Review

Therapeutic targeting of human immunodeficiency virus type-1 latency: current clinical realities and future scientific possibilities

Salvatore T. Butera *

HIV and Retrovirology Branch, Division of AIDS, STD, and TB Laboratory Research, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA

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Abstract

Factors affecting HIV-1 latency present formidable obstacles for therapeutic intervention. As these obstacles have become a clinical reality, even with the use of potent anti-retroviral regimens, the need for novel therapeutic strategies specifically targeting HIV-1 latency is evident. However, therapeutic targeting of HIV-1 latency requires an understanding of the mechanisms regulating viral quiescence and activation. These mechanisms have been partially delineated using chronically infected cell models and, clearly, HIV-1 activation from latency involves several key viral and cellular components. Among these distinctive therapeutic targets, cellular factors involved in HIV-1 transcription especially warrant further consideration for rational drug design. Exploring the scientific possibilities of new therapies targeting HIV-1 latency may hold new promise of eventual HIV-1 eradication. © 2000 Published by Elsevier Science B.V. All rights reserved.

Keywords: HIV-1 latency; Therapeutic intervention; Cell models; Viral transcription

1. Introduction

Without question, the development and clinical introduction of highly active anti-retroviral therapy (HAART, regimens including various combinations of nucleoside, non-nucleoside, and protease inhibitors) have provided unequivocal benefits to infected individuals by controlling the replication of human immunodeficiency virus type-1 (HIV-1). These clinical benefits include dramatic and sustained reductions in the level of circulating HIV-1 (viral load) and a halting of the progressive CD4 T-lymphocyte loss that accompanies HIV-1 disease progression (Gulick et al., 1997; Hammer et al., 1997; Gulick et al., 2000). Furthermore, HAART implementation has resulted in substantial reductions in morbidity and...
mortality from various opportunistic infections that characterize acquired immunodeficiency syndrome (AIDS), thus often prolonging of survival (Detels et al., 1998; Mocroft et al., 1998; Palella et al., 1998).

However, these clinical benefits of HAART still hold limitations. First, the high cost and limited availability of drugs used in HAART make the clinical benefits largely available only to persons in developed countries. Second, HAART regimens require patients to take many tablets per day. Furthermore, the potential side effects and the risk of developing HIV-1 resistant to any of the antiretroviral compounds is concerning and makes long-term maintenance on HAART unrealistic. Lastly, the initial hope that prolonged and maintained suppression of HIV-1 load by HAART regimens alone could result in the eventual clearance or eradication of virus from an individual (Perelson et al., 1997) is now clearly improbable. It soon became apparent that issues related to HIV-1 latency would limit the possibility of complete viral clearance and that latent HIV-1 reservoirs would have to be addressed therapeutically if virus eradication was to become a reality. These subjects are the nature of this review.

2. What has HAART taught us?

In the mid-1990s, when HAART regimens first became available (as a result of the clinical introduction of HIV-1 protease inhibitors), there was continuing uncertainty surrounding the mechanisms by which HIV-1 led to the development of AIDS. This uncertainty arose from the observations that only a small percentage of peripheral T-cells harbor HIV-1 in infected individuals (Brinchmann et al., 1991) and from the inability to define completely mechanisms by which HIV-1 infection results in progressive T-cell loss. The introduction of effective viral suppression under HAART regimens firmly established that preventing continued high-level viral replication will impede progressive immunologic dysfunction and reduce AIDS mortality (Gulick et al., 1997; Hammer et al., 1997; Detels et al., 1998; Mocroft et al., 1998).

The widespread use of HAART for treating advanced HIV-1 disease among persons in developed countries has demonstrated both the restorative capacity of the adult immune system as well as its limitations. In many cohorts that have been studied to date, HIV-1-infected persons receiving HAART have responded with partial restoration of immune cell populations (Bohler et al., 1999; Hengel et al., 1999), immune functions (Autran et al., 1997; Arno et al., 1998; Dam Nielsen et al., 1998; Kroon et al., 1998; Lederman et al., 1998), and homeostasis (Andersson et al., 1998; Bouscarat et al., 1998; Evans et al., 1998; Dyrhol-Riiise et al., 1999). However, immune recovery generally appears incomplete and variable (Connors et al., 1997; Fleury et al., 1998; Pakker et al., 1999; Zaunders et al., 1999). Although an extensive detailing of immune restoration resulting from HAART regimens is beyond the scope of this review (reviewed by Autran et al., 1999), this subject is relevant and discussed below because such restoration may hold promise for eventual viral eradication.

The complete suppression of ongoing viral replication in patients taking HAART also permitted the ground-breaking work to describe HIV-1 viral dynamics and calculate the half-lives of free virions and infected cells actively replicating virions (Ho et al., 1995; Wei et al., 1995). Furthermore, the suppression of continued HIV-1 replication afforded an opportunity to document additional reservoirs of viral replication. Amid the early excitement surrounding the apparent clinical success of antiretroviral combination therapies was the growing theoretical prediction that, given complete suppression of HIV-1 replication and sufficient time, complete clearance of HIV-1 was possible (Perelson et al., 1997). These predictions were based on the finite life span of infected cells and, as a worse-case scenario, the belief that infected cells of the macrophage lineage require up to 3 years to completely turnover. Many studies have now shown ongoing viral replication during HAART (Gunthard et al., 1998; Lafeuil-lade et al., 1998; Dornadula et al., 1999; Ferguson et al., 1999; Furtado et al., 1999; Gunthard et al., 1999; Martinez et al., 1999; Natarajan et al., 1999; Zhang et al., 1999a; Sharkey et al., 2000) which
most likely arises due to a persistent latent population (Chun et al., 1997a; Finzi et al., 1997; Wong et al., 1997; reviewed in Finzi and Siliciano, 1998) and contributes to continued HIV-1 dissemination (Grossman et al., 1998, 1999). Remarkably, decay estimates now suggest that the HIV-1 latent population may persist for up to 60 years in infected individuals receiving HAART (Finzi et al., 1999; Ramratnam et al., 2000).

3. HIV-1 latency defined

The concept of HIV-1 latency can be considered on several levels and must be precisely defined to avoid misunderstanding. First, HIV-1 latency can be regarded on a clinical level as the extended and variable asymptomatic period after acute infection but prior to the onset of AIDS-defining immune dysfunction. But, as discussed below, HIV-1 infection does not assume a state of true latency, in which cessation of viral replication accounts for the lack of disease progression (Garcia-Blanco and Cullen, 1991), and to apply the term latency in this manner is imprecise. However, as we have learned from the advent of HAART, HIV-1 latency on a cellular level does have clinical ramifications thus the two concepts are inherently linked.

On a cellular level, HIV-1 latency can exist during either the afferent or the efferent portion of the viral life cycle (Fig. 1). The afferent portion of the HIV-1 life cycle includes the necessary events between extracellular binding to the surface co-receptors and eventual integration of the reverse-transcribed viral genome into the host cell chromosomes. However, when HIV-1 enters an unactivated CD4 T-lymphocyte, the afferent portion of the life cycle is not completed and a temporally labile, partially reverse-transcribed complex is created in the cytoplasm of the host cell (Stevenson et al., 1990; Zack et al., 1990; Bukrinsky et al., 1991). This situation is considered pre-integration latency. If cellular activation occurs with hours to days of HIV-1 entry and before to the degradation of the pre-integration complex, then reverse transcription and integration ensue to complete the afferent portion of the life cycle. Therefore, resting CD4+ lymphocytes can serve as a latent viral reservoir by maintaining HIV-1 in the pre-integrated state until cellular activation (Bukrinsky et al., 1992; Zack et al., 1992).

The efferent portion of the HIV-1 life cycle begins at the point of stable proviral integration and includes subsequent viral transcription, translation, and assembly to produce infectious viral progeny. At this point of the life cycle, HIV-1 latency is defined by the transcriptional state of the integrated provirus (reviewed in McCune,
Table 1  
Characteristics of cellular models of latent and constitutive HIV-1 infection

<table>
<thead>
<tr>
<th>Cell derivation</th>
<th>Viral status</th>
<th>No. proviruses</th>
<th>Viral defect</th>
</tr>
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<tbody>
<tr>
<td><strong>T-cells (parental)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8E5 (CEM)</td>
<td>Constitutive</td>
<td>1</td>
<td>Stop codon in pol</td>
</tr>
<tr>
<td>ACH-2 (CEM)</td>
<td>Latent</td>
<td>1</td>
<td>TAR mutations</td>
</tr>
<tr>
<td>J1 (Jurkat)</td>
<td>Latent</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>J-delta-k (Jurkat)</td>
<td>Latent</td>
<td>Unknown</td>
<td>NF-kB deletions</td>
</tr>
<tr>
<td><strong>Promyelocytic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OM-10.1 (HL-60)</td>
<td>Latent</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>PLB-IIIB (PLB-985)</td>
<td>Constitutive</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td><strong>Promonocytic</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>U1 (U937)</td>
<td>Latent</td>
<td>2</td>
<td>Tat mutations</td>
</tr>
<tr>
<td>U33 (U937)</td>
<td>Constitutive</td>
<td>1</td>
<td>Stop codon in env</td>
</tr>
<tr>
<td><strong>Monocytic</strong></td>
<td></td>
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<tr>
<td>THP-1-derived</td>
<td>Latent and restricted</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td><strong>B-cells (EBV-transformed)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL58</td>
<td>Latent</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
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</table>

1995). In some instances, the integrated HIV-1 provirus is transcriptionally dormant, regardless of the activation status of the host cell. This condition, termed absolute latency, appears to be a dead-end for viral replication and may result from the integration of a transcriptionally defective provirus. However, the best characterized and clinically most concerning state of post-integration latency exists when the HIV-1 provirus is transcriptionally dormant until the host cell becomes activated by any number of first, second, or third messenger pathways (discussed below). Viral activation from this state, termed microbiologic latency, results in the production of infectious HIV-1 progeny to complete the life cycle and further propagate infection and disease. As issues related to viral eradication have evolved, HIV-1 microbiologic latency has come to the forefront of clinical and therapeutic considerations regarding HAART.

4. History of HIV-1 latency

HIV-1 latency was first demonstrated in experiments examining various cellular outcomes of acute HIV-1 infection. Working with transformed cell lines in vitro, cells that survived the cytopathic effects of acute HIV-1 replication could be identified and cloned. Although some of these cloned populations maintained chronic high-level viral replication (Folks et al., 1986), others were observed to express little or no viral proteins unless activated by exogenous stimuli (Folks et al., 1987, 1988, 1989; Clouse et al., 1989). This state of HIV-1 microbiologic latency was subsequently established using transformed cells representing several different cellular lineages (Tozzi et al., 1989; Mikovits et al., 1990; Butera et al., 1991; Perez et al., 1991) (Table 1). A large body of literature quickly became established concerning the spectrum of soluble mediators and cytokines that could activate HIV-1 expression from latency and the intracellular mechanisms regulating this process (reviewed in Butera and Folks, 1992). Cells that harbored an integrated HIV-1 provirus but could not be activated using the established stimuli could also be identified. These cells may harbor a dormant or transcriptionally defective HIV-1 provirus or respond to extracellular stimuli other than that tested (Mikovits et al., 1990).

By extension, the state of HIV-1 microbiologic latency seemed to reasonably explain the clinically asymptomatic period between acute HIV-1 infec-
tion and the onset of AIDS-defining illnesses and suggested that HIV-1 enters into a state of clinical latency. In fact, during this early time period, a reservoir of virally infected cells was detected in peripheral blood CD4\(^+\) T-lymphocytes (Schnittman et al., 1989). Since HIV-1 replication was known to down-modulate cell surface CD4 expression via active expression of envelope or other viral proteins (Butera et al., 1991), it was considered probable that HIV-1 was transcriptionally dormant in these infected lymphocytes that retained surface CD4 expression. Shortly following these observations, the lymph nodes were firmly established as a major site of HIV-1 infection (Pantaleo et al., 1991) and were examined for the extent of viral replication. Using more sophisticated in situ technologies, researchers found that lymphoid tissues from HIV-1 infected individuals during the clinically asymptomatic phase showed a large percentage of cells that harbored proviral DNA without evidence of viral RNA (Embretson et al., 1993). However, it also became quite clear that HIV-1 expression was active at all stages of disease progression, including the clinically asymptomatic period, both in the lymph nodes (Pantaleo et al., 1993) and the peripheral blood (Michael et al., 1992; Piatak et al., 1993). Therefore, HIV-1 did not enter a state of true clinical latency, in which cessation of viral replication correlated with asymptomatic infection. These landmark findings raised an aura of doubt over HIV-1 latency as being a possible artifact of in vitro infections, the use of transformed and cloned cell lines, or a virologic phenomenon without any real clinical relevance.

These perceptions of HIV-1 latency dramatically changed again with the advent of HAART. Although HAART maintenance held the initial promise of HIV-1 eradication, levels of detectable provirus remained for extended periods of time in individuals undergoing HAART. Furthermore, the detectable provirus was integrated into the host genome and could be activated in vitro to generate infectious virus particles. These features implied that this was not dead-end defective provirus but rather replication-competent HIV-1 existing in a state of microbiologic latency in vivo. It is now well accepted that HIV-1 latency is a clinical reality and a new therapeutic challenge to possible viral eradication.

5. Clinical complications of HIV-1 latency — what do we know?

Recent technological advances have enabled researchers to address the impact of HIV-1 latency on a clinical level. Much of the difficulty in detecting and quantitating this viral reservoir arose from the large amount of patient material required and the low frequency at which these latently infected cells exist. Techniques were first established to demonstrate the existence of this population even before HAART was available to reduce the background of active HIV-1 replication (Chun et al., 1995). These pioneering studies assumed that HIV-1 microbiologic latency would exist in unactivated T-lymphocytes that retain surface CD4 expression and rigorously purified resting cells from the CD4\(^+\) T-cell population by selecting against those expressing activation surface markers (HLA-DR, CD25, etc.). A culture period of several days was also implemented to permit the degradation of temporally labile, HIV-1 pre-integration complexes. PCR-based techniques were then applied that detected HIV-1 proviral DNA only when integrated into cellular DNA.

In the first patient populations examined, the frequency of resting CD4 lymphocytes that harbored HIV-1 in a state of microbiologic latency was estimated at less than 1:2000. An even smaller proportion of these cells (1:10 000–1:1 000 000) harbored a replication-competent HIV-1 provirus that could generate infectious progeny virions upon cellular activation with a T-cell mitogen (Chun et al., 1995, 1997b). These findings placed the total body burden of resting CD4 T-lymphocytes harboring integrated HIV-1 at \(\sim 10^7\) and those harboring replication-competent HIV-1 in a state of microbiologic latency at between \(10^5\) and \(10^6\). The frequency of these cells was largely consistent among individuals examined and did not correlate with other clinical parameters of disease progression or therapy. Early studies on patient material also established that the main
cellular phenotype harboring latent HIV-1 provirus was the memory T-cell expressing the CD45RO surface antigen (as opposed to the naïve T-cell expressing CD45RA) and that the frequencies of these cells were similar in blood and lymph nodes (Chun et al., 1997b).

When these techniques were applied to patients receiving HAART (Chun et al., 1997a; Finzi et al., 1997; Wong et al., 1997), a more complete appreciation of the clinical complications arising from HIV-1 microbiologic latency became apparent. Although early predictions estimated that the decay of infected cells occurred ~3 years after effectively halting HIV-1 replication, the decay of the latently HIV-1 reservoir was determined to be extremely protracted in all individuals examined. It is now estimated that complete eradication of the estimated less than 1 million cells harboring replication-competent latent HIV-1 will require up to 60 years of continued viral suppressive therapy (Finzi et al., 1999; Ramratnam et al., 2000). The mean half-life for this cellular reservoir was estimated between 6 and 44 months, with the rate of decay possibly influenced by the effectiveness of suppressive HIV-1 therapy in a given individual (Finzi et al., 1999; Ramratnam et al., 2000).

Equally sobering was the finding that the population of cells harboring HIV-1 in a state of microbiologic latency was established very early, during primary HIV-1 infection (Chun et al., 1998a). In patients initiating suppressive HAART regimens within 10 days of the onset of HIV-related acute retroviral syndrome (probably 2–3 weeks post-exposure but after the burst of viremia and viral dissemination), the latent population of cells had already been established. Therefore, even with effective intervention at the earliest recognition of symptoms, latent HIV-1 infection will present a significant therapeutic challenge. Furthermore, in vitro experiments with latently infected cells from patient material indicated that HIV-1 expression could be activated either by direct stimulation of the T-cell receptor (via anti-CD3 cross-linking) or by the addition of a combination of proinflammatory and T-cell activating cytokines (Chun et al., 1998b). These conditions are most likely to continually exist within the normal cellular milieu and microenvironment of lymph nodes and further emphasize the challenges facing HIV-1 interventions.

Although the latent HIV-1 reservoir constitutes less than 1 million cells in almost all individuals examined, the protracted half-life may be due to several nonmutually exclusive mechanisms. During suppressive HAART regimens that result in a lack of detectable viremia, continued low-level HIV-1 replication has been noted by several groups (Lafeuillade et al., 1998; Dornadula et al., 1999; Ferguson et al., 1999; Furtado et al., 1999; Natarajan et al., 1999; Zhang et al., 1999a; Ramratnam et al., 2000; Sharkey et al., 2000). This low-level viral replication may permit ongoing reseeding and renewal of the latent reservoir. Evidence of continued viral evolution during suppressive HAART regimens (Gunthard et al., 1999; Martinez et al., 1999) further underscores the fact that HIV-1 replication continues even under the most effective therapies. However, continued viral replication during HAART does not appear to be related to the development of therapeutic resistance (Gunthard et al., 1998), suggesting that HIV-1 replication and possible renewal of the latent pool persists in sanctuary sites that are unavailable to or largely unaffected by current therapies (discussed below). The protracted decay of the latent HIV-1 population may also result, in part, from the natural stability of the CD45RO memory T-cell population. The normal life span of CD45RO T-cells is measured in months or years (McLean and Michie, 1995) and long-term survival of memory cells ensures immunologic protection against previously encountered pathogens. Finally, it also remains possible that HIV-1 latency is established in a unique population of host T-cells that are not susceptible to the cytopathic effects normally associated with viral replication in lymphocytes. Such a virus-host T-cell relationship, although not yet described, might allow repeated rounds of antigen stimulation and HIV-1 expression without loss of cells from the latently infected population. Latent infection of a unique population of T-cells (unique in its response to antigen stimulation, antigen specificity, or ability to accommodate active HIV-1 replication) might also explain why only a limited num-
ber of latently infected cells (between $10^5$ and $10^6$) are observed in almost all individuals examined thus far, regardless of stage of disease (Chun et al., 1997a; Finzi et al., 1997; Wong et al., 1997).

Ongoing viral replication during highly suppressive therapy also suggests the presence of sequestered sites unavailable or insensitive to the effects of antiretroviral drugs (reviewed in Hoetelmans 1998; Schrager and D’Souza 1998; Clarke et al., 2000). Several anatomical sites are suspected to support ongoing viral replication during HAART regimens; however, the lymphoid compartments are especially concerning (Cavert et al., 1997; Lafeuillade et al., 1997; Rosok et al., 1997a; Stellbrink et al., 1997; Pantaleo et al., 1998; Perrin et al., 1998; Tenner-Racz et al., 1998) because they contribute extensively to HIV-1 pathogenesis (Embretson et al., 1993; Pantaleo et al., 1993; Hufert et al., 1997). The lymphoid microenvironment also provides a rich source of soluble factors capable of perpetuating HIV-1 replication (Rieckmann et al., 1991; Schnittman et al., 1991). Furthermore, the follicular dendritic cell network with lymphoid compartments may serve as an additional reservoir for HIV-1, even if these cells are not productively infected (Cameron et al., 1992; Spiegel et al., 1992; Heath et al., 1995). Other anatomical HIV-1 reservoirs of concern include the rectal mucosa (Kotler et al., 1998), the genital tract (Byrn and Kiessling, 1998; Coombs et al., 1998; Cu-Uvin et al., 1998; Mayer et al., 1999), and the central nervous system (Bagasra et al., 1996; Cinque et al., 1998), the latter being complicated by issues of drug penetration.

Furthermore, the numerous cellular reservoirs of latent HIV-1 infection during HAART are important obstacles for viral eradication. The complexity of this issue was recently made apparent by the demonstration that the reemergence of HIV-1 replication upon intermittent cessation of HAART may not correspond in some individuals to expression of virus sequestered in resting CD4 lymphocytes (Chun et al., 2000). These findings imply that latent HIV-1 may persist in other cellular compartments, including infected cells of the monocyte lineage. Circulating monocytes and tissue macrophages are a critical component of HIV-1 pathogenesis and can serve as an important source of virus (Orenstein et al., 1997; Lewin et al., 1998; Lawn et al., 2000). These infected cells are long-lived, and HIV-1 replication does not induce cytopathic effects as observed with infected T-lymphocytes — features that originally placed infected monocytes as a primary concern for HIV-1 eradication by HAART (Perelson et al., 1997). Although residual HIV-1 has been detected in circulating monocytes from individuals on HAART regimens (Lambotte et al., 2000), methods for quantitating latent virus in monocytes have not been developed and refined as have those for resting T-cells. Quantitating latent virus in macrophages is further complicated by the long-term persistence of the majority of these cells as tissue macrophages, unavailable to sampling via the peripheral blood. While it is difficult to ascertain the magnitude of latent infection among macrophages, HIV-1 latency in this population of cells has been documented (Mikovits et al., 1992; Lambotte et al., 2000) and should be considered in therapeutic strategies for HIV-1 eradication. Latent HIV-1 may also persist in some cellular reservoirs that are either not generally considered primary targets of active infection, such as CD8+ T-lymphocytes (Semenzato et al., 1998; Yang et al., 1998), or not yet described.

6. Mechanisms regulating HIV-1 expression, latency, and activation

6.1. Mechanisms controlling HIV-1 expression

Key to the development of therapeutic targeting for HIV-1 latency is a basic understanding of the viral, cellular, and immune mechanisms regulating HIV-1 proviral expression. Once HIV-1 establishes an integrated provirus as a necessary component of its life cycle, viral expression is regulated, as are many host genes, by the influence of cellular transcription factors. Furthermore, HIV-1 expression is regulated by host factors that participate in cellular activation (reviewed in Gaynor, 1992), thereby inherently linking these processes. This link of expression at the level of necessary transcription factors is one means by which HIV-1 latency persists in resting
CD4 lymphocytes and by which cellular activation can result in renewed viral expression.

The HIV-1 core promoter, contained in the 5’-long terminal repeat (LTR), contains a consensus TATA sequence and three binding sites for the constitutive cellular transcription factor Sp1 (Fig. 2). This region is flanked upstream by the viral enhancer region that contains binding regions for several well-recognized host transcription factors. Most notable among these transcription factors is nuclear factor kappa B (NF-κB), a heterodimeric inducible enhancer that exists in the host cell cytoplasm as an inactive complex with its inhibitor, IκB. Upon cellular activation via several first and second messenger pathways involving cellular kinases (Kinter et al., 1990; Critchfield et al., 1997), phosphorylation of IκB results in its proteolytic degradation. As a result, active NF-κB is liberated and translocates to the nucleus to stimulate expression of genes that contain NF-κB-specific binding sites in the enhancer (Fig. 3). Activation of HIV-1 expression via the NF-κB response pathway is a well-characterized aspect of viral regulation and latency (Duh et al., 1989; Griffin et al., 1989; Osborn et al., 1989). Furthermore, cooperative interaction between NF-κB and other elements, especially Sp1 (Perkins et al., 1993), may be required for full proviral expression.

Fig. 2. A schematic representation of the HIV-1 genome and 5’ long terminal repeat (LTR) promoter, indicating recognized sites of interaction for DNA and RNA binding proteins. HIV-1 proteins Tat and Rev are translated from spliced mRNAs which combine 2 coding exons, as indicated. Sites are depicted only for the major DNA binding proteins: activator protein (AP-1), nuclear factor of activated T-cells (NF-AT), nuclear factor-κB (NF-κB), Sp1, and TATA.
The studies cited above all suggest that cellular factors in addition to NF-κB are necessary for HIV-1 regulation and activation from latency. Other transcription factor binding sites contained in the enhancer region of the HIV-1 LTR include that for activation protein-1 (AP-1) and nuclear factors of activated T-cells (NF-AT). Some evidence for the contribution of these factors in regulating HIV-1 expression has been established (Kinoshita et al., 1998; Navarro et al., 1998). HIV-1 expression is also responsive to a variety of other identified cellular factors, including the c-myc proto-oncogene (Sun and Clark, 1999), the tumor suppressor protein p53 (Duan et al., 1994a; Gualberto et al., 1995), and p38 mitogen-activated protein (MAP) kinase (Cohen et al., 1997; Shapiro et al., 1998a). Furthermore, depending upon the primary cellular stimulus, HIV-1 expression may be responsive to still poorly characterized factors (Couvronnere and Devaux, 1998; Briant et al., 1999) or factors that regulate viral replication in a cell lineage-selective manner (Henderson and Calame, 1997). Therefore, the activation of HIV-1 expression is intricately linked to cellular activation, involves a variety of second messenger kinase and third messenger nuclear factor signals, and can be regulated in a cell type-specific manner.

Through the molecular manipulation of the LTR and transient transfection experiments using LTR-reporter gene constructs, numerous other binding regions for a variety of cellular factors have been implicated in and around the LTR (reviewed in Gaynor, 1992). Delineating all the cellular pathways and components involved in regulating HIV-1 expression and establishing their importance using latently infected cells derived from patient material ex vivo will be a necessary undertaking. Each cellular component involved in regulating HIV-1 expression is a potential therapeutic target for blocking HIV-1 activation from latency (reviewed in Baba, 1997). Therapeutic targeting of cellular factors has proven effective using a variety of agents that inhibit NF-κB-mediated HIV-1 expression (Mihm et al., 1991; Li et al., 1993; Biswas et al., 1994; Navarro et al., 1996) and with HIV-1 proviruses deleted of the NF-κB binding regions (Antoni et al., 1994).

Translocation of NF-κB to the nucleus also stimulates renewed expression of its inhibitor, I-κB, to limit the impact of the original cellular stimulus (Sun et al., 1993); however, this regulatory mechanism does not appear to restrict continued HIV-1 expression (Butera et al., 1995). Furthermore, although NF-κB is recognized as a critical element for HIV-1 activation, binding of NF-κB to the HIV-1 enhancer without the availability of additional cellular and viral components is not sufficient to activate HIV-1 expression (Doppler et al., 1992; Butera et al., 1995). Activation of HIV-1 expression has also been observed with stimulating agents that do not involve NF-κB as a third messenger component (Stanley et al., 1990; Vlach and Pitha, 1993; Antoni et al., 1994; Laughlin et al., 1995; Hashimoto et al., 1995) and with HIV-1 proviruses deleted of the NF-κB binding regions (Antoni et al., 1994).
This conceptual approach, which deserves renewed attention for rational drug design, has the distinct advantage of limiting the opportunity for the emergence of viral escape mutants. However, for targeting of cellular signaling pathways and transcription factors to become a therapeutic reality for inhibiting HIV-1 activation from latency, a great degree of specificity for HIV-1 expression will have to be achieved. Even under circumstances where redundant cellular pathways exist, these cellular components are involved in vital cellular gene expression and inhibitors that block HIV-1 expression may also result in unforeseen cellular toxicities.

Regulation of HIV-1 transcription also critically involves the participation of a virally encoded transactivation protein, Tat. The primary contribution of Tat is during the process of HIV-1 transcriptional elongation (rather than transcriptional initiation, reviewed in Jones, 1997). Promoter-proximal HIV-1 transcripts have been detected in peripheral blood cells from infected individuals, suggesting a block of the Tat-transactivation axis to maintain latency in vivo (Adams et al., 1994). Tat functions by physically interacting with a hairpin loop structure, known as the TAR (transactivation response) element, in the proximal portion of the nascent viral RNA transcript (Fig. 2). Tat also physically interacts with cellular factors necessary for HIV-1 transcriptional elongation, thereby recruiting them to the HIV-1 promoter. From a multitude of site-directed mutagenesis studies on the TAR element, a consensus binding pattern was established in which Tat binds to a bulge in the stem of the hairpin loop and Tat-associated cellular factors bind to a loop at the distal end (Fig. 4; reviewed in Jones, 1997).

By all indications, the cellular components that participate in Tat-directed HIV-1 transcriptional elongation include a kinase that functions to hyperphosphorylate the carboxy-terminal domain (CTD) of cellular RNA polymerase II (pol II) and permit polymerase processivity. Within recent years, the identity of such a Tat-associated kinase (TAK) was described as the positive transcription elongation factor-b (P-TEFb) (Mancebo et al., 1997; Zhu et al., 1997), a finding that has been confirmed by several additional studies (Flores et al., 1999; Kanazawa et al., 2000). P-TEFb is a complex comprised of multiple cellular proteins, one of which, CDK9, is a kinase capable of CTD hyperphosphorylation resulting in HIV-1 transcriptional elongation. This kinase demonstrates cell lineage-selective regulation (Herrmann et al., 1998) which could once again account for differences in the regulation of HIV-1 latency in monocytes and T-lymphocytes.

Tat-dependent HIV-1 transactivation also involves several other key cellular proteins. The interaction between Tat and P-TEFb is mediated by an additional cellular protein, Cyclin T1, that directly interacts with Tat and allows for high-affinity binding of the complex to TAR RNA (Wei et al., 1998). Furthermore, other cellular transcription complexes and associated kinases interact with Tat and participate in HIV-1 promoter-directed transcription. Among these, the...

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**Fig. 4.** Proposed model of Tat interaction with Cyclin T1 and P-TEFb to permit CDK9 mediated hyperphosphorylation of the carboxy-terminal domain of RNA polymerase II (conversion from pol IIa to pol IIo) and subsequent HIV-1 transcriptional elongation. Other proposed transcription factor involvement is indicated (adopted from Jones, 1997).
multi-subunit transcription complex, TFIIH, and its kinase subunit, CDK7, demonstrate enhanced CTD phosphorylation in vitro via an interaction with Tat (Cupec et al., 1997). However, this interaction does not satisfy all the necessary aspects of Tat-directed HIV-1 transcriptional elongation and may be more critically involved in the process of transcriptional initiation (reviewed in Jones, 1997).

As with the cellular second and third messenger signaling components involved in HIV-1 activation, understanding the full complement of host cell factors required for Tat activity will identify potential new targets for therapeutic intervention against HIV-1 latency. The involvement of these cellular components in the regulation of HIV-1 latency from material derived from infected individuals still awaits confirmation. However, in this situation, the issue of drug specificity and cellular toxicity may be of slightly less concern because of the ability to target a process involving a virally encoded protein with no known cellular homologue.

6.2. Mechanisms maintaining HIV-1 latency

The potential mechanisms regulating the state of HIV-1 latency are numerous, diverse, and involve both cellular and viral factors (reviewed in Bednarik and Folks, 1992). Regulatory events involving the HIV-1 LTR promoter may be of primary importance in controlling the latent state. Key to this process is chromatin remodeling of the HIV-1 promoter region to permit an open and transcriptionally active configuration. A single nucleosome (termed nuc-1) positioned in the region immediately after the HIV-1 transcription start site was conserved in a number of different latently HIV-1-infected cell lines and disrupted upon HIV-1 transcriptional activation (Verdin et al., 1993). Additional reconstitution studies (Pazin et al., 1996) and studies demonstrating HIV-1 transcriptional activation following chromatin modification (histone acetylation, Van Lint et al., 1996) further support the importance of this regulatory process. HIV-1 latency may be further regulated by proviral integration into a transcriptionally dormant region of the host genome (Winslow et al., 1993) or by the extent of DNA methylation in the HIV-1 LTR (Bednarik et al., 1991).

HIV-1 encodes several accessory proteins (Nef, Vpu, and Vpr) that have been implicated in controlling both the establishment of cellular latency upon infection and the subsequent conversion to productive viral expression (reviewed in Ikuta et al., 1997). Mutations in these viral regulatory genes are associated with decreased HIV-1 cytopathicity, viral persistence, and a more rapid conversion to a nonproductive viral infection (Kishi et al., 1995; Song et al., 1996; Kawano et al., 1997). Furthermore, extracellular Vpr can directly activate latent HIV-1 from several cellular models examined and serum antibodies against this viral protein may prevent viral activation in vivo (Levy et al., 1994). The contribution of Nef to viral latency may be related to its ability to permit HIV-1 infection of resting CD4 lymphocytes (Miller et al., 1994; Spina et al., 1994) and the unique viral-host cell relationship that is established under these circumstances (Spina et al., 1995).

The regulatory proteins of HIV-1 can also be major determinants of viral latency. As might be anticipated, defects in the coding region for Tat (Emiliante et al., 1998) or mutations that disrupt binding to TAR (Emiliante et al., 1996) restrict HIV-1 transcriptional activity, as observed in several chronically infected cell lines (discussed below). Interestingly, these chronically infected cell lines can still undergo transcriptional activation and respond differently to the intracellular introduction of exogenous Tat (Duan et al., 1994b).

A second HIV-1 regulatory protein, Rev, was also implicated in controlling the balance between productive and dormant infection via a regulation of HIV-1 mRNA processing (Pomerantz et al., 1990a). Rev interacts with Rev-response elements (RRE) in HIV-1 mRNA to protect full-length messages from nuclear RNA splicosomes and allow transport into the cytoplasm. In the absence of Rev, full-length HIV-1 mRNA is multiply spliced into products encoding the regulatory proteins (Tat and Rev). Thus, a critical threshold of Rev accumulation is required to protect HIV-1 mRNA from splicing and permit subsequent pro-
duction of viral structural proteins and progeny virions (Pomerantz et al., 1992). This mechanism, termed blocked early-stage latency, can be observed as an accumulation of multiply spliced HIV-1 mRNA transcripts and was first documented using chronically infected cell lines (Pomerantz et al., 1990a). Although not a consistent mechanism of latency in several additional cell lines examined (Butera et al., 1994), evidence for blocked early-stage latency has been demonstrated using material from HIV-1-infected individuals (Seshamma et al., 1992).

6.3. Mechanisms of HIV-1 activation from latency

During the past decade, an enormous body of literature has accumulated from studies conducted in vitro and in vivo to examine the participation of various factors and soluble products in regulating the activation of HIV-1 expression. While truly beyond the scope of this review, several key points concerning the mechanisms of HIV-1 activation are pertinent. Most importantly, immune activation at all levels is a driving force behind HIV-1 expression (reviewed in Wahl and Orenstein, 1997) and, most likely, behind activation of HIV-1 from latency. This concept is based on findings from initial studies performed using chronically infected cell models demonstrating that the proinflammatory cytokines, in particular, tumor necrosis factor-alpha (TNF-α), stimulate HIV-1 expression from latency (Folks et al., 1987; Clouse et al., 1989; Folks et al., 1989; Poli et al., 1990a; Butera et al., 1993). These findings were subsequently extended to other proinflammatory cytokines (Poli et al., 1990b, 1994; Granowitz et al., 1995; Marshall et al., 1999) and a host of other cytokines and monokines (Broor et al., 1994; Smithgall et al., 1996; Shapiro et al., 1998b; Klein et al., 2000; reviewed in Butera, 1993). Mechanistically, the proinflammatory cytokines activate HIV-1 expression from latency via receptor-mediated second messenger signal transduction that ultimately results in NF-κB activation (Duh et al., 1989; Osborn et al., 1989; Beg et al., 1993). Production of endogenous TNF-α during acute HIV-1 infection in vitro may also affect the establishment of the latent state (Fujinaga et al., 1998).

The positive impact of TNF-α upon HIV-1 replication has also been documented in primary human peripheral blood mononuclear cells (Vyakarnam et al., 1990; Munoz-Fernandez et al., 1997), although TNF-α may also directly stimulate cell death in these populations (Klein et al., 1996; Lazdins et al., 1997). TNF-α was among a small group of cytokines, that also included the T-cell activating cytokine interleukin-2 (IL-2), which is capable of stimulating ex vivo HIV-1 expression from latently infected resting CD4 cells derived from infected individuals (Chun et al., 1998b). Importantly, elevated systemic TNF-α levels also correlate with treatment failure in individuals receiving HAART (Aukrust et al., 1999).

It is necessary, however, to consider the impact of proinflammatory mediators upon HIV-1 replication in the context of the complex cytokine network (Butera, 1994; Kinter et al., 1995a, 1996; Goletti et al., 1998). Synergistic activity among cytokines for HIV-1 activation has been documented (Poli et al., 1990b; Finnegan et al., 1996; Rabbi et al., 1998). Furthermore, inhibitory cytokines (Poli et al., 1991; Truong et al., 1999) and antagonistic cytokine relationships (Weissman et al., 1994; Kubo et al., 1997; Moriuchi et al., 1998) have been described. Systemic immune activation by a variety of means will result in the liberation of a spectrum of soluble mediators, the balance of which will ultimately determine HIV-1 activation (Rieckmann et al., 1991; Bollinger et al., 1993; Staprans et al., 1995; Moriuchi et al., 1999).

Certain immune stimuli can selectively activate discrete populations of immune cells, which could result in HIV-1 activation from different cellular compartments. Soluble products from activated T-lymphocytes may more selectively activate latent HIV-1 from resting CD4 lymphocytes (Bollinger et al., 1993; Harrer et al., 1993). Liberation of proinflammatory cytokines from activated macrophages, especially following bacterial antigen stimulation, may stimulate multiple cellular compartments of latent HIV-1 infection (Toossi et al., 1997; Goletti et al., 1998) and this has been observed using latently infected resting CD4 lymphocytes ex vivo (Moriuchi et al., 2000).
Furthermore, soluble bacterial products may directly activate HIV-1 expression from latently infected macrophages (Pomerantz et al., 1990b; Toossi et al., 1997; Goletti et al., 1998) via signal transduction through the CD14 cell surface molecule (Bagasra et al., 1992).

Other non-cytokine agents have been described that directly activate latent HIV-1 in vitro. In fact, one of the first observations of HIV-1 activation from latency involved the use of chemical agents belonging to the phorbol ester family (Folks et al., 1988) and mediated via activation of NF-κB (Griffin et al., 1989). As mentioned previously, other non-NF-κB-related chemical agents (Vlach and Pitha, 1993; Antoni et al., 1994; Laughlin et al., 1995) and cellular pathways (Stanley et al., 1990; Hashimoto et al., 1996; Nagai et al., 1997; Cole et al., 1998) have since been described. While the use of these agents have helped dissect discrete intracellular pathways involved in signal transduction and resultant HIV-1 activation (Kinter et al., 1990; Critchfield et al., 1997; Cole et al., 1998), their application to HIV-1 latency on a clinical level remains to be established.

7. Cell models representing latent HIV-1 infection

To decipher the cellular and molecular events that regulate HIV-1 latency and transcriptional activation, cell models representing several different lineages have been developed. As previously mentioned and detailed in Table 1, most of these cell models were obtained after an acute infection of a transformed cell line and the surviving cells were expanded and cloned. Generally, among the clonal populations derived in this manner are some that maintain HIV-1 expression at various levels, from a high constitutive expression to absolute latency. These cell models provide convenient and coordinated systems in which to study the events of HIV-1 activation and therapeutic interventions (reviewed in Butera and Folks, 1992).

Among the first models to be characterized were the constitutively expressing 8.E5 line (Folks et al., 1986) and the latently infected ACH-2 line (Clouse et al., 1989), both developed after an acute HIV-1 infection of a derivative of the transformed T-cell line, CEM. Concurrently, a latently infected promonocytic line, U1 (from the parental line U937), was developed and further characterized (Folks et al., 1987, 1988). These lines are noteworthy because much of the early information concerning cytokine regulation of HIV-1 expression and the intracellular signaling pathways and the molecular events resulting in virus expression were generated by studying coordinated activation in these systems.

Other cell systems of HIV-1 latency with various features have since been characterized and allow for important comparative studies. A promyelocytic cell model, OM-10.1 (HL-60 derived) that maintains surface CD4 expression until HIV-1 activation was described (Butera et al., 1991) and found to be convenient and informative in regard to studying therapeutic interventions (Feorino et al., 1993; Butera, 1998). Two cell lines with unique features were developed using the Jurkat transformed T-cell line, J1 and J-delta-K. J1 (Perez et al., 1991) is a latent cell line that maintains T-cell receptor surface expression and can be used to study signaling and viral-induced defects involving this receptor. J-delta-K (Antoni et al., 1994) harbors an HIV-1 provirus from which the NF-κB binding sites of the LTR have been deleted and can be used to study third messenger requirements for HIV-1 activation via selected stimuli.

It should be emphasized that comparative studies using these various cell models should be considered whenever approaching therapeutic intervention for latency because the mechanisms controlling the latent state may be quite different (Butera et al., 1994). This could be accomplished by testing compounds on cells of both T-cell and monocytic lineages. Along the monocytic lineage, comparing the promyelocytic OM-10.1 and the promonocytic U1 lines and lines developed from infected monocytic THP-1 cells (Mikovits et al., 1990) could be particularly informative with regard to the impact of monocytic differentiation. The OM-10.1 and U1 cell lines can also be treated with agents that induce cellular differentiation prior to HIV-1 activation (Bagasra et al., 1992;
Promyelocytic OM-10.1 cells may also be compared with constitutively expressing promyelocytes, PLB-IIIB (derived from transformed PLB-985 cells; Roulston et al., 1992), just as a comparison of ACH-2 and 8E.5 T-cell lines would be informative.

However, while the transformed cell models of HIV-1 latency are convenient due to coordinated expression, how they relate to the mechanisms controlling latency in resting CD4 T-lymphocytes and latently infected macrophages in vivo is not at all certain. In fact, some of the cell models harbor defective proviruses that confound their use as representatives of latently infected cells in vivo (Duan et al., 1994b; Emiliani et al., 1996, 1998). Other groups have attempted to develop in vitro cell systems of dormant post-integrated HIV-1 infection using highly purified resting T-lymphocytes (Spina et al., 1995; Tang et al., 1995). This method, however, has not always been successful (Chou et al., 1997) and mild cytokine activation of the cells (Unutmaz et al., 1999) may be required to complete the afferent portion of the HIV-1 life cycle. However, with resting CD4 cells also capable of supporting viral replication in vivo (Zhang et al., 1999b), infection of these cells in vitro may not result in a viable model of in vivo post-integration HIV-1 latency. This makes it difficult to determine whether any of the current in vitro models accurately reflect the mechanisms controlling in vivo latency.

What is unique about latently infected cells in vivo that permits the establishment of this state and their longevity? Currently, only speculation is available. It has been proposed that these cells become activated during the process of HIV-1 infection to complete the afferent viral life cycle and then revert to a resting state to establish viral latency, only to express virus upon reactivation (Finzi and Siliciano, 1998). If HIV-1 expression does occur during the first activation period, these cells would have to survive the cytopathic effects of viral replication and immune surveillance mechanisms. These cells may be unique in their differential susceptibility to viral cytopathic effects (Chun et al., 1997c) or may benefit from a viral-induced change in cellular processes regulating cell death and survival (Aillet et al., 1998). It is also curious that the frequency of these cells is roughly similar in most individuals and does not increase in individuals along with increasing time of infection. Does this suggest that these cells are indeed unique in their survival capacity or, possibly, their antigen specificity rather than being simply slow in their kinetics of turn-over and replacement? Is it possible that latent cells develop during the process of antigen activation in naïve T-cells, known to be unique in their requirements for HIV-1 infection (Roederer et al., 1997; Spina et al., 1997; Woods et al., 1997; Ostrowski et al., 1999), that results in a latently infected memory cell of predetermined antigen specificity? Until experimental data are available to address some of these issues, determining whether any of the current in vitro models accurately reflect the mechanisms controlling in vivo latency will be difficult.

8. Current attempts at clearing latently HIV-1 infected cells

8.1. HAART + IL-2 regimens

With the demonstrated success of IL-2 therapy in promoting immune recovery during HIV-1 disease (Kovacs et al., 1996; Bartlett et al., 1998; Carr et al., 1998; Arno et al., 1999; Aladdin et al., 2000) and the knowledge that IL-2 can stimulate HIV-1 expression from infected T-cells and promote other protective responses (Kinter et al., 1995b), studies of combination regimens of HAART + IL-2 were undertaken (Hengge et al., 1998; Stellbrink et al., 1998; Chun et al., 1999a; De Paoli et al., 1999; Pandolfi et al., 2000). Some
parameters of immune function were further enhanced by this combined antiviral and immune-based treatment; however, the hope was that IL-2 immunotherapy would stimulate latent HIV-1 and accelerate the clearance of this population of cells. In the studies that examined the impact upon the latent pool, these cells were observed to slowly decline with therapy (Stellbrink et al., 1998) and in several individuals receiving HAART plus IL-2, no recoverable latent virus could be found under extreme culture conditions (Chun et al., 1999a). While these results seemed initially promising, the clinical reality was that when the individuals showing clearance of latent virus went off antiretroviral therapy, reemergence of HIV-1 in the blood was quickly observed (Chun et al., 1999b). Other attempts at immune-based therapy to eradicate HIV-1 during HAART have been implemented (Prins et al., 1999) or proposed (Pantaleo, 1997) based on the accumulative effects provided by additional T-cell activating cytokines (Bayard-McNeeley et al., 1996; Al-Harthi et al., 1998). These approaches may be more effective when combined with immunotoxin-based agents that specifically target HIV-1 activated cells (Goldstein et al., 2000) or nonselectively target latently infected cells (McCoig et al., 1999) and induce cytolysis.

8.2. Structured therapy interruptions

Another means for enhancing the elimination of latently infected cells in vivo currently under evaluation is through structured antiretroviral therapy interruptions (STIs). Although the safety, efficacy, and limits of applicability have not been firmly established in clinical trials, this is a rapidly emerging therapeutic approach that aims to enhance HIV-specific immunity in individuals by periodically allowing viral rebound and immune recognition (reviewed in Garcia et al., 2000). The use of STIs in this manner would essentially be a form of immune-based therapy or natural therapeutic vaccination. While it is quite clear that nearly all individuals will experience a rebound of virus upon stopping therapy (Davey et al., 1999; Garcia et al., 1999; Harrigan et al., 1999) and that this rebound has little or no deleterious effects if monitored and kept in check (Neumann et al., 1999), it is not clear if periodic exposure to viral antigens will bolster immune clearance of HIV-1. Further complicating this issue, HIV-1-specific and -nonspecific immune responses decline during prolonged suppressive therapy (Kalams et al., 1999; Pitcher et al., 1999; Wilkinson et al., 1999) and may not be adequate to restrict HIV-1 replication when therapy is interrupted. Initiation of HAART early in infection has attempted to preserve HIV-1-specific T-helper cell responses (Rosenberg et al., 1997) but may actually restrict development of this response (Plana et al., 1998) or the diversity of T-cell clones responding to infection (Souden et al., 2000). More information on STIs and the impact of this approach on the latent HIV-1 pool is certain to emerge in the near future.

8.3. Other attempts at immune activation-nonspecific versus specific (therapeutic vaccination)

Many additional approaches have been suggested in an attempt to bolster immune recovery during HAART, including ex vivo expansion of T-cell populations (Levine et al., 1996; Nielsen et al., 1997; Riley et al., 1998), the use of colony-stimulating factors to expand T-cells in vivo (Nielsen et al., 1998), and the use of novel chemical agents (Achour et al., 1998) or cell-signal cascade antagonists (Aandahl et al., 1999) to restore T-cell responsiveness. While these approaches do not target latently HIV-1 infected cells directly, they all aim to enhance immune responsiveness in infected individuals to more rapidly and effectively eliminate latent cells upon reactivation of HIV-1 expression. It has also been proposed that the use of chemical agents which inhibit T-cell activation, and thereby place direct or indirect limitations on the completion of the HIV-1 life cycle, may be advantageous adjuncts to anti-HIV-1 therapies (Thali, 1995; Chapuis et al., 2000). Although this may dampen overall immune responsiveness to antigenic stimulation, these agents may, in theory, restrict the development of new latently infected cells as the pool turns-over and thereby increase the decay of this population.
Various forms of HIV-1-specific therapeutic vaccinations have been proposed (Gotch et al., 1999) or implemented (Calarota et al., 1999) to further enhance immune clearance of latently infected cells upon reactivation. While this approach seems reasonable and promising, the proof-of-concept experiments for many of these therapeutic vaccine candidates await a more complete evaluation.

8.4. Natural suppressive agents

Since its description more than a decade ago, a soluble factor produced by CD8 T-lymphocytes and capable of suppressing HIV-1 replication (Walker and Levy, 1989; Brinchmann et al., 1990) has remained somewhat of a mystery. While very little biochemical information on this novel suppressive agent, including its identification, has become available, several groups have confirmed production of this activity (Moriuchi et al., 1996; Rosok et al., 1997b; Leith et al., 1999; Wilkinson et al., 1999). It is clear that this CD8-cell-derived soluble factor does not function via a cytotoxic mechanism (Walker et al., 1991; Leith et al., 1999) and is distinct from CD8-cell-derived chemokines that function to inhibit HIV-1 entry into cells (Moriuchi et al., 1996; Barker et al., 1998). Available data further suggests that CD8-derived suppressive activity functions to restrict HIV-1 transcription (Leith et al., 1999; Mackewicz et al., 2000) making this factor a putative natural therapy to block activation of latent HIV-1 expression. With the development and characterization of transformed CD8 T-cell lines that produce such an activity (Mackewicz et al., 1997; Moriuchi et al., 1998), possible therapeutic application of such activity remains feasible.

Other approaches to modulating the activity of natural HIV-1 suppressive agents have been proposed or attempted. Data from several groups indicate that a newly characterized interleukin, IL-16, holds promise as a natural suppressive agent against HIV-1 replication (Viglianti et al., 1997; Idziorek et al., 1998; Truong et al., 1999; Zhou et al., 1999), possibly acting at the level of viral transcription or mRNA stability (Zhou et al., 1997). Furthermore, interferons as a family of soluble mediators show antiviral activity, and type I interferon has been proven to be particularly effective against HIV-1 replication (Lapenta et al., 1999). Chemical agents (Navarro et al., 1996; Dezube et al., 1997; Biswas et al., 1998; Moulton et al., 1998; Traber et al., 1999) and receptor antagonists (Poli et al., 1994; Granowitz et al., 1995) designed to limit the activity of HIV-1 activating cytokines in vivo may also prove to be useful adjuncts to restrict viral activation from latency. Identifying additional agents that function in this manner should be a goal of further therapeutic development, especially if specific response pathways can be targeted.

9. Experimental approaches targeting HIV-1 latency

In theory, any therapeutic approach targeting a component of the efferent portion of the HIV-1 life cycle could interfere with viral expression from latently infected cells. For example, protease inhibitors (which act to hinder HIV-1 protein processing after proviral activation and expression) show activity against the production of mature progeny virions from latently infected cells (Al-Harthi et al., 1997). Other compounds that affect post-activation viral protein processing (San Jose et al., 1997) or progeny virion assembly (Mlynar et al., 1997) have also been described and could be worthwhile adjuncts in blocking steps of the efferent HIV-1 life cycle. However, while these compounds are effective in this manner in vitro, the clinical reality is that individuals receiving treatment regimens that include protease inhibitors still maintain latently infected cells with a decay half-life that may approach 60 years. As discussed earlier, the lack of clinical benefit of protease inhibitors in eliminating latently infected cells may be due to a variety of non-mutually exclusive explanations. However, these observations clearly demonstrate that novel and innovative approaches to clearing latently infected cells are necessary for HIV-1 eradication to become feasible.
Table 2
Characteristics of described inhibitors of HIV-1 transcription

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical class</th>
<th>Mechanism of action</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>RD6-5071</td>
<td>Acridone derivative</td>
<td>Protein kinase inhibition</td>
<td>Fujiwara et al., 1999</td>
</tr>
<tr>
<td>Gö 6976</td>
<td>Indolocarbazole</td>
<td>Protein kinase inhibition</td>
<td>Qatsha et al., 1993</td>
</tr>
<tr>
<td>EM2487</td>
<td><em>Strep spp</em> product</td>
<td>Tat inhibition</td>
<td>Baba et al., 1999</td>
</tr>
<tr>
<td>Topotecan</td>
<td>Camptothecins</td>
<td>DNA modification?</td>
<td>Zhang et al., 1997</td>
</tr>
<tr>
<td>Cepharanthine</td>
<td>Biscoclaurine alkaloid</td>
<td>Tat inhibition</td>
<td>Okamoto et al., 1998</td>
</tr>
<tr>
<td>Pentoxifylline</td>
<td>Dimethylxanthine</td>
<td>TNF-κ/αNF-κB inhibition</td>
<td>Navarro et al., 1996</td>
</tr>
<tr>
<td>K-12 and</td>
<td>Fluorquinolones</td>
<td>Unknown</td>
<td>Baba et al., 1997</td>
</tr>
<tr>
<td>K-37</td>
<td></td>
<td>Tat inhibition?</td>
<td>Witvrouw et al., 1998</td>
</tr>
<tr>
<td>Temacrazine</td>
<td>Bistriazoloacridone</td>
<td>Unknown</td>
<td>Turpin et al., 1998</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>Anthracycline</td>
<td>Tat inhibition</td>
<td>Jeyaseelan et al., 1996</td>
</tr>
<tr>
<td>DRB and T174557</td>
<td>Ribofuranosyl benzimidazole</td>
<td>Tat inhibition via CDK9 inhibition</td>
<td>Braddock et al., 1991</td>
</tr>
<tr>
<td>Chrysine</td>
<td>Flavonoid</td>
<td>CDK9 inhibition?</td>
<td>Mancebo et al., 1997</td>
</tr>
<tr>
<td>PD144795</td>
<td>Benzothiophenes</td>
<td>CDK9 inhibition?</td>
<td>Critchfield et al., 1996</td>
</tr>
<tr>
<td>H-7 and HA100</td>
<td>Isoquinoline sulfonamide</td>
<td>CDK9 inhibition</td>
<td>Critchfield et al., 1997</td>
</tr>
<tr>
<td>T172298</td>
<td>Benzimidazole</td>
<td>Transcript. elongation</td>
<td>Critchfield et al., 1999</td>
</tr>
</tbody>
</table>

Approaches involving gene therapy specifically targeting steps along the efferent HIV-1 life cycle have shown initial promise in preventing viral expression from latency in vitro. Some of these approaches transfer genes that encode inhibitors of the HIV-1 regulatory proteins, Rev (Ho et al., 1998) and Tat (Rossi et al., 1997; Marasco et al., 1999; Mhashilkar et al., 1999; Li et al., 2000), to restrict viral production from infected cells, including those that are latently infected. Other approaches transfer genes encoding RNA-cleaving ribozymes with specificity for HIV-1 mRNA (Ramezani et al., 1997; Wong-Staal et al., 1998) to eliminate these products prior to viral translation or virion assembly. This application has moved beyond in vitro testing and has undergone initial clinical evaluation for safety (Wong-Staal et al., 1998). Still other novel gene transfer approaches use either inhibitors of HIV-1 activation (Muto et al., 1999) or viral delivery systems to attack HIV-1-expressing cells (Schnell et al., 1997). While these attempts have all shown efficacy in vitro, the clinical reality of delivering the gene product to cells that harbor a dormant HIV-1 provirus or protecting all cells susceptible to HIV-1 infection still faces difficult hurdles.

9.1. Targeting cellular pathways: advantages and disadvantages

As a therapeutic approach to inhibit HIV-1 activation and expression from latently infected cells, targeting the cellular components involved in this process has many distinct advantages (reviewed in Baba, 1997) that should be exploited and at the forefront of new drug design. HIV-1 has commandeered discrete cellular elements for its own survival, possibly with little available biologic redundancy, and selective inhibition of these cellular elements could severely hinder viral replication. Such an approach would also limit the generation of viral escape mutants because the inhibitory pressure would be applied to a cellular rather than viral component. Generally, biologic redundancy of the cellular pathways would permit continuation of cellular activity. However, the specificity and selectivity of inhibitors targeting cellular components (Table 2) must be a primary consideration. As more extensive information is generated in regard to the specific cellular components and mechanisms involved in HIV-1 activation and expression, more selective therapeutic cellular targets should emerge.
The cellular second messenger kinase pathways involved in HIV-1 activation from latency are attractive therapeutic targets. It is critical to determine which extracellular stimuli are involved in HIV-1 activation, especially from latently infected cells in vivo, and which cellular signaling pathways mediate the process. Since HIV-1 expression from latently infected cells obtained from infected individuals can be stimulated with cytokines (Chun et al., 1998b), much of what has been learned from TNF-α activation of HIV-1 expression in cell models may be directly applicable in vivo. Cell model studies using novel inhibitory compounds suggest that selected serine-threonine kinases (Qatsha et al., 1993; Biswas et al., 1994; Critchfield et al., 1997; Sato et al., 1998; Fujiwara et al., 1999), but not tyrosine kinases (Critchfield et al., 1999), are involved in signal transduction resulting in HIV-1 expression. Several studies have suggested that protein kinase C is an important kinase in this signaling cascade (Kinter et al., 1990; Qatsha et al., 1993; Fujiwara et al., 1999); however, some chemical inhibitors selective for this kinase showed no activity in blocking HIV-1 expression (Critchfield et al., 1999). Therefore, although cellular serine-threonine kinases are indeed involved in HIV-1 activation following extracellular cytokine stimulation, the identity of these kinases remains to be fully resolved.

Furthermore, the kinases involved in signaling via other extracellular stimuli that result in HIV-1 activation from latency need to be delineated, and the in vivo relevance of these findings needs to be confirmed. As several kinase inhibitors (Table 2) have already proven effective and selective against HIV-1 activation (Qatsha et al., 1993; Biswas et al., 1994; Critchfield et al., 1997; Sato et al., 1998; Critchfield et al., 1999; Fujiwara et al., 1999), this line of drug discovery appears to hold great promise. At the level of second-messenger signal transduction, biologic redundancy may be appropriately extensive so that a selective inhibitor would not severely compromise normal cellular functions.

9.2. HIV-1 transcriptional inhibitors — what is known?

Therapeutic targeting of HIV-1 replication at the level of transcriptional activation also holds great potential for further attempts at clearing viral latency. Inhibitors of HIV-1 transcription would prevent continued low-level viral replication by maintaining infected cells in a state of absolute latency and permit gradual depletion of this reservoir. Again, this approach would necessitate specific inhibitors of cellular transcription components critically involved in HIV-1 transcriptional activation or elongation (Table 2). The specificity and selectivity of these inhibitors would be of even greater concern than those targeting cellular second messenger pathways because biologic redundancy at the level of transcriptional promoters may be limited. Even though these concerns seem restrictive, selective inhibitors targeting several different cellular components involved in HIV-1 transcription have been described. These initial proof-of-concept compounds show limited cellular toxicity and establish a foundation for the development of future selective agents, as mechanisms of action become fully appreciated.

Each cellular component involved in the steps of HIV-1 transcriptional activation could serve as a putative target for inhibition. Nucleosomal unraveling, specifically nuc-1 disruption (Verdin et al., 1993; Van Lint et al., 1996), remains an important aspect of HIV-1 transcriptional activation and a potential target for HIV-1 intervention if approaches with appropriate HIV-1 specificity are exploited. A mechanism involving a yet uncharacterized DNA-modifying enzyme may be involved in the HIV-1 inhibitory action of topotecan. Although this compound is characterized as an inhibitor of topoisomerase I, the ability of topotecan to limit HIV-1 replication was found to be via a mechanism not directly involving topoisomerase activity (Zhang et al., 1997).

Several compounds that inhibit HIV-1 transcriptional activation target the release or biologic functionality of NF-κB (Mihm et al., 1991; Roederer et al., 1991; Li et al., 1993; Kopp and Ghosh, 1994; Okamoto et al., 1998). As discussed earlier,
NF-κB is an important contributor to HIV-1 transcriptional activation, and compounds that restrict the activity of this element would markedly limit HIV-1 replication. However, NF-κB-independent HIV-1 replication has been observed in a variety of systems (Stanley et al., 1990; Vlach and Pitha, 1993; Antoni et al., 1994; Laughlin et al., 1995; Hashimoto et al., 1996).

Furthermore, inhibition of HIV-1 expression by one of the putative NF-κB inhibitors (Okamoto et al., 1998) was observed in only cells of monocytic lineage. Because NF-κB is a critical transcription element for many cellular genes, a high degree of specificity for HIV-1 activation would also have to be considered.

Several classes of selective HIV-1 transcriptional inhibitors that act via a putative cellular factor without interrupting NF-κB activation or function were identified using latently infected cell systems (Table 2). These agents, including benzothiophene derivatives (Butera et al., 1995), naturally occurring flavonoids (Critchfield et al., 1996), and isoquinoline sulphonamide derivatives (Critchfield et al., 1999), prevent HIV-1 transcription without inhibiting cellular differentiation or transcription of other selected cellular genes. Furthermore, these agents induced a state of HIV-1 latency in cells that were actively expressing virus, even under culture conditions of continued extracellular stimulation (Butera et al., 1995; Critchfield et al., 1996). The ability to inhibit HIV-1 in this manner suggests that the target of these compounds is critical for both HIV-1 activation and continued viral replication and that therapeutically induced viral latency is achievable. Several of these compounds displayed selective inhibition of the cellular kinase, casein kinase II (CKII, Critchfield et al., 1997). Although other classic inhibitors of CKII also inhibited HIV-1 activation (Critchfield et al., 1997) and Tat-induced transactivation (Braddock et al., 1991), a direct link between CKII inhibition and HIV-1 inhibition could not be established. With a better resolution of their mechanism of action (discussed below), these agents or related analogs may prove to be important therapeutic agents for blocking HIV-1 activation from latency as well as continued low-level replication in vivo during HAART.

Other selective and very potent inhibitors of HIV-1 transcription, including a bistriazoloacridone analog, temacrazine (Turpin et al., 1998), and fluoroquinoline derivatives, K-12 and K-37 (Baba et al., 1997, 1998), have been described and warrant further development. These agents demonstrate inhibitory activity against HIV-1 activation from latency, in addition to inhibition of acute and chronic HIV-1 infections, at drug concentrations in the nanomolar range. Although both classes of agents selectively inhibit HIV-1 transcription (Turpin et al., 1998; Witvrouw et al., 1998), the precise molecular target, most certainly a cellular component involved in viral transcription, remains to be identified. Based on the spectrum of activity of these agents, their mechanisms of action and cellular targets may differ (Turpin et al., 1998). Efforts to demonstrate a direct effect of these agents on Tat activity have either been unsuccessful (Baba et al., 1997; Turpin et al., 1998) or have required the use of unique assay conditions (Witvrouw et al., 1998), implying that the effect of these agents is via a poorly characterized component not fully appreciated in standard transient transfection assay systems. In the effort to develop new compounds that can inhibit HIV-1 transcription and prevent HIV-1 activation from latency, these agents appear among the most promising and have demonstrated synergistic HIV-1 inhibitory activity when combined with other clinically available antiretroviral agents (Okamoto et al., 1999).

9.3. Tat as a target

Because of the viral dependence upon Tat for high level transcription, therapeutic targeting of the Tat transactivation process as a means to inhibit HIV-1 activation from latency should be a primary line of investigation. As previously discussed, many of the molecular details and cellular components involved in Tat transactivation and HIV-1 transcriptional elongation have recently been resolved. Although the need to identify selective inhibitors of transactivation remains a princi-
pal concern, the participation of both cellular and viral components provides an opportunity for selective disruption of this process.

Although first-generation inhibitors of Tat transactivation came to the forefront nearly a decade ago (Hsu et al., 1991), they have remained poorly characterized with regard to molecular target and mechanism of action. In recent years, other compounds have been described that either alter the binding of Tat or cellular cofactors to the viral TAR element (Jeyaseelan et al., 1996; Hamy et al., 1997) or act via a poorly resolved mechanism (Baba et al., 1999). However, as the molecular details and cellular components of Tat transactivation have been brought into focus, new classes of compounds that selectively inhibit this process have been identified.

Identification of additional compounds with therapeutic potential against HIV-1 transcription (Table 2) was accomplished in studies of cellular cofactors involved in Tat transactivation (Mancebo et al., 1997). As a common feature, these agents inhibited the enzymatic activity of CDK9, the kinase associated with P-TEFb. Among this panel of compounds, the isoquinoline sulphonamide derivatives previously shown to inhibit HIV-1 activation from latency (Butera et al., 1991; Critchfield et al., 1999) also demonstrated marked inhibitory activity against CDK9 enzymatic activity. These agents prevented HIV-1 transcriptional elongation after activation (Critchfield et al., 1999), consistent with a mechanism of action against CDK9-mediated hyperphosphorylation of the carboxy-terminal domain (CTD) of cellular RNA polymerase II. Furthermore, HIV-1-inhibitory compounds found previously to inhibit CKII (Braddock et al., 1991; Critchfield et al., 1997) also showed inhibitory activity against CDK9 enzymatic activity (Flores et al., Personal communication; Yamaguchi et al., 1999). It is now becoming clear that inhibition of CDK9 enzymatic activity, thereby preventing pol II CTD hyperphosphorylation, allows negative regulatory elements to remain associated with the polymerase and retards HIV-1 transcriptional elongation (Yamaguchi et al., 1999).

While these accumulative studies suggest that the CDK9 enzymatic subunit of P-TEFb may be a universal target of multiple HIV-1 transcriptional inhibitors, many other steps required for the transcriptional activation process could hold potential for therapeutic intervention. For instance, CDK9 itself may be subject to regulation via phosphorylation or other mechanisms and inhibitors that interfere with these regulatory events would display a similar inhibitory activity against HIV-1 transcription. CKII and other cellular factors may be intricately involved at this level. Furthermore, CDK9 and cyclin T1 protein levels are regulated in response to extracellular stimuli (Herrmann et al., 1998) and inhibitors that block these induction pathways could ultimately affect HIV-1 transcription. As a case in point, the novel HIV-1 transcriptional inhibitor, K-12 (Baba et al., 1998), was found to inhibit neither CKII (Butera et al., Unpublished observations) nor CDK9 (Flores et al., Personal communication) enzymatic activity.

10. Is complete HIV-1 clearance feasible?

Although the initial optimism of HIV-1 eradication has faded, there is still reason for hope that complete viral clearance is possible. Without question, the obstacles to HIV-1 eradication are numerous and formidable (reviewed in Chun and Fauci, 1999); however, our knowledge base of viral reservoirs and mechanisms regulating HIV-1 latency in vivo are largely incomplete. If HIV-1 eradication is not clinically achievable by available therapeutic approaches, then maintaining virus in a permanently state of absolute latency, effectively blocking HIV-1 activation and continued low-level HIV-1 replication in these cells, may hasten the decay of this reservoir.

The transformed cell models of latent HIV-1 infection are useful reagents for dissecting many of the mechanisms regulating viral latency and identifying cellular components involved in viral activation. However, new in vitro cell systems of HIV-1 latency in primary T-cell and macrophage cultures must be developed to better represent the in vivo circumstance. Although ex vivo studies using latent cells derived from infected individuals are possible, these cells are present in such limited
numbers in vivo that the opportunities to study molecular mechanisms in this manner are restricted. A large body of information concerning mechanisms regulating HIV-1 latency and therapeutic intervention could be quickly generated if such a representative cell system were available.

Therapeutic efforts to eliminate latently HIV-1-infected cells in vivo are in their infancy. Immune stimulating cytokines and STI regimens are reasonable starting points for evaluation, but limitations have become apparent since their inception. Development of new therapeutics to inhibit low-level HIV-1 replication during HAART are imperative. Cellular factors involved at several points of the efferent component of the HIV-1 life cycle appear to be attractive therapeutic targets, if specificity for HIV-1 replication can be achieved. Among potential candidate steps, inhibition of HIV-1 transcription by novel therapeutic compounds has proven extremely effective and selective when tested on cellular models of latent HIV-1 infection in vitro.

The process of Tat transactivation provides a mechanism of selective inhibition of HIV-1 activation from latency. As this mechanism becomes better understood, new therapeutic targets for rational drug design most certainly will become known. Recent studies indicate that the association of Tat with P-TEFb changes the substrate specificity of CDK9, the kinase component of P-TEFb (Zhou et al., 2000), most likely through a change in protein conformation. These remarkable findings further suggest that novel inhibitors might be selectively effective against this altered enzyme conformational state. The specific physical interaction between Tat and Cyclin T1 (Wei et al., 1998) seems to present a reasonable target for intervention designed to interfere with this interaction. Furthermore, the Tat-Cyclin T1 interaction may result in a conformational change in Tat to promote interaction with TAR RNA. Designing novel compounds capable of recognizing the alterations in Tat conformation may also prove worthwhile. As our understanding of the process of Tat transactivation expands, our ability to evaluate the possible utility of novel therapeutic targets also will improve.

Other issues related to the clinical reality of HIV-1 latency will provide additional obstacles to viral eradication. HIV-1 latency in cells of macrophage lineage poses a particularly difficult problem, and the ability to quantify viral latency in this long-lived lineage is currently not available. Although believed to constitute only a small proportion of infected cells, the non-cytopathic infection of monocytic cells has remained a concern since originally proposed (Perelson et al., 1997). In addition, HIV-1 latency may be under very different regulatory mechanisms in macrophages and respond to different stimulatory signals as compared to cells of lymphocytic derivation. Using in vitro models of latent infection, most HIV-1 transcriptional inhibitors have thus far proven equally effective in cells representing these lineages, and universal efficacy may be mediated by blocking a very basic but critical component of HIV-1 replication. Identifying these basic elements should generate new enthusiasm for the real possibility of maintaining infected cells in a state of induced absolute viral latency and provide further progress towards eventual HIV-1 eradication.

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