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Strategies to Target Non-T cell HIV Reservoirs

Jonah B. Sacha, PhD[1,2] and **Lishomwa C. Ndhlovu, MD, PhD**[3,4]

[1]Vaccine and Gene Therapy Institute, Oregon Health & Science University, 185th Avenue, Beaverton, OR 97006, USA

[2]Oregon National Primate Research Center, Oregon Health & Science University, 185th Avenue, Beaverton, OR 97006, USA, Tel: 503 418-2700; Fax: 503 418-2701; sacha@ohsu.edu

[3]Department of Tropical Medicine, University of Hawaii, 651 Ilalo St, Honolulu, Hawaii, USA.

[4]Hawaii Center for AIDS, University of Hawaii, 651 Ilalo St, Honolulu, Hawaii, USA. Tel: 808-692-1604; Fax: 808-692-1984; lndhlovu@hawaii.edu

Abstract

Purpose—A central question for the HIV cure field is to determine new ways to target clinically relevant, latently and actively replicating HIV-infected cells beyond resting memory CD4+ T cells, particularly in anatomical areas of low drug penetrability.

Recent findings—HIV eradication strategies being positioned for targeting HIV for extinction in the CD4 T cell compartment may also show promise in non-CD4 T cells reservoirs. Furthermore, several exciting novel therapeutic approaches specifically focused on HIV clearance from non-CD4 T cells populations are being developed.

Summary—Although reservoir validity in these non-CD4 T cells continues to remain debated, this review will highlight recent advances and make an argument as to their clinical relevancy as we progress towards an HIV cure.

Keywords

HIV; Reservoirs; Eradication

Introduction

Despite the unquestionable success of either prolonged or early institution of combined antiretroviral therapy (cART), a cure for Human immunodeficiency virus (HIV) remains elusive. With the exception of rare cases such as Timothy Brown, aka the Berlin patient, cessation of cART invariably leads to HIV reemergence[1-3]. Most studies have designed HIV eradication strategies focused primarily on targeting resting memory CD4+ T cells[4]. Although latently infected resting memory CD4+ T cells represent the predominate reservoir, non-CD4+ T cells reservoirs in blood such as monocytes and other T cells, as well as tissue macrophages in the lung[5,6], adipose tissue[7], gut associated lymphoid tissue (GALT), genital tract, semen, bone marrow, and central nervous system (CNS) cells

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including both microglia and astrocytes in brain must not be discounted and instead given attention as targets for elimination. Indeed, comparison of virus archived in resting CD4+ T cells during cART to virus rebounding following cART cessation indicates the presence of another, non-lymphocyte pool of persistent virus[8].

Given recent data demonstrating HIV replication persists in lymphoid tissue with low cART penetration, ongoing low-level HIV replication may continue and contribute to the viral reservoir in both CD4 T cells and non-CD4 T cells[9]. There is a need to not only identify and characterize non-CD4+ T cells in blood and tissues, but also to consider strategies targeted specifically for these populations if we are to effectively ensure no viral rebound after ART cessation.

Advances in Targeting non-CD4+ T cells sources of HIV persistence: Relevance of Location

There are a variety of attributes that make monocyte and tissue macrophages, including microglia, candidates for contributing to the HIV reservoir, both as carriers and replenishers of the viral reservoir. The macrophage reservoir half-life has historically been underdetermined, yet in the presence of cART, macrophages from SIV infected rhesus macaques can sustain viremia for several months[10-12]. Furthermore, myeloid cells are relatively more resistant to apoptosis induced by HIV infection[13], and virus produced by macrophages may be more infectious than virus originating from CD4+ T cells[14]. Monocytes and macrophages disseminate into most tissues in the body and therefore have the widest capacity for mediating HIV spread. These sites can be difficult to access with conventional ART. Indeed, a well-discussed site is the CNS. Post-mortem brain tissue analysis has revealed that viral DNA is present in 3% to 19% of astrocytes[15] despite astrocyte infection being both relatively infrequent and unproductive[16]. Moreover, our group has used next generation *in situ* hybridization RNAScope to identify HIV vRNA in cerebellum macrophages of in an infected individual who died with no detectable viral load[17]. Therefore, it is of the utmost importance to consider the contribution of non CD4+ T cells as reservoirs and sources of HIV viremia.

Another unique non-CD4+ T cell population has recently been demonstrated by DeMaster *et al* as being both CD4 negative and CD8 negative T cells that are positive for Gag expression with integrated proviral DNA[18]. DeMaster *et al* show that these CD4/CD8 “double negative” T cells do not display activation markers and therefore are a quiescent population[18]. The double negative T cell phenotype was also observed in peripheral blood of cART treated HIV-infected patients[18]. Although Gag+ cells may be short lived, their low-level capability of producing replication competent virus may contribute new virus to replenish the reservoir, although the longevity of these cells has yet to be extensively studied and may prove to be an under-appreciated cellular reservoir for HIV.

i. Peripheral blood: Monocytes

A new study has shown that the CD52 receptor is retained on HIV infected immune cells[19]. The authors further demonstrate that targeting CD52 *in vitro* with a monoclonal antibody

(alemtuzamab) induced the depletion of HIV-infected immune cells including peripheral monocytes. This was achieved by rendering infected cells sensitive to complement-mediated lysis, a key event induced by alemtuzamab that may improve its effectiveness in vivo (Figure 1).

ii. Central Nervous System (CNS): Perivascular Macrophages, Microglia and Astrocytes

Perivascular macrophages have a half-life of ~3 months[20] while microglia have a half-life of months-years to years-lifetime[21]. Therefore, both CNS perivascular macrophages and microglia need to be considered possible long-lived HIV reservoirs, particularly because parenchymal microglia have been shown to be two-thirds of the infected cells in the brains of patients with encephalitis[22]. Thus, the HIV reservoir in the CNS could be the largest obstacle to achieving complete HIV elimination. Recently, McFarren *et al* have demonstrated that ^{213}Bi-2556 mAb, a radio-labeled monoclonal antibody to HIV gp41, crossed an *in vitro* human blood brain barrier and killed significantly more transmigrated HIV-infected PBMCs and monocytes in comparison to an irrelevant ^{213}Bi-1418 mAb or uninfected cells[23]. Although promising, cytotoxic bystander effects need to be characterized as we may require curative measures that avoid depleting minimally-replenished cells of the brain.

The neutrophil chemotaxis recruiter, interleukin-8 (IL-8) has been shown *in vitro* in monocyte-derived macrophages (MDM) to not only enhance CCL2-mediated monocyte migration but also enhance HIV replication in both macrophages and T cells[24,25]. Curative measures that intervene by modulating cell chemotaxis could avoid potentially detrimental cell depletion approaches. Interestingly, IL-8 also is correlated with 2-LTR circle formations, a marker of nuclear import of viral DNA or enhanced infectivity, again in MDM and primary human microglia[26].

Methods to target infected macrophages that are in the CNS could develop from the immunotargeting drug liposomal alendronate, which can reduce circulating and disseminated macrophage frequency. Burwitz *et al* have demonstrated that liposomal alendronate safely and effectively depletes peripheral and tissue macrophages in nonhuman primates followed by an increase in bone marrow macrophage precursor frequency[27]. Afergan *et al* used liposomal delivery to target brain monocytes for transport of encapsulated serotonin by negatively charge nano-sized liposomes across the blood brain barrier[28]. The use of the liposomal alendronate system could provide a more sophisticated approach if used in conjunction with cART to increase drug concentration in tissues with poor cART penetrance. For example, to deliver anatomically relevant cART to the CNS, liposomal alendronate could be paired with the recent advent of cART regimens that have effective CNS-penetration, known as neurocART.

Beyond parenchymal macrophages and microglia, astrocytes are the other predominant cell type that comes into contact with virus-infected cells migrating from the blood to the CNS. The protein kinase C (PKC) activator Bryostatin could become a possible adjunct of CNS targeted HIV-1 elimination therapy. Bryostatin has been shown to have anti-HIV-1 activity by downregulating CXCR4 receptors or indirectly inhibiting HIV-1 Tat function by dephosphorylating a kinase necessary for RNA polymerase-II functional initiation.

Furthermore, Bryostatin has been shown to have moderate HIV latency reversing agent (LRA) activity in astrocyte cell lines *in vitro* and in cultured primary astrocytes by inducing HIV-1 expression through NF-kB activation[29].

iii. Macrophages in Adipose Tissue

Adipose tissue is comprised of adipocytes and stromal vasculature. In macaques infected with SIV, adipose density was elevated as well as enhanced immune-activated profiles. Damouche *et al* demonstrate that HIV-1 DNA was detected in both CD4+ T cells and sorted CD206+ CD14+ macrophages in adipose tissue samples from ART-treated HIV-infected donors[7]. A phage system that displays a visceral adipose tissue-specific peptide ligand (TDA1) has been developed by Lee *et al*, which could form the homing moiety basis for a delivery system to target adipose macrophages[30].

iv. Lymph Nodes; Follicular dendritic cells (FDC)

are located in the B cell follicle and recently have been shown to harbor infectious HIV virions within a non-degradative cycling compartment[31]. Heesters and colleagues further demonstrate that virion uptake by FDC appears to be regulated and retained through the complement receptor CD21. Thus exploiting CD21 with targeted reagents such as a soluble CD21-Ig could provide adjunctive therapy to cART or broadly neutralizing antibodies for the elimination of this reservoir compartment.

Current Eradication Approaches Redirected towards Non CD4+ T cell Reservoirs

Several insights have been gained from HIV cure strategies targeting the CD4 T cell reservoir compartment, which could be redirected to other cellular HIV viral sanctuaries (Figure. 2).

i Limiting the viral reservoir size by early ART

*V*ery early introduction of cART soon after infection is hypothesized to achieve HIV remission. This has been observed in principle in adults in the Visconti cohort[1], which is comprised of HIV controllers who are able to spontaneously control HIV replication following cART cession. This phenomenon was also transiently observed in the infant referred to as “the Mississippi baby” who received cART 30 hours after birth. Ongoing studies evaluating early initiation of cART in several cohorts will provide further data on such an approach[32-34]. However, viral rebound did occur in the Mississippi baby suggesting that early cART intervention alone may not be sufficient as a curative strategy. To what extent the non-CD4 T cell reservoir compartments contributed to viral recrudescence is unclear. Current studies by our group are assessing the myeloid reservoir soon after infection and determining the efficacy of early mega cART interventions in those captured in the early acute stages of infection.

ii. ART intensification

The inability of current antiretroviral drugs to sufficiently suppress virus within circulating monocytes and tissue macrophages including the CNS is an area needing continued investigation. Several *in vitro* studies have demonstrated varying efficacies of existing cART drugs against HIV infection in macrophages[35]. Both Maraviroc and Raltegravir have been tested under cART intensification. We have shown that intensification with Maraviroc for 24 weeks in HIV-infected cART treated participants leads to a reduction in monocyte HIV DNA[36]. Lafeuillade *et al* showed that from 10 PBMC and gut biopsy samples of HIV-infected patients on either Truvada® or Kaletra® with further intensified antiretroviral therapy by taking Maraviroc and Raltegravir showed no appreciable proviral DNA reservoir reduction in blood or gut biopsies, except in a few patient cases where proviral DNA in gut biopsies showed a modest reduction[37]. More data from such intensification studies may be useful to develop a systematic clinical intervention sequence of ART therapeutics designed for each reservoir compartment.

iii. Shock and Kill approach

Drugs that are already in clinical trials which target the HIV-1 reservoir exploit a strategy known as ‘shock and kill’ whereby the integrated pro-viral DNA is kicked into transcriptional activity by a latency reversing agent; the virally active cells will subsequently be eliminated with the continuation of antiretroviral therapy during this ‘kill’ phase. Although histone deacetylase inhibitors (HDACi) decrease HIV release from macrophages, the HDAC inhibitors may not alter the initial susceptibility of macrophages to HIV infection[38]. Churchill *et al* show that in human primary astrocyte cell lines transfected with patient-derived HIV-1 LTR, treatment with HDACi panobinostat, trichostatin A, vorinostat, and entinostat activated HIV LTRs[39].

Simian immunodeficiency virus (SIV)-specific CD8+ T cells (CTL) can efficiently kill SIV-infected CD4+ T cells but not SIV-infected macrophages[40]. Nef downregulates MHC-I production and enhances CD8+ T cell evasion. Rainho *et al* show that CTL mediated killing of CD4+ T cells and monocyte derived macrophages infected with SIV nef variants was more efficient when targeting CD4+ T cells than macrophages[41] suggesting alternate mechanisms for macrophage resistance to CTL killing[41]. Finding strategies to enhance CTL activity against infected macrophages are therefore important. Pegu *et al* have shown that a bi-specific immunomodulatory protein that stimulates CD8+ T-cell effector function thereby initiating latent-infected cell lysis through recognition of Env[42] may be retargeted towards HIV infected macrophages. Studies evaluating immunotherapies targeting negative checkpoint receptors[43-45] to improve CTL activity as well as harnessing NK cells[46] are needed and may serve as alternate strategies to overcome myeloid cell resistance to CTL killing.

iv. Stem Cell Transplantation

The successful homozygous CCR5 delta32 stem-cell transplant into an HIV-1 infected individual with acute myeloid leukemia resulted in an apparent cure whereby Timothy Brown, the ‘Berlin Patient’, could stop antiretroviral treatment without subsequent viremic rebound[47]. The exact mechanisms for the apparent curative approach remain undefined. In

multiple samplings of non-CD4 T cell populations no virus was detected using several different assays[48]. However, overcoming the HIV reservoir in macrophages by stem cell transplantation is still unresolved. In the case of the 'Berlin Patient' prior administration of gemtuzumab (anti-CD33 mAb) may have targeted myeloid cells for depletion. It is unclear whether or not lack of myeloid-targeted depletion contributed to the incomplete reservoir elimination and viral reemergence in other human[2] and non-human[49] stem cell transplantation cases tested thereafter. Stem cell transplantation remains an understudied, yet highly multifaceted approach to understanding and clearing the latent viral reservoir.

v. Gene Therapy

The CRISPR-Cas9 system is an alternate approach that uses guide RNA instead of custom proteins to home in on target DNA. Zhang *et al* have reported using a dCas9-synergistic activation mediator (dCas9-SAM) system to reactivate HIV-1 in both CD4+ T cell and microglial cell lines[50]. The possible advantage of a dCas9-SAM system is that it may minimally affect localized HIV-negative cells. Zhang *et al* report finding two MS2-mediated single guide RNA that direct the dCas9-SAM system to potently target specific HIV-infected cells[50]. However, the most promising results are presented by Hu *et al* where in latently infected microglial, promonocytes, and T cell lines they developed a Cas9/guide RNA system to eradicate the HIV-1 genome and immunize target cells against HIV-1 reactivation[51]. However, studies using patient-derived myeloid cells have not been reported yet. Studies by the Churchill group and others show CNS viral strains are genotypically distinct from those found in the peripheral blood; in particular the long terminal repeat, also known as the viral promoter region is varied[52,53]. Therefore, a Cas9 gene therapy system may need to be tailored to each compartmentalized viral reservoir.

vi. Permanent HIV Suppression

Activation of positive transcriptional elongation factor b (p-TEFb) has recently been established as the mechanism by which the LRAs, vorinostat and panobinostat work to activate latent HIV from CD4+ T cells[54]. However, because p-TEFb is required for RNA polymerase II activation and therefore viral production, p-TEFb could also be a possible target for permanent HIV-1 suppression. Heat shock protein 90 (Hsp90) inhibitors can block triggering the NF-kB pathway and thus suppress HIV-1 transcription[55]. In addition PIM-1 inhibitors can block HIV-1 reactivation making it a possible permanent suppression therapeutic[56]. Whether these novel approaches can be extended to non-CD4 T cell reservoirs is untested and should be evaluated further as alternative strategies.

Conclusion and Future Direction

The breadth of possible reservoirs considered here makes the prospect of HIV elimination seem daunting if not impossible; it may be tempting to limit our consideration to only one reservoir at a time, to address only the clinically relevant first—that once we find moderate success with the most clinically relevant reservoir, our success could translate to other cell types. However, the evidence presented here suggests a unique solution may be required for each cell type, and delivery mechanisms or targeting strategies will need to be tissue-specific tailored. New therapeutic strategies that place emphasis on targeting non-CD4+ T cell

reservoir contributions to the HIV-1 reservoir will be imperative on the road to HIV eradication.

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SUMMARY

- Cessation of antiretroviral therapy invariably leads to HIV reemergence
- HIV eradication strategies being positioned for targeting HIV for extinction in the CD4 T cell reservoir may also show promise in other cellular HIV viral sanctuaries.
- Several exciting novel therapeutic approaches specifically and broadly focused on HIV clearance from non-CD4 T cells populations such as, monocytes, macrophages, astrocytes and follicular dendritic cells are being developed.

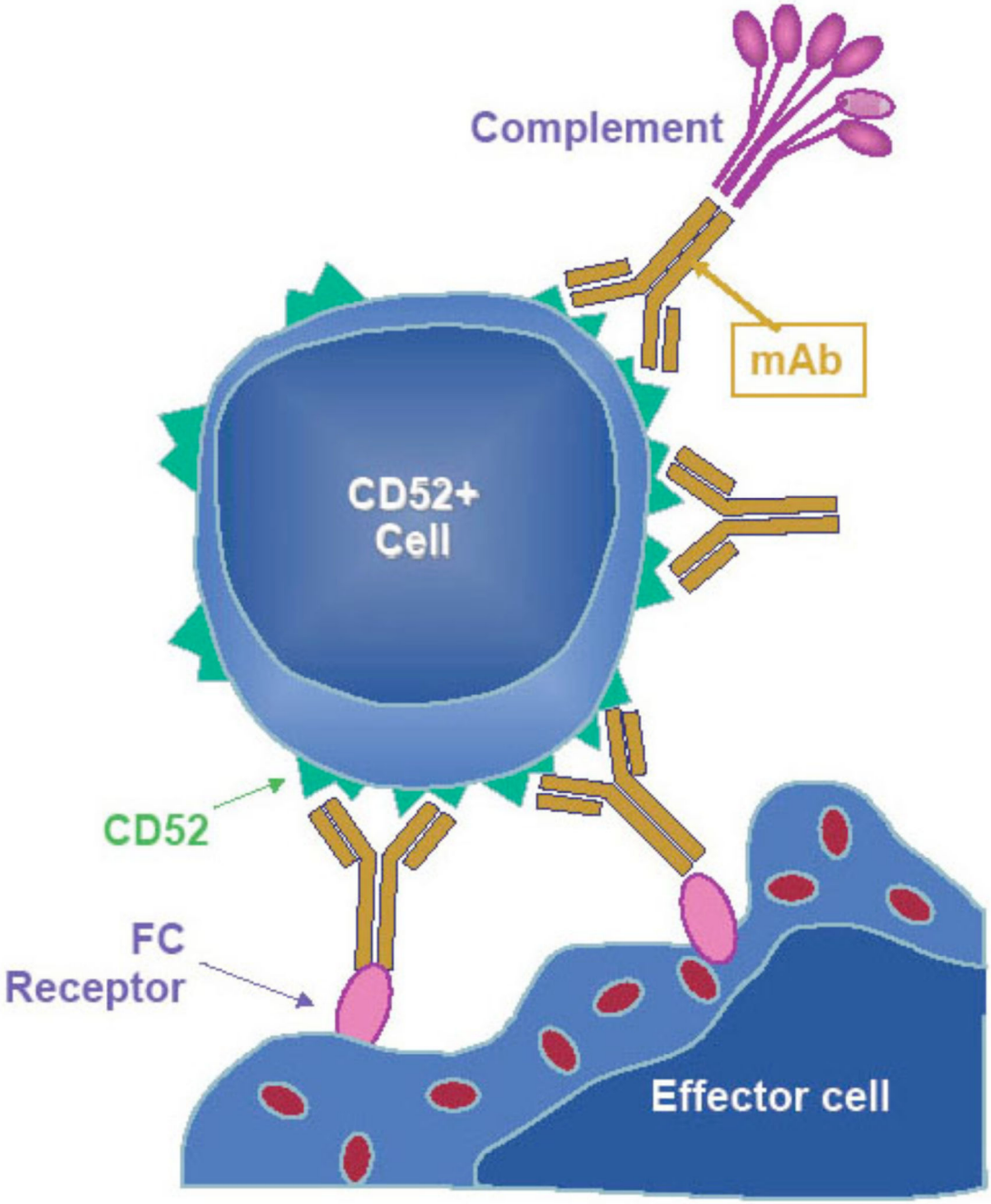

Figure 1. Mechanism of action of alemtuzumab

Alemtuzumab lyses not only lymphocytes but monocytes and other non-CD4 T cells presumably via complement fixation, antibody-dependent cell-mediated cytotoxicity (ADCC), and by induction of apoptosis. Adapted with permission from Journal of Virus Eradication 2016; **2**: 12–18.

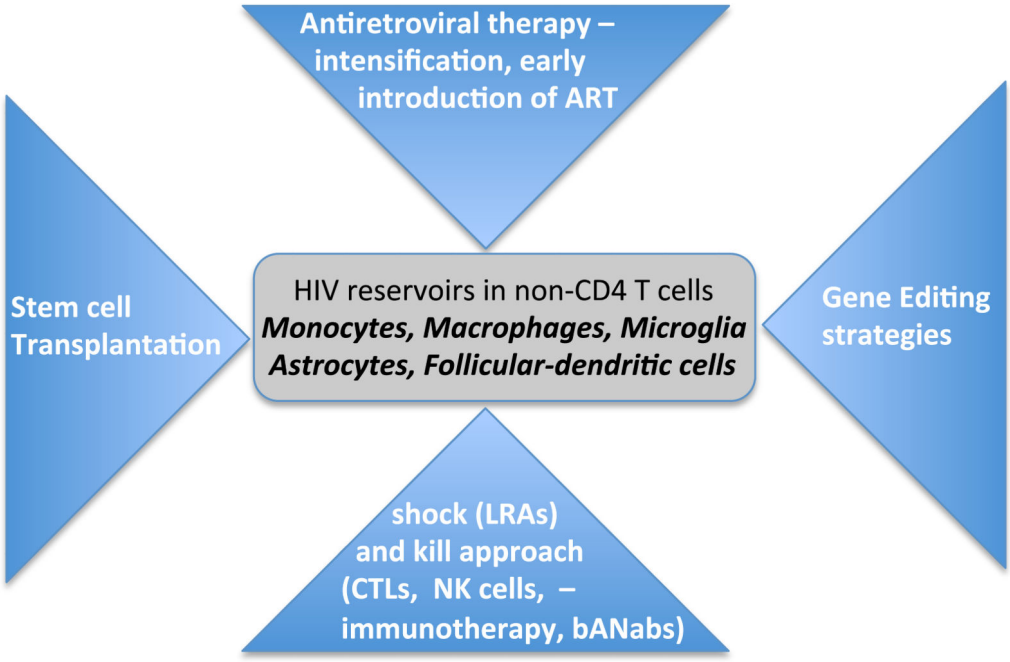

Figure 2. Current HIV eradication approaches: Focused on non-CD4+ T cell reservoirs

Overview of cure strategies that are being actively considered for CD4 T cell HIV clearance of relevance to non-CD4 T cell populations