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Innate and Adaptive Factors Regulating Human Immunodeficiency Virus Type 1 Genomic Activation

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Abstract

Over the past decade, antiretroviral therapy targeting the viral entry process, reverse transcriptase, integrase, and protease, has prolonged the lives of people infected with human immunodeficiency virus type 1 (HIV-1). However, despite the development of more effective therapeutic strategies, reservoirs of viral infection remain. This review discusses molecular mechanisms surrounding the development of latency from the site of integration to pre- and post-integration maintenance of latency, including epigenetic factors. In addition, an overview of innate and adaptive cells important to HIV-1 infection are examined from the viewpoint of cytokines released and cytokines that act on these cells to explore an overall understanding of HIV-1 proviral genome activation.

Finally, this review is discussed from the viewpoint of how an understanding of the interplay of all of these factors will help guide the next generation of therapies.

Keywords

HIV-1; transcription; latency; immune factors

Introduction

Globally, there are approximately 33.5 million people currently infected with human immunodeficiency virus type 1 (HIV-1) (WHO/UNAIDS 2007), and there are over 1 million new people on therapy as of 2008 (Harris and Bolus 2008). Between 2005 and 2007, mortality from infection was reduced by 200,000 people (Harris and Bolus 2008). Over the past decade, highly active antiretroviral therapy (HAART) targeting the viral entry process, reverse transcriptase, integrase, and protease, has prolonged the lives of people infected with HIV-1. However, despite the advances in antiretroviral therapy and its ability to dramatically reduce mortality rates of those infected (Palella et al. 1998), there is still no therapeutic regimen to cure chronic HIV-1 infection. Although HAART can reduce plasma viral loads to undetectable levels, the development of drug resistance or the discontinuation of therapy can result in a rebound of viremia, within weeks, due to the reactivation of latent viral reservoirs.

A major obstacle in the eradication of HIV-1 disease is the establishment of latent infection by the virus. Viral reservoirs are established early during infection, and because infectious viral particles are not actively being produced, these reservoirs remain unaffected by antiretroviral therapies (Finzi et al. 1999) and are able to reinitiate systemic infection upon interruption of HAART, or following the development of resistance (Marcello 2006; Suyama et al. 2009). HIV-1 is able to establish chronic, non-productive, or low-level persistent reservoirs in cells of the monocyte-macrophage lineage (Biswas et al. 1992, 1994; Coleman and Wu 2009; Folks et al. 1988a), dendritic cells (Coleman and Wu 2009; Marcello 2006), tissues such as the male urogenital tract (Zhu et al. 1996) and central nervous system (Cheng-Mayer et al. 1989), CD34⁺CD38⁺ bone marrow progenitor cells (Alexaki et al. 2008; Alexaki and Wigdahl 2008; Folks et al. 1988b), and CD4⁺ memory T cells (Chun et al. 1995, 1997; Finzi et al. 1997; Siliciano et al. 2003; Wong et al. 1997). Infected bone marrow progenitor cells differentiate into daughter cells (like their uninfected counterparts) that include T cells and cells of the monocyte-macrophage lineage, and harbor endogenous provirus, capable of effectively reseeding infection into the periphery of an infected individual for indefinite periods of time (Dong et al. 2009; Folks et al. 1988b; McElrath et al. 1989), in the presence or absence of viral replication. In addition, infection of memory CD4⁺ T cells may result in a long-term silent infection in quiescent cells, or if the cells become activated, a highly productive viral infection (Bukrinsky et al. 1992; Folks et al. 1988b; Han et al. 2007; Ho et al. 1995; Marcello 2006; McElrath et al. 1989; Zack et al. 1990). Cells of the monocyte-macrophage lineage have also been proposed as a site of latent virus infection due to their long-lived nature (especially as tissue macrophages), relative resistance to the cytopathic effects of HIV-1 gene products (Ho et al. 1986; Nicholson et al. 1986), and ability to support a continuous albeit low-level of virus production throughout their lifetime due to several host-cell replication blocks such as barriers in reverse transcription (O'Brien 1994) and nuclear import (Diamond et al. 2004), all contributing to inefficient expression of viral antigens, allowing the virus to escape host immune surveillance mechanisms. This review focuses on understanding HIV-1 integration as it pertains to transcriptional regulation. It also reviews the mechanisms that have been studied thus far with respect to HIV-1 latency. Finally, it covers the known innate and

adaptive immune factors that contribute to HIV-1 proviral genome activation and viral replication (Table 1).

Integration of viral DNA into the host genome

Integration is a key step in the HIV-1 viral lifecycle and is defined as the insertion of the proviral DNA template into the host cell DNA. Integration is required for efficient production of viable progeny (Liszewski et al. 2009) utilizing the host cell transcription and translation machinery. After the virion enters the host cell through interactions of the viral envelope glycoprotein 120 with the primary receptor CD4, and a coreceptor, either CCR5 or CXCR4, the viral RNA is reverse transcribed into complementary DNA (cDNA) and transported to the nuclear membrane as a pre-integration complex (PIC) (Fig. 1). This complex contains the viral proteins integrase, matrix, viral protein R, and host cell proteins such as the histone acetyltransferase p300 (Cereseto et al. 2005; Farnet and Bushman 1997; Miller et al. 1997), which assist in the nuclear import of the viral genome. Retroviral integration occurs in two steps, referred to as 3'-processing and strand transfer (Van Maele et al. 2006). During the 3'-processing, integrase removes a pGT dinucleotide at the 3' end of each long terminal repeat (LTR) that neighbors a highly conserved CA dinucleotide (Engelman et al. 1991). Following nuclear import of the PIC, strand transfer occurs, whereby integrase controls a nucleophilic attack by the 3'-hydroxyl residues of the viral DNA on phosphodiester bridges located on both sides of the major groove in the targeted DNA site (Van Maele et al. 2006). The processed CA-3'-OH viral DNA ends are ligated to the 5'-O-phosphate ends of the target DNA in a single transesterification reaction, and the integration process is completed by the cleavage of the unpaired dinucleotides from the 5' ends of the viral DNA and repair of the gaps created between the viral and target DNA is mediated by host DNA repair enzymes (Van Maele et al. 2006; Yoder and Bushman 2000).

In addition to chromatin structure around the proviral DNA discussed below, the location of viral integration site has been shown to play a role in controlling the transcriptional activity of the integrated proviral genome. Actively replicating HIV-1 viral DNA preferentially integrates into active host genes (Schroder et al. 2002). This has been shown to be true for both resting and activated CD4⁺ T cells (Barr et al. 2005; Berry et al. 2006; Bushman et al. 2005; Lewinski et al. 2006; Marshall et al. 2007; Mitchell et al. 2004; Wang et al. 2007; Wu et al. 2003). Transcriptionally silent (latent), and low-expressing provirus, which is capable of being reactivated, has been found in alphoid repeats, gene deserts, centromeric heterochromatin, and highly expressed cellular genes (Han et al. 2007; Lewinski et al. 2005). More recently, studies have shown that integration sites in activated CD4⁺ T cells were more often found in genomic regions that were dense in genes, dense in CpG islands, and enriched in G/C content while integration in resting CD4⁺ T cells occurred more often in less gene-dense regions that may be less optimal for expression due to epigenetic features common to heterochromatin and gene repression, such as trimethylation of lysine 27 of histone 3 (Brady et al. 2009).

Establishment and persistence of latent viral reservoirs in CD4⁺ memory T cells

The memory CD4⁺ T cell has been the most widely studied latently infected cell phenotype and has provided extensive knowledge to how HIV-1 remains latent in viral reservoir cells. There are two forms of HIV-1 latency in CD4⁺ memory T cells: (1) pre-integration latency and (2) post-integration latency (Fig. 1).

Factors that regulate pre-integration latency

Pre-integration latency is predominant in patients that are not on therapy and consists of full-length, linear unintegrated viral DNA that represents the final product of reverse transcription (Lassen et al. 2004; Zack et al. 1990). The pre-integration form of latent proviral DNA is extremely labile, and the efficiency of complete reverse transcription before the onset of the decay process is only about 50% (Zhou et al. 2005; Pierson et al. 2002). Even after reverse transcription, the viral cDNA has a narrow window of time ($t_{1/2}=1-5$ days) before degradation begins, which is related to a number of factors (Koelsch et al. 2008; Pierson et al. 2002). The first factor is the presence of low deoxyribonucleotide triphosphate (dNTP) pools resulting in very slow rates of reverse transcription because the cells are metabolically inert (Korin and Zack 1999). The second block involves the low levels of ATP present in memory CD4⁺ T cells, which is needed for transport of the viral PIC from the cytoplasm into the nucleus (Bukrinsky et al. 1991, 1992; Zack et al. 1990). As a result of this block, the proviral genome cannot enter the nucleus and integrate into the host genome. Overall, although pre-integration latency is more frequent than post-integration latency, its contribution to long-term persistence of the virus and progressive HIV disease is likely of less clinical importance. However, when the cell is activated, the PIC can enter the nucleus, and viral DNA can integrate into the host genome and subsequently serve as a transcription template guiding the production of viral RNAs and proteins necessary for the production of progeny virus (Bukrinsky et al. 1992; Korin and Zack 1999; Lassen et al. 2004).

Other host factors, which form integral components of the host innate immune arsenal, also serve to specifically inhibit HIV-1 replication in susceptible cells by acting at the level of viral cDNA synthesis and/or viral cDNA nuclear import. One such group of cellular antiviral response factors belongs to the apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like (APOBEC) family of polynucleotide-cytidine deaminases. Studies performed with HIV-1 strains deficient in the viral infectivity factor (*Δvif*) identified APOBEC3G as a specific inhibitor of viral infection in non-permissive cells like primary human CD4⁺ T cells. Additionally, resistance of peripheral blood monocytes and immature dendritic cells to HIV-1 has been attributed, in part, to high levels of APOBEC3G; whereas establishment of macrophages as receptive hosts is consistent with low levels of this enzyme (Peng et al. 2007). In turn, HIV-1 *vif* counteracts the action of APOBEC3G (Sheehy et al. 2002), which is consistent with the fact that *Δvif* HIV-1 viruses are able to replicate in permissive cells having very low levels of APOBEC3G. Among the human APOBEC proteins, APOBEC3G and APOBEC3F are the most potent antagonists of HIV-1 infectivity (Alce and Popik 2004; Fan and Peden 1992; Madani and Kabat 2000; Simon et al. 1998).

Formation of latent memory CD4⁺ T cells

Post-integration latency occurs when activated effector CD4⁺ T cells become infected and then revert back to the resting memory state. The formation of memory T cells begins with antigen presentation by professional antigen presenting cells (APC) such as B cells, macrophages, and dendritic cells to the naïve CD4⁺ T cell, resulting in an interaction between the T cell receptor/CD3 complex and the antigen/major histocompatibility class II complex from the professional APC. This interaction stimulates the naïve T cell to undergo blast transformation into an activated effector T cell with subsequent proliferation and generation of clones. Once the antigen is cleared from the system, a small subset of the activated T cells revert to a resting state which generates a set of long-lived memory cells that can be reactivated to respond to the same antigen again in the future. During HIV-1 infection, the virus primarily infects and replicates in activated CD4⁺ T cells; however, infected CD4⁺ T cells are short lived. Despite the short lifespan of infected CD4⁺ T cells, a

small fraction of infected activated T cells survive long enough to return back to a resting memory state, in which the virus can no longer replicate. This results in a stable, persistent viral infection that is transcriptionally silent until the cell is reactivated. Memory CD4⁺ T cells have dense heterochromatic nuclei, and the silencing of genes in T cells involves changes in the chromatin structure and/or repositioning of heterochromatic regions (Festenstein et al. 2003; Smale 2003). The non-productive nature of HIV-1 infection of memory CD4⁺ T cells occurs because viral DNA integrates into areas of the chromatin that are or become transcriptionally inactive (Jordan et al. 2003; Lewinski et al. 2005).

Following HIV-1 integration into host cell DNA, the promoter region of the viral genome, designated the long terminal repeat (LTR), can become transcriptionally silent in the absence of appropriate stimulation (Archin et al. 2009; Pazin et al. 1996; Van Lint et al. 1996). HIV-1 gene expression in CD4⁺ T cells and other susceptible cells is critically dependent on regulatory elements contained within the LTR that drive the synthesis of viral RNAs, proteins, and infectious virus. Additionally, the integrated proviral genome is regulated by the same epigenetic controls that manage host genes such as histone acetylation, methylation, and ubiquitination (Marzio and Giacca 1999). The importance of this viral reservoir rests in the ability to be reactivated from the latent state and re-seed virus systemically. How the latent virus is reactivated will be discussed below.

Epigenetics and chromatin modification

The human genome is comprised of approximately 2 m of DNA and requires significant condensation so that it can be contained within the nucleus. This is accomplished through the formation of nucleosomes and other structures that combine and fold together to eventually form a chromosome, which adds another area of regulatory control to ensure correct gene expression. Nucleosomes carry epigenetically inherited information in the form of covalent modifications of their core histones. The nucleosome consists of DNA wrapped around a histone octamer comprised of duplicate copies of the core histones H2A, H2B, H3, and H4, while the H1 histone acts as a linker between nucleosomes. Nucleosomes allow cells to tightly condense DNA within the nucleus to assist in the control of gene expression by making the DNA less accessible to factors that promote the initiation and elongation of transcription (Marzio and Giacca 1999; Turner 1993).

Following integration of viral DNA into the host genome, HIV-1 becomes subject to host factors that regulate chromatin organization and gene transcription. Studies concerning viral transcription have shown that the LTR interacts with the nucleosomes nuc-1 and nuc-0 regardless of the integration site (Marzio and Giacca 1999; Steger and Workman 1997; Verdin 1991; Verdin et al. 1993). In a transcriptionally latent state, nuc-0 (positioned at nucleotide (nt) -405 to -245 relative to the transcriptional start site) and the nuc-1 (positioned at nt +20 to +165 relative to the transcriptional start site) define two open nucleosome-free regions in the viral DNA, extending from -244 to +19 and from +166 to +256 relative to the transcription start site (Fig. 1). These open regions include the HIV-1 LTR modulatory region (transcription factor binding sites (TFBs) such as CCAAT/enhancer binding protein (C/EBP) site I and activating transcription factor/cAMP response element binding protein (ATF/CREB)), enhancer/core region (TFBs for nuclear factor-kappa B (NF-κB) and Sp factors), TFBs for AP3-L, Sp1 (Verdin et al. 1993) and upstream stimulatory factor (USF) (Jones and Peterlin 1994), the transactivating response (TAR) element region, and a region overlapping the primer-binding site immediately downstream of the 5' LTR (Van Lint et al. 1997). Promoter bending by these proteins (d'Adda di Fagagna et al. 1995; Ikeda et al. 1993) has been thought to keep this stretch of DNA nucleosome free by hindering histone assembly. Genomic footprinting studies have confirmed that independent of the activation state, most of these *cis*-acting elements are always occupied by cognate

cellular transcription factors, indicating that transcriptional activation is more likely to be dependent on the nucleosomal conformation rather than binding site accessibility (Demarchi et al. 1993). In fact, it has been proposed that displacement of nuc-1 is a prerequisite for HIV-1 transcription (Verdin et al. 1993) as observed in response to T cell activation stimuli (Verdin et al. 1993).

One mechanism by which HIV-1 latency is maintained is through the action of histone deacetylases (HDACs) at the HIV-1 LTR. HDACs repress transcription through their ability to covalently modify the lysine tail of core histones through deacetylation, thereby decreasing the access of transcription factors to the DNA. They are divided into three categories designated class I, class II, and class III HDACs. Class I HDACs consist of HDAC 1, HDAC 2, HDAC 3, and HDAC 8 and are very effective inducers of virus outgrowth from resting CD4⁺ T cells of aviremic patients (Archin et al. 2009) compared to class II or class III HDACs. NF- κ B, Sp1, AP-1, YY-1/LBP-1, and Tat recruit chromatin-modifying complexes to the LTR (Deng et al. 2001; He and Margolis 2002; Marzio et al. 1998; Schroder et al. 2002). HDAC 1 is recruited to the LTR by transcription factors such as LSF/YY1, AP-4, NF- κ B p50 homodimers, C-myc with Sp1, and CBF-1 by binding near the NF- κ B/nuclear factor of activated T cells (NFAT) binding site (Coull et al. 2000; Imai and Okamoto 2006; Jiang et al. 2007; Tyagi and Karn 2007; Williams et al. 2006). In addition, NF- κ B p50-p50 homodimers recruit HDAC 3 (Hoberg et al. 2006; Williams et al. 2006), thereby deacetylating the chromatin and making it transcriptionally inactive. Conversely, histone acetyltransferases (HATs) transfer an acetyl group from acetyl-CoA to the lysine tail of histones, thereby leading to greater accessibility of transcription factors and other proteins to the DNA (Marzio and Giacca 1999; van Holde and Zlatanova 1996; Wolffe 1994; Wolffe and Pruss 1996), thereby making the DNA accessible to transcription factors and RNA polymerase to facilitate viral transcription and subsequent translation. Activating complexes may recruit HATs to the DNA and contribute to a stabilization of the transcriptionally active state of HIV-1 in either a Tat independent or dependent manner (Burnett et al. 2009). The NF- κ B p50-RelA heterodimers interact with HATs such as p300 (Gerritsen et al. 1997; Perkins et al. 1997), which are necessary for full Tat activity (Kaehlcke et al. 2003; Kiernan et al. 1999), as well as P-TEFb recruitment (Barboric et al. 2001). P-TEFb phosphorylates and aids in the function of RNA polymerase II, which will in turn produce fully elongated, productive transcripts (Kim et al. 2006; Williams et al. 2007).

CpG methylation of the HIV-1 LTR is another mechanism of transcriptional silencing of the promoter that contributes to limited access of transcription factors to target DNA (Singh and Pauza 1992). This form of transcriptional silencing occurs by specific methyltransferases that are directed to the target DNA by the methylation of lysine 9 of histone H3 through histone methyltransferases (Marcello 2006). The attenuation of LTR expression may be explained by the binding of methyl-CpG-binding protein 1 complex and methyl-CpG-binding protein 2 to methylated Sp1 sites, thereby inhibiting the binding of Sp1 transcription factors (Joel et al. 1993; Shao 1997). The transcription factors NF- κ B and USF lose affinity for their methylated LTR binding sites as well (Pion et al. 2003). Another mechanism of histone modification occurs through ubiquitination mainly found in the monoubiquitinated form. Sequential ubiquitination and deubiquitination through the histone H2B ubiquitin protease leads to transcriptional activation of genes and is possibly also mediated through histone methylation (Henry et al. 2003).

The role of HIV-1 LTR genetic variation and genome activation

The HIV-1 LTR is the driver of viral transcription and subsequent macromolecular synthesis including the dynamics of its viral life cycle involving gene expression, repression, and ultimately the establishment of a latent state that can be reactivated with appropriate

stimulation. Following proviral integration into the host chromatin, transcription factor binding sites in the LTR recruit both activating and repressing host cell transcription factors that will determine how basal viral transcription will occur, the highest inducible rate of viral expression, and the dynamics of switching between these two states (Burnett et al. 2009; Jordan et al. 2001; Mitchell et al. 2004; Schroder et al. 2002; Weinberger et al. 2005). The HIV-1 genotype and resultant phenotype, which change over the course of infection and alter disease progression, are important variables that impact both viral replication and disease progression (Koot et al. 1993; Nielsen et al. 1993; Quinones-Mateu et al. 1998). Genotypic variants of the virus occur because of the low fidelity of reverse transcriptase, a lack of proofreading ability by viral polymerase, and selective pressures the virus faces in vivo (Coffin 1995; Gao et al. 1996), leading to the development of viral quasiespecies. HIV-1 viral quasiespecies contain sequence variation throughout the viral genome, including the LTR. Due to sequence variation within the LTR, *cis*-acting transcription factor binding sites in the LTR may be functionally altered during the evolution of quasiespecies, resulting in altered promoter activity (Berkhout and Jeang 1992; Quinones-Mateu et al. 1998; Zhang et al. 1998) and altered transcription factor binding (Burdo et al. 2004a, b; Nonnemacher et al. 2004; Ross et al. 2001a, ^b).

Sequence variation within *cis*-acting regulatory elements of the LTR and alteration of the quantity and specific activities of cellular transcription factors, such as the Sp family of proteins, NF- κ B, members of the C/EBP family, and others during the course of cellular differentiation and activation are also factors that influence the level of HIV-1 LTR-driven transcription. Specific nucleotide changes within the LTR, such as a C-to-T mutation at nucleotide position 3 of C/EBP site I or position 5 of the Sp III binding site, abrogates binding of cognate transcription factors to their corresponding binding site (Burdo et al. 2004b; Hogan et al. 2003a, b; Nonnemacher et al. 2004; Ross et al. 2001a, b). C/EBP β binds to at least two upstream LTR sites and has been shown to activate the LTR in transient transfection and stable expression studies (Tesmer et al. 1993). Additional studies have shown that one of the two functional C/EBP sites was required for replication of an HIV-1 infectious molecular clone (IMC) in U-937 cells and in primary cells of the monocyte–macrophage lineage. However, these sites were dispensable for HIV-1 IMC replication in T cell lines and primary T cells (Henderson and Calame 1997; Henderson et al. 1995, 1996). A number of studies have also shown that mutations in the Sp regions of the viral promoter result in altered rates of viral gene expression and viral replication (Berkhout et al. 1990; Gomez-Gonzalo et al. 2001; Harrich et al. 1990; Leonard et al. 1989; McAllister et al. 2000; Ross et al. 1991). The Sp1 transcription factor plays a significant role in the transcriptional activation of the LTR, and particularly, Sp site III has a slightly larger contribution to transcriptional activation than the other two adjacent Sp sites (Burnett et al. 2009). In addition, mutational loss of Sp sites within the LTR lead to a destabilized, transactivated expression state, which also demonstrated that the Sp sites appear to control transcriptional noise and stochastic switching between transcriptionally active and transcriptionally inactive states (Burnett et al. 2009). This phenomenon occurs through a dynamic interplay between its association with HDACs and HATs as discussed above. This implicates Sp1 sites as significant factors in the establishment and maintenance of viral latency and C/EBP in viral transcription enhancement. Specific sequence variations may facilitate the maintenance of HIV-1 latency and subsequent viral reactivation, and the site of integration and the host cell phenotype may alter the impact of these changes.

Immune response to HIV-1

The host immune system functions through two arms: the innate immune response and the adaptive immune response. The innate immune response serves as the first line of defense against insults such as bacterial, parasitic, or viral infections, and defends the host in a non-

specific short-term manner. This response system is comprised of the inflammatory response, the complement system, leukocytes such as natural killer (NK) cells, mast cells, eosinophils, and basophils, and phagocytes such as macrophages, neutrophils, and dendritic cells. Damaged or infected cells will release cytokines, which are chemical mediators, to activate and recruit the immune cells to the site of infection. Released cytokines can both activate and inactivate the immune response and control the cells that become activated or inactivated.

Innate immune response

Monocyte–macrophage

It has been shown that HIV-1 infects cells of the innate immune system, namely cells of the monocyte–macrophage lineage (Biswas et al. 1992, 1994; Coleman and Wu 2009; Folks et al. 1988a). Infection of cells of the monocyte–macrophage lineage by HIV-1, which is not cytotoxic for these cells in comparison to infection of CD4⁺ T cells, results in a chronic, persistent low-level infection. The level of genomic activation of integrated viral DNA in these cells can be manipulated by cytokine interactions with these cells or cytokine interactions with other cells of the innate immune system.

Interleukin (IL)-1, IL-18, and tumor necrosis factor (TNF)- α strongly upregulate HIV-1 viral replication kinetics in infected monocytes and macrophages (Granowitz et al. 1995; Imamichi et al. 2008; Shapiro et al. 1998; von Briesen et al. 1991). IL-1 also induces HIV-1 expression in chronically infected U1 cells and interestingly is elevated in AIDS patients as it seems to be released from blood monocytes of these patients in higher amounts compared with uninfected individuals (Merrill et al. 1989). IL-18 induces HIV-1 replication in chronically infected monocyte–macrophages through the release of TNF- α and IL-6 (Shapiro et al. 1998). TNF- α , a potent activator of NF- κ B, signals through a cascade that activates the I κ B kinase complex which subsequently phosphorylates I κ B resulting in the release of NF- κ B and its translocation into the nucleus. Consequently, the NF- κ B p50-relA heterodimer is believed to interact with HATs, thereby modifying the host chromatin to make the HIV-1 LTR more accessible to host transcription factors and viral transactivators such as Tat, resulting in viral transcription. Release of IL-6 by monocyte–macrophages due to TNF- α stimulation directly feeds back on these cells and has been shown to increase HIV-1 production fourfold in U1 cells, directly upregulate production of HIV in acutely as well as in chronically infected cells of monocytic lineage, and synergizes with TNF- α in the induction of latent HIV expression (Poli et al. 1990; Shapiro et al. 1998). Unlike TNF- α , upregulation of viral expression induced by IL-6 alone does not occur at the transcriptional level, and it is not associated with accumulation of HIV RNA. When IL-6 and TNF- α synergistically stimulate HIV production, accumulation of HIV RNA and increased transcription are observed, indicating that IL-6 affects HIV expression at multiple levels (Poli et al. 1990; Shapiro et al. 1998).

In contrast, the cytokines interferon (IFN)- α , IFN- β , and IL-27 act to inhibit viral expression from infected monocyte–macrophages (Imamichi et al. 2008; von Briesen et al. 1991). IFN- α activates a series of genes often referred to as IFN-inducible genes (IFIG) and inhibits viral replication through multiple pathways including the RNA-dependent protein kinase-eukaryotic initiation factor 2 α kinase pathway, the oligoadenylate synthetase–RNaseL pathway, and the myxovirus protein–guanosine triphosphatase pathway (Imamichi et al. 2008). It is also able to induce apolipoprotein B messenger RNA-editing enzyme-catalytic polypeptide-like 3G (APOBEC3G) (Chen et al. 2006; Imamichi et al. 2008; Peng et al. 2006; Sheehy et al. 2003). APOBEC3G, as described above, is one of the innate cellular factors that has been shown to act in degradation of viral DNA and regulate pre-integration latency. IL-27, which belongs to the IL-6/IL-12 family, has also been shown to induce IFIG

like IFN- α (Imamichi et al. 2008) acting through activation of STAT1 and STAT3 inhibiting the production of pro-inflammatory cytokines (Alfano et al. 2008; Holscher et al. 2005; Imamichi et al. 2008). Interestingly, there is conflicting data on the effect of IFN- γ on viral replication in infected monocytes-macrophages. When the promonocytic U1 cell line was stimulated for 24 h with 10–1,000 U/mL IFN- γ , HIV-1 RT activity increased in a dose-dependent manner (Biswas et al. 1992). However, in another study in which monocyte-macrophages were isolated from PBMCs of HIV-1-positive individuals, and then cultured in the presence of IFN- γ (0–30 U/mL) for 7 days, a dose-dependent suppression of viral p24 antigen was observed (von Briesen et al. 1991).

NK cells

HIV-1 infection induces a very typical pattern of immune responses, including an early induction of type 1 interferons (IFN- α and IFN- β) secreted by dendritic cells, increased expression of interleukin-15, and a proliferation of NK cells (Alter and Altfeld 2009). NK cells mediate non-specific lysis of target cells and function as a first line of defense in the control of viral infections. Subsequently, large quantities of Th1 type 1 cytokines (IFN- γ , TNF- α , and several chemokines) are released, which drive a strong Th1-type adaptive immune response, followed by a rapid proliferation of T cells, resulting in the clearance of the viral infection or a reduction in viral replication in persistent viral infections (Alter and Altfeld 2009). When stimulated with IL-2 or IL-15, NK cells increase production of the chemokines RANTES, MIP-1 α , and MIP-1 β , thereby resulting in both suppression of viral replication in CD8⁺ T cells and NK cell depleted PBMCs, and inhibition of MT-2-negative HIV-1 BaL in the PM1 T cell line (Oliva et al. 1998). In addition, IL-15 also increases NK cell survival and activity in infected individuals (Rodriguez et al. 2007). IL-18 induces IFN- γ production from NK cells (Alfano et al. 2008) which could result in IFN- γ -mediated inhibition of viral replication in infected monocytes and macrophages, potentially resulting in a step towards viral latency.

Adaptive immune response

The adaptive immune response, composed of CD4⁺ T cells, CD8⁺ T cells, and B cells, is the second line of defense of the immune system. This response helps the host fight infection and disease in an antigen-specific manner. The adaptive immune response also creates longterm memory, so a more rapid response to the pathogen can occur the next time the host is infected by the pathogen.

CD4⁺ T cells

CD4 T cells differentiate into a number of different effector T cells with a variety of functions. The main functional subset of CD4 effector T cells are Th1, Th2, Th17, and regulatory T cells. These subsets are defined on the basis of the different cytokines they secrete. All effector T cells are involved in activating their target cells to make responses that help clear pathogens from the body. IL-2, which is secreted by Th1 cells, induces an upregulation of HIV-1 replication in infected mitogen-stimulated PBMCs and an IL-2 dependent T cell line, Kit 225-K6 (Al-Harhi et al. 1998). In a counteractive manner, IL-2 stimulation of CD8⁺ T cells results in the suppression of HIV-1 replication in CD4⁺ T cells (Kinter et al. 1995). Other cytokines that induce HIV-1 replication in PBMCs, CD4⁺ T cells, and/or T cell lines include IL-7, IL-12, IL-15, IL-18, TNF- α , and IFN- γ . IL-7 has been shown to reactivate HIV-1 from latent T cell reservoirs, reduce apoptosis in CD4⁺ T cells, and enhance X4-utilizing viral replication in resting naïve CD4⁺ T cells (Alfano et al. 2008; Managlia et al. 2006; Wang et al. 2005). IL-7 upregulates the nuclear factor of activated T cells (NFAT) (Managlia et al. 2006), which has two binding sites in the HIV-1 LTR, potentially resulting in an increase in transcription as mediated by the LTR. IL-12 induces

HIV-1 replication in PBMCs, CD4⁺ T cells, and/or T cell lines. IL-12 production has been observed during the course of chronic HIV infection, and acute/early HIV infection is associated with heightened, maximal, IL-12-productive capacity ex vivo and increased IL-12 production in vivo (Byrnes et al. 2008). IL-15, involved in T cell proliferation and differentiation like IL-2, was also found to induce HIV-1 replication in PBMCs and Kit 225-K6 T cells. IL-7 and IL-15 bind to the IL-2 receptor and initiate the janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway (Alfano et al. 2008) which can lead to an increase in viral transcription through STAT binding to the LTR. The viral protein Nef induces IL-18 production from the THP-1 monocytic cell line (Shapiro et al. 1998), and the cytokine has also been shown to induce viral replication in the HUT78 T cell line (Klein et al. 2000). IL-18 impacts HIV-1 replication possibly through IL-18-induced release of TNF- α and IL-6, which act through the activation of p38 MAP kinase and NF- κ B (Shapiro et al. 1998). NF- κ B, as described above, binds to the core/enhancer region and is integral in reactivation of cells from post-integration latency as well as general HIV-1 genome activation. TNF- α has been shown to reactivate latent HIV-1 in chronically infected Jurkat T cell lines (Jordan et al. 2001, 2003) through the activation of NF- κ B and possibly through its interaction with DNA methylation inhibitors also resulting in increases in viral gene expression (Kauder et al. 2009).

Expression of particular cytokines can also result in the suppression of viral gene expression. These cytokines include IL-16, IL-27, and IFN- α . IL-16 pretreatment of CD4⁺ T cells resulted in an inhibition of Tat activation and a suppression of LTR activity (Maciaszek et al. 1997); in addition, IL-16 was shown to inhibit HIV-1 entry in CD4⁺ T cells (Truong et al. 1999). IL-27 and IFN- α both inhibit replication in T cells functioning through the JAK/STAT pathway to interact with STAT5 (Imamichi et al. 2008). Inhibition of HIV-1 replication by IFN- α also occurs through suppression of reverse transcriptase (Shirazi and Pitha 1992).

Discussion

Although the current antiretroviral therapy (ART) regimen against HIV-1 is extremely effective in eliminating plasma viral burden by impeding ongoing viral replication in infected cells, complete eradication remains an elusive therapeutic goal due to the persistence of the latent virus pool. Latently infected T cells are rare, to the order of one in a million resting CD4⁺ T cells (Chun et al. 1997). These cells are long-lived with a slow decay rate (half life= 44 months in HAART patients) (Finzi et al. 1999), enabling these cells to persist as long as 60 years for people on consistent therapy (Finzi et al. 1999; Siliciano et al. 2003), helping the virus to stay in a state of reversible genomic activation. In the light of these practical challenges, novel treatment strategies have started focusing towards purging latent virus. Some of the initial approaches focused on activation of the resting CD4⁺ T cell population with IL-2 (Chun et al. 1999; Prins et al. 1999; Stellbrink et al. 2002), OKT-3 (Fraser et al. 2000; van Praag et al. 2001), and IL-7 (Wang et al. 2005) along with intensified ART. Although still in its infancy, structured or supervised treatment interruption (STI) strategies have generated a lot of interest and involve cycles of treatment withdrawal followed by re-initiation (Paci et al. 2009; Volberding et al. 2009). Besides allowing temporary respite from the side effects of continual drug use, the rationale behind such intermittent breaks in treatment is that it will allow rapid replication of HAART-suppressed viral quasispecies as well as re-emergence of latent viral strains, which can then be targeted by resumption of therapy. In the absence of HAART, cells harboring archived latent drug-resistant strains may also get transiently activated with subsequent production, processing, and presentation of viral antigens. As a result, they might become more susceptible to cytolytic T cell-mediated lysis. However, efficacy of STI has been debatable till date, especially in managing acute infection and highlights the need to conceive optimal strategies

on an individual basis depending on the clinical correlates of HIV-1 disease severity. Given the more recent understanding of the epigenetic factors associated with HIV-1 latency, HDAC inhibitors such as valproic acid (Lehrman et al. 2005; Siliciano et al. 2007; Wiech et al. 2009) and vorinostat (suberoylanilide hydroxamic acid) (Edelstein et al. 2009; Heider et al. 2009) have been and are being developed to try and initiate release of proviral DNA from latency. In addition, non-coding small RNAs have been and are being developed to target post-transcriptional gene silencing (Boden et al. 2003; Coburn and Cullen 2002; Jacque et al. 2002) and transcriptional gene silencing (Lim et al. 2008; Suzuki et al. 2008; Suzuki et al. 2005; Yamagishi et al. 2009). As the knowledge of HIV-1 latency and transcriptional control continues to be explored, more targets for potential therapeutics will be elucidated. Included in these new targets will be cells of the central nervous system (CNS), which have been shown to be infected early in the course of disease (An et al. 1999; Davis et al. 1992), such as perivascular macrophages, microglial cells, and astrocytes. Perivascular macrophages are found on the parenchymal side of the blood–brain barrier (BBB) adjacent to the microvasculature and are therefore much more likely to come into direct contact with HIV-1-infected cells of the monocyte–macrophage lineage traversing the BBB into the brain. The “Trojan Horse” hypothesis suggests that HIV-1-infected monocytes in the periphery continuously replenish the pool of perivascular macrophages, thereby acting as carriers of HIV-1 into the brain (Haase 1986; Peluso et al. 1985). In comparison, microglia are found dispersed throughout the brain and have slower turnover rates (Hickey et al. 1992; Lassmann et al. 1993). Although proximity to the periphery makes perivascular macrophages more amenable to infection and subsequent virion production (Cosenza et al. 2002; Fischer-Smith et al. 2004; Takahashi et al. 1996; Wiley et al. 1986; Williams et al. 2001), this does not minimize the role played by long-lived microglia in amplifying the neuropathological cascade. Studies have also established that microglia might serve as a potential reservoir for latent HIV-1, capable of infectious virus production under activation conditions (Albright et al. 2004; Cosenza et al. 2002; Watkins et al. 1990). Astrocytes are the most abundant cell type in the CNS and are critical regulators of normal brain homeostasis. The extent of their susceptibility to HIV-1 in vivo is controversial as a result of the trace amounts of surface CD4. However, persistent infection of astrocytes has been documented in in vitro cell culture models with production of low levels of infectious virus (Brack-Werner et al. 1992; Kramer-Hammerle et al. 2005; Wang et al. 2004). Detection of viral DNA and RNA in postmortem brain tissues of acquired immunodeficiency syndrome (AIDS) patients (Takahashi et al. 1996; Trillo-Pazos et al. 2003; Wang et al. 2004) but rarely viral antigens, suggests that astrocytes may harbor latent virus. Under pro-inflammatory stimuli, treatment of astrocytes with cytokines like tumor necrosis factor (TNF)- α and IL-1 β that are abundant in late stage disease could activate virus from latency and initiate new rounds of infection (Tornatore et al. 1994). Given this, the next generation of therapeutics for HIV-1-infected individuals will need to continue to target these silent/latent and/or chronically infected cellular reservoirs in the peripheral blood and cells of the CNS with a combination of ART and potentially cytokine treatments to attempt to eradicate replication competent virus.

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Fig. 1.

Regulators of pre- and post-integration latency. Pre-integration is regulated as the viral RNA is reverse transcribed into the proviral DNA (1). This is controlled by the availability of the nucleotide pool, half life of the forming cDNA copy of the proviral DNA, and the interaction of the viral protein Vif with the cellular antiviral protein APOBEC, especially family members 3G and 3F. It is also regulated at the step of transport across the nuclear membrane through the availability of ATP as the process requires energy. Within activated cells, such as CD4⁺ T cells, the intracellular levels of dNTPs and ATP are sufficient to allow the preintegration complex (PIC) to be transported across the nuclear membrane (2). In resting cells, the levels of dNTPs and ATP are reduced and therefore do not allow for efficient reverse transcription of the viral RNA or transfer of the PIC across the nuclear membrane. This is known as preintegration latency (3). In addition, during the resting state, the presence of APOBEC proteins enhances degradation of the viral DNA. If the resting cell returns to an activated state within a sufficient time period, the increased levels of dNTPs and ATP can allow the translocation of the PIC into the nucleus to commence. Once within the nucleus, the viral DNA becomes integrated into the host genomic DNA, aided by the viral integrase protein. Once in a resting state, this integrated proviral DNA becomes latent (4). The latent LTR remains transcriptional silent due to recruitment of histone deacetylase-1 (HDAC-1) by NF-κB homodimers p50 and/or by LSF and YY1, which maintains the nucleosomes, nuc-0 and nuc-1, in a deacetylated state, keeping the chromatin in a condensed state and preventing the recruitment of RNA polymerase (a). Basal transcription driven by the LTR occurs in the absence of the viral protein Tat. It is driven primarily through the upregulation of cellular transcription factors such as NFAT and the p65 subunit of NF-κB, which help recruit the RNAPol II complex to the transcriptional start site. However, in the absence of Tat, transcription from the HIV-1 LTR produces predominantly short RNA as a result of the hypophosphorylated state of RNAPol II and the activity of 5, 6-dichloro-1-beta-D-ribofuranosylbenzimidazole sensitivity-inducing factor (DSIF) and negative elongation factor complex (NELF), which bind to hypophosphorylated RNAPol II and inhibit transcriptional elongation (b). However, there are a number of longer transcripts that do provide a build up of viral regulatory proteins, especially Tat, that eventually feedback into the next stage of viral transcription, Tat-mediated transcription (c). Tat binds to the trans-activation response region (TAR) structure on the viral RNA and recruits positive transcription elongation factor b (P-TEFb) (the Cyclin dependent kinase 9 (Cdk9) and cyclin T1 (CycT1) complex) through binding to cyclin T1. Recruitment of P-TEFb to TAR induces hyperphosphorylation of CTD by Cdk9 and results in the dissociation of DSIF and NELF. Acetylation of Tat at Lys 50 creates a binding site for p300/CREB binding protein-associated factor (PCAF) and promotes the formation of a ternary complex of Tat-PCAF and P-TEFb. The interaction of PCAF with acetylated Tat competes against TAR RNA binding of Lys50-acetylated Tat, and causes its dissociation from TAR RNA, thereby enhancing the transcriptional elongation of HIV-1. The binding and subsequent signaling of TNF-α has been shown to reactivate latently infected cells. The binding and signaling of TNF-α through the TNF-α receptor activates a cascade, which results in the production and nuclear translocation of NF-κB. This NF-κB binds to its cognate binding sites on the HIV-1 LTR, thereby activating transcription.

Table 1

Innate and adaptive immune cytokines that effect HIV-1 genome activation

Cytokine	Cells secreted by	Cells acted on	Mode of action
IL-1	Monocytes, macrophages, neutrophils	Monocytes and macrophages	<ul style="list-style-type: none"> Strongly upregulates HIV-1 viral replication kinetics in infected monocytes and macrophages Induces HIV-1 expression in chronically infected U1 cells Is reportedly elevated in AIDS patients, as it seems to be released from blood monocytes of these patients in higher amounts compared to normal
IL-2	T cells	NK cells, T cells	<ul style="list-style-type: none"> Causes NK cells to secrete chemokines such as RANTES, MIP-1α, and MIP-1β resulting in both suppression of viral replication in CD8⁺ and NK cell depleted PBMCs, and inhibition of MT-2 negative HIV-1 BaL in the PM1 T cell line IL-2 induces an upregulation of HIV-1 replication in infected mitogen-stimulated PBMCs and an IL-2 dependent T cell line, Kit 225-K6 IL-2 stimulation of CD8⁺T cells results in the suppression of HIV-1 replication in CD4⁺T cells
IL-6	T cells, macrophages	Monocytes, macrophages	<ul style="list-style-type: none"> Exogenous IL-6 added to U1 cells increased HIV-1 production four-fold over control Directly upregulates production of HIV in acutely as well as in chronically infected cells of monocytic lineage Synergizes with TNF-α in the induction of latent HIV expression Unlike TNF-α, upregulation of viral expression induced by IL-6 alone does not occur at the transcriptional level and it is not associated with accumulation of HIV RNA When IL-6 and TNF-α synergistically stimulate HIV production, accumulation of HIV RNA and increased transcription are observed, indicating that IL-6 affects HIV expression at multiple levels
IL-7	Non-T cells	T cells	<ul style="list-style-type: none"> Shown to reactivate HIV-1 from latent T cell reservoirs, reduce apoptosis in CD4⁺T cells, and enhance X4-utilizing viral replication in resting naïve CD4⁺T cells Upregulates NFAT, which has two binding sites on the HIV-1 LTR, potentially resulting in an increase in transcription as mediated by the LTR Can bind to the IL-2 receptor and initiate the JAK/STAT pathway, which can lead to an increase in viral transcription through STAT binding to the LTR
IL-12	Macrophages, dendritic cells	T cells	<ul style="list-style-type: none"> Induces HIV-1 replication in PBMCs, CD4⁺T cells, and/or T cells lines IL-12 potently induces IFN-γ production from lymphocytes and NK cells IL-12 productive capacity observed with chronic HIV infection, acute/early HIV infection is associated with heightened, maximal, IL-12-productive capacity ex vivo and increased IL-12 in vivo
IL-15	Many non-T cells	NK cells, T cells	<ul style="list-style-type: none"> Causes NK cells to secrete chemokines such as RANTES, MIP-1α, and MIP-1β resulting in both suppression of viral replication in CD8⁺ and NK cell depleted PBMCs, and inhibition of MT-2 negative HIV-1 BaL in the PM1 T cell line Increases NK cell survival and activity in infected individuals

Cytokine	Cells secreted by	Cells acted on	Mode of action
IL-16	T cells, mast cells, eosinophils	T cells	<ul style="list-style-type: none"> Found to induce HIV-1 replication in PBMCs and Kit 225-K6 T cells Can bind to the IL-2 receptor and initiate the JAK/STAT pathway, which can lead to an increase in viral transcription through STAT binding to the LTR Pretreatment of CD4⁺ T cells resulted in an inhibition of Tat activation and a suppression of LTR activity Shown to inhibit HIV-1 entry in CD4⁺ T cells
IL-18	Activated macrophages	Monocytes, macrophages NK cells	<ul style="list-style-type: none"> Induces HIV-1 replication through the release of TNF-α and IL-6 Induces IFN-γ production from NK cells, which could result in IFN-γ mediated inhibition of viral replication in infected monocytes and macrophages Induced by Nef in the THP-1 monocytic cell line and has also been shown to induce viral replication in the HUT78 T cell line Acts on HIV-1 replication possibly through the release of TNF-α and IL-6, which act through the activation of p38 MAP kinase and NF-κB
IL-27	Monocytes, macrophages, dendritic cells	T cells, monocytes, macrophages	<ul style="list-style-type: none"> Inhibits replication in T cells Works through the JAK/STAT pathway to interact STAT5 Acts to inhibit viral expression from infected monocytes/macrophages
IFN- γ	T cells, NK cells	Monocytes and macrophages	<ul style="list-style-type: none"> In vitro, the promonocytic U1 cell line was stimulated with IFN-γ and HIV-1 RT activity increased in a dose-dependent manner
IFN- α	Leukocytes, dendritic cells	T cells, monocytes, macrophages	<ul style="list-style-type: none"> Inhibits replication in T cells Works through the JAK/STAT pathway to interact STAT5 Inhibition of HIV-1 replication also occurs through suppression of reverse transcriptase Act to inhibit viral expression from infected monocytes/macrophages Acts through activation of STAT1 and STAT3
TNF- α	Macrophages, NK cells, T cells	Monocytes and macrophages	<ul style="list-style-type: none"> A potent activator of NF-κB Once in the nucleus, the NF-κB p50-reIA heterodimer is believed to interact with HATS, thereby modifying the host chromatin to make the HIV-1 LTR more accessible to host transcription factors and viral transactivators such as Tat, resulting in viral transcription