the eradication of HIV reservoirs, more and more knowledge about HIV cure and viral reservoirs will help us on the road to reduce or eradicate HIV-1 reservoirs.

*This work was supported by grants from State Key Laboratory for Infectious Disease Prevention and Control (2011SKLID102), National Nature Science Foundation of China (81172733).

#Correspondence should be addressed to MA Li Ying, Tel: 86-10-58900976, E-mail: mal@chinaaids.cn

Biographical note of the first author: YIN Qian Qian, female, born in 1988, PhD candidate, majoring in pathogen biology.

Received: May 6, 2014;

Accepted: May 21, 2014

REFERENCES

1. Allers K, Hutter G, Hofmann J, et al. Evidence for the cure of HIV infection by CCR5Delta32/Delta32 stem cell transplantation. Blood, 2011; 117, 2791-9.
2. Ruelas DS and Greene WC. An integrated overview of HIV-1 latency. Cell, 2013; 155, 519-29.
3. Josefsson L, Stockenstroma SV, Fariac NR, et al. The HIV-1 reservoir in eight patients on long-term suppressive antiretroviral therapy is stable with few genetic changes over time. PNAS, 2013; 110, 4987-96.
4. Buzon MJ, Sun H, Li C, et al. HIV-1 persistence in CD4+ T cells with stem cell-like properties. Nat Med, 2014; 20, 139-42.
5. Zaikos TD and Collins KL. Long-lived reservoirs of HIV-1. Trends Microbiol, 2014; 22, 173-5.
6. Ho YC, Shan L, Hosmane NN, et al. Replication-Competent Noninduced Proviruses in the Latent Reservoir Increase Barrier to HIV-1 Cure. CELL, 2013; 155, 540-51.
7. Holt N, Wang J, Kim K, et al. Human hematopoietic stem/progenitor cells modified by zinc-finger nucleases targeted to CCR5 control HIV-1 in vivo. Nat Biotechnol, 2010; 28, 839-47.
8. Tebas P, Stein D, Tang WW, et al. Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV. N Engl J Med, 2014; 370, 901-10.
9. Didigu CA, Wilen CB, Wang J, et al. Simultaneous zinc-finger nuclease editing of the HIV coreceptors ccr5 and cxcr4 protects $CD4^+$ T cells from HIV-1 infection. Blood, 2014; 123, 61-9.
10. Persaud D, Gay H, Ziemniak C, et al. Absence of detectable HIV-1 viremia after treatment cessation in an infant. N Engl J Med, 2013; 369, 1828-35.
11. Sáez-Cirión A, Bacchus C, Hocqueloux L, et al. Post-treatment HIV-1 controllers with a long-term virological remission after the interruption of early initiated antiretroviral therapy ANRS VISCONTI Study. PLoS Pathog, 2013; 9, e1003211.
12. Deeks SG, Autran B, Berkhout B, et al. Towards an HIV cure: a global scientific strategy. Towards an HIV cure: a global scientific strategy, 2012; 12, 607-14.
13. Katlama C, Deeks SG, Autran B, et al. Barriers to a cure for HIV: new ways to target and eradicate HIV-1 reservoirs. Lancet, 2013; 381, 2109-17.
14. Wei DG, Chiang V, Fyne E, et al. Histone Deacetylase Inhibitor Romidepsin Induces HIV Expression in CD4 T Cells from Patients on Suppressive Antiretroviral Therapy at Concentrations Achieved by Clinical Dosing. PLoS Pathog, 2014; 10, e1004071.
15. Miller LK, Kobayashi Y, Chen CC, et al. Proteasome inhibitors act as bifunctional antagonists of human immunodeficiency virus type 1 latency and replication. Retrovirology, 2013; 10, 120.