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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.

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

* Tel.: +1-404-6391033; fax: +1-404-6391174.
E-mail address: stb3@cdc.gov (S.T. Butera).

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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-Riise 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; Lafeuillade 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,

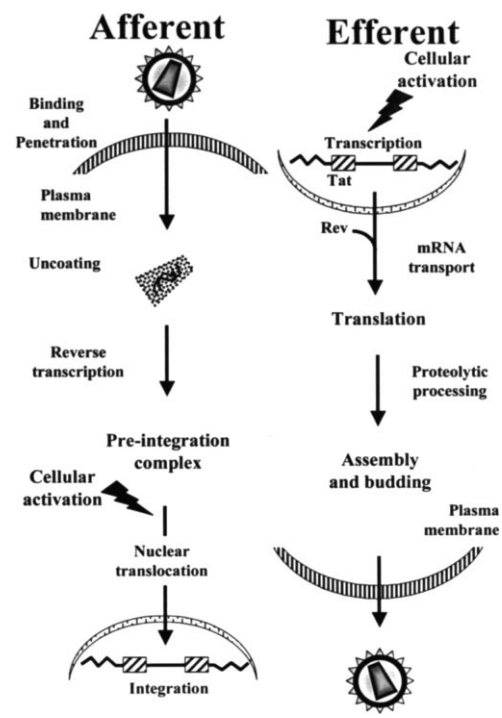

Fig. 1. The HIV-1 life cycle can theoretically be divided into afferent and efferent components. The afferent component includes the initial steps of virus binding, penetration, reverse transcription, and integration and occurs fairly rapidly under normal conditions. A state of pre-integration latency is established in the absence of cellular activation. The efferent component of the HIV-1 life cycle includes those steps necessary to produce viral progeny and may require an extracellular signal to activate the integrated provirus after a prolonged period of latency. The development of effective therapeutics for events during the efferent component of the HIV-1 life cycle, especially at the level of viral transcription, has been hampered by an incomplete understanding of the mechanisms controlling HIV-1 latency and activation.

Table 1
Characteristics of cellular models of latent and constitutive HIV-1 infection

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

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, I-κB. Upon cellular activation via several first and second messenger pathways involving cellular kinases (Kinter et al., 1990; Critchfield et al., 1997), phosphorylation of I-κB 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.

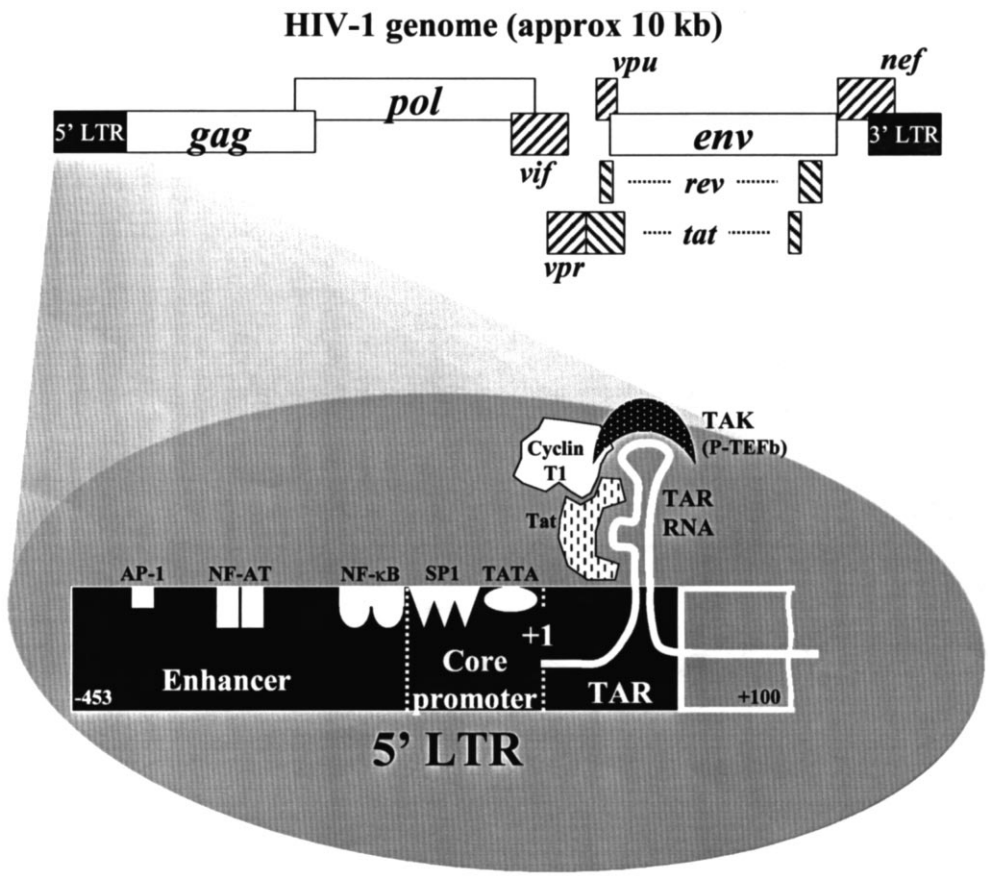

Fig. 2. A schematic representation of the HIV-1 genome and 5′ long terminal repeat (LTR) promoter, indicating recognized sites of interaction for DNA and RNA binding proteins. HIV-1 proteins Tat and Rev are translated from spliced mRNAs which combine 2 coding exons, as indicated. Sites are depicted only 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.

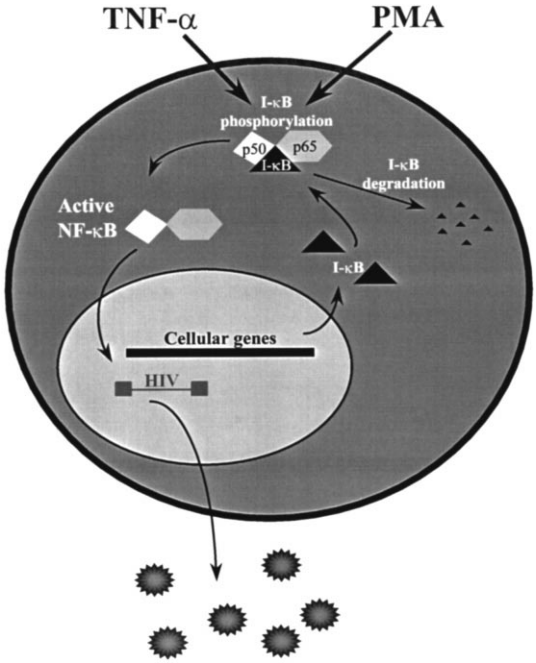

Fig. 3. A schematic of nuclear factor-κB (NF-κB) activation. The NF-κB heterodimer (p50 and p65) is present in the cytoplasm associated with its inhibitor, I-κB. Cellular stimulation by TNF-α, phorbol esters (PMA), or other agents that activate the appropriate cellular kinase cascades results in the phosphorylation-directed dissociation and degradation of I-κB. As a result, active NF-κB is translocated to the nucleus where it stimulates selected gene expression by binding to a motif in the promoter of responsive genes. NF-κB-responsive genes include its inhibitor, I-κB, and HIV-1.

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., 1996) and with HIV-1 proviruses deleted of the NF-κB binding regions (Antoni et al., 1994).

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 (Coudronniere 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.,

1998). 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

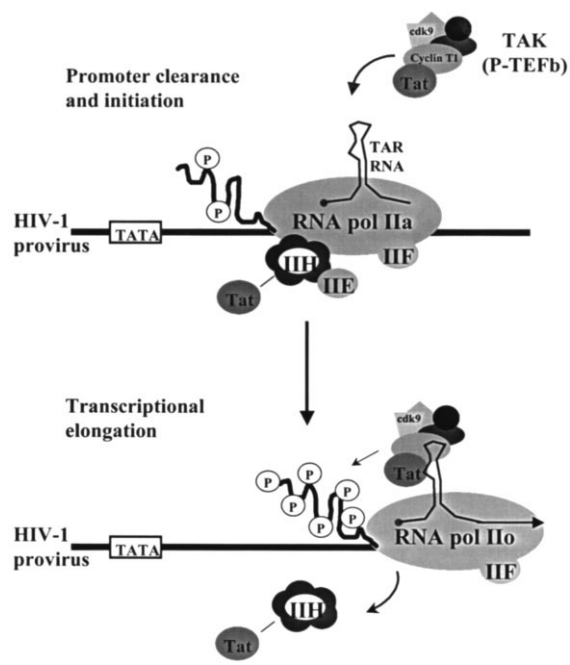

Fig. 4. Proposed model of Tat interaction with Cyclin T1 and P-TEFb to permit CDK9 mediated hyperphosphorylation of the carboxy-terminal domain of RNA polymerase II (conversion from pol IIa to pol IIo) and subsequent HIV-1 transcriptional elongation. Other proposed transcription factor involvement is 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 (Cujec 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 deceased 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 (Emiliani et al., 1998) or mutations that disrupt binding to TAR (Emiliani 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;

Shattock et al., 1996). 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. Development of such an in vitro cell system that accurately reflects the state of HIV-1 latency in vivo would be an important advance in understanding the mechanisms controlling HIV-1 expression in these cells and testing putative therapeutics for activity. It may be possible to use novel infection systems involving dendritic-T-cell interactions (Weissman, et al., 1996), discrete populations of T-cells with unique functional properties (Marodon et al., 1999; Wallace et al., 2000), or inhibitors during the acute infection process (Korin and Zack, 1999) to better establish an in vitro system of HIV-1 latency in primary cells for use in evaluating therapeutic interventions.

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 (Soudeyns 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

Compound	Chemical class	Mechanism of action	Reference
RD6-5071	Acridone derivative	Protein kinase inhibition	Fujiwara et al., 1999
Gö 6976	Indolocarbazole	Protein kinase inhibition	Qatsha et al., 1993
EM2487	*Strep spp* product	Tat inhibition	Baba et al., 1999
Topotecan	Camptothecins	DNA modification?	Zhang et al., 1997
Cepharanthine	Biscoclaurine alkaloid	NF-κB inhibition	Okamoto et al., 1998
Pentoxifylline	Dimethylxanthine	TNF-α/NF-κB inhibition	Navarro et al., 1996
K-12 and	Fluoroquinolones	Unknown	Baba et al., 1997
K-37		Tat inhibition?	Witvrouw et al., 1998
Temacrazine	Bistriazoloacridone	Unknown	Turpin et al., 1998
Doxorubicin	Anthracycline	Tat inhibition	Jeyaseelan et al., 1996
DRB and T174557	Ribofuranosyl benzimidazole	Tat inhibition via CDK9 inhibition	Braddock et al., 1991 Mancebo et al., 1997
Chrysin	Flavonoid	CDK9 inhibition? CKII inhibition	Critchfield et al., 1996 Critchfield et al., 1997
PD144795	Benzothiophenes	CDK9 inhibition? CKII inhibition	Butera et al., 1995 Critchfield et al., 1997
H-7 and HA100	Isoquinoline sulfonamide	CDK9 inhibition Transcript. elongation	Mancebo et al., 1997 Critchfield et al., 1999
T172298	Benzimidazole	CDK9 inhibition	Mancebo et al., 1997

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 fully be 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. This finding may indicate cell lineage-specific requirements for HIV-1 transcriptional elements or be related to issues of pharmacology and metabolism. 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.

References

Aandahl, E.M., Aukrust, P., Muller, F., Hansson, V., Tasken, K., Froland, S.S., 1999. Additive effects of IL-2 and protein kinase A type I antagonist on function of T cells from HIV-infected patients on HAART. AIDS 13, F109–F114.

Achour, A., Landureau, J.C., Salerno-Concalves, R., Maziere, J.C., Zagury, D., 1998. Restoration of immune response by a cationic amphiphilic drug (AY 9944) in vitro: a new approach to chemotherapy against human immunodeficiency virus type1. Antimicrob. Agents Chemother. 42, 2482–2491.

Adams, M., Sharmeen, L., Kimpton, J., Romeo, J.M., Garcia, J.V., Peterlin, B.M., Groudine, M., Emerman, M., 1994. Cellular latency in human immunodeficiency virus-infected individuals with high CD4 levels can be detected by the presence of promoter-proximal transcripts. Proc. Natl. Acad. Sci. USA 91, 3862–3866.

Aillet, F., Masutani, H., Elbim, C., Raoul, H., Chene, L., Nugeyre, M.T., Paya, C., Barre-Sinoussi, F., Gougerot-Pocidalo, M.A., Israel, N., 1998. Human immunodeficiency virus induces a dual regulation of Bcl-2, resulting in persistent infection of CD4(+) T- or monocytic cell lines. J. Virol. 72, 9698–9705.

Aladdin, H., Larsen, C.S., Moller, B.K., Ullum, H., Buhl, M.R., Gerstoft, J., Skinho, J.P., Pedersen, B.K., 2000. Effects of subcutaneous interleukin-2 therapy on phenotype and function of peripheral blood mononuclear cells in human immunodeficiency virus infected patients. Scand. J. Immunol. 51, 168–175.

Al-Harthi, L., Roebuck, K.A., Landay, A., 1998. Induction of HIV-1 replication by type 1-like cytokines, interleukin (IL)-12 and IL-15, effect on viral transcriptional activation, cellular proliferation, and endogenous cytokine production. J. Clin. Immunol. 18, 124–131.

Al-Harthi, L., Roebuck, K.A., Kessler, H., Landay, A., 1997. Inhibition of cytokine-driven human immunodeficiency virus type 1 replication by protease inhibitor. J. Infect. Dis. 176, 1175–1179.

Andersson, J., Fehniger, T.E., Patterson, B.K., Pottage, J., Agnoli, M., Jones, P., Behbahani, H., Landay, A., 1998. Early reduction of immune activation in lymphoid tissue following highly active HIV therapy. AIDS 12, F123–F129.

Antoni, B.A., Rabson, A.B., Kinter, A., Bodkin, M., Poli, G., 1994. NF-kappa B-dependent and -independent pathways of HIV activation in a chronically infected T cell line. Virology 202, 684–694.

Arno, A., Ruiz, L., Juan, M., Zayat, M.K., Puig, T., Balague, M., Romeu, J., Pujol, R., O'Brien, W.A., Clotet, B., 1998. Impact on the immune system of undetectable plasma HIV-1 RNA for more than 2 years. AIDS 12, 697–704.

Arno, A., Ruiz, L., Juan, M., Jou, A., Balague, M., Zayat, M.K., Marfil, S., Martinez-Picado, J., Martinez, M.A., Romeu, J., Pujol-Borrell, R., Lane, C., Clotet, B., 1999. Efficacy of low-dose subcutaneous interleukin-2 to treat advanced human immunodeficiency virus type 1 in persons with $</=250$/microL CD4 T cells and undetectable plasma virus load. J. Infect. Dis. 180, 56–60.

Aukrust, P., Muller, F., Lien, E., Nordoy, I., Liabakk, N.B., Kvale, D., Espevik, T., Froland, S.S., 1999. Tumor necrosis factor (TNF) system levels in human immunodeficiency virus-infected patients during highly active antiretroviral therapy: persistent TNF activation is associated with virologic and immunologic treatment failure. J. Infect. Dis. 179, 74–82.

Autran, B., Carcelain, G., Li, T.S., Blanc, C., Mathez, D., Tubiana, R., Katlama, C., Debre, P., Leibowitch, J., 1997. Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. Science 277, 112–116.

Autran, B., Carcelaint, G., Li, T.S., Gorochov, G., Blanc, C., Renaud, M., Durali, M., Mathez, D., Calvez, V., Leibowitch, J., Katlama, C., Debre, P., 1999. Restoration of the immune system with anti-retroviral therapy. Immunol. Lett. 66, 207–211.

Baba, M., 1997. Cellular factors as alternative targets for inhibition of HIV-1. Antiviral Res. 33, 141–152.

Baba, M., Okamoto, M., Makino, M., Kimura, Y., Ikeuchi, T., Sakaguchi, T., Okamoto, T., 1997. Potent and selective inhibition of human immunodeficiency virus type 1 transcription by piperazinyloxoquinoline derivatives. Antimicrob. Agents Chemother. 41, 1250–1255.

Baba, M., Okamoto, M., Kawamura, M., Makino, M., Higashida, T., Takashi, T., Kimura, Y., Ikeuchi, T., Tetsuka, T., Okamoto, T., 1998. Inhibition of human immunodeficiency virus type 1 replication and cytokine production by fluoroquinoline derivatives. Mol. Pharm. 53, 1097–1103.

Baba, M., Okamoto, M., Takeuchi, H., 1999. Inhibition of human immunodeficiency virus type 1 replication in acutely and chronically infected cells by EM2487, a novel substance produced by a Streptomyces species. Antimicrob. Agents Chemother. 43, 2350–2355.

Bagasra, O., Lavi, E., Bobroski, L., Khalili, K., Pestaner, J.P., Tawadros, R., Pomerantz, R.J., 1996. Cellular reservoirs of HIV-1 in the central nervous system of infected individuals: identification by the combination of in situ polymerase chain reaction and immunohistochemistry. AIDS 10, 573–585.

Bagasra, O., Wright, S.D., Seshamma, T., Oakes, J.W., Pomerantz, R.J., 1992. CD14 is involved in control of human immunodeficiency virus type 1 expression in latently infected cells by lipopolysaccharide. Proc. Natl. Acad. Sci. USA 89, 6285–6289.

Barker, E., Bossart, K.N., Levy, J.A., 1998. Primary CD8+ cells from HIV-infected individuals can suppress productive infection of macrophages independent of beta-chemokines. Proc. Natl. Acad. Sci. USA 95, 1725–1729.

Bartlett, J.A., Berend, C., Petroni, G.R., Ottinger, J., Tyler, D.L., Pettinelli, C., Weinhold, K.J., 1998. Coadministration of zidovudine and interleukin-2 increases absolute CD4 cells in subjects with Walter Reed stage 2 human immunodeficiency virus infection: results of ACTG protocol 042. J. Infect. Dis. 178, 1170–1173.

Bayard-McNeeley, M., Doo, H., He, S., Hafner, A., Johnson, W.D., Jr, Ho., J.L., 1996. Differential effects of interleukin-12, interleukin-15, and interleukin-2 on human immunodeficiency virus type 1 replication in vitro. Clin. Diagn. Lab. Immunol. 3, 547–553.

Bednarik, D.P., Folks, T.M., 1992. Mechanisms of HIV-1 latency. AIDS 6, 3–16.

Bednarik, D.P., Duckett, C., Kim, S.U., Perez, V.L., Griffis, K., Guenthner, P.C., Folks, T.M., 1991. DNA CpG methylation inhibits binding of NF-kappa B proteins to the HIV-1 long terminal repeat cognate DNA motifs. New Biol. 3, 969–976.

Beg, A.A., Finco, T.S., Nantermet, P.V., Baldwin, A.S., Jr, 1993. Tumor necrosis factor and interleukin-1 lead to phosphorylation and loss of IκBα: a mechanism for NF-κB activation. Mol. Cell. Biol. 13, 3301–3310.

Biswas, D.K., Ahlers, C.M., Dezube, B.J., Pardee, A.B., 1994. Pentoxifylline and other protein kinase C inhibitors down-regulate HIV-LTR NF-kappa B induced gene expression. Mol. Med. 1, 31–43.

Biswas, D.K., Mhashilkar, A.M., Ewaniuk, D.S., Pezza, J.A., Oh, L.M., Kannangara, G.S., Tius, M.A., Pardee, A.B., 1998. Inhibition of HIV-1 replication by combination of a novel inhibitor of TNF-alpha with AZT. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. 18, 426–434.

Bohler, T., Walcher, J., Holzl-Wenig, G., Geiss, M., Buchholz, B., Linde, R., Debatin, K.M., 1999. Early effects of antiretroviral combination therapy on activation, apoptosis and regeneration of T cells in HIV-1-infected children and adolescents. AIDS 13, 779–789.

Bollinger, R.C., Quinn, T.C., Liu, A.Y., Stanhope, P.E., Hammond, S.A., Viveen, R., Clements, M.L., Siliciano, R.F., 1993. Cytokines from vaccine-induced HIV-1 specific cytotoxic T lymphocytes: effects on viral replication. AIDS Res. Hum. Retroviruses 9, 1067–1077.

Bouscarat, F., Levacher, M., Landman, R., Muffat-Joly, M., Girard, P.M., Saimot, A.G., Brun-Vezinet, F., Sinet, M., 1998. Changes in blood CD8+ lymphocyte activation status and plasma HIV RNA levels during antiretroviral therapy. AIDS 12, 1267–1273.

Braddock, M., Thorburn, A.M., Kingsman, A.J., Kingsman, S.M., 1991. Blocking of Tat-dependent HIV-1 RNA modification by an inhibitor of RNA polymerase II processivity. Nature 350, 439–441.

Briant, L., Reynes, J., Coudronniere, N., Benezech, J.P., Devaux, C., 1999. HIV-1 reactivation in resting peripheral blood mononuclear cells of infected adults upon in vitro CD4 cross-linking by ligands of the CDR2-loop inextracellular domain 1. J. Acquir. Immune Defic. Syndr. 21, 9–19.

Brinchmann, J.E., Gaudernack, G., Vartdal, F., 1990. CD8+ T cells inhibit HIV replication in naturally infected CD4+ T cells. Evidence for a soluble inhibitor. J. Immunol. 144, 2961–2966.

Brinchmann, J.E., Albert, J., Vartdal, F., 1991. Few infected CD4+ T cells but a high proportion of replication-competent provirus copies in asymptomatic human immunodeficiency virus type 1 infection. J. Virol. 65, 2019–2023.

Broor, S., Kusari, A.B., Zhang, B., Seth, P., Richman, D.D., Carson, D.A., Wachsman, W., Lotz, M., 1994. Stimulation of HIV replication in mononuclear phagocytes by leukemia inhibitory factor. J. Acquir. Immune Defic. Syndr. 7, 647–654.

Bukrinsky, M.I., Stanwick, T.L., Dempsey, M.P., Stevenson, M., 1991. Quiescent T lymphocytes as an inducible virus reservoir in HIV-1 infection. Science 254, 423–427.

Bukrinsky, M.I., Sharova, N., Dempsey, M.P., Stanwick, T.L., Bukrinskaya, A.G., Haggerty, S., Stevenson, M., 1992. Active nuclear import of human immunodeficiency virus type 1 preintegration complexes. Proc. Natl. Acad. Sci. USA 89, 6580–6584.

Butera, S.T., 1993. Cytokine involvement in viral permissiveness and the progression of HIV disease. J. Cell. Biochem. 53, 336–342.

Butera, S.T., 1994. Interrelationships between cytokines and the human immunodeficiency virus. In: Griffin, G. (Ed.), Cytokines in Infection, Baillière's Clinical Infectious Diseases. Baillière Tindall, London, pp. 109–126.

Butera, S.T., 1998. Evaluation of compounds that prevent reactivation of HIV-1 in OM-10.1 cells. In: Kinchington, D., Schinazi, R. (Eds.), Methods in Cellular and Molecular Biology: Antiviral Evaluation. Humana Press Inc., Totowa, NJ, pp. 201–206.

Butera, S.T., Folks, T.M., 1992. Application of latent HIV-1 infected cellular models to therapeutic intervention. AIDS Res. Hum. Retroviruses 8, 991–995.

Butera, S.T., Perez, V.L., Wu, B.-Y., Nabel, G.J., Folks, T.M., 1991. Oscillation of the human immunodeficiency virus surface receptor is regulated by the state of viral activation in a CD4+ model of chronic infection. J. Virol. 65, 4645–4653.

Butera, S.T., Roberts, B.D., Folks, T.M., 1993. Regulation of HIV-1 expression by cytokine networks in a CD4+ model of chronic infection. J. Immunol. 150, 625–634.

Butera, S.T., Roberts, B.D., Lam, L., Hodge, T., Folks, T.M., 1994. Human immunodeficiency virus type 1 RNA expression by four chronically infected cell lines indicates multiple mechanisms of latency. J. Virol. 68, 2726–2730.

Butera, S.T., Roberts, B.D., Critchfield, J.W., Fang, G., McQuade, T., Gracheck, S.J., Folks, T.M., 1995. Compounds that target novel cellular components involved in HIV-1 transcription. Mol. Med. 1, 758–767.

Byrn, R.A., Kiessling, A.A., 1998. Analysis of human immunodeficiency virus in semen: indications of a genetically distinct virus reservoir. J. Reprod. Immunol. 41, 161–176.

Calarota, S.A., Leandersson, A.C., Bratt, G., Hinkula, J., Klinman, D.M., Weinhold, K.J., Sandstrom, E., Wahren, B., 1999. Immune responses in asymptomatic HIV-1-infected patients after HIV-DNA immunization followed by highly active antiretroviral treatment. J. Immunol. 163, 2330–2338.

Cameron, P.U., Forsum, U., Teppler, H., Granelli-Piperno, A., Steinman, R.M., 1992. During HIV-1 infection most blood dendritic cells are not productively infected and can induce allogeneic CD4+ T cells clonal expansion. Clin. Exp. Immunol. 88, 226–236.

Carr, A., Emery, S., Lloyd, A., Hoy, J., Garsia, R., French, M., Stewart, G., Fyfe, G., Cooper, D.A., 1998. Outpatient continuous intravenous interleukin-2 or subcutaneous, polyethyleneglycol-modified interleukin-2 in human immunodeficiency virus-infected patients:a randomized, controlled, multicenter study. Australian IL-2 Study Group. J. Infect. Dis. 178, 992–999.

Cavert, W., Notermans, D.W., Staskus, K., Wietgrefe, S.W., Zupancic, M., Gebhard, K., Henry, K., Zhang, Z.Q., Mills, R., McDade, H., Schuwirth, C.M., Goudsmit, J., Danner, S.A., Haase, A.T., 1997. Kinetics of response in lymphoid tissues to antiretroviral therapy of HIV-1 infection. Science 276, 960–964.

Chapuis, A.G., Rizzardi, G.P., D'Agostino, C., Attinger, A., Knabenhans, C., Fleury, S., Acha-Orbea, H., Pantaleo, G., 2000. Effects of mycophenolic acid on human immunodeficiency virus infection in vitro and in vivo. Nat. Med. 6, 762–768.

Chou, C.S., Ramilo, O., Vitetta, E.S., 1997. Highly purified CD25-resting T cells cannot be infected de novo with HIV-1. Proc. Natl. Acad. Sci. USA 94, 1361–1365.

Chun, T.W., Fauci, A.S., 1999. Latent reservoirs of HIV: obstacles to the eradication of virus. Proc. Natl. Acad. Sci. USA 96, 10958–10961.

Chun, T.W., Finzi, D., Margolick, J., Chadwick, K., Schwartz, D., Siliciano, R.F., 1995. In vivo fate of HIV-1-infected T cells: quantitative analysis of the transition to stable latency. Nat. Med. 1, 1284–1290.

Chun, T.W., Stuyve, L., Mizell, S.B., Ehler, L.A., Mican, J.A., Baseler, M., Lloyd, A.L., Nowak, M.A., Fauci, A.S., 1997a. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. Proc. Natl. Acad. Sci. USA 94, 13193–13197.

Chun, T.W., Carruth, L., Finzi, D., Shen, X., Di Giuseppe, J.A., Taylor, H., Hermankova, M., Chadwick, K., Margolick, J., Quinn, T.C., Kuo, Y.H., Brookmeyer, R., Zeiger, M.A., Barditch-Crovo, P., Siliciano, R.F., 1997b. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. Nature 387, 183–188.

Chun, T.W., Chadwick, K., Margolick, J., Siliciano, R.F., 1997c. Differential susceptibility of naive and memory CD4+ T cells to the cytopathic effects of infection with human immunodeficiency virus type 1 strain LAI. J. Virol. 71, 4436–4444.

Chun, T.W., Engel, D., Berrey, M.M., Shea, T., Corey, L., Fauci, A.S., 1998a. Early establishment of a pool of latently infected, resting CD4(+) T cells during primary HIV-1 infection. Proc. Natl. Acad. Sci. USA 95, 8869–8873.

Chun, T.W., Engel, D., Mizell, S.B., Ehler, L.A., Fauci, A.S., 1998b. Induction of HIV-1 replication in latently infected CD4+ T cells using a combination of cytokines. J. Exp. Med. 188, 83–91.

Chun, T.W., Engel, D., Mizell, S.B., Hallahan, C.W., Fischette, M., Park, S., Davey, R.T., Jr, Dybul, M., Kovacs, J.A., Metcalf, J.A., Mican, J.M., Berrey, M.M., Corey, L., Lane, H.C., Fauci, A.S., 1999a. Effect of interleukin-2 on the pool of latently infected, resting CD4+ T cells in HIV-1-infected patients receiving highly active anti-retroviral therapy. Nat. Med. 5, 651–655.

Chun, T.W., Davey, R.T., Jr, Engel, D., Lane, H.C., Fauci, A.S., 1999b. Re-emergence of HIV after stopping therapy. Nature 401, 874–875.

Chun, T.W., Davey, R.T., Jr, Ostrowski, M., Justement, J.S., Engel, D., Mullins, J.I., Fauci, A.S., 2000. Relationship between pre-existing viral reservoirs and the re-emergence of plasma viremia after discontinuation of highly active anti-retroviral therapy. Nat. Med. 6, 757–761.

Cinque, P., Vago, L., Mengozzi, M., Torri, V., Ceresa, D., Vicenzi, E., Transidico, P., Vagani, A., Sozzani, S., Mantovani, A., Lazzarin, A., Poli, G., 1998. Elevated cerebrospinal fluid levels of monocyte chemotactic protein-1 correlate with HIV-1 encephalitis and local viral replication. AIDS 12, 1327–1332.

Clarke, J.R., White, N.C., Weber, J.N., 2000. HIV compartmentalization: pathogenesis and clinical implications. AIDS Rev. 2, 15–21.

Clouse, K.A., Powell, D., Washington, I., Poli, G., Strebel, K., Farrar, W., Barstad, P., Kovacs, J., Fauci, A.S., Folks, T.M., 1989. Monokine regulation of human immunodeficiency virus-1 expression in a chronically infected human T cell clone. J. Immunol. 142, 431–438.

Cohen, P.S., Schmidtmayerova, H., Dennis, J., Dubrovsky, L., Sherry, B., Wang, H., Bukrinsky, M., Tracey, K.J., 1997. The critical role of p38 MAP kinase in T cell HIV-1 replication. Mol. Med. 3, 339–346.

Cole, S.W., Korin, Y.D., Fahey, J.L., Zack, J.A., 1998. Norepinephrine accelerates HIV replication via protein kinase A-dependent effects on cytokine production. J. Immunol. 161, 610–616.

Connors, M., Kovacs, J.A., Krevat, S., Gea-Banacloche, J.C., Sneller, M.C., Flanigan, M., Metcalf, J.A., Walker, R.E., Falloon, J., Baseler, M., Feuerstein, I., Masur, H., Lane, H.C., 1997. HIV infection induces changes in CD4+ T-cell phenotype and depletions within the CD4+ T-cell repertoire that are not immediately restored by antiviral or immune-based therapies. Nat. Med. 3, 533–540.

Coombs, R.W., Speck, C.E., Hughes, J.P., Lee, W., Sampoleo, R., Ross, S.O., Dragavon, J., Peterson, G., Hooton, T.M., Collier, A.C., Corey, L., Koutsky, L., Krieger, J.N., 1998. Association between culturable human immunodeficiency virus type 1 (HIV-1) in semen and HIV-1 RNA levels in semen and blood: evidence for compartmentalization of HIV-1 between semen and blood. J. Infect. Dis. 177, 320–330.

Coudronniere, N., Devaux, C., 1998. A novel complex of proteins binds the HIV-1 promoter upon virus interaction with CD4. J. Biomed. Sci. 5, 281–289.

Critchfield, J.W., Butera, S.T., Folks, T.M., 1996. Inhibition of HIV activation in latently infected cells by flavonoid compounds. AIDS Res. Hum. Retroviruses 12, 39–46.

Critchfield, J.W., Coligan, J.E., Folks, T.M., Butera, S.T., 1997. Casein kinase II is a selective target of HIV-1 transcriptional inhibitors. Proc. Natl. Acad. Sci. USA 94, 6110–6115.

Critchfield, J.W., Ho, O., Roberts, B.D., Van Lint, C., Verdin, E., Butera, S.T., 1999. Isoquinolinesulphonamide derivatives inhibit transcriptional elongation of human immunodeficiency virus type 1 RNA in a promyelocytic model of latency. Antiviral Chem. Chemother. 10, 275–284.

Cujec, T.P., Okamoto, H., Fujinaga, K., Meyer, J., Chamberlin, H., Morgan, D.O., Peterlin, B.M., 1997. The HIV transactivator TAT binds to the CDK-activating kinase and activates the phosphorylation of the carboxy-terminal domain of RNA polymerase II. Genes Dev. 11, 2645–2657.

Cu-Uvin, S., Caliendo, A.M., Reinert, S., Chang, A., Juliano-Remollino, C., Flanigan, T.P., Mayer, K.H., Carpenter, C.C., 1998. HIV-1 in the female genital tract and the effect of antiretroviral therapy. AIDS 12, 826–827.

Dam Nielsen, S., Kjaer Ersboll, A., Mathiesen, L., Nielsen, J.O., Hansen, J.E., 1998. Highly active antiretroviral therapy normalizes the function of progenitor cells in human immunodeficiency virus-infected patients. J. Infect. Dis. 178, 1299–1305.

Davey, R.T., Jr, Bhat, N., Yoder, C., Chun, T.W., Metcalf, J.A., Dewar, R., Natara, V., Lempicki, R.A., Adelsberger, J.W., Miller, K.D., Kovacs, J.A., Polis, M.A., Walker,

R.E., Falloon, J., Masur, H., Gee, D., Baseler, M., Dimitrov, D.S., Fauci, A.S., Lane, H.C., 1999. HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. Proc. Natl. Acad. Sci. USA 96, 15109–15114.

De Paoli, P., Zanussi, S., Caggiari, L., Bortolin, M.T., D'Andrea, M., Simonelli, C., Tirelli, U., 1999. Kinetics of lymphokine production in HIV+ patients treated with highly active antiretroviral therapy and interleukin 2. J. Clin. Immunol. 19, 317–325.

Detels, R., Munoz, A., McFarlane, G., Kingsley, L.A., Margolick, J.B., Giorgi, J., Schrager, L.K., Phair, J.P., 1998. Effectiveness of potent antiretroviral therapy on time to AIDS and death in men with known HIV infection duration. Multicenter AIDS Cohort Study Investigators. J. Am. Med. Assoc. 280, 1497–1503.

Dezube, B.J., Lederman, M.M., Chapman, B., Georges, D.L., Dogon, A.L., Mudido, P., Reis-Lishing, J., Cheng, S.L., Silberman, S.L., Crumpacker, C.S., 1997. The effect of tenidap on cytokines, acute-phase proteins, and virus load in human immunodeficiency virus (HIV)-infected patients: correlation between plasma HIV-1 RNA and proinflammatory cytokine levels. J. Infect. Dis. 176, 807–810.

Doppler, C., Schalasta, G., Amtmann, E., Sauer, G., 1992. Binding of NF-κB to the HIV-1 LRT is not sufficient to induce HIV-1 LTR activity. AIDS Res. Hum. Retroviruses 8, 245–252.

Dornadula, G., Zhang, H., Van Uitert, B., Stern, J., Livornese, L., Jr, Ingerman, M.J., Witek, J., Kedanis, R.J., Natkin, J., DeSimone, J., Pomerantz, R.J., 1999. Residual HIV-1 RNA in blood plasma of patients taking suppressive highly active antiretroviral therapy. J. Am. Med. Assoc. 282, 1627–1632.

Duan, L., Ozaki, I., Oakes, J.W., Taylor, J.P., Khalili, K., Pomerantz, R.J., 1994a. The tumor suppressor protein p53 strongly alters human immunodeficiency virus type 1 replication. J. Virol. 68, 4302–4313.

Duan, L., Oakes, J.W., Ferraro, A., Bagasra, O., Pomerantz, R.J., 1994b. Tat and rev differentially affect restricted replication of human immunodeficiency virus type 1 in various cells. Virology 199, 474–478.

Duh, E.J., Maury, W., Folks, T.M., Fauci, A.S., Rabson, A.B., 1989. Tumor necrosis factor-alpha activates human immunodeficiency virus-1 through induction of nuclear factor binding to the NF-κB sites in the long terminal repeat. Proc. Natl. Acad. Sci. USA 86, 5974–5978.

Dyrhol-Riise, A.M., Voltersvik, P., Olofsson, J., Asjo, B., 1999. Activation of CD8 T cells normalizes and correlates with the level of infectious provirus in tonsils during highly active antiretroviral therapy in early HIV-1infection. AIDS 13, 2365–2376.

Embretson, J., Zupancic, M., Ribas, J.L., Burke, A., Racz, P., Tenner-Racz, K., Haase, A.T., 1993. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. Nature 362, 359–362.

Emiliani, S., Van Lint, C., Fischle, W., Paras, P., Jr, Ott, M., Brady, J., Verdin, E., 1996. A point mutation in the HIV-1 Tat responsive element is associated with postintegration latency. Proc. Natl. Acad. Sci. USA 93, 6377–6381.

Emiliani, S., Fischle, W., Ott, M., Van Lint, C., Amella, C.A., Verdin, E., 1998. Mutations in the tat gene are responsible for human immunodeficiency virus type 1 postintegration latency in the U1 cell line. J. Virol. 72, 1666–1670.

Evans, T.G., Bonnez, W., Soucier, H.R., Fitzgerald, T., Gibbons, D.C., Reichman, R.C., 1998. Highly active antiretroviral therapy results in a decrease in CD8+ T cell activation and preferential reconstitution of the peripheral CD4+ T cell population with memory rather than naive cells. Antiviral Res. 39, 163–173.

Feorino, P.M., Butera, S.T., Folks, T.M., Schinazi, R.F., 1993. Prevention of activation of HIV-1 by antiviral agents in OM-10.1 cells. Antiviral Chem. Chemother. 4, 55–63.

Ferguson, N.M., de Wolf, F., Ghani, A.C., Fraser, C., Donnelly, C.A., Reiss, P., Lange, J.M., Danner, S.A., Garnett, G.P., Goudsmit, J., Anderson, R.M., 1999. Antigen-driven CD4+ T cell and HIV-1 dynamics: residual viral replication under highly active antiretroviral therapy. Proc. Natl. Acad. Sci. USA 96, 15167–15172.

Finnegan, A., Roebuck, K.A., Nakai, B.E., Gu, D.S., Rabbi, M.F., Song, S., Landay, A.L., 1996. IL-10 cooperates with TNF-alpha to activate HIV-1 from latently and acutely infected cells of monocyte/macrophage lineage. J. Immunol. 156, 841–851.

Finzi, D., Siliciano, R.F., 1998. Viral dynamics in HIV-1 infection. Cell 93, 665–671.

Finzi, D., Hermankova, M., Pierson, T., Carruth, L.M., Buck, C., Chaisson, R.E., Quinn, T.C., Chadwick, K., Margolick, J., Brookmeyer, R., Gallant, J., Markowitz, M., Ho, D.D., Richman, D.D., Siliciano, R.F., 1997. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science 278, 1295–1300.

Finzi, D., Blankson, J., Siliciano, J.D., Margolick, J.B., Chadwick, K., Pierson, T., Smith, K., Lisziewicz, J., Lori, F., Flexner, C., Quinn, T.C., Chaisson, R.E., Rosenberg, E., Walker, B., Gange, S., Gallant, J., Siliciano, R.F., 1999. Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. Nat. Med. 5, 512–517.

Fleury, S., de Boer, R.J., Rizzardi, G.P., Wolthers, K.C., Otto, S.A., Welbon, C.C., Graziosi, C., Knabenhans, C., Soudeyns, H., Bart, P.A., Gallant, S., Corpataux, J.M., Gillet, M., Meylan, P., Schnyder, P., Meuwly, J.Y., Spreen, W., Glauser, M.P., Miedema, F., Pantaleo, G., 1998. Limited CD4+ T-cell renewal in early HIV-1 infection: effect of highly activeantiretroviral therapy. Nat. Med. 4, 794–801.

Flores, O., Lee, G., Kessler, J., Miller, M., Schlief, W., Tomassini, J., Hazuda, D., 1999. Host-cell positive transcription elongation factor b kinase activity is essential and limiting for HIV type 1 replication. Proc. Natl. Acad. Sci. USA 96, 7208–7213.

Folks, T.M., Powell, D., Lightfoote, M., Koenig, S., Fauci, A.S., Benn, S., Rabson, A., Daugherty, D., Gendelman, H.E., Hoggan, M.D., Venkatesan, S., Martin, M.A., 1986. Biological and biochemical characterization of a cloned Leu-3$^-$ cell surviving infection with the acquired immune deficiency syndrome retrovirus. J. Exp. Med. 164, 280–290.

Folks, T.M., Justement, J., Kinter, A., Dinarello, C.A., Fauci, A.S., 1987. Cytokine-induced expression of HIV-1 in a chronically infected promonocyte cell line. Science 238, 800–802.

Folks, T.M., Justement, J., Kinter, A., Schnittman, S., Orenstein, J., Poli, G., Fauci, A.S., 1988. Characterization of a promonocyte clone chronically infected with HIV and inducible by 13-phorbol-12-myristate acetate. J. Immunol. 140, 1117–1122.

Folks, T.M., Clouse, K.A., Justement, J., Rabson, A., Duh, E., Kehrl, J.H., Fauci, A.S., 1989. Tumor necrosis factor alpha induces expression of human immunodeficiency virus in a chronically infected T-cell clone. Proc. Natl. Acad. Sci. USA 86, 2365–2368.

Fujinaga, K., Nakaya, T., Ikuta, K., 1998. Generation of endogenous tumour necrosis factor-alpha in MOLT-4 cells during the acute replication phase of human immunodeficiency virus type 1 determines the subsequent latent infection. J. Gen. Virol. 79, 221–229.

Fujiwara, M., Okamoto, M., Okamoto, M., Watanabe, M., Machida, H., Shigeta, S., Konno, K., Yokota, T., Baba, M., 1999. Acridone derivatives are selective inhibitors of HIV-1 replication in chronically infected cells. Antiviral Res. 43, 189–199.

Furtado, M.R., Callaway, D.S., Phair, J.P., Kunstman, K.J., Stanton, J.L., Macken, C.A., Perelson, A.S., Wolinsky, S.M., 1999. Persistence of HIV-1 transcription in peripheral-blood mononuclear cells in patients receiving potent antiretroviral therapy. N. Engl. J. Med. 340, 1614–1622.

Garcia, F., Plana, M., Vidal, C., Cruceta, A., O'Brien, W.A., Pantaleo, G., Pumarola, T., Gallart, T., Miro, J.M., Gatell, J.M., 1999. Dynamics of viral load rebound and immunological changes after stopping effective antiretroviral therapy. AIDS 13, F79–F86.

Garcia, F., Miró, J.M., Gatell., J.M., 2000. Structured antiretroviral therapy interruption as a form of immune-based therapy in HIV-1 infection. AIDS Rev. 2, 3–8.

Garcia-Blanco, M.A., Cullen, B.R., 1991. Molecular basis of latency in pathogenic human viruses. Science 254, 815–820.

Gaynor, R., 1992. Cellular transcription factors involved in the regulation of HIV-1 gene expression. AIDS 6, 347–363.

Goldstein, H., Pettoello-Mantovani, M., Bera, T.K., Pastan, I.H., Berger, E.A., 2000. Chimeric toxins targeted to the human immunodeficiency virus type 1 envelope glycoprotein augment the in vivo activity of combination antiretroviral therapy in thy/liv-SCID-Hu mice. J. Infect. Dis. 181, 921–926.

Goletti, D., Weissman, D., Jackson, R.W., Collins, F., Kinter, A., Fauci, A.S., 1998. The in vitro induction of human immunodeficiency virus (HIV) replication in purified protein derivative-positive HIV-infected persons by recall antigen response to Mycobacterium tuberculosis is the result of a balance of the effects of endogenous interleukin-2 and proinflammatory and antiinflammatory cytokines. J. Infect. Dis. 177, 1332–1338.

Gotch, F., Hardy, G., Imami, N., 1999. Therapeutic vaccines in HIV-1 infection. Immunol. Rev. 170, 173–182.

Granowitz, E.V., Saget, B.M., Wang, M.Z., Dinarello, C.A., Skolnik, P.R., 1995. Interleukin 1 induces HIV-1 expression in chronically infected U1 cells: blockade by interleukin 1 receptor antagonist and tumor necrosis factor binding protein type 1. Mol. Med. 1, 667–677.

Griffin, G.E., Leung, K., Folks, T.M., Kunkel, S., Nabel, G.J., 1989. Activation of HIV gene expression during monocytic differentiation by induction of NF-κB. Nature 339, 70–73.

Grossman, Z., Feinberg, M.B., Paul, W.E., 1998. Multiple modes of cellular activation and virus transmission in HIV infection: a role for chronically and latently infected cells in sustaining viral replication. Proc. Natl. Acad. Sci. USA 95, 6314–6319.

Grossman, Z., Polis, M., Feinberg, M.B., Grossman, Z., Levi, I., Jankelevich, S., Yarchoan, R., Boon, J., de Wolf, F., Lange, J.M., Goudsmit, J., Dimitrov, D.S., Paul, W.E., 1999. Ongoing HIV dissemination during HAART. Nat. Med. 5, 1099–1104.

Gualberto, A., Hixon, M.L., Finco, T.S., Perkins, N.D., Nabel, G.J., Baldwin, A.S., Jr, 1995. A proliferative p53-responsive element mediates tumor necrosis factor alpha induction of the human immunodeficiency virus type 1 long terminal repeat. Mol. Cell. Biol. 15, 3450–3459.

Gulick, R.M., Mellors, J.W., Havlir, D., Eron, J.J., Gonzalez, C., McMahon, D., Richman, D.D., Valentine, F.T., Jonas, L., Meibohm, A., Emini, E.A., Chodakewitz, J.A., 1997. Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. N. Engl. J. Med. 337, 734–739.

Gulick, R.M., Mellors, J.W., Havlir, D., Eron, J.J., Meibohm, A., Condra, J.H., Valentine, F.T., McMahon, D., Gonzalez, C., Jonas, L., Emini, E.A., Chodakewitz, J.A., Isaacs, R., Richman, D.D., 2000. Three-year suppression of HIV viremia with indinavir, zidovudine, and lamivudine. Ann. Intern. Med. 133, 35–39.

Gunthard, H.F., Frost, S.D., Leigh-Brown, A.J., Ignacio, C.C., Kee, K., Perelson, A.S., Spina, C.A., Havlir, D.V., Hezareh, M., Looney, D.J., Richman, D.D., Wong, J.K., 1999. Evolution of envelope sequences of human immunodeficiency virus type 1 in cellular reservoirs in the setting of potent antiviral therapy. J. Virol. 73, 9404–9412.

Gunthard, H.F., Wong, J.K., Ignacio, C.C., Guatelli, J.C., Riggs, N.L., Havlir, D.V., Richman, D.D., 1998. Human immunodeficiency virus replication and genotypic resistance in blood and lymph nodes after a year of potent antiretroviral therapy. J. Virol. 72, 2422–2428.

Hammer, S.M., Squires, K.E., Hughes, M.D., Grimes, J.M., Demeter, L.M., Currier, J.S., Eron, J.J., Jr, Feinberg, J.E., Balfour, H.H., Jr, Deyton, L.R., Chodakewitz, J.A., Fischl, M.A., 1997. A controlled trial of two nucleoside analogues plus indinavir in persons with hman immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team. N. Engl. J. Med. 337, 725–733.

Hamy, F., Felder, E.R., Heizmann, G., Lazdins, J., Aboul-ela, F., Varani, G., Karn, J., Klimkait, T., 1997. An inhibitor of the Tat/TAR RNA interaction that effectively suppresses HIV-1 replication. Proc. Natl. Acad. Sci. USA 94, 3548–3553.

Harrer, T., Jassoy, C., Harrer, E., Johnson, R.P., Walker, B.D., 1993. Induction of HIV-1 replication in a chronically infected T-cell line by cytotoxic T lymphocytes. J. Acquir. Immune Defic. Syndr. 6, 865–871.

Harrigan, P.R., Whaley, M., Montaner, J.S., 1999. Rate of HIV-1 RNA rebound upon stopping antiretroviral therapy. AIDS 13, F59–F62.

Hashimoto, K., Baba, M., Gohnai, K., Sato, M., Shigeta, S., 1996. Heat shock induces HIV-1 replication in chronically infected promyelocyte cell line OM 10.1. Arch. Virol. 141, 439–447.

Heath, S.L., Tew, J.G., Tew, J.G., Szakal, A.K., Burton, G.F., 1995. Follicular dendritic cells and human immunodeficiency virus infectivity. Nature 377, 740–744.

Henderson, A.J., Calame, K.L., 1997. CCAAT/enhancer binding protein (C/EBP) sites are required for HIV-1 replication in primary macrophages but not CD4(+) T cells. Proc. Natl. Acad. Sci. USA 94, 8714–8719.

Hengel, R.L., Jones, B.M., Kennedy, M.S., Hubbard, M.R., McDougal, J.S., 1999. Lymphocyte kinetics and precursor frequency-dependent recovery of CD4(+)CD45RA(+) CD62L(+) naive T cells following triple-drug therapy for HIV type 1 infection. AIDS Res. Hum. Retroviruses 15, 435–443.

Hengge, U.R., Goos, M., Esser, S., Exner, V., Dotterer, H., Wiehler, H., Borchard, C., Muller, K., Beckmann, A., Eppner, M.T., Berger, A., Fiedler, M., 1998. Randomized, controlled phase II trial of subcutaneous interleukin-2 in combination with highly active antiretroviral therapy (HAART) in HIV patients. AIDS 12, F225–F234.

Herrmann, C.H., Carroll, R.G., Wei, P., Jones, K.A., Rice, A.P., 1998. Tat-associated kinase, TAK, activity is regulated by distinct mechanisms in peripheral blood lymphocytes and promonocytic cell lines. J. Virol. 72, 9881–9888.

Ho, D.D., Neumann, A.U., Perelson, A.S., Chen, W., Leonard, J.M., Markowitz, M., 1995. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature 373, 123–126.

Ho, W.Z., Lai, J.P., Bouhamdan, M., Duan, L., Pomerantz, R.J., Starr, S.E., 1998. Inhibition of HIV type 1 replication in chronically infected monocytes and lymphocytes by retrovirus-mediated gene transfer of anti-Rev single-chain variable fragments. AIDS Res. Hum. Retroviruses 14, 1573–1580.

Hoetelmans, R.M., 1998. Sanctuary sites in HIV-1 infection. Antivir. Ther. 3, 13–17.

Hsu, M.C., Schutt, A.D., Holly, M., Slice, L.W., Sherman, M.I., Richman, D.D., Potash, M.J., Volsky, D.J., 1991. Inhibition of HIV replication in acute and chronic infections in vitro by a Tat antagonist. Science 254, 1799–1802.

Hufert, F.T., van Lunzen, J., Janossy, G., Bertram, S., Schmitz, J., Haller, O., Racz, P., von Laer, D., 1997. Germinal centre CD4+ T cells are an important site of HIV replication in vivo. AIDS 11, 849–857.

Idziorek, T., Khalife, J., Billaut-Mulot, O., Hermann, E., Aumercier, M., Mouton, Y., Capron, A., Bahr, G.M., 1998. Recombinant human IL-16 inhibits HIV-1 replication and protects against activation-induced cell death (AICD). Clin. Exp. Immunol. 112, 84–91.

Ikuta, K., Kameoka, M., Luftig, R.B., 1997. AIDS pathogenesis: the role of accessory gene mutations, leading to formation of long-lived persistently infected cells and/or apoptosis-inducing HIV-1 particles. Virus Res. 52, 145–156.

Jeyaseelan, R., Kurabayashi, M., Kedes, L., 1996. Doxorubicin inhibits Tat-dependent transactivation of HIV type 1 LTR. AIDS Res. Hum. Retroviruses 12, 569–576.

Jones, K.A., 1997. Taking a new TAK on tat transactivation. Genes Dev. 11, 2593–2599.

Kalams, S.A., Goulder, P.J., Shea, A.K., Jones, N.G., Trocha, A.K., Ogg, G.S., Walker, B.D., 1999. Levels of human immunodeficiency virus type 1-specific cytotoxic T-lymphocyte effector and memory responses decline after suppression of viremia with highly active antiretroviral therapy. J. Virol. 73, 6721–6728.

Kanazawa, S., Okamoto, T., Peterlin, B.M., 2000. Tat competes with CIITA for the binding to P-TEFb and blocks the expression of MHC class II genes in HIV infection. Immunity 12, 61–70.

Kawano, Y., Tanaka, Y., Misawa, N., Tanaka, R., Kira, J.I., Kimura, T., Fukushi, M., Sano, K., Goto, T., Nakai, M., Kobayashi, T., Yamamoto, N., Koyanagi, Y., 1997. Mutational analysis of human immunodeficiency virus type 1 (HIV-1) accessory genes: requirement of a site in the nef gene for HIV-1 replication in activated CD4+ T cells in vitro and in vivo. J. Virol. 71, 8456–8466.

Kinoshita, S., Chen, B.K., Kaneshima, H., Nolan, G.P., 1998. Host control of HIV-1 parasitism in T cells by the nuclear factor of activated T cells. Cell 95, 595–604.

Kishi, M., Zheng, Y.H., Bahmani, M.K., Tokunaga, K., Takahashi, H., Kakinuma, M., Lai, P.K., Nonoyama, M., Luftig, R.B., Ikuta, K., 1995. Naturally occurring accessory gene mutations lead to persistent human immunodeficiency virus type 1 infection of CD4-positive T cells. J. Virol. 69, 7507–7518.

Kinter, A.L., Poli, G., Maury, W., Folks, T., Fauci, A.S., 1990. Direct and cytokine-mediated activation of protein kinase C induces human immunodeficiency virus expression in chronically infected promonocytic cells. J. Virol. 64, 4306–4312.

Kinter, A.L., Ostrowski, M., Goletti, D., Oliva, A., Weissman, D., Gantt, K., Hardy, E., Jackson, R., Ehler, L., Fauci, A.S., 1996. HIV replication in CD4+ T cells of HIV-infected individuals is regulated by a balance between the viral suppressive effects of endogenous beta-chemokines and the viral inductive effects of other endogenous cytokines. Proc. Natl. Acad. Sci. USA 93, 14076–14081.

Kinter, A.L., Poli, G., Fox, L., Hardy, E., Fauci, A.S., 1995a. HIV replication in IL-2-stimulated peripheral blood mononuclear cells is driven in an autocrine/paracrine manner by endogenous cytokines. J. Immunol. 154, 2448–2459.

Kinter, A.L., Bende, S.M., Hardy, E.C., Jackson, R., Fauci, A.S., 1995b. Interleukin 2 induces CD8+ T cell-mediated suppression of human immunodeficiency virus replication in CD4+ T cells and this effect overrides its ability to stimulate virus expression. Proc. Natl. Acad. Sci. USA 92, 10985–10989.

Klein, S.A., Dobmeyer, J.M., Dobmeyer, T.S., Falke, D., Kabelitz, D., Friese, K., Helm, E.B., Hoelzer, D., Rossol-Voth, R., 1996. TNF-alpha mediated apoptosis of CD4 positive T-lymphocytes. A model of T-cell depletion in HIV infected individuals. Eur. J. Med. Res. 1, 249–258.

Klein, S.A., Klebba, C., Kauschat, D., Pape, M., Ozmen, L., Hoelzer, D., Ottmann, O.G., Kalina, U., 2000. Interleukin-18 stimulates HIV-1 replication in a T-cell line. Eur. Cytokine Netw. 11, 47–52.

Kopp, E., Ghosh, S., 1994. Inhibition of NF-κB by sodium salicylate and aspirin. Science 265, 956–959.

Korin, Y.D., Zack, J.A., 1999. Nonproductive human immunodeficiency virus type 1 infection in nucleoside-treated G0 lymphocytes. J. Virol. 73, 6526–6532.

Kotler, D.P., Shimada, T., Snow, G., Winson, G., Chen, W., Zhao, M., Inada, Y., Clayton, F., 1998. Effect of combination antiretroviral therapy upon rectal mucosal HIV RNA burden and mononuclear cell apoptosis. AIDS 12, 597–604.

Kovacs, J.A., Vogel, S., Albert, J.M., Falloon, J., Davey, R.T., Jr, Walker, R.E., Polis, M.A., Spooner, K., Metcalf, J.A., Baseler, M., Fyfe, G., Lane, H.C., 1996. Controlled trial of interleukin-2 infusions in patients infected with the human immunodeficiency virus. N. Engl. J. Med. 335, 1350–1356.

Kroon, F.P., Rimmelzwaan, G.F., Roos, M.T., Osterhaus, A.D., Hamann, D., Miedema, F., van Dissel, J.T., 1998. Restored humoral immune response to influenza vaccination in HIV-infected adults treated with highly active antiretroviral therapy. AIDS 12, F217–F223.

Kubo, M., Ohashi, T., Fujii, M., Oka, S., Iwamoto, A., Harada, S., Kannagi, M., 1997. Abrogation of in vitro suppression of human immunodeficiency virus type 1 (HIV-1) replication mediated by CD8+ T lymphocytes of asymptomatic HIV-1 carriers by staphylococcal enterotoxin B and phorbol esters through induction of tumor necrosis factor alpha. J. Virol. 71, 7560–7566.

Lafeuillade, A., Poggi, C., Tamalet, C., Profizi, N., 1997. Human immunodeficiency virus type 1 dynamics in different lymphoid tissue compartments. J. Infect. Dis. 176, 804–806.

Lafeuillade, A., Chollet, L., Hittinger, G., Profizi, N., Costes, O., Poggi, C., 1998. Residual human immunodeficiency virus type 1 RNA in lymphoid tissue of patients with sustained plasma RNA of <200 copies/ml. J. Infect. Dis. 177, 235–238.

Lambotte, O., Taoufik, Y., de Goër, M.G., Wallon, C., Goujard, C., Delfraissy, J.F., 2000. Detection of infectious HIV in circulating monocytes from patients on prolonged highly active antiretroviral therapy. J. Acquir. Immune Defic. Syndr. 23, 114–119.

Lapenta, C., Santini, S.M., Proietti, E., Rizza, P., Logozzi, M., Spada, M., Parlato, S., Fais, S., Pitha, P.M., Belardelli, F., 1999. Type I interferon is a powerful inhibitor of in vivo HIV-1 infection and preserves human CD4(+) T cells from virus-induced depletion in SCID mice transplanted with human cells. Virology 263, 78–88.

Laughlin, M.A., Chang, G.Y., Oakes, J.W., Gonzalez-Scarano, F., Pomerantz, R.J., 1995. Sodium butyrate stimulation of HIV-1 gene expression: a novel mechanism of induction independent of NF-kappa B. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. 9, 332–339.

Lawn, S.D., Roberts, B.D., Griffin, G.E., Folks, T.M., Butera, S.T., 2000. Cellular compartments of human immunodeficiency virus type 1 replication in vivo: determination by presence of virion-associated host proteins and impact of opportunistic infection. J. Virol. 74, 139–145.

Lazdins, J.K., Grell, M., Walker, M.R., Woods-Cook, K., Scheurich, P., Pfizenmaier, K., 1997. Membrane tumor necrosis factor (TNF) induced cooperative signaling of TNFR60 and TNFR80 favors induction of cell death rather than virus production in HIV-infected T cells. J. Exp. Med. 185, 81–90.

Lederman, M.M., Connick, E., Landay, A., Kuritzkes, D.R., Spritzler, J., St. Clair, M., Kotzin, B.L., Fox, L., Chiozzi, M.H., Leonard, J.M., Rousseau, F., Wade, M., Roe, J.D., Martinez, A., Kessler, H., 1998. Immunologic responses associated with 12 weeks of combination antiretroviral therapy consisting of zidovudine, lamivudine, and ritonavir: results of AIDS Clinical Trials Group Protocol 315. J. Infect. Dis. 178, 70–79.

Leith, J.G., Copeland, K.F., McKay, P.J., Bienzle, D., Richards, C.D., Rosenthal, K.L., 1999. T cell-derived suppressive activity: evidence of autocrine noncytolytic control of HIV type 1 transcription and replication. AIDS Res. Hum. Retroviruses 15, 1553–1561.

Levine, B.L., Mosca, J.D., Riley, J.L., Carroll, R.G., Vahey, M.T., Jagodzinski, L.L., Wagner, K.F., Mayers, D.L., Burke, D.S., Weislow, O.S., St. Louis, D.C., June, C.H., 1996. Antiviral effect and ex vivo CD4+ T cell proliferation in HIV-positive patients as a result of CD28 costimulation. Science 272, 1939–1943.

Levy, D.N., Refaeli, Y., MacGregor, R.R., Weiner, D.B., 1994. Serum Vpr regulates productive infection and latency of human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. USA 91, 10873–10877.

Lewin, S.R., Kirihara, J., Sonza, S., Irving, L., Mills, J., Crowe, S.M., 1998. HIV-1 DNA and mRNA concentra-

tions are similar in peripheral blood monocytes and alveolar macrophages in HIV-1-infected individuals. AIDS 12, 719–727.

Li, C.J., Zhang, L.J., Dezube, B.J., Crumpacker, C.S., Pardee, A.B., 1993. Three inhibitors of type 1 human immunodeficiency virus long terminal repeat-directed gene expression and virus replication. Proc. Natl. Acad. Sci. USA 90, 1839–1842.

Li, Y., Starr, S.E., Lisziewicz, J., Ho, W.Z., 2000. Inhibition of HIV-1 replication in chronically infected cell lines and peripheral blood mononuclear cells by retrovirus-mediated anti-tat gene transfer. Gene Ther. 7, 321–328.

Mackewicz, C.E., Orque, R., Jung, J., Levy, J.A., 1997. Derivation of herpesvirus saimiri-transformed CD8+ T cell lines with noncytotoxic anti-HIV activity. Clin. Immunol. Immunopathol. 82, 274–281.

Mackewicz, C.E., Patterson, B.K., Lee, S.A., Levy, J.A., 2000. CD8(+) cell noncytotoxic anti-human immunodeficiency virus response inhibits expression of viral RNA but not reverse transcription or provirus integration. J. Gen. Virol. 81, 1261–1264.

Mancebo, H.S.Y., Lee, G., Flygare, J., Tomassini, J., Luu, P., Zhu, Y., Peng, J., Blau, C., Hazuda, D., Price, D., Flores, O., 1997. P-TEFb kinase is required for HIV Tat transcriptional activation in vivo and in vitro. Genes Dev. 11, 2633–2644.

Marasco, W.A., LaVecchio, J., Winkler, A., 1999. Human anti-HIV-1 tat sFv intrabodies for gene therapy of advanced HIV-1-infection and AIDS. J. Immunol. Methods 231, 223–238.

Marodon, G., Warren, D., Filomio, M.C., Posnett, D.N., 1999. Productive infection of double-negative T cells with HIV in vivo. Proc. Natl. Acad. Sci. USA 96, 11958–11963.

Marshall, W.L., Brinkman, B.M., Ambrose, C.M., Pesavento, P.A., Uglialoro, A.M., Teng, E., Finberg, R.W., Browning, J.L., Goldfeld, A.E., 1999. Signaling through the lymphotoxin-beta receptor stimulates HIV-1 replication alone and in cooperation with soluble or membrane-bound TNF-alpha. J. Immunol. 162, 6016–6023.

Martinez, M.A., Cabana, M., Ibanez, A., Clotet, B., Arno, A., Ruiz, L., 1999. Human immunodeficiency virus type 1 genetic evolution in patients with prolonged suppression of plasma viremia. Virology 256, 180–187.

Mayer, K.H., Boswell, S., Goldstein, R., Lo, W., Xu, C., Tucker, L., DePasquale, M.P., D'Aquila, R., Anderson, D.J., 1999. Persistence of human immunodeficiency virus in semen after adding indinavir to combination antiretroviral therapy. Clin. Infect. Dis. 28, 1252–1259.

McCoig, C., Van Dyke, G., Chou, C.S., Picker, L.J., Ramilo, O., Vitetta, E.S., 1999. An anti-CD45RO immunotoxin eliminates T cells latently infected with HIV-1 in vitro. Proc. Natl. Acad. Sci. USA 96, 11482–11485.

McCune, J.M., 1995. Viral latency in HIV disease. Cell 82, 183–188.

McLean, A.R., Michie, C.A., 1995. In vivo estimates of division and death rates of human T lymphocytes. Proc. Natl. Acad. Sci. USA 92, 3707–3711.

Mhashilkar, A.M., LaVecchio, J., Eberhardt, B., Porter-Brooks, J., Boisot, S., Dove, J.H., Pumphrey, C., Li, X., Weissmahr, R.N., Ring, D.B., Ramstedt, U., Marasco, W.A., 1999. Inhibition of human immunodeficiency virus type 1 replication in vitro in acutely and persistently infected human CD4+ mononuclear cells expressing murine and humanized anti-human immunodeficiency virus type 1 Tat single-chain variable fragment intrabodies. Hum. Gene Ther. 10, 1453–1467.

Michael, N.L., Vahey, M., Burke, D.S., Redfield, R.R., 1992. Viral DNA and mRNA expression correlate with the stage of human immunodeficiency virus (HIV) type 1 infection in humans: evidence for viral replication in all stages of HIV disease. J. Virol. 66, 310–316.

Mihm, S., Ennen, J., Pessara, U., Kurth, R., Droge, W., 1991. Inhibition of HIV-1 replication and NF-κB activity by cysteine and cysteine derivatives. AIDS 5, 497–503.

Miller, M.D., Warmerdam, M.T., Gaston, I., Greene, W.C., Feinberg, M.B., 1994. The human immunodeficiency virus-1 nef gene product: a positive factor for viral infection and replication in primary lymphocytes and macrophages. J. Exp. Med. 179, 101–113.

Mikovits, J.A., Raziuddin, Gonda, M., Ruta, M., Lohrey, N.C., Kung, H.F., Ruscetti, F.W., 1990. Negative regulation of human immune deficiency virus replication in monocytes. Distinctions between restricted and latent expression in THP-1 cells. J. Exp. Med. 171, 1705–20.

Mikovits, J.A., Lohrey, N.C., Schulof, R., Courtless, J., Ruscetti, F.W., 1992. Activation of infectious virus from latent human immunodeficiency virus infection of monocytes in vivo. J. Clin. Invest. 90, 1486–1491.

Mlynar, E., Bevec, D., Billich, A., Rosenwirth, B., Steinkasserer, A., 1997. The non-immunosuppressive cyclosporin A analogue SDZ NIM 811 inhibits cyclophilin A incorporation into virions and virus replication in human immunodeficiency virus type 1-infected primary and growth-arrested T cells. J. Gen. Virol. 78, 825–835.

Mocroft, A., Vella, S., Benfield, T.L., Chiesi, A., Miller, V., Gargalianos, P., d'Arminio Monforte, A., Yust, I., Bruun, J.N., Phillips, A.N., Lundgren, J.D., 1998. Changing patterns of mortality across Europe in patients infected with HIV-1. EuroSIDA Study Group. Lancet 352, 1725–1730.

Moriuchi, H., Moriuchi, M., Combadiere, C., Murphy, P.M., Fauci, A.S., 1996. CD8+ T-cell-derived soluble factor(s), but not beta-chemokines RANTES., MIP-1 alpha, and MIP-1 beta, suppress HIV-1 replication in monocyte/macrophages. Proc. Natl. Acad. Sci. USA 93, 15341–15345.

Moriuchi, H., Moriuchi, M., Fauci, A.S., 1998. Factors secreted by human T lymphotropic virus type I (HTLV-I)-infected cells can enhance or inhibit replication of HIV-1 in HTLV-I-uninfected cells: implications for in vivo coinfection with HTLV-I and HIV-1. J. Exp. Med. 187, 1689–1697.

Moriuchi, H., Moriuchi, M., Fauci, A.S., 1999. Induction of HIV-1 replication by allogeneic stimulation. J. Immunol. 162, 7543–7548.

Moriuchi, H., Moriuchi, M., Mizell, S.B., Ehler, L.A., Fauci, A.S., 2000. In vitro reactivation of human immunodeficiency virus 1 from latently infected, resting CD4+ T cells after bacterial stimulation. J. Infect. Dis. 181, 2041–2044.

Moulton, S., Pantazis, P., Epstein, J.S., Sadaie, M.R., 1998. 9-Nitrocamptothecin inhibits tumor necosis factor-mediated activation of human immunodeficiency virus type 1 and enhances apoptosis in a latently infected T cell clone. AIDS Res. Hum. Retroviruses 14, 39–49.

Munoz-Fernandez, M.A., Navarro, J., Garcia, A., Punzon, C., Fernandez-Cruz, E., Fresno, M., 1997. Replication of human immunodeficiency virus-1 in primary human T cells is dependent on the autocrine secretion of tumor necrosis factor through the control of nuclear factor-kappa B activation. J. Allergy Clin. Immunol. 100, 838–845.

Muto, N.F., Martinand-Mari, C., Adelson, M.E., Suhadolnik, R.J., 1999. Inhibition of replication of reactivated human immunodeficiency virus type 1 (HIV-1) in latently infected U1 cells transduced with an HIV-1 long terminal repeat-driven PKR cDNA construct. J. Virol. 73, 9021–9028.

Nagai, K., Wong, A.H., Li, S., Tam, W.N., Cuddihy, A.R., Sonenberg, N., Mathews, M.B., Hiscott, J., Wainberg, M.A., Koromilas, A.E., 1997. Induction of CD4 expression and human immunodeficiency virus type 1 replication by mutants of the interferon-inducible protein kinase PKR. J. Virol. 71, 1718–1725.

Natarajan, V., Bosche, M., Metcalf, J.A., Ward, D.J., Lane, H.C., Kovacs, J.A., 1999. HIV-1 replication in patients with undetectable plasma virus receiving HAART. Lancet 353, 119–120.

Navarro, J., Punzon, C., Jimenez, J.L., Fernandez-Cruz, E., Pizarro, A., Fresno, M., Munoz-Fernandez, M.A., 1998. Inhibition of phosphodiesterase type IV suppresses human immunodeficiency virus type 1 replication and cytokine production in primary T cells: involvement of NF-kappaB and NFAT. J. Virol. 72, 4712–4720.

Navarro, J., Punzon, M.C., Pizarro, A., Fernandez-Cruz, E., Fresno, M., Munoz-Fernandez, M.A., 1996. Pentoxifylline inhibits acute HIV-1 replication in human T cells by a mechanism not involving inhibition of tumour necrosis factor synthesis or nuclear factor-kappa B activation. AIDS 10, 469–475.

Neumann, A.U., Tubiana, R., Calvez, V., Robert, C., Li, T.S., Agut, H., Autran, B., Katlama, C., 1999. HIV-1 rebound during interruption of highly active antiretroviral therapy has no deleterious effect on reinitiated treatment. Comet Study Group. AIDS 13, 677–683.

Nielsen, S.D., Nielsen, J.O., Hansen, J.E., 1997. In vitro separation and expansion of CD4 lymphocytes from HIV-infected individuals without activation of HIV infection. J. Immunol. Methods 200, 107–112.

Nielsen, S.D., Afzelius, P., Dam-Larsen, S., Nielsen, C., Nielsen, J.O., Mathiesen, L., Hansen, J.E., 1998. Effect of granulocyte colony-stimulating factor (G-CSF) in human immunodeficiency virus-infected patients: increase in numbers of naive CD4 cells and CD34 cells makes G-CSF a candidate for use in gene therapy or to support antiretroviral therapy. J. Infect. Dis. 177, 1733–1736.

Okamoto, M., Ono, M., Baba, M., 1998. Potent inhibition of HIV type 1 replication by an antiinflammatory alkaloid, cepharanthine, in chronically infected monocytic cells. AIDS Res. Hum. Retroviruses 14, 1239–1245.

Okamoto, M., Okamoto, T., Baba, M., 1999. Inhibition of human immunodeficiency virus type 1 replication by combination of transcription inhibitor K-12 and other antiretroviral agents in acutely and chronically infected cells. Antimicrob. Agents Chemother. 43, 492–497.

Orenstein, J.M., Fox, C., Wahl, S.M., 1997. Macrophages as a source of HIV during opportunistic infections. Science 276, 1857–1861.

Osborn, L., Kunkel, S., Nabel, G.J., 1989. Tumor necrosis factor α and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor κB. Proc. Natl. Acad. Sci. USA 86, 2336–2340.

Ostrowski, M.A., Chun, T.W., Justement, S.J., Motola, I., Spinelli, M.A., Adelsberger, J., Ehler, L.A., Mizell, S.B., Hallahan, C.W., Fauci, A.S., 1999. Both memory and CD45RA+/CD62L+ naive CD4(+) T cells are infected in human immunodeficiency virus type 1-infected individuals. J. Virol. 73, 6430–6435.

Pakker, N.G., Kroon, E.D., Roos, M.T., Otto, S.A., Hall, D., Wit, F.W., Hamann, D., van der Ende, M.E., Claessen, F.A., Kauffmann, R.H., Koopmans, P.P., Kroon, F.P., ten Napel, C.H., Sprenger, H.G., Weigel, H.M., Montaner, J.S., Lange, J.M., Reiss, P., Schellekens, P.T., Miedema, F., 1999. Immune restoration does not invariably occur following long-term HIV-1 suppression during antiretroviral therapy. INCAS Study Group. AIDS 13, 203–212.

Palella, F.J., Jr, Delaney, K.M., Moorman, A.C., Loveless, M.O., Fuhrer, J., Satten, G.A., Aschman, D.J., Holmberg, S.D., 1998. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N. Engl. J. Med. 338, 853–860.

Pandolfi, F., Pierdominici, M., Marziali, M., Livia Bernardi, M., Antonelli, G., Galati, V., D'Offizi, G., Aiuti, F., 2000. Low-dose IL-2 reduces lymphocyte apoptosis and increases naive CD4 cells in HIV-1 patients treated with HAART. Clin. Immunol. 94, 153–159.

Pantaleo, G., 1997. How immune-based interventions can change HIV therapy. Nat. Med. 3, 483–486.

Pantaleo, G., Graziosi, C., Butini, L., Pizzo, P.A., Schnittman, S.M., Kotler, D.P., Fauci, A.S., 1991. Lymphoid organs function as major reservoirs for human immunodeficiency virus. Proc. Natl. Acad. Sci. USA 88, 9838–9842.

Pantaleo, G., Graziosi, C., Demarest, J.F., Butini, L., Montroni, M., Fox, C.H., Orenstein, J.M., Kotler, D.P., Fauci, A.S., 1993. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. Nature 362, 355–358.

Pantaleo, G., Cohen, O.J., Schacker, T., Vaccarezza, M., Graziosi, C., Rizzardi, G.P., Kahn, J., Fox, C.H., Schnittman, S.M., Schwartz, D.H., Corey, L., Fauci, A.S., 1998. Evolutionary pattern of human immunodeficiency virus (HIV) replication and distribution in lymph nodes

following primary infection: implications for antiviral therapy. Nat. Med. 4, 341–345.

Pazin, M.J., Sheridan, P.L., Cannon, K., Cao, Z., Keck, J.G., Kadonaga, J.T., Jones, K.A., 1996. NF-kappa B-mediated chromatin reconfiguration and transcriptional activation of the HIV-1 enhancer in vitro. Genes Dev. 10, 37–49.

Perelson, A.S., Essunger, P., Cao, Y., Vesanen, M., Hurley, A., Saksela, K., Markowitz, M., Ho, D.D., 1997. Decay characteristics of HIV-1-infected compartments during combination therapy. Nature 387, 188–191.

Perez, V.L., Rowe, T., Justement, J.S., Butera, S.T., June, C.H., Folks, T.M., 1991. An HIV-1-infected T cell clone defective in IL-2 production and Ca^{2+} mobilization after CD3 stimulation. J. Immunol. 147, 3145–3148.

Perkins, N.D., Edwards, N.L., Duckett, C.S., Agranoff, A.B., Schmid, R.M., Nabel, G.J., 1993. A cooperative interaction between NF-κB and Sp1 is required for HIV-1 enhancer activation. EMBO J. 12, 3551–3558.

Perrin, L., Yerly, S., Marchal, F., Schockmel, G.A., Hirschel, B., Fox, C.H., Pantaleo, G., 1998. Virus burden in lymph nodes and blood of subjects with primary human immunodeficiency virus type 1 infection on bitherapy. J. Infect. Dis. 177, 1497–1501.

Piatak, M., Jr, Saag, M.S., Yang, L.C., Clark, S.J., Kappes, J.C., Luk, K.C., Hahn, B.H., Shaw, G.M., Lifson, J.D., 1993. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. Science 259, 1749–1754.

Pitcher, C.J., Quittner, C., Peterson, D.M., Connors, M., Koup, R.A., Maino, V.C., Picker, L.J., 1999. HIV-1-specific CD4 + T cells are detectable in most individuals with active HIV-1infection, but decline with prolonged viral suppression. Nat. Med. 5, 518–525.

Plana, M., Garcia, F., Gallart, T., Miro, J.M., Gatell, J.M., 1998. Lack of T-cell proliferative response to HIV-1 antigens after 1 year of highly active antiretroviral treatment in early HIV-1 disease. Immunology Study Group of Spanish EARTH-1 Study. Lancet 352, 1194–1195.

Poli, G., Kinter, A., Justement, J.S., Kehrl, J.H., Bressler, P., Stanley, S., Fauci, A.S., 1990a. Tumor necrosis factor alpha functions in an autocrine manner in the induction of human immunodeficiency virus expression. Proc. Natl. Acad. Sci. USA 87, 782–785.

Poli, G., Bressler, P., Kinter, A., Duh, E., Timmer, W.C., Rabson, A., Justement, J.S., Stanley, S., Fauci, A.S., 1990b. Interleukin 6 induces human immunodeficiency virus expression in infected monocytic cells alone and in synergy with tumor necrosis factor alpha by transcriptional and post-transcriptional mechanisms. J. Exp. Med. 172, 151–158.

Poli, G., Kinter, A.L., Justement, J.S., Bressler, P., Kehrl, J.H., Fauci, A.S., 1991. Transforming growth factor beta suppresses human immunodeficiency virus expression and replication in infected cells of the monocyte/macrophage lineage. J. Exp. Med. 173, 589–597.

Poli, G., Kinter, A.L., Fauci, A.S., 1994. Interleukin 1 induces expression of the human immunodeficiency virus alone and in synergy with interleukin 6 in chronically infected U1 cells: inhibition of inductive effects by the interleukin 1 receptor antagonist. Proc. Natl. Acad. Sci. USA 91, 108–112.

Pomerantz, R.J., Trono, D., Feinberg, M.B., Baltimore, D., 1990a. Cells nonproductively infected with HIV-1 exhibit an aberrant pattern of viral RNA expression: a molecular model for latency. Cell 61, 1271–1276.

Pomerantz, R.J., Feinberg, M.B., Trono, D., Baltimore, D., 1990b. Lipopolysaccharide is a potent monocyte/macrophage-specific stimulator of human immunodeficiency virus type 1 expression. J. Exp. Med. 172, 253–261.

Pomerantz, R.J., Seshamma, T., Trono, D., 1992. Efficient replication of human immunodeficiency virus type 1 requires a threshold level of Rev: potential implications for latency. J. Virol. 66, 1809–1813.

Prins, J.M., Jurriaans, S., van Praag, R.M., Blaak, H., van Ri, J.R., Schellekens, P.T., ten Berge, I.J., Yong, S.L., Fox, C.H., Roos, M.T., de Wolf, F., Goudsmit, J., Schuitemaker, H., Lange, J.M., 1999. Immuno-activation with anti-CD3 and recombinant human IL-2 in HIV-1-infected patients on potent antiretroviral therapy. AIDS 13, 2405–2410.

Qatsha, K.A., Rudolph, C., Marme, D., Schachtele, C., May, W.S., 1993. Gö 6976, a selective inhibitor of protein kinase C, is a potent antagonist of human immunodeficiency virus 1 induction from latent/low-level-producing reservoir cells in vitro. Proc. Natl. Acad. Sci. USA 90, 4674–4678.

Rabbi, M.F., Finnegan, A., Al-Harthi, L., Song, S., Roebuck, K.A., 1998. Interleukin-10 enhances tumor necrosis factor-alpha activation of HIV-1 transcription in latently infected T cells. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. 19, 321–331.

Ramezani, A., Ding, S.F., Joshi, S., 1997. Inhibition of HIV-1 replication by retroviral vectors expressing monomeric and multimeric hammerhead ribozymes. Gene Ther. 4, 861–867.

Ramratnam, B., Mittler, J.E., Zhang, L., Boden, D., Hurley, A., Fang, F., Macken, C.A., Perelson, A.S., Markowitz, M., Ho, D.D., 2000. The decay of the latent reservoir of replication-competent HIV-1 is inversely correlated with the extent of residual viral replication during prolonged anti-retroviral therapy. Nat. Med. 6, 82–85.

Rieckmann, P., Poli, G., Kehrl, J.H., Fauci, A.S., 1991. Activated B lymphocytes from human immunodeficiency virus-infected individuals induce virus expression in infected T cells and a promonocytic cell line, U1. J. Exp. Med. 173, 1–5.

Riley, J.L., Levine, B.L., Craighead, N., Francomano, T., Kim, D., Carroll, R.G., June, C.H., 1998. Naive and memory CD4 T cells differ in their susceptibilities to human immunodeficiency virus type 1 infection following CD28 costimulation: implications for transmission and pathogenesis. J. Virol. 72, 8273–8280.

Roederer, M., Raju, P.A., Staal, F.J., Herzenberg, L.A., Herzenberg, L.A., 1991. N-acetyl cysteine inhibits latent HIV expression in chronically infected cells. AIDS Res. Hum. Retroviruses 7, 563–567.

Roederer, M., Raju, P.A., Mitra, D.K., Herzenberg, L.A., Herzenberg, L.A., 1997. HIV does not replicate in naive CD4 T cells stimulated with CD3/CD28. J. Clin. Invest. 99, 1555–1564.

Rosenberg, E.S., Billingsley, J.M., Caliendo, A.M., Boswell, S.L., Sax, P.E., Kalams, S.A., Walker, B.D., 1997. Vigorous HIV-1-specific CD4 + T cell responses associated with control of viremia. Science 278, 1447–1450.

Rosok, B., Brinchmann, J.E., Voltersvik, P., Olofsson, J., Bostad, L., Asjo, B., 1997a. Correlates of latent and productive HIV type-1 infection in tonsillar CD4(+) T cells. Proc. Natl. Acad. Sci. USA 94, 9332–9336.

Rosok, B., Voltersvik, P., Larsson, B.M., Albert, J., Brinchmann, J.E., Asjo, B., 1997b. CD8 + T cells from HIV type 1-seronegative individuals suppress virus replication in acutely infected cells. AIDS Res. Hum. Retroviruses 13, 79–85.

Rossi, C., Balboni, P.G., Betti, M., Marconi, P.C., Bozzini, R., Grossi, M.P., Barbanti-Brodano, G., Caputo, A., 1997. Inhibition of HIV-1 replication by a Tat transdominant negative mutant in human peripheral blood lymphocytes from healthy donors and HIV-1-infected patients. Gene Ther. 4, 1261–1269.

Roulston, A., D'Addario, M., Boulerice, F., Caplan, S., Wainberg, M.A., Hiscott, J., 1992. Induction of monocytic differentiation and NF-kappa B-like activities by human immunodeficiency virus 1 infection of myelomonoblastic cells. J. Exp. Med. 175, 751–763.

San Jose, E., Munoz-Fernandez, M.A., Alarcon, B., 1997. Megalomicin inhibits HIV-1 replication and interferes with gp160 processing. Virology 239, 303–314.

Sato, T., Asamitsu, K., Yang, J.P., Takahashi, N., Tetsuka, T., Yoneyama, A., Kanagawa, A., Okamoto, T., 1998. Inhibition of human immunodeficiency virus type 1 replication by a bioavailable serine/threonine kinase inhibitor, fasudil hydrochloride. AIDS Res. Hum. Retroviruses 14, 293–298.

Schnell, M.J., Johnson, J.E., Buonocore, L., Rose, J.K., 1997. Construction of a novel virus that targets HIV-1-infected cells and controls HIV-1 infection. Cell 90, 849–857.

Schnittman, S.M., Psallidopoulos, M.C., Lane, H.C., Thompson, L., Baseler, M., Massari, F., Fox, C.H., Salzman, N.P., Fauci, A.S., 1989. The reservoir for HIV-1 in human peripheral blood is a T cell that maintains expression of CD4. Science 245, 305–308.

Schnittman, S.M., Singer, K.H., Greenhouse, J.J., Stanley, S.K., Whichard, L.P., Le, P.T., Haynes, B.F., Fauci, A.S., 1991. Thymic microenvironment induces HIV expression. Physiologic secretion of IL-6 by thymic epithelial cells up-regulates virus expression in chronically infected cells. J. Immunol. 147, 2553–2558.

Schrager, L.K., D'Souza, M.P., 1998. Cellular and anatomical reservoirs of HIV-1 in patients receiving potent antiretroviral combination therapy. J. Am. Med. Assoc. 280, 67–71.

Semenzato, G., Agostini, C., Chieco-Bianchi, L., De Rossi, A., 1998. HIV load in highly purified CD8 + T cells retrieved from pulmonary and blood compartments. J. Leukoc. Biol. 64, 298–301.

Seshamma, T., Bagasra, O., Trono, D., Baltimore, D., Pomerantz, R.J., 1992. Blocked early-stage latency in the peripheral blood cells of certain individuals infected with human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. USA 89, 10663–10667.

Shapiro, L., Heidenreich, K.A., Meintzer, M.K., Dinarello, C.A., 1998a. Role of p38 mitogen-activated protein kinase in HIV type 1 production in vitro. Proc. Natl. Acad. Sci. USA 95, 7422–7426.

Shapiro, L., Puren, A.J., Barton, H.A., Novick, D., Peskind, R.L., Shenkar, R., Gu, Y., Su, M.S., Dinarello, C.A., 1998b. Interleukin 18 stimulates HIV type 1 in monocytic cells. Proc. Natl. Acad. Sci. USA 95, 12550–12555.

Sharkey, M.E., Teo, I., Greenough, T., Sharova, N., Luzuriaga, K., Sullivan, J.L., Bucy, R.P., Kostrikis, L.G., Haase, A., Veryard, C., Davaro, R.E., Cheeseman, S.H., Daly, J.S., Bova, C., Ellison 3rd, R.T., Mady, B., Lai, K.K., Moyle, G., Nelson, M., Gazzard, B., Shaunak, S., Stevenson, M., 2000. Persistence of episomal HIV-1 infection intermediates in patients on highly active anti-retroviral therapy. Nat. Med. 6, 76–81.

Shattock, R.J., Rizzardi, G.P., Hayes, P., Griffin, G.E., 1996. Engagement of adhesion molecules (CD18, CD11a, CD45, CD44, and CD58) enhances human immunodeficiency virus type 1 replication in monocytic cells through a tumor necrosis factor-modulated pathway. J. Infect. Dis. 174, 54–62.

Smithgall, M.D., Wong, J.G., Critchett, K.E., Haffar, O.K., 1996. IL-7 up-regulates HIV-1 replication in naturally infected peripheral blood mononuclear cells. J. Immunol. 156, 2324–2330.

Song, S.K., Li, H., Cloyd, M.W., 1996. Rates of shutdown of HIV-1 into latency: roles of the LTR and tat/rev/vpu gene region. Virology 225, 377–386.

Soudeyns, H., Campi, G., Rizzardi, G.P., Lenge, C., Demarest, J.F., Tambussi, G., Lazzarin, A., Kaufmann, D., Casorati, G., Corey, L., Pantaleo, G., 2000. Initiation of antiretroviral therapy during primary HIV-1 infection induces rapid stabilization of the T-cell receptor beta chain repertoire and reduces the level of T-cell oligoclonality. Blood 95, 1743–1751.

Spiegel, H., Herbst, H., Niedobitek, G., Foss, H.D., Stein, H., 1992. Follicular dendritic cells are a major reservoir for human immunodeficiency virus type 1 in lymphoid tissues facilitating infection of CD4 + T-helper cells. Am. J. Pathol. 140, 15–22.

Spina, C.A., Kwoh, T.J., Chowers, M.Y., Guatelli, J.C., Richman, D.D., 1994. The importance of nef in the induction of human immunodeficiency virus type 1 replication from primary quiescent CD4 lymphocytes. J. Exp. Med. 179, 115–123.

Spina, C.A., Guatelli, J.C., Richman, D.D., 1995. Establishment of a stable, inducible form of human immunodeficiency virus type 1 DNA in quiescent CD4 lymphocytes in vitro. J. Virol. 69, 2977–2988.

Spina, C.A., Prince, H.E., Richman, D.D., 1997. Preferential replication of HIV-1 in the CD45RO memory cell subset of

primary CD4 lymphocytes in vitro. J. Clin. Invest. 99, 1774–1785.

Stanley, S.K., Bressler, P.B., Poli, G., Fauci, A.S., 1990. Heat shock induction of HIV production from chronically infected promonocytic and T cell lines. J. Immunol. 145, 1120–1126.

Staprans, S.I., Hamilton, B.L., Follansbee, S.E., Elbeik, T., Barbosa, P., Grant, R.M., Feinberg, M.B., 1995. Activation of virus replication after vaccination of HIV-1-infected individuals. J. Exp. Med. 182, 1727–1737.

Stellbrink, H.J., van Lunzen, J., Hufert, F.T., Froschle, G., Wolf-Vorbeck, G., Zollner, B., Albrecht, H., Greten, H., Racz, P., Tenner-Racz, K., 1997. Asymptomatic HIV infection is characterized by rapid turnover of HIV RNA in plasma and lymph nodes but not of latently infected lymph-node CD4+ T cells. AIDS 11, 1103–1110.

Stellbrink, H.J., Hufert, F.T., Tenner-Racz, K., Lauer, J., Schneider, C., Albrecht, H., Racz, P., van Lunzen, J., 1998. Kinetics of productive and latent HIV infection in lymphatic tissue and peripheral blood during triple-drug combination therapy with or without additional interleukin-2. Antivir. Ther. 3, 209–214.

Stevenson, M., Stanwick, T.L., Dempsey, M.P., Lamonica, C.A., 1990. HIV-1 replication is controlled at the level of T cell activation and proviral integration. EMBO J. 9, 1551–1560.

Sun, S.-C., Ganchi, A., Ballard, D.W., Green, W.C., 1993. NF-κB controls expression of inhibitor IκBα: evidence for an inducible autoregulatory pathway. Science 259, 1912–1915.

Sun, Y., Clark, E.A., 1999. Expression of the c-myc proto-oncogene is essential for HIV-1 infection in activated T cells. J. Exp. Med. 189, 1391–1398.

Tang, S., Patterson, B., Levy, J.A., 1995. Highly purified quiescent human peripheral blood CD4+ T cells are infectible by human immunodeficiency virus but do not release virus after activation. J. Virol. 69, 5659–5665.

Tenner-Racz, K., Stellbrink, H.J., van Lunzen, J., Schneider, C., Jacobs, J.P., Raschdorff, B., Grosschupff, G., Steinman, R.M., Racz, P., 1998. The unenlarged lymph nodes of HIV-1-infected, asymptomatic patients with high CD4 T cell counts are sites for virus replication and CD4 T cell proliferation. The impact of highly active antiretroviral therapy. J. Exp. Med. 187, 949–959.

Thali, M., 1995. Cyclosporins: immunosuppressive drugs with anti-HIV-1 activity. Mol. Med. Today 1, 287–291.

Toossi, Z., Nicolacakis, K., Xia, L., Ferrari, N.A., Rich, E.A., 1997. Activation of latent HIV-1 by Mycobacterium tuberculosis and its purified protein derivative in alveolar macrophages from HIV-infected individuals in vitro. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. 15, 325–331.

Tozzi, V., Britton, S., Ehrnst, A., Lenkei, R., Strannegard, O., 1989. Persistent productive HIV infection in EBV-transformed B lymphocytes. J. Med. Virol. 27, 19–24.

Traber, K.E., Okamoto, H., Kurono, C., Baba, M., Saliou, C., Soji, T., Packer, L., Okamoto, T., 1999. Anti-rheumatic compound aurothioglucose inhibits tumor necrosis factor-alpha-induced HIV-1 replication in latently infected OM10.1 and Ach2 cells. Int. Immunol. 11, 143–150.

Truong, M.J., Darcissac, E.C., Hermann, E., Dewulf, J., Capron, A., Bahr, G.M., 1999. Interleukin-16 inhibits human immunodeficiency virus type 1 entry and replication in macrophages and in dendritic cells. J. Virol. 73, 7008–7013.

Turpin, J.A., Buckheit, R.W., Jr, Derse., D., Hollingshead, M., Williamson, K., Palamone, C., Osterling, M.C., Hill, S.A., Graham, L., Schaeffer, C.A., Bu, M., Huang, M., Cholody, W.M., Michejda, C.J., Rice, W.G., 1998. Inhibition of acute-, latent-, and chronic-phase human immunodeficiency virus type 1 (HIV-1) replication by a bistriazoloacridone analog that selectively inhibits HIV-1 transcription. Antimicrob. Agents Chemother. 42, 487–494.

Unutmaz, D., KewalRamani, V.N., Marmon, S., Littman, D.R., 1999. Cytokine signals are sufficient for HIV-1 infection of resting human T lymphocytes. J. Exp. Med. 189, 1735–1746.

Van Lint, C., Emiliani, S., Ott, M., Verdin, E., 1996. Transcriptional activation and chromatin remodeling of the HIV-1 promoter in response to histone acetylation. EMBO J. 15, 1112–1120.

Verdin, E., Paras, P., Jr, Van Lint, C., 1993. Chromatin disruption in the promoter of human immunodeficiency virus type 1 during transcriptional activation. EMBO J. 12, 3249–3259.

Viglianti, G.A., Parada, N.A., Maciaszek, J.W., Kornfeld, H., Center, D.M., Cruikshank, W.W., 1997. IL-16 anti-HIV-1 therapy. Nat. Med. 3, 938.

Vlach, J., Pitha, P.M., 1993. Hexamethylene bisacetamide activates the human immunodeficiency virus type 1 provirus by an NF-kappa B-independent mechanism. J. Gen. Virol. 74, 2401–2408.

Vyakarnam, A., McKeating, J., Meager, A., Beverley, P.C., 1990. Tumour necrosis factors (alpha, beta) induced by HIV-1 in peripheral blood mononuclear cells potentiate virus replication. AIDS 4, 21–27.

Wahl, S.M., Orenstein, J.M., 1997. Immune stimulation and HIV-1 viral replication. J. Leukoc. Biol. 62, 67–71.

Walker, C.M., Levy, J.A., 1989. A diffusible lymphokine produced by CD8+ T lymphocytes suppresses HIV replication. Immunology. 66, 628–630.

Walker, C.M., Erickson, A.L., Hsueh, F.C., Levy, J.A., 1991. Inhibition of human immunodeficiency virus replication in acutely infected CD4+ cells by CD8+ cells involves a noncytotoxic mechanism. J. Virol. 65, 5921–5927.

Wallace, D.L., Matear, P.M., Davies, D.C., Hicks, R., Lebosse, C., Eyeson, J., Beverley, P.C., Vyakarnam, A., 2000. CD7 expression distinguishes subsets of CD4(+) T cells with distinct functional properties and ability to support replication of HIV-1. Eur. J. Immunol. 30, 577–585.

Wei, P., Garber, M.E., Fang, S.M., Fischer, W.H., Jones, K.A., 1998. A novel CDK9-associated C-type cyclin interacts directly with HIV-1 Tat and mediates its high-affinity, loop-specific binding to TAR RNA. Cell 92, 451–462.

Wei, X., Ghosh, S.K., Taylor, M.E., Johnson, V.A., Emini, E.A., Deutsch, P., Lifson, J.D., Bonhoeffer, S., Nowak, M.A., Hahn, B.H., Shaw, G.M., 1995. Viral dynamics in human immunodeficiency virus type 1 infection. Nature 373, 117–122.

Weissman, D., Poli, G., Fauci, A.S., 1994. Interleukin 10 blocks HIV replication in macrophages by inhibiting the autocrine loop of tumor necrosis factor alpha and interleukin 6 induction of virus. AIDS Res. Hum. Retroviruses 10, 1199–1206.

Weissman, D., Daucher, J., Barker, T., Adelsberger, J., Baseler, M., Fauci, A.S., 1996. Cytokine regulation of HIV replication induced by dendritic cell-CD4-positive T cell interactions. AIDS Res. Hum. Retroviruses 12, 759–767.

Wilkinson, J., Zaunders, J.J., Carr, A., Cooper, D.A., 1999. CD8 + anti-human immunodeficiency virus suppressor activity (CASA) in response to antiretroviral therapy: loss of CASA is associated with loss of viremia. J. Infect. Dis. 180, 68–75.

Winslow, B.J., Pomerantz, R.J., Bagasra, O., Trono, D., 1993. HIV-1 latency due to the site of proviral integration. Virology 196, 849–854.

Witvrouw, M., Daelemans, D., Pannecouque, C., Neyts, J., Andrei, G., Snoeck, R., Vandamme, A.M., Balzarini, J., Desmyter, J., Baba, M., De Clercq, E., 1998. Broad-spectrum antiviral activity and mechanism of antiviral action of the fluoroquinolone derivative K-12. Antiviral Chem. Chemother. 9, 403–411.

Wong, J.K., Hezareh, M., Gunthard, H.F., Havlir, D.V., Ignacio, C.C., Spina, C.A., Richman, D.D., 1997. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. Science 278, 1291–1295.

Wong-Staal, F., Poeschla, E.M., Looney, D.J., 1998. A controlled, Phase 1 clinical trial to evaluate the safety and effects in HIV-1 infected humans of autologous lymphocytes transduced with a ribozyme that cleaves HIV-1 RNA. Hum. Gene Ther. 9, 2407–2425.

Woods, T.C., Roberts, B.D., Butera, S.T., Folks, T.M., 1997. Loss of inducible virus in CD45RA naive cells after human immunodeficiency virus-1 entry accounts for preferential viral replication in CD45RO memory cells. Blood 89, 1635–1641.

Yamaguchi, Y., Takagi, T., Wada, T., Yano, K., Furuya, A., Sugimoto, S., Hasegawa, J., Handa, H., 1999. NELF, a multisubunit complex containing RD, cooperates with DSIF to repress RNA polymerase II elongation. Cell 97, 41–51.

Yang, L.P., Riley, J.L., Carroll, R.G., June, C.H., Hoxie, J., Patterson, B.K., Ohshima, Y., Hodes, R.J., Delespesse, G., 1998. Productive infection of neonatal CD8 + T lymphocytes by HIV-1. J. Exp. Med. 187, 1139–1144.

Zack, J.A., Arrigo, S.J., Weitsman, S.R., Go, A.S., Haislip, A., Chen, I.S., 1990. HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. Cell 61, 213–222.

Zack, J.A., Haislip, A.M., Krogstad, P., Chen, I.S., 1992. Incompletely reverse-transcribed human immunodeficiency virus type 1 genomes in quiescent cells can function as intermediates in the retroviral life cycle. J. Virol. 66, 1717–1725.

Zaunders, J.J., Cunningham, P.H., Kelleher, A.D., Kaufmann, G.R., Jaramillo, A.B., Wright, R., Smith, D., Grey, P., Vizzard, J., Carr, A., Cooper, D.A., 1999. Potent antiretroviral therapy of primary human immunodeficiency virus type 1 (HIV-1) infection: partial normalization of T lymphocyte subsets and limited reduction of HIV-1 DNA despite clearance of plasma viremia. J. Infect. Dis. 180, 320–329.

Zhang, J.L., Sharma, P.L., Li, C.J., Dezube, B.J., Pardee, A.B., Crumpacker, C.S., 1997. Topotecan inhibits human immunodeficiency virus type 1 infection through a topoisomerase-independent mechanism in a cell line with altered topoisomerase I. Antimicrob. Agents Chemother. 41, 977–981.

Zhang, L., Ramratnam, B., Tenner-Racz, K., He, Y., Vesanen, M., Lewin, S., Talal, A., Racz, P., Perelson, A.S., Korber, B.T., Markowitz, M., Ho, D.D., 1999a. Quantifying residual HIV-1 replication in patients receiving combination antiretroviral therapy. N. Engl. J. Med. 340, 1605–1613.

Zhang, Z., Schuler, T., Zupancic, M., Wietgrefe, S., Staskus, K.A., Reimann, K.A., Reinhart, T.A., Rogan, M., Cavert, W., Miller, C.J., Veazey, R.S., Notermans, D., Little, S., Danner, S.A., Richman, D.D., Havlir, D., Wong, J., Jordan, H.L., Schacker, T.W., Racz, P., Tenner-Racz, K., Letvin, N.L., Wolinsky, S., Haase, A.T., 1999b. Sexual transmission and propagation of SIV and HIV in resting and activated CD4 + T cells. Science 286, 1353–1357.

Zhou, M., Halanski, M.A., Radonovich, M.F., Kashanchi, F., Peng, J., Price, D.H., Brady, J.N., 2000. Tat modifies the activity of CDK9 to phosphorylate serine 5 of the RNA polymerase II carboxyl-terminal domain during human immunodeficiency virus type 1 transcription. Mol. Cell. Biol. 20, 5077–5086.

Zhou, P., Goldstein, S., Devadas, K., Tewari, D., Notkins, A.L., 1997. Human CD4 + cells transfected with IL-16 cDNA are resistant to HIV-1 infection: inhibition of mRNA expression. Nat. Med. 3, 659–664.

Zhou, P., Devadas, K., Tewari, D., Jegorow, A., Notkins, A.L., 1999. Processing, secretion, and anti-HIV-1 activity of IL-16 with or without a signal peptide in CD4 + T cells. J. Immunol. 163, 906–912.

Zhu, Y., Pe'ery, T., Peng, J., Ramanathan, Y., Marshall, N., Marshall, T., Amendt, B., Mathews, M.B., Price, D.H., 1997. Transcription elongation factor P-TEFb is required for HIV-1 tat transactivation in vitro. Genes Dev. 11, 2622–2632.