Negative Regulation of HIV Expression in Monocytes

By

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DEDICATION

To my mother, Gloria Furr-Fornshill, who gave us the freedom to pursue individual goals and the support to accomplish them and to my mentor, Francis W. Ruscetti, a risk taker, with an unwaivering passion for science and uncompromising pursuit of excellence, without whom I would have neither undertaken nor accomplished this goal.

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ABSTRACT

The retrovirus, human immunodeficiency virus 1 (HIV-1), the causative agent of AIDS is a member of the nontransforming and cytopathic animal lentivirus family. A characteristic feature of AIDS is the long latency period prior to the onset of clinical symptoms. As part of the overall objective of this research, a model of latent infection was developed in the monocytoid cell line, THP-1. In THP-1, viral expression can be regulated in several ways: (a) latency (no viral expression); (b) restricted expression (chronic low level viral expression with little or no detectable virus released); and (c) continuous production. In cells with restricted HIV expression, nuclear factor(s) were found that blocked DNA binding complex formation indicating that viral transcription was negatively regulated. Also, viral particles were seen budding into and accumulating within intracytoplasmic vacuoles with little virus released, suggesting multiple levels of regulation. These cultures with restricted expression had no detectable viral antigens on the cell surface and were not lysed by IL-2 activated large granular lymphocyte (LGL), while the productively infected cells were efficiently lysed. Cells with restricted expression could cause viral-mediated T cell cytolysis in cell-cell assays suggesting that monocytes with restricted expression can transmit virus and evoke T cell pathology upon cell contact, while evading the immune

systems. In addition, cells with latent HIV were identified which could still produce infectious virus after 5-azacytidine exposure or Con A activated T cell coculture. LPS and other treatments could increase viral production in cells with restricted but not latent expression. Thus, both restricted and latent HIV expression can occur in monocytes, probably by distinct mechanisms, leading to viral persistance.

The ability of Con A-activated T cells to induce HIV expression from latent THP-1 afforded us the opportunity to examine the physiologic relevance of this model. Using PCR analysis, results showed that monocytes from 3 of 21 patients had both HIV DNA and RNA, 6 contained HIV DNA but no detectable RNA and 12 contained neither HIV DNA nor RNA. After co-culture of these monocytes with Con A-activated T cells from HIV-negative normal donors, the 6 samples and THP-1 containing HIV DNA but no RNA were now positive for RNA and p24. Further, 8/12 samples in which HIV DNA originally could not be detected now expressed both HIV RNA and p24. Neither Con A by itself, resting T cells nor conA treated T cell supernatants induced HIV expression. Cell-cell contact was required since activated T cells, separated from monocytes by a membrane, failed to induce HIV expression. Plasma membranes from Con A-activated T cells stimulated HIV expression, suggesting cell contact induces factor(s) in monocytes capable of overcoming latency. Thus,

monocytes in AIDS patients can harbor latent HIV, which can be induced by T cells during an immune response. The ability of HIV produced by such monocytes to infect T cells leading to viral-induced pathology, suggests that latently infected monocytes play a role in the slow, chronic pathogenesis of AIDS.

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ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
AZT	Azidothymidine
BSA	bovine serum albumin
CAEV	Caprine arthritis encephalitis virus
CON A	Conconavalin A
CPM	counts per minute
cDNA	copy deoxyribonucleic acid
DEAE	diethylaminoethyl
DNA	deoxyribonucleic acid
EIAV	equine infectious anemia virus
FACS	fluorescent activated cell sorter
FCS	fetal calf serum
FITC	fluorescein isothiocyanate
GM-CSF	granulocyte/macrophage colony stimulating factor
HIV-1	human immunodeficiency virus-1
HIV-2	human immunodeficiency virus-2
IgG	immunoglobulin
IFN	interferon
NK	natural killer cell
LGL	large granular lymphocyte
LPS	lipopolysaccaride
LTHP-1	HIV infected THP-1 with latent expression
LTR	long terminal repeat
MOI	multiplicity of infection
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-

	diphenyltetrazoliumbromide
NFκB	nuclear factor kappa B
NMDA	N-methyl-D-aspartate
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PHA	phytohemataglutinin
PIPES	piperazine-N,N'-bis(2-ethanesulfonic acid)
PMA	Phorbol myristic acid
RNA	ribonucleic acid
RT	reverse transcriptase
RTHP-1	HIV infected THP-1 with restriced expression
RPMI 1640	Roswell Park Memorial Institute, media formulation #1640
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TAQ	thermus aquaticus
TAR	transactivation response element
TNF	tumor necrosis factor
TCID-50	tissue culture infective dose 50% endpoint
XTT	(2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide)

INTRODUCTION

Retroviruses are a large family of RNA viruses that cause a number of pathologic disorders. The Retrovirus family is composed of three subfamilies: Oncoviruses, Spumaviruses, and Lentiviruses (1). Retroviruses differ from other viruses in that they replicate via a DNA intermediate. The provirus can be stably integrated into the chromosomes of somatic or germ line cells. Unlike other retroviruses, which require dividing cells to replicate, ungulate lentiviruses such as visna-maedi, caprine arthritis-encephalitis virus and equine infectious anemia virus, replicate well in non-dividing end stage cells both in vivo and in vitro. Another significant difference in replication between oncogenic retroviruses and these lentiviruses is that the oncogenic retroviruses synthesize proviral DNA in the cytoplasm while lentiviruses synthesize proviral DNA almost exclusively in the nucleus.

Retroviruses have the unique ability to transduce host cellular genes and rely on host mechanisms under the direction of viral genes to regulate viral gene expression. They are the only RNA viruses known to cause neoplasias. The oncoviruses, such as Rous sarcoma virus, induce neoplasias acutely through oncogenes. Oncogenesis occurs by transduction or insertional mutation of normal cellular genes which are involved in normal growth control (2). Other retroviruses, such as HTLV-1 (3) induce neoplasias

chronically by indirect mechanisms which are not well understood. Spumaviruses have not as yet been associated with human disease, while members of the lentivirus family cause various chronic diseases in their hosts. Retroviruses are classified on the basis of their biology, electron microscopy, and genomic structure.

Lentiviruses are exogenous, cytopathic and non-oncogenic (4). These viruses, such as visna in sheep (5), equine infectious anemia virus (EIAV) in horses (6), and caprine arthritis encephalitis virus (CAEV) in goats (7), cause chronic diseases affecting the lungs, joints, nervous, hematopoietic and immune systems in their hosts. Recently, several new members of the lentivirus family have been isolated and characterized. These viruses, including simian immunodeficiency virus (SIV) (8,9,10), feline immunodeficiency virus (FIV) (11), and human immunodeficiency virus (HIV) (12,13,14), are closely related and all cause a profound immunodeficiency in the infected host. On the basis of many parameters, HIV, (the etiologic agent of AIDS), has been classified as a member of the lentivirus subfamily. First, the biological effects of infection are similar to other members of the lentiviral family. These include: slow infection; the time from initial infection to clinically observed symptoms is usually measured in years; and pathogenesis involving both immunological and neurological manifestations. In addition,

HIV has other biological properties in common with visna such as syncytia formation and cytopathic effects in tissue culture. On the basis of morphological analysis by electron microscopy, the HIV retroviral particle with its bar-shaped dense central core structure, whose structural elements are encoded by the gag gene, most closely resembles members of the lentivirus family, such as visna and equine infectious anemia virus. In addition, these viruses all share similar polypeptide composition, large envelope glycoproteins, antigenic determinants in the major structural protein (gag), and similar size and structure of their genomes. Further, the HIV provirus contains the unusual $tRNA^{lys}$ primer binding site, which is used as a primer for DNA synthesis, a feature shared by the visna virus. Most convincing, nucleotide sequence analysis of the complete genomes of visna, EIAV, and HIV show significant nucleotide and amino acid homologies mainly in conserved regions of gag-pol (15) leading to the conclusion that HIV is a member of the lentivirus family of retroviruses.

Retroviral Life Cycle. The extracellular virus is composed of two copies of a single-stranded RNA genome wrapped in a core of viral proteins. This core is surrounded by an envelope studded with viral glycoproteins derived from the membranes of previous host cells. The lifecycle of a retrovirus involves binding of the virus to specific receptors on the cell surface by viral envelope proteins.

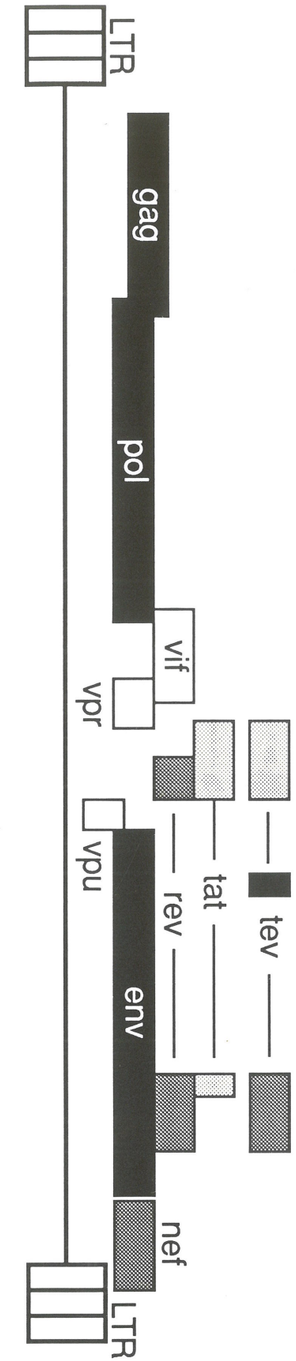
In the case of HIV, viral infection begins with the envelope proteins gp120 and gp41 binding to the CD4 antigen (16,17,18) on uninfected cells. Next, the viral lipid bilayer fuses with the lipid bilayer of the uninfected cell. The virus enters the cell through membrane-to-membrane fusion, in a mechanism similar to receptor mediated endocytosis. At this time, the virion and possibly the capsid itself are expelled into the cell. The virion is uncoated by a proteolytic event directed by the viral protease. The RNA, nucleocapsid proteins, reverse transcriptase (RT), protease, and integrase are released into the cytoplasm where the retroviral RNA is converted into double stranded DNA in a process executed by two enzymes, polymerase and ribonuclease (1). Next, the DNA usually integrates into the host's chromosomal DNA, a process carried out by the integrase enzyme. Once the provirus is integrated, the virus becomes highly dependent on the host cell's machinery for functions such as, transcription of the provirus by RNA polymerase II; processing of RNA transcripts by mechanisms normally used to cap, polyadenylate, and splice host RNA; and translation of resulting messenger RNAs by host cell polyribosomes. The primary RNA transcript of the provirus made by the host polymerse II provides both the mRNA for the synthesis of viral polyproteins and the viral RNA which will be incorporated into new virions. The RNA and core proteins

associate with envelope glycoproteins embedded in an area of the plasma membrane of the host cell that is highly concentrated in viral elements. This area then evaginates from the cell and is released as small densely packed spherical particles, 0.1 micron in diameter, in a process known as budding, resulting in the production of new virions. Subsequently, in another envelope protein dependent process, infected cells can fuse with uninfected cells leading to multinucleated giant cell formation and cell death.

Genomic Organization of Lentiviruses. The genomes of lentiviruses are quite complex. The most complex genome at present, HIV is known to contain seven additional regulatory genes (19). A schematic of the organization of the HIV-1 genome is shown in Figure 1.

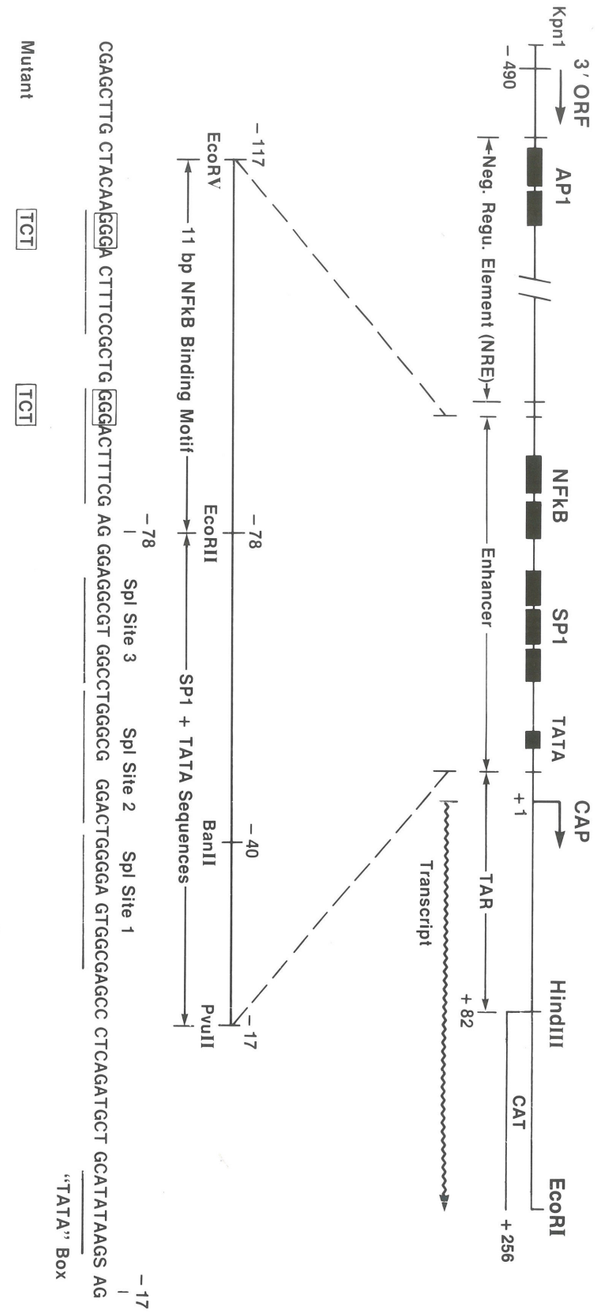
The HIV provirus is 9.7 kB in length. Its contains structural genes in the order 5'-gag-pol-env-3'. Although all replication competent retroviruses contain these genes, each family of retroviruses is unique in its structural features and mechanisms of gene expression. The proviral DNA is terminally redundant. Each end contains repeats called long terminal repeats (LTRs). LTRs contain viral regulatory elements including promoter sequences and comprise U3, R, and U5 (unique 3', repeat, unique 5') sequences. The HIV LTR contains R and U5 regions of 98 and 84 bp respectively (20). In the R region at both the 5' and

Figure 1. Schematic representation of the HIV-1 proviral genome.



3' ends of the RNA are sequences that contain the cap site, the polyadenylation signal (AATAAA) and the termination signal for viral RNA transcription. A schematic of the HIV-1 LTR is shown in Figure 2. Sequences at the 3' end of the viral RNA in the U3 region contain enhancer-promoter elements for initiation of RNA transcription. Transcription begins in the 5'LTR enhancer region of HIV-1. A TATA sequence 22-27 bp 5' to the RNA start site is the recognition signal (promoter) for RNA transcription. In addition to the TATA box, the HIV-1 enhancer region also contains two copies of the 11-base pair (bp) repeat which binds NF-κB (-117 to -78) (21), three SP1 sites (-45 to -77) (22). Unlike several other LTRs, the HIV LTR contains no CAAT sequence. Further upstream of the enhancer region in the HIV-1 LTR a negative regulatory element (NRE) is located (-340 to -156). An AP-1 site overlaps the NRE (-340 to -278). Downstream of the transcription start site within the 5' untranslated region are the leader region (-2 to +21), which has been shown to interact with several cellular proteins (23) and the transactivation response element (tar, +19 to +42) which is required for the action of the virally encoded tat protein (24). Recently, a sequence has been identified (-5 to +82) termed inducer of short transcripts (IST), which significantly enhances the activity of the HIV-1 promoter (25). The interactions of viral and cellular proteins with these regions of the LTR regulate expression

Figure 2. A schematic representation of HIV-1 LTR sequences. Three separate regions are defined within the LTR: NRE (nt -350 to -137), enhancer (nt -117 to -17) and tar (-17 to +82). Then NRE contains AP1 and IL-2/IFNα-responsive sequences. The enhancer contains NFκB, SP1 and TATA sequences and the tar region contains numerous sequences which recognize transcriptional activators.



of HIV-1.

The HIV gag gene (1536 nucleotides) encodes three proteins, p24, p17 and p15 which are synthesized as a 55kD polypeptide precursor and proteolytically cleaved by the virally encoded protease into the individual gag proteins. The pol gene (3045 nucleotides) overlaps gag and is in a different reading frame. It is translated only as a gag/pol fusion polyprotein p160. p160 is made as a result of a frameshift in a region following p24 at a specific TTA sequence located 79 nucleotides upstream of the gag termination codon. This frameshift occurs approximately 10% of the time (20). It encodes a protease, RT (p68/51), endonuclease (p34) and integrase enzymes. The pol and env genes do not overlap and are separated by a segment of DNA with several open reading frames. The envelope protein is made by a single splicing event as a single, large polypeptide which contains 28 glycosylation sites and, thus, is heavily glycosylated by the cellular machinery. It is cleaved intracellularly from gp160 into an extracellular gp120 and a transmembrane gp41 (which are not covalently associated) and then transported to the plasma membrane of infected cells, where viral budding results in its presentation on the outer surface of the mature virion. Cellular enzymes execute post-translational modifications of the polyproteins such as glycosylation of the env proteins and myristoylation of the gag p17 protein. These

modifications are essential for the attachment of these proteins to the cell membrane. The viral lipid membrane is lined on the inner surface by the gag encoded p17 protein.

HIV Regulatory Genes. Regulation of viral replication involves the interplay between viral genes and host cell genes. HIV contains several novel regulatory proteins encoded by the open reading frames between pol and env. Two of these genes encode small nuclear regulatory proteins, Tat and Rev, which are essential for virus replication (26,27). Tat is a 16 kilodalton 86 amino acid protein which is produced from overlapping reading frames by multiply spliced messenger RNAs (mRNAs) (Figure 1). The tat gene is derived from sequences at the 5' end of the genome, the region immediately following sor (vif/vpr) and preceding env and from sequences in the gp41 region of env. The Tat protein can be divided into three distinct domains: an acidic domain at the amino terminus, a cysteine rich region consisting of seven cysteine residues, and a positively charged region rich in arginine and lysine residues. Mutations in any of these domains abolish Tat activity. Tat increases the levels of all HIV-1 structural mRNAs (28). A cis-acting sequence in the LTR of HIV is essential for the function of the Tat protein. This Tat responsive sequence known as tar is localized to nucleotides +1 to +42 (29,30). Tar RNA forms a stable stem loop structure. Tat binds to a 3-nucleotide bulge in tar and cellular factors bind to a 6-

nucleotide loop. Tat-RNA binding has been shown to be important for transactivation (31,32), while the significance of cellular protein binding is yet to be determined. There is some evidence that tat regulates viral expression both transcriptionally and post-transcriptionally by association of Tat with tar RNA (33,34). It is thought that this allows Tat to align itself in order to optimally activate the DNA promoter sequences (35,36). Recent evidence strongly suggests that Tat acts mainly at the transcriptional level and that the increase in viral mRNAs results primarily from increased efficiency of elongation (37). While a large body of data concerning Tat and tar exists, the precise mechanism of transactivation remains elusive. It is probable that Tat acts at several levels of control including transcription and translation mainly through interactions with cellular proteins (38).

A second essential regulatory gene in HIV-1 is known as rev (39,40). Rev is mainly produced from doubly or triply spliced mRNAs which contain the rev open reading frame (orf) as the first orf. It is derived from sequences immediately following the short open reading frame (sor) between the pol and env genes and from sequences in the gp41 region of env. Rev encodes a small (19 kD) nuclear/nucleolar protein which interacts in trans through a cis-acting sequence, the rev responsive element (rre) (41). This element is located in a 210 nucleotide region of the env gene. In a manner similar

to the Tat/tar interaction, the rre is necessary and sufficient to mediate Rev function. Rev induces the cytoplasmic translocation of unspliced mRNAs that encode the HIV structural proteins. While the mechanism of action is unclear, evidence indicates Rev induces HIV-1 structural gene expression by activating sequence specific nuclear export of incompletely spliced HIV-1 RNA species (42,43) and is thought to exert its effects through association with RNA (44). Thus, during the viral replication cycle, Tat and Rev act together resulting in sequential changes in both the amount and type of viral genes expressed. Initially after infection, the HIV-1 provirus gives rise to a low level of viral RNA expression that is exclusively the short, multiply spliced, 2 kb transcripts that encode the viral regulatory proteins Tat and Rev. Tat increases the expression of these 2 kb transcripts. Through increased transport, Rev mediates a switch in viral mRNA expression to the unspliced (9kb) and singly spliced (4kb) mRNAs that encode the viral structural proteins. Thus, Rev function can be thought of as a feedback mechanism of regulation of HIV expression. Increasing the transport of unspliced RNAs to the cytoplasm results in a decrease of multiply spliced mRNAs and a balanced production of viral structural and regulatory proteins necessary for production of infectious HIV.

In addition to the essential regulatory genes, tat and rev, several non-essential regulatory genes play a role in

the regulation of viral replication. The conservation of these genes in different lentiviruses of different species suggests an important role for these genes in the viral life cycle. Among these, the nef gene may be involved in negative regulation of viral mRNA expression (45,46), though conflicting reports regarding the role of this gene product on the rate or extent of viral replication exist. The nef gene is made from sequences in the 3' end of the env gene and a small open reading frame (3'orf) which overlaps the 3' LTR. The product of this gene is a 27 kD myristylated cytoplasmic protein (47) which has recently been shown to have autophosphorylating activities essential for its function (48). While the mechanism of Nef function is unclear, it has been reported to down regulate transcription from the viral LTR promoter (46,49). Recently, differences in Nef function in vivo vs. in vitro have been demonstrated (50). These studies strongly argue that Nef is required to maintain high virus titers during persistent infection in vivo. Further, while nef can be deleted without abrogating the ability of the virus to replicate in vitro, it may be necessary for full pathogenic potential of the virus in vivo. The resolution of these conflicting data and the role of Nef in HIV-1 regulation await further study.

A viral gene which may be involved in negatively regulating HIV-1 is known as vpu. The vpu gene is located in the 5' region of the gp120 region of the env gene. Its

product, viral protein U, is thought to effect viral release (51). Two additional non-essential genes have recently been described which are thought to upregulate viral expression. These overlapping genes are made from the sor region located in the DNA segment between the pol and env gene and overlaps the endonuclease domain of pol. The vif gene encodes a cytoplasmic protein which increases the infectivity of the virus particle (52,53). Vpr (54, viral protein R, encodes a 96 amino acid 15kD protein, which reportedly acts in trans to increase the rate of replication and the cytopathic affect of the virus in T cells (54). Importantly, Vpr has recently been shown to be virion associated. As the only regulatory gene associated with the virion, vpr could play an important role in the initial steps of viral replication.

The production of these gene products from the HIV promoter is achieved by three different mechanisms (24): ribosomal frameshifting, alternative splicing, and production of bicistronic mRNAs (55). While all three mechanisms are used in other retroviruses, only the lentiviruses make such extensive use of alternative splicing and production of bicistronic mRNAs. The net effect is the ability of the virus to produce several additional proteins which regulate expression of the virus consistent with host environment.

Cellular regulation of HIV-1 transcription. A second level of regulation of HIV-1 expression occurs at the

cellular level. Many cellular transcription factors have been reported to contribute to the replication of HIV. A schematic of the HIV-1 LTR and the structural arrangement of HIV-1 promoter/enhancer-specific transcription factors is shown in Figure 2. The HIV-1 LTR contains a typical RNA pol II promoter that contains several motifs common to other pol II promoters such as TATA, SP1 (3 sites, -43 to -83), AP1 (-340 to -278), NFκB (-117 to -78). The enhancer region of the HIV-1 LTR is critical for both basal and tat induced transcriptional activation in both T cells and nonlymphoid cell lines. Mutations in this region abolish transcriptional activation. A wide range of nuclear factors have been shown to bind to the HIV-1 LTR including the rel family of transriptional activators, which bind the two kB enhancer motifs (AGGGACTTTCC) in the LTR (23).

Other cellular transcription factors such as, leader binding protein (LBP-1 (-16 to +27 includes TATA), and untranslated leader binding protein UBP-1 which bind to the leader region (+3 to +57) downstream of the transcription start site within the 5' untranslated region may play a role in attenuation of the HIV-1 promoter. In addition, several other factors which interact with the HIV-1 promoter such as CTF/NF-1(+32 to +52), and EPB-1 (23), have been identified. Whether they play a role (either positive or negative) in the regulation of HIV expression is yet to be determined. Thus, the leader region constitutes an important control

element of the HIV-1 promoter. HIV-1 is a strong promoter. Attenuation of activity is achieved through regulation in both the leader region and the tar region the tar region (56,57). The tar sequence forms a stable stem loop structure. The double stranded portion of the leader has been shown to inhibit protein synthesis through the activation of double stranded RNA-dependent kinases and by the activation of (2'-5') oligoadenylate (2-5A) synthetase (57).

Further, tissue specific transcription factors can control cell specific regulation of HIV expression. For example, the protooncogene c-myb has been shown to bind with high affinity to sequences (-151 to -156) in the negative regulatory element (NRE) located -419 to -147 just upstream from the enhancer region in the 5' end of the LTR (58). Deletion of the NRE markedly augments viral replication in both T cells and monocyte cell lines. Myb is expressed in high levels in CD4+ T cells and myeloid cells. Its expression is associated with the proliferation of these cells following mitogen and antigenic stimulation. Thus, the binding of c-myb to the negative regulatory element could be involved in suppressing the negative regulation effect of the NRE. Furthermore, the existence of cell-type and differentiation state specific transcription factors, such as the T-cell specific activating factor, NFAT-1(-260 to -290)(23), rpt-1 (59), and TCF-1 (60), suggests that like

enhancer activity, viral promoter activity can be differentially regulated in a cell-specific manner and providing an additional explanation for differences in HIV-1 expression among T cells and monocytes. Promoter-specific transcription factors can be involved in several aspects of RNA metabolism, such as initiation, cellular localization of transcripts, the integration of initiation with attenuation, and RNA 3' end processing (23,24,61).

HIV-1 is inducible by agents such as phorbol esters or mitogens which induce T cell or monocyte activation factors. Examples of cytokines that have been shown to upregulate viral expression include TNF, GMCSF and IL-6 (62-64). Others, such as αIFN, have been shown to restrict viral production in monocytes (65). Many reagents have been shown to regulate HIV-1 replication. Most of these have been shown to function through the modulation of DNA binding proteins. These, in turn, activate the T cell resulting not only in increased transcription of the virus but concomitant increased spread of infection as these same activation signals are important in establishing a productive infection of T lymphocytes (66,67). In addition, other viruses can modulate HIV-1 replication. This is usually mediated by other viral regulatory proteins such as the adenovirus E1A protein, Herpes simplex virus (HSV) immediate early gene products, (68) or the cytomegalovirus (CMV) immediate early gene product (69). In addition this induction has been

demonstrated to be mediated by transcription factors such as NFκB (21,70), AP-1 (71) and SP-1. For example, the HSV-1 induced activation site on the HIV-1 LTR has been shown to include the SP-1 binding sites and to be independent of the tat responsive region, tar (68). HSV-1 has also been shown to induce a DNA binding protein that interacts with NFκB sequences in the enhancer region of the HIV-1 LTR (72). Additional studies found the EIA induced activation involved the TATA sequences (21), while other viral gene induced activation was independent of any specific site on the HIV-1 LTR. Thus, in vivo activation signals can provide two important functions in the pathogenesis of AIDS: conversion of a latent infection to a productive one and activation of target cells which facilitates viral spread.

Cellular Tropism of lentivirus infections.

Lentiviruses preferentially infect cells of the immune system in vivo, particularly monocytes and macrophages (73). In contrast to the more recently diverged immunodeficiency viruses including HIV and SIV, lentiviruses are not generally immunosuppressive, although most have been shown to infect T cells (74). They cause pathogenic effects in the lungs and central nervous system (CNS), which probably reflect the ability of the virus to infect the monocyte/macrophage. The course of disease progression in the prototypic lentivirus, visna, shares several features with that of HIV-1. Following infection, the spread of

virus continues in the blood despite a strong immune response mounted by the host. The virus persists despite the presence not only of neutralizing antibodies but also cellular effector functions such as antibody-dependent cellular cytotoxicity (75), natural killer (NK) activity (76), and cytotoxic T lymphocyte (CTL) activity (77,78). Pathological effects seen in lentiviral infections are primarily mediated by the inflammatory response of the host (15). In visna, at least one infected cell type is the oligodendrocyte in the brain. Because this cell type is responsible for myelination, the end result is demyelination. In the lungs, monocyte and T cell infiltration impairs gas exchange. In the joints, inflammatory cell infiltration leads to destruction of cartilage. In equine infectious anemia, lymphoproliferative changes and immune complexes play a major role in the pathogenesis (6). HIV differs most profoundly from the lentiviruses of animals in its effects on the immune system. HIV infects all CD4+ cells of the immune system including T cells, monocyte/macrophages, follicular dendritic cells, langerhan's cells, as well as retinal cells, and colonic mucosal cells. This results in progressive defects of both humoral and cell mediated immunity. Unlike infection of monocytes, HIV infection is highly cytopathic to T-cells. The dominant immunologic feature of HIV infection is the progressive depletion of the CD4+ subset of T lymphocytes.