

HIV Infection and Adipose Tissue Resident Stem Cells: Their Involvement in Pathology and Treatment

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Abstract

The HIV reservoir creates a true challenge for eradicating the virus from infected patients. Current Highly Active Antiretroviral Therapy (HAART) is very effective in controlling active viral replication in the periphery, although the drugs may not penetrate efficiently in all cellular and anatomical reservoirs. In these reservoirs, the already established HIV proviruses are stably integrated into the host cell genome and insensitive to antiviral therapy. The anatomical HIV reservoirs in the brain, lymph nodes and other compartments have been well described, but many questions remain on the actual cell types that constitute this reservoir. Recent advances in basic and clinical research have provided a better understanding of Adipose Tissue Resident Stem Cells (ASC) as possible HIV reservoir. Although ASC do not support active viral replication, the cells differentiating from ASC are susceptible to viral infection. A number of approaches have been proposed to characterize the virus from ASC and other cellular reservoirs. A detailed characterization of ASC and its association with HIV may elucidate new cellular targets for therapeutic intervention. Moreover, the current HAART treatment also affects ASC cell growth and division in adipose tissue. Laboratory and animal studies have shown a strong correlation between HAART and lipodystrophy in HIV infected patients treated with

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antiretroviral drugs. The current review describes disease progression during HIV infection and antiretroviral treatment, with a particular focus on the possible role of ASC as viral reservoir. These observations may suggest future treatment options to obtain better control of this chronic infectious disease.

Adipose Tissue and Immune System

Human Adipose Tissue Resident Stem Cell (ASC) Localization, Phenotype and Characterization

Adipose tissue is specialized connective tissue that functions as the major storage site for fat in the form of triglycerides. In addition to this role as major energy storage depot, adipose tissue is an endocrine organ that is involved in synthesizing and secreting cytokines, chemokines, and hormones such as adiponectin and resistin. These regulatory proteins and hormones are involved in numerous physiological functions including inflammation, immunity and metabolism. Adipose tissue is composed of many different cell types including adipocytes, pericytes, monocytes, macrophages, cells of endothelium (endothelial and vascular smooth muscle cells), and mesenchymal stem cells (MSC). Adipocytes are the most prominent cell types in this tissue and are differentiated from adipocyte progenitor cells (Maumus et al. 2011). MSC possess adipogenic, osteogenic, chondrogenic, myogenic and neurogenic potential. Adipose tissue represents an abundant and accessible source of adult stem cells that can differentiate along multiple lineages. Within the adipose tissue, the generation of adipocytes is through a sequential pathway of differentiation under the guidance of adipogenic micro-environmental factors, which include metabolites like glucose or lipids and other signaling molecules (Laharrague and Casteilla 2010). ASC are mainly CD90⁺ and CD105⁺ cells lacking the markers of hematopoietic (CD14, CD45) and endothelial (CD31) cells (Maumus et al. 2011).

Adipose Tissue and the Immune Response

Adipocytes, the dominant cell types within adipose tissue, participate in the regulation of pro-inflammatory cytokines and the generation of hematopoietic lineage specific cells. Recently, it has been shown that *in vitro* expanded ASC are capable of generating functional macrophages indicating the potential for hematopoietic lineage differentiation (Freisinger et al. 2010). Adipose tissue also contains cells referred to as the stromal vascular fraction (SVF). Several populations of cells within the SVF of adipose tissue contain hematopoietic markers. Considering the hematopoietic potential of ASC, it has been concluded that adipose tissue plays a role in the immune response. The mechanisms by which adipose tissue contributes to the immune response may be (I) through direct effects of resident immune cells within adipose tissues (II) through indirect effects whereby adipocytes modulate immune cell function in an endocrine or paracrine fashion or (III) through generation of hematopoietic cells from ASC. Adipose tissue most likely contributes to the immune response through all these mechanisms, but more studies are required to determine their relative importance. Cellular components of adipose tissue are functionally active and exert potent effects on adipocyte metabolism and endocrine function. Macrophages accumulate in adipose tissue during inflammation, which correlates with increased expression of cytokines and chemokines including tumor necrosis factor alpha (TNF- α), interleukine-1 β (IL-1 β), IL-6, IL-8, monocyte chemoattractant protein-1 (MCP1), and IL-18 (Weisberg et al. 2003; Whang et al. 1998; Whigham et al. 2007). The increased expression of cytokines has been correlated with enhanced hematopoietic differentiation of ASCs, decreased insulin sensitivity, increased lipolysis and increased leptin production (Trujillo et al. 2006). It has also been shown that adipocytes are highly responsive to endotoxins released from bacterial infections and that they produce high levels of proinflammatory cytokines (Lin et al. 2000).

Adipose Tissue and HIV Infection

HIV infection causes numerous metabolic abnormalities including dyslipidemia, insulin resistance, fat loss, lipodystrophy, lipoatrophy, and fat accumulation. The idea that adipocytes may play a role in HIV infection was suggested because of the significant changes in adipose tissue morphology and metabolism in HIV-infected individuals. The HIV infection lipodystrophy syndrome is particularly prevalent in patients on antiretroviral therapy and is also associated with other metabolic complications, including insulin resistance, dyslipidemia, cholesterol and fat redistribution. Within the adipose tissue all immune cells can serve as primary targets for HIV infection. Infection of lymphoid and myeloid lineages is mediated by recognition of CD4 and the chemokine co-receptor CXCR4 or CCR5 (Moore et al. 2004). These receptors promote viral attachment and fusion to cellular membranes, thus facilitating entry into the cell (Zaitseva et al. 2003). It has been shown that the receptors for HIV entry, CD4, CXCR4 and CCR5, are expressed on preadipocytes and adipocytes (Hazan et al. 2002). However, *in vitro* infection of adipose tissue with the virus was not successful as these receptors on ASCs did not support cellular entry of the virus (Munier et al. 2003). HIV exposure to hematopoietic cells may cause changes in the tissue microenvironment, which may alter the differentiation process of ASC. As mentioned earlier, macrophages are one of the main targets for HIV infection. Macrophages also play an important role in viral latency and the recurrence of infection upon stopping of therapy. Furthermore, progenitor cells differentiating towards macrophages have been documented to be susceptible to HIV infection (Duncan and Sattentau 2011).

Numerous research efforts have focused on whether ASC serve as HIV reservoir. Nazari-Shafti et al. 2011 measured significant expression of certain markers in hematopoietic differentiated (HD) cells derived from ASC. In the initial assessment, it was observed that HD cells express the HIV receptors CD4, CXCR4 and CCR5,

unlike undifferentiated ASC (Fig. 2.1a). HD cells also express certain genes that have been implicated in regulating HIV infection, which includes IL-8, SERPINA1, CCL8, CD69 and the interleukins 2, 10 and 16 (Fig. 2.1b). However, this study did not address whether ASC could harbor latent HIV-1 proviruses and serve as reservoir. Munier et al. (2003) investigated the biopsies from patients for the level of expression of the HIV entry receptors (CD4, CXCR4 and CCR5) on ASC. Expression of CD4 and CCR5 was not detected, and CXCR4 expression was variable on those biopsy samples. On the other hand, early research indicated that bone marrow derived CD34+ progenitor cells from HIV infected patients are infected (Folks et al. 1988). More recently, HIV infection and killing of hematopoietic progenitor cells (HPC) has been demonstrated both *in vitro* and *in vivo* (Carter et al. 2010). The possible reason could be that HIV can affect HPC and induce cell death by affecting their hematopoietic potential (Iglesias-Ussel and Romerio 2011). Overall, several investigations suggested that HPC and ASC could contribute to the HIV reservoir (Lafeuillade and Stevenson 2011). It would be interesting to study whether CD34 cells produce any viral proteins in case of HAART treated HIV patients with viral levels staying below the detection limit. *In vivo* studies have thus far not provided evidence of ASC as viral reservoir, but further characterization of ASC in HIV infected patients may help to elucidate the contribution of these cells to the total viral reservoir.

Effect of HIV on ASC

HIV-1 predominantly infects hematopoietic cell types such as helper T lymphocytes, monocytes and macrophages. Infection of lymphoid and myeloid cells is mediated by the receptor CD4 (Nazari-Shafti et al. 2011). Although adipocytes also express CD4 that may facilitate viral entry, no evidence of viral replication in human adipocytes has been reported *in vitro* (Sankale et al. 2006). Hazan et al. (2002) demonstrated the

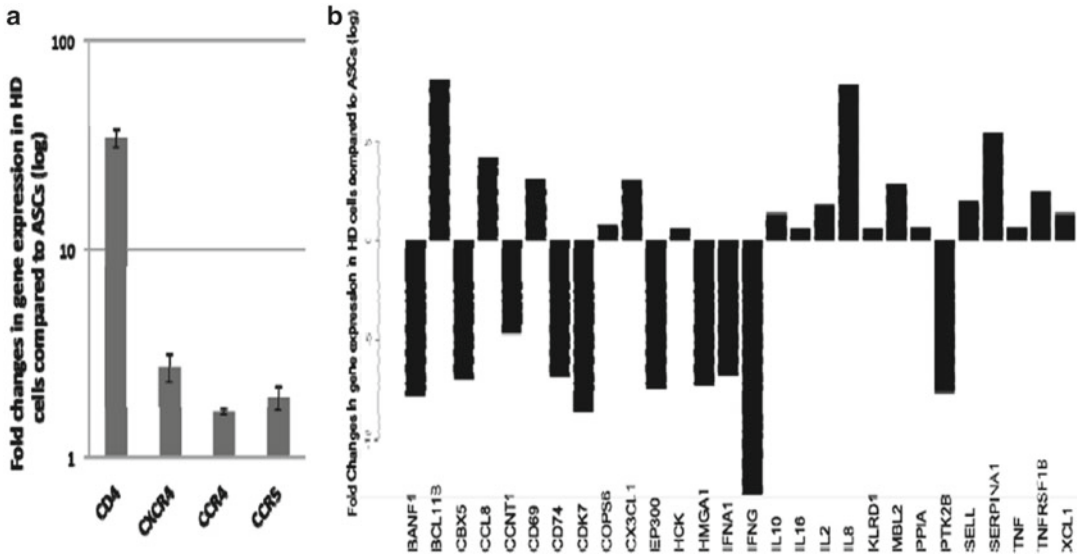


Fig. 2.1 Gene expression analysis of HD cells following hematopoietic differentiation. (a) Fold change expression of HIV receptor gene HD cells following differentiation compared to ASCs. (b) Expression of several genes involved in innate and adaptive immune reaction. Relative

expression of cell cycle regulator genes in HD cells compared to ASCs. The fold change was used to select genes ($p < 0.05$). All values are normalized to ASCs (Adapted from Nazari-Shafti et al. (2011) and permission obtained from Biomed Central)

presence of the CXCR4 and CCR5 co-receptors on human adipocytes, supporting the possibility of viral entry into these cells and further evidence for this was obtained by PCR methods. Along the same line, Nazari-Shafti et al. (2011) observed that HIV exposure can significantly alter the expression of cell cycle and apoptosis regulatory genes in HD cells. Immunocytochemical analysis indicated profound expression of CCR4, CCR5, NOS2 and CXCR4 protein on HIV-exposed HD cells, although CD4 expression was undetectable (Fig. 2.2). Additionally, HIV may facilitate the macrophage type commitment of ASC, which may also support productive viral infection in differentiated cells. During differentiation of ASC into certain stromal cell lineages, the cells get more susceptible to viral infection (Nazari-Shafti et al. 2011). The bone marrow (BM) stroma is a major component of the microenvironment that regulates the hematopoietic activity. Stroma is a heterogeneous mixture of cells including fibroblast, macrophages, endothelial cells, adipocytes and other cell types. HIV infection of some of these cell types may thus directly influence the

hematopoietic cell microenvironment. Primary human stroma appears to be susceptible to *in vitro* infection with the HIV-1_{ADA} strain (Cheng et al. 2013). The causal relationship between infected stroma cells and the loss of hematopoietic cells is still unresolved. There are at least two possible causes of the reduction of stem cell numbers in HIV infected patients. Either there is inhibition of a cellular factor that stimulates hematopoiesis or there is induction of cytokines that inhibit the hematopoiesis process (Bahner et al. 1997).

It is noteworthy that HIV-1 is able to induce transforming growth factor beta (TGF- β) expression in other cell types like macrophages and hematopoietic stem cells (HSC). TGF- β is a pleiotropic cytokine that negatively regulates hematopoiesis and induces apoptosis. A recently characterized member of the TNF family, known as proliferation-inducing ligand (APRIL), positively regulates the proliferation of megakaryocytes (MK) during differentiation. In fact, TGF- β and APRIL work together in regulating hematopoiesis and MK cell replication. In an

HIV infected environment, the HIV gp120 Envelope protein interacts with the CD4 receptor to down regulate APRIL and TGF- β . In this regard, the HIV gp120 protein potently down regulates the differentiation process towards MK cells (Gibellini et al. 2007). Fc epsilon Receptor 1 (Fc ϵ R1) that is present on hematopoietic cells induces the synthesis and release of IL4 in HIV infected tissue. The virus sheds HIV gp120 molecules that bind to IgE/Fc ϵ R1 complexes on hematopoietic cells to induce IL-4 secretion. This event may contribute to the initiation of the gradual immune deficiency in patients (Becker 2004). The HIV-1 regulatory protein Tat has been suggested to play a role in AIDS pathogenesis by interaction with CD34 progenitor cells (Gibellini et al. 2003). Tat is an early transcriptional trans-activator protein that is released from HIV infected cells and readily taken up by uninfected cells. Tat has the potential to induce a large number of host cellular genes and to initiate various signal transduction pathways. CXCR4 is a member of the transmembrane G protein family that is present on many cells including CD34 haematopoietic cells and CXCR4 has a high affinity for the chemokine stromal cell derived factor-1 alpha (SDF-1 α). A possible scenario for cell apoptosis is thus Tat-induced CXCR4 expression and subsequent induction of SDF-1 α that may contribute to the gradual loss of stem cells in HIV infected patients. It was confirmed that CD34 $^{+}$ cells from patients with HIV infection are committed to apoptosis (Gibellini et al. 2003). In general, HAART treatment improves the CD34 cell viability and function in HIV infected patients, although the underlying mechanism is not yet clear. The HIV-1 Protease Inhibitors (PIs) atazanavir (ATV) and lopinavir (LPV), frequently used in HAART regimens, can reduce the resistance of CD34 $^{+}$ cells to an apoptotic stimulus even in healthy adults. RTV has no effect on CD34 $^{+}$ cell apoptosis when used in combination with ATV or LPV. The combined data suggested that certain PI drugs and the HIV gp120 protein may increase the apoptotic susceptibility of CD34 $^{+}$ hematopoietic progenitor cells (MacEneaney et al. 2011).

Effect of Antiretroviral Therapy on ASC

HIV infected patients, particularly those on HAART, are frequently characterized by adipose dysregulation, dyslipidemia and insulin resistance, which are the hallmarks of HIV related lipodystrophy. Lipodystrophy is often regarded as toxicity attributed to various antiretroviral drugs used in HAART therapy. The specific mechanisms are not yet known, but it was observed that viral exposure dramatically increases the secretion of adiponectin from human adipocytes, even without an active infection of these cell types (Sevastianova et al. 2008). HIV significantly affects adiponectin endocrine regulation that cannot be physiologically sustained even though the viral loads are down due to HAART (Sankale et al. 2006). It has also been demonstrated that HIV infected lipodystrophy patients show 40 % reduction in plasma adiponectin levels compare to patients without lipodystrophy (Vernochet et al. 2005). The symptoms develop with the increased use of antiviral PI drugs. Nonetheless, recent clinical trials indicated that lipodystrophy is observed in PI-naïve patients and patients treated with nucleoside reverse transcriptase inhibitors (NRTIs). Currently, five PIs are approved for AIDS therapy: amprenavir (APV), indinavir (IDV), nelfinavir (NFV), ritonavir (RTV) and saquinavir (SQV). These antiviral drugs significantly reduce the viral load, but also interfere with adipocyte and/or fat cell differentiation. That in turn affects the adipose tissue and its body distribution, resulting in changes in lipid metabolism or adipogenesis.

Adipogenesis is mainly controlled by two receptors, peroxisome proliferator activated receptor gamma (PPAR- γ) and retinoid X receptor alpha (RXR- α), which form a heterodimer and affect cellular gene expression. It has been observed that SQV, NFV and RTV alter the fat metabolism in murine mesenchymal stem cells (C3H10T1/2). These PIs inhibit the conversion of stem cells to adipocytes. Interestingly, other than SQV, none of the PIs bound to PPAR- γ . On the other hand, APV and IDV have very little effect on adipogenesis. Recent data have shown

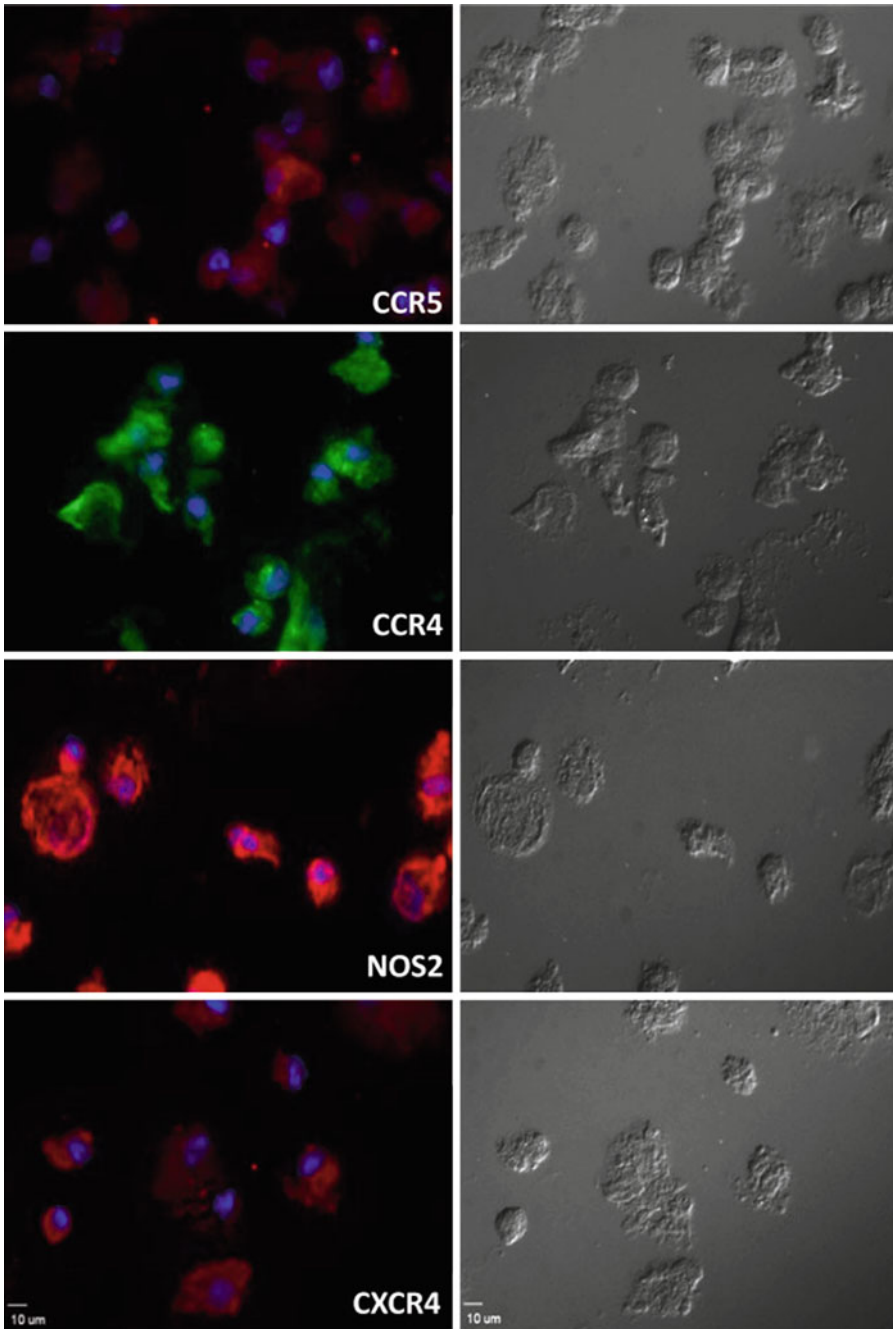


Fig. 2.2 Expression of hematopoietic markers in HD cells following HIV infection. *Left panel:* immunohistochemistry of HIV-exposed HD cells indicating the expression of CCR5, CCR4, NOS2 and CXCR4. *Right panel:* Differential Interference Contrast (DIC) images of the

identical fields. Images were obtained with the Leica TCS SP-2 confocal microscope. Scale bar 10 μm (Adapted from Nazari-Shafti et al. (2011) and permission obtained from Biomed Central)

that 50 μ M IDV and 10 μ M SQV inhibit the differentiation of primary human adipocytes (Lenhard et al. 2000). IDV also impairs differentiation at an early stage of adipose conversion, probably involving the process that controls the intracellular localization of sterol regulatory element binding protein-1 (SREBP-1). SREBP-1 is a well-known regulator of multiple genes that are involved in the metabolism of cholesterol, triglycerides and fatty acids, particularly in the liver. SREBP-1 also controls the expression of PPAR- γ , which is an important adipocyte differentiation factor. In fact, SREBP-1 mediates the effect of insulin by increasing the expression of insulin target genes such as PPAR- γ . The matrix metalloproteinase protein (MMP) family plays a major role in the adipocyte differentiation process. Bouloumié et al. (2001) have shown that human adipocytes and preadipocytes produce and release two members of the MMP family, MMP-2 and -9. The use of MMP inhibitors can decrease the rate of adipocyte differentiation in rats, suggesting that MMP activities are required for adipogenesis in rodents. Furthermore, the broad-spectrum MMP inhibitor Batimastat strongly inhibits human adipocyte differentiation. PIs like IDV, RTV, SQV and NFV also lead to a strong reduction of the human adipocytes differentiation process by a mechanism that involves MMP-9 inhibition. The decrease in MMP-9 secretion might be linked to the reduced MMP-9 gene expression observed in pre-adipocytes following a PI-containing treatment regime. Interestingly, the effect of most of the PIs (IDV, NFV, SQV, and RTV) on the differentiation process was similar. The mechanism by which the PIs affect MMP-9 expression in human pre-adipocytes may involve the degradation of I-kappaB (IKb) molecule that sequesters the NFkB transcription factor. While reducing MMP secretion, PIs may also alter the proteolytic cleavage of several circulating, cell surface and pericellular proteins, which regulate cell behaviors in numerous way. The extracellular level and activity of adipocytokines (IL-8, IL-1b, IL-6) and transforming growth factor- β

(TGF- β) may be regulated by MMPs, which can affect adipocyte differentiation (Bourlier et al. 2005).

In clinical practice, it was observed that long-term PI usage may cause a loss of fat from the face and limbs, but an increase of fat at the abdomen and the back of the neck (Caron et al. 2001). The molecular mechanism behind this process may include the inhibition of one of the main receptors, PPAR- γ or RXRalpha (Zhang et al. 1999). PI and non-nucleoside reverse transcriptase inhibitors (NNRTI) were detected in the adipose tissue of patients, but a direct effect of these drugs on the metabolism of adipocytes has not been established. Adipose tissue was also described to play a critical role in insulin resistance through the expression of tumor necrosis factor- α (TNF- α), IL-6 and adiponectin (Vernochet et al. 2005). Increased accumulation of macrophages in subcutaneous adipose tissue was observed by CD68 cell staining. These tissue macrophages are involved in several immune functions, including phagocytosis of cellular debris and triggering of immune response via cytokine release.

Increased expression of macrophage markers and inflammatory cytokines in the liver of HIV infected lipodystrophic patients is well documented (Sebastianova et al. 2008). In adipocytes, RTV was able to up-regulate TNF-alpha, IL-6 and adiponectin. As a result of up-regulation of IL6 and TNF- α , insulin resistance occurs in lipodystrophic patients on HAART (Vernochet et al. 2005). Some HIV drugs could inhibit the differentiation of precursor cells isolated from human adipose tissue. Concerning the lipodystrophy associated with the use of PIs, alternative therapeutic regimens based on NNRTIs efavirenz (EFV) or nevirapine (NFV) have been proposed. However, recent studies indicated that EFV can also accumulate in adipose tissue (Dupin et al. 2002), although the intracellular accumulation is low compared to PIs. Of note, NFV does not interfere with lipid accumulation during adipocyte development in human ASCs (Vernochet et al. 2005).

Present Clinical Scenarios

The introduction of antiretroviral therapy (ART) has changed the morbidity and mortality of HIV disease. In the era of successful HAART, HIV-infected individuals have a near normal life expectancy, with relatively minor medical complications. However, the effective drug treatment does not eradicate the virus from HIV reservoir organs in the body like the central nervous system (CNS), lymph nodes, testis etc. (Pippi 2008). This is due to integration of the HIV proviral genome into the host cell DNA genome in long-lived cellular reservoir like resting T cells.

The concept of HSC based gene therapy for HIV is gaining popularity in pre-clinical cure research because of its potential to address the difficult issue of viral reservoirs and possible virus eradication (Deeks and McCune 2010). Initial attempts of engineering HIV-resistant haematopoietic progenitor cells (HPS) faced many hurdles because of general toxicity of the gene therapy *in vivo*, which includes immune suppression and induction of leukemia (Tamhane and Akkina 2008). Over the past two decades, investigators have focused mostly on bone marrow and adipose derived MSC to optimize the efficacy and safety of stem cell based gene therapy. This includes challenges of development of *in vitro* protocols for clinical grade cell preparation and *in vivo* studies to probe the long-term adverse effects. These multiple lines of research have generated a wealth of basic and clinical research data documenting the potential of MSC therapy, bringing the gap from bench to bed side (Gimble et al. 2010). Two recent developments in this field are worth mentioning. The major cell surface receptor (CCR5) for HIV seems an ideal target for drug treatment or gene therapy because it has no obvious role in human physiology (Contento et al. 2008; Deeks and McCune 2010). In 2008, an HIV infected patient who was treated in a Berlin clinic was declared virus-free some 20 months after a bone marrow transplant with cells from a CCR5-negative donor and upon discontinuation of HAART (Hutter et al. 2009).

Chemokine Receptors

Chemokine receptors are known for their role in cell migration and the significant contribution to host defence in case of inflammation and infection. There are two primary receptors involved in HIV infection besides CD4: CCR5 and CXCR4, which the virus exploits to enter host cells (O'Hayre et al. 2010). The majority of transmitted HIV variants use CCR5 (R5 variants). As disease progresses, the virus mutates and starts recognizing the CXCR4 receptor in some patients (Contento et al. 2008). The development of the CCR5 antagonist Maraviroc and the fusion inhibitor T20 demonstrated the value of blocking cell entry for the treatment of HIV infection (O'Hayre et al. 2010). In case of the Berlin patient, the donor of the bone marrow transplant carried a 32-base pair deletion in the CCR5 gene that leads to a protein production defect. The deletion of base pair 32 within the coding region of CCR5 gene results in a frameshift during translation and the synthesis of a non-functional receptor that does not support HIV infection (Samson et al. 1996). This rare kind of genetic mutation is found in only 1–3 % of the population of northern European ancestry, but is absent in the population from western and central Africa and Japan (Hutter and Thiel 2011; Samson et al. 1996). The homozygous delta-32 population is generally protected against HIV infection, whereas heterozygous persons exhibit a slightly slower disease progression. These important observations triggered experimentalists to design a therapy based on the genetic engineering of cells that would make the patient at least partially resistant to HIV. At the same time, pharmaceutical companies had invested their resources to discover antibody or small molecule CCR5 inhibitors. In both cases, a considerable amount of success has been achieved over the years (Deeks and McCune 2010).

The Berlin Patient

A 40-year-old man was presented with acute myeloid leukemia (AML) at Berlin's Charite

University Medical Center in February 2007. He had been diagnosed with HIV infection for some 10 years and had been on HAART for four years prior to AML diagnosis. Seven months later, when his leukemia relapsed, he underwent an allogeneic hematopoietic stem cell transplantation using progenitor cells from a donor with the homozygous CCR5-delta32 deletion. Most importantly, after this treatment the patient did not show any sign of viral replication in blood and organs even after discontinuation of HAART. Moreover, his CD4 counts increased more than 800 cells/ul and his entire hematopoietic stem cell compartment consisted of CCR5 negative cells (Hutter et al. 2009; Hutter and Thiel 2011).

Presently, researchers are trying to create the same resistance to HIV by alternative means. This includes the silencing of CCR5 expression by the RNA interference (RNAi) mechanism or even the complete removal of the CCR5 gene from the host cell genome by sequence-specific endonucleases. In such an *ex vivo* gene therapy scenario one could target the mature T cells or the hematopoietic precursor cells. It should be mentioned that the Berlin patient represents only a single case and this approach should be repeated in a larger cohort, combined with long term monitoring. Very recently, two more transplantation successes were reported, but surprisingly with cells that encode a functional CCR5 receptor (Hutter and Thiel 2011). In contrast, allogeneic stem cell transplantation with a wild-type CCR5 gene was reported not to be successful (Hutter et al. 2009). These conflicting studies indicate the need to develop standardized protocols and to organize larger clinical trials. Anyhow, it is likely that the approach will not help patients that already developed an X4-using virus variant.

A recent report provided an update on the status of the Berlin patient, indicating the complete systemic recovery of CD4⁺ T-cells. After stem cell therapy the expansion of activated CD4⁺ T-cells usually enriches the pool of target cells of HIV infection. In this patient, an approximately normal number of CD4⁺ T-cells were recovered, but HIV remained undetectable. Although this patient still remains susceptible to X4-using HIV

strains, the results indicate that these strains did not evolve (Benito 2011). This result revived hope that one could achieve the same effect with a gene therapy, which would also open new avenues for a complete cure of HIV infection. This remains an important future goal as current HAART therapy helps to control the infection by keeping the viral load low, but it is not able to achieve complete viral eradication from the reservoirs.

Gene Therapy

Gene therapy includes the introduction of a functional gene (transgene) in certain cells of the body to combat a persisting virus infection. The transgene is expressed in the target cells and as a consequence it will rescue a genetic defect or provide the cell with a new property, e.g. durable resistance against viral infection. Ideal target cells for such a gene delivery will be the stem cells. These cells have self-renewal and differentiation capacity that will allow the expression of the antiviral transgene in all progeny cells. Human mesenchymal stem cells (MSC) have been recognized as attractive targets for gene therapy because of their multilineage differentiation potential and *ex vivo* expansion capacity.

Retroviral and lentiviral vector systems are commonly used for gene therapy applications because these vectors integrate into the host cell genome, thus achieving permanent gene transduction. For instance, a lentiviral vector can be used to express certain transgene proteins from a housekeeping gene promoter. It was shown that mesenchymal progenitor cells from adipose tissue can maintain transgene expression during lineage-specific differentiation, which seems essential for a durable therapeutic effect (Morizono et al. 2003). Alternative viral vector systems have been developed (e.g. based on Adenovirus and Adeno-associated virus) and transposon-mediated gene therapy has been proposed in combination with a HIV-resistance gene. The Sleeping Beauty (SB) transposon system offers a non-viral vector for gene transfer

that bypasses the risk of vector-induced oncogenesis.

The SB system has been used to evaluate the stable gene transfer of CCR5 and CXCR4 *in vitro*. This system consists of a synthetic transposon and an associated transposase. Gene transposition is initiated by recognition of a short direct repeat sequence and excision by the transposon. Subsequently, the transposon gets attached to the target DNA at sites with the TA-dinucleotide sequence. In the GHOST-R3/X4/R5 cell culture model that expresses both the CXCR4 and CCR5 receptors, 94 % down-regulation of both receptors after SB mediated small interfering RNA (siRNA) gene transfer was observed. The SB system needs to be evaluated further in CD34 progenitor cells *in vitro* to compare the efficacy with vector-mediated gene transfer (Tamhane and Akkina 2008). Other antiviral approaches that have been proposed include diverse RNA interference (RNAi) based antivirals (Liu et al. 2009; ter Brake et al. 2009), the use of the human TRIM (tripartite motif)5 α gene that encodes a potent HIV restriction factor and the *Herpesvirus saimiri* subgroup C transformation associated protein (StpC) that modulates HIV replication (Pham et al. 2010; Turner et al. 2006).

As a first step towards such a stem cell based gene therapy protocol, patients will be administered the *ex vivo* modified hematopoietic progenitor cells expressing multiple RNA-based anti-HIV moieties (e.g. short hairpin RNA (shRNA), TAR decoy, CCR5 ribozyme) or inhibitory proteins such as restriction factors. It is important to attack the virus with a combinatorial approach to avoid the evolution of escape variants (ter Brake et al. 2006; von Eije et al. 2008). In the first clinical trial, the lentiviral vector-modified cells were transplanted in autologous HIV-positive non-Hodgkin lymphoma patients and these cells showed sustained expression of the shRNA and ribozyme inhibitors for up to 24 months (DiGiusto et al. 2010). This clinical trial presents a milestone for cell based gene therapy for HIV infection.

Another retrovirus vector-based clinical trial demonstrated safety, although no therapeutic effect was scored. An anti-HIV ribozyme gene

made up the antiviral payload, but it was observed that hematopoietic stem cells produce six times less ribozyme over a period of 6 months than expected (Mitsuyasu et al. 2009). The goal of ongoing research is to introduce an effective anti-HIV gene into progenitor stem cells or mature T cells. The progenitor cells will continuously produce HIV-resistant T cells, macrophages and dendritic cells to provide a long-term immune reconstitution. Because HIV-infection will trigger the removal of non-modified cells, the expectation is that the genetically modified cells will preferentially survive because they resist HIV infection, leading to a (slow and partial) reconstitution of the immune system (Bandi and Akkina 2008).

New Treatment Strategies

In recent years anti-HIV drug discovery efforts have included the chemokine receptors. Most approaches focus on small molecule inhibitors, but monoclonal antibodies and peptide analogs are in different stages of development. The first FDA-approved CCR5-specific antagonist for HIV infection that arrived in 2007 is Selzentry (Maraviroc, Pfizer), which can block R5-tropic HIV variants (O'Hayre et al. 2010). Based on a similar concept, the second approved drug was Mozobil (AMD3100, Genzyme) in 2008, which targets CXCR4 and also mobilizes hematopoietic stem cells in infected patients. The clinical evaluation of AMD3100 confirmed that the drug does mobilize CD34 cells. In a murine model, a dose of 5 mg/kg AMD3001 mobilized HPC within 1 h after injection (Broxmeyer et al. 2005). A level of complexity that may hinder the path of drug discovery is the existence of homo-, hetero-dimeric and higher order oligomeric receptor complexes. Recent findings indicated that CCR2, CCR5 and CXCR4 form functional homo-dimers and hetero-dimers on T cells (Contento et al. 2008). Evidence was also presented for the formation of CCR2, CCR5 and CXCR4 hetero-oligomeric complexes when recombinantly expressed on HEK293 cells. These complexes were also

reported to exist when endogenously expressed on primary leukocytes (Sohy et al. 2009).

As CCR5 is the most important co-receptor for HIV infection, blocking CCR5 on human CD34 stem cells would give rise to a polyclonal multi-lineage progeny cells in which CCR5 will be permanently disrupted. Engineered zinc finger nuclease (ZFN), which comprise of series of linked zinc fingers domains especially designed to recognize specific DNA sequences, can be designed to delete the CCR5 gene. CD4 T cells modified to express CCR5-targeting ZFNs are currently under investigation in clinical trials (Holt et al. 2010). Deletion of the CCR5 gene in stem cells may provide the most durable anti-viral effect that is transferred to CCR5-negative lymphoid and myeloid cells, but the virus may still escape through CXCR4-usage. The safety of genome manipulation by CCR5-specific ZFNs in modified T lymphocytes is currently under investigation. Such gene therapeutic approaches can form a back-up plan for HAART therapy, especially for patients that develop resistance against most antiviral drugs. That use of CCR5-specific ZFNs may help to repopulate the CD4 cell compartment that was seriously affected by HIV infection (Holt et al. 2010; Lafeuillade and Stevenson 2011).

These new strategies are now being investigated along with current HAART regimens to achieve viral eradication from its tissue reservoir. The current approaches are to exhaust, shock-and-kill or to permanently silence the latent HIV reservoir (Frater 2011). While HAART keeps the viral load below the detection level, Immune Activation Therapy (IAT) could be used to stimulate the latent HIV-1 reservoir. As virus resides in resting memory CD4 T cells, the strategy is focused on activation of these cells to produce more virus. Virus-producing cells will be recognized and killed by cytotoxic T lymphocytes. Antiviral agents that produced promising *in vitro* results include histone deacetylase inhibitors, methylation inhibitors and NFkB activators. These agents are very effective in triggering the production of virus from a latent reservoir (Frater 2011). In an animal model, it was observed that IAT combined with HAART treatment triggered

the complete removal of virus (Pippi 2008). The recent success with HIV-based vectors for a gene therapy against leukemia caught the attention of many researchers and may also stimulate investigations on the path towards a molecular gene therapy of HIV. In this particular case, a HIV-based lentiviral vector was used to infect T cells of Chronic Lymphoid Leukemia patients (Berkhout 2013; Porter et al. 2011). The complete recovery of these patients nicely adds to the control of both HIV and leukemia in the “Berlin patient”. As mentioned earlier, transplantation with CCR5delta 32/delta32 stem cells demonstrated a successful reconstitution of CD4 T cells at the systemic level (Hutter et al. 2009). As immune reconstitution is a major hurdle in stem cell transplantation, the systemic recovery of CD4 cells after CCR5 delta32/delta32 stem cell transplantation is a major success (Allers et al. 2011). It should be noticed that the delta32 deletion is not common in HIV infected patients across the world, and the proposed stem cell gene therapy will not form a universal solution. However, these examples provide hope that HIV will eventually become a curable virus infection.

Conclusions

This review describes the complex HIV-host interaction as it occurs *in vivo*, which remains partially understood. Although the currently available drug cocktails have dramatically improved the life expectancy of HIV-infected individuals, a complete cure is not within easy reach. We described several novel treatment approaches that have provided the proof of concept of durable HIV-1 inhibition in the laboratory, but these results need to be verified in *in vivo* model systems and subsequently translated towards clinical application. In this regard, stem cell based gene therapy recently received much attention due to its success in some patients (Aiuti et al. 2013; Biffi et al. 2013). However, it is noteworthy that stem cell therapy for HIV infection may not be an immediate solution because of its side effects, which include teratoma formation, disease progression and genomic stability

(Benderitter et al. 2014). It is likely that routine drug therapy should be combined with novel therapeutic strategies to purge HIV-1 from reservoirs and to reach a functional cure, which means that the patient can discontinue regular drug treatment. It is important to realize that such patients will still harbor integrated copies of the HIV provirus in some of their cells. A functional cure without complete virus eradication seems the next goal in clinical HIV research.

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References

- Aiuti A, Biasco L, Scaramuzza S, Ferrua F et al (2013) Lentiviral hematopoietic stem cell gene therapy in patients with Wiskott-Aldrich syndrome. *Science* 341:1233151-1-9
- Allers K, Hutter G, Hofmann J, Loddenkemper C et al (2011) Evidence for the cure of HIV infection by CCR5Delta32/Delta32 stem cell transplantation. *Blood* 117:2791–2799
- Bahner I, Kearns K, Coutinho S, Leonard EH et al (1997) Infection of human marrow stroma by human immunodeficiency virus-1 (HIV-1) is both required and sufficient for HIV-1-induced hematopoietic suppression in vitro: demonstration by gene modification of primary human stroma. *Blood* 90:1787–1798
- Bandi S, Akkina R (2008) Human embryonic stem cell (hES) derived dendritic cells are functionally normal and are susceptible to HIV-1 infection. *AIDS Res Ther* 5:1–9
- Becker Y (2004) HIV-1 gp120 binding to dendritic cell receptors mobilize the virus to the lymph nodes, but the induced IL-4 synthesis by FcepsilonRI+ hematopoietic cells damages the adaptive immunity—a review, hypothesis, and implications. *Virus Genes* 29:147–165
- Benderitter M, Caviggioli F, Chapel A, Coppes R et al (2014) Stem cell therapies for the treatment of radiation-induced normal tissue side effects. *Antioxid Redox Signal* 21(2):338–355
- Benito JM (2011) HIV cure following CCR5 delta 32 stem cell transplantation-an update. *AIDS Rev* 13:58
- Berkhout B (2013) HIV, leukemia, and new horizons in molecular therapy. *J Formos Med Assoc* 112:441–444
- Biffi A, Montini E, Lorioli L, Cesani M et al (2013) Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy. *Science* 341:1233158
- Bouloumié A, Sengenès C, Portolan G, Galitzky J, Lafontan M (2001) Adipocyte produces matrix metalloproteinases 2 and 9: involvement in adipose differentiation. *Diabetes* 50(9):2080–2086
- Bourlier V, Zakaroff-Girard A, De Barros S, Pizzacalla C, de Saint Front VD, Lafontan M, Bouloumié A, Galitzky J (2005) Protease inhibitor treatments reveal specific involvement of matrix metalloproteinase-9 in human adipocyte differentiation. *J Pharmacol Exp Ther* 312:1272–1279
- Broxmeyer HE, Orschell CM, Clapp DW, Hangoc G et al (2005) Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. *J Exp Med* 201:1307–1318
- Caron M, Auclair M, Vigouroux C, Glorian M, Forest C, Capeau J (2001) The HIV protease inhibitor indinavir impairs sterol regulatory element-binding protein-1 intranuclear localization, inhibits preadipocyte differentiation, and induces insulin resistance. *Diabetes* 50:1378–1388
- Carter CC, Onafuwa-Nuga A, McNamara LA, Riddell JT et al (2010) HIV-1 infects multipotent progenitor cells causing cell death and establishing latent cellular reservoirs. *Nat Med* 16:446–451
- Cheng K, Rai P, Lan X, Plagov A et al (2013) Bone-derived mesenchymal stromal cells from HIV transgenic mice exhibit altered proliferation, differentiation capacity and paracrine functions along with impaired therapeutic potential in kidney injury. *Exp Cell Res* 319:2266–2274
- Contento RL, Molon B, Boularan C, Pozzan T et al (2008) CXCR4-CCR5: a couple modulating T cell functions. *Proc Natl Acad Sci U S A* 105:10101–10106
- Deeks SG, McCune JM (2010) Can HIV be cured with stem cell therapy? *Nat Biotechnol* 28:807–810
- DiGiusto DL, Krishnan A, Li L, Li H et al (2010) RNA-based gene therapy for HIV with lentiviral vector-modified CD34(+) cells in patients undergoing transplantation for AIDS-related lymphoma. *Sci Transl Med* 2:36.1–12
- Duncan CJ, Sattentau QJ (2011) Viral determinants of HIV-1 macrophage tropism. *Viruses* 3:2255–2279
- Dupin N, Buffet M, Marcelin AG, Lamotte C, Gorin I et al (2002) HIV and antiretroviral drug distribution in plasma and fat tissue of HIV-infected patients with lipodystrophy. *AIDS* 16:2419–2424
- Folks TM, Kessler SW, Orenstein JM, Justement JS, Jaffe ES, Fauci AS (1988) Infection and replication of HIV-1 in purified progenitor cells of normal human bone marrow. *Science* 242:919–922
- Frater J (2011) New approaches in HIV eradication research. *Curr Opin Infect Dis* 24:593–598
- Freisinger E, Cramer C, Xia X, Murthy SN et al (2010) Characterization of hematopoietic potential of mesenchymal stem cells. *J Cell Physiol* 225:888–897

- Gibellini D, Re MC, Vitone F, Rizzo N et al (2003) Selective up-regulation of functional CXCR4 expression in erythroid cells by HIV-1 Tat protein. *Clin Exp Immunol* 131:428–435
- Gibellini D, Vitone F, Buzzi M, Schiavone P et al (2007) HIV-1 negatively affects the survival/maturation of cord blood CD34(+) hematopoietic progenitor cells differentiated towards megakaryocytic lineage by HIV-1 gp120/CD4 membrane interaction. *J Cell Physiol* 210:315–324
- Gimble JM, Guilak F, Bunnell BA (2010) Clinical and preclinical translation of cell-based therapies using adipose tissue-derived cells. *Stem Cell Res Ther* 1:19
- Hazan U, Romero IA, Canello R, Valente S et al (2002) Human adipose cells express CD4, CXCR4, and CCR5 [corrected] receptors: a new target cell type for the immunodeficiency virus-1? *FASEB J* 16:1254–1256
- Holt N, Wang J, Kim K, Friedman G et al (2010) Human hematopoietic stem/progenitor cells modified by zinc-finger nucleases targeted to CCR5 control HIV-1 in vivo. *Nat Biotechnol* 28:839–847
- Hutter G, Thiel E (2011) Allogeneic transplantation of CCR5-deficient progenitor cells in a patient with HIV infection: an update after 3 years and the search for patient no. 2. *AIDS* 25:273–274
- Hutter G, Nowak D, Mossner M, Ganepola S et al (2009) Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. *N Engl J Med* 360:692–698
- Iglesias-Ussel MD, Romero F (2011) HIV reservoirs: the new frontier. *AIDS Rev* 13:13–29
- Lafeuillade A, Stevenson M (2011) The search for a cure for persistent HIV reservoirs. *AIDS Rev* 13:63–66
- Laharrague P, Casteilla L (2010) The emergence of adipocytes. *Endocr Dev* 19:21–30
- Lenhard JM, Furfine ES, Jain RG, Ittoop O et al (2000) HIV protease inhibitors block adipogenesis and increase lipolysis in vitro. *Antiviral Res* 47:121–129
- Lin Y, Lee H, Berg AH, Lisanti MP et al (2000) The lipopolysaccharide-activated toll-like receptor (TLR)-4 induces synthesis of the closely related receptor TLR-2 in adipocytes. *J Biol Chem* 275:24255–24263
- Liu YP, von Eije KJ, Schopman NC, Westerink JT et al (2009) Combinatorial RNAi against HIV-1 using extended short hairpin RNAs. *Mol Ther* 17:1712–1723
- MacEaney OJ, Connick E, DeSouza CA (2011) Effects of HIV-1 gp120 and protease inhibitors on apoptotic susceptibility of CD34+ hematopoietic progenitor cells. *J Acquir Immune Defic Syndr* 56:e49–e50
- Maumus M, Peyrafitte JA, D'Angelo R, Fournier-Wirth C et al (2011) Native human adipose stromal cells: localization, morphology and phenotype. *Int J Obes (Lond)* 35(9):1141–1153
- Mitsuyasu RT, Merigan TC, Carr A, Zack JA et al (2009) Phase 2 gene therapy trial of an anti-HIV ribozyme in autologous CD34+ cells. *Nat Med* 15:285–292
- Moore JP, Kitchen SG, Pugach P, Zack JA (2004) The CCR5 and CXCR4 coreceptors – central to understanding the transmission and pathogenesis of human immunodeficiency virus type 1 infection. *AIDS Res Hum Retroviruses* 20:111–126
- Morizono K, De Ugarte DA, Zhu M, Zuk P et al (2003) Multilineage cells from adipose tissue as gene delivery vehicles. *Hum Gene Ther* 14:59–66
- Munier S, Borjabad A, Lemaire M, Mariot V, Hazan U (2003) In vitro infection of human primary adipose cells with HIV-1: a reassessment. *AIDS* 17:2537–2539
- Nazari-Shafti TZ, Freisinger E, Roy U, Bulot CT et al (2011) Mesenchymal stem cell derived hematopoietic cells are permissive to HIV-1 infection. *Retrovirology* 8:3.1–12
- O'Hayre M, Salanga CL, Handel TM, Hamel DJ (2010) Emerging concepts and approaches for chemokine-receptor drug discovery. *Expert Opin Drug Discov* 5:1109–1122
- Pham QT, Bouchard A, Grutter MG, Berthouix L (2010) Generation of human TRIM5alpha mutants with high HIV-1 restriction activity. *Gene Ther* 17:859–871
- Pippi F (2008) A novel approach to HIV therapy: highly active antiretroviral therapy and autologous hematopoietic cell transplantation. *Med Hypotheses* 70:291–293
- Porter DL, Levine BL, Kalos M, Bagg A, June CH (2011) Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med* 365:725–733
- Samson M, Libert F, Doranz BJ, Rucker J et al (1996) Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. *Nature* 382:722–725
- Sankale JL, Tong Q, Hadigan CM, Tan G et al (2006) Regulation of adiponectin in adipocytes upon exposure to HIV-1. *HIV Med* 7:268–274
- Sevastianova K, Sutinen J, Kannisto K, Hamsten A et al (2008) Adipose tissue inflammation and liver fat in patients with highly active antiretroviral therapy-associated lipodystrophy. *Am J Physiol Endocrinol Metab* 295:E85–E91
- Sohy D, Yano H, de Nadai P, Urizar E et al (2009) Hetero-oligomerization of CCR2, CCR5, and CXCR4 and the protean effects of “selective” antagonists. *J Biol Chem* 284:31270–31279
- Tamhane M, Akkina R (2008) Stable gene transfer of CCR5 and CXCR4 siRNAs by sleeping beauty transposon system to confer HIV-1 resistance. *AIDS Res Ther* 5:16.1–9
- ter Brake O, Konstantinova P, Ceylan M, Berkhout B (2006) Silencing of HIV-1 with RNA interference: a multiple shRNA approach. *Mol Ther* 14:883–892
- ter Brake O, Legrand N, von Eije KJ, Centlivre M et al (2009) Evaluation of safety and efficacy of RNAi against HIV-1 in the human immune system (Rag-2(–/–)gammac(–/–)) mouse model. *Gene Ther* 16:148–153

- Trujillo ME, Lee MJ, Sullivan S, Feng J et al (2006) Tumor necrosis factor alpha and glucocorticoid synergistically increase leptin production in human adipose tissue: role for p38 mitogen-activated protein kinase. *J Clin Endocrinol Metab* 91:1484–1490
- Turner LS, Tsygankov AY, Henderson EE (2006) StpC-based gene therapy targeting latent reservoirs of HIV-1. *Antiviral Res* 72:233–241
- Vernochet C, Azoulay S, Duval D, Guedj R et al (2005) Human immunodeficiency virus protease inhibitors accumulate into cultured human adipocytes and alter expression of adipocytokines. *J Biol Chem* 280:2238–2243
- von Eije KJ, ter Brake O, Berkhout B (2008) Human immunodeficiency virus type 1 escape is restricted when conserved genome sequences are targeted by RNA interference. *J Virol* 82:2895–2903
- Weisberg SP, McCann D, Desai M, Rosenbaum M et al (2003) Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 112:1796–1808
- Whang KT, Steinwald PM, White JC, Nylen ES et al (1998) Serum calcitonin precursors in sepsis and systemic inflammation. *J Clin Endocrinol Metab* 83:3296–3301
- Whigham LD, Dhurandhar NV, Rahko PS, Atkinson RL (2007) Comparison of combinations of drugs for treatment of obesity: body weight and echocardiographic status. *Int J Obes (Lond)* 31:850–857
- Zaitseva M, Peden K, Golding H (2003) HIV coreceptors: role of structure, posttranslational modifications, and internalization in viral-cell fusion and as targets for entry inhibitors. *Biochim Biophys Acta* 1614:51–61
- Zhang B, MacNaul K, Szalkowski D, Li Z et al (1999) Inhibition of adipocyte differentiation by HIV protease inhibitors. *J Clin Endocrinol Metab* 84:4274–4277

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