Table 10

Cell-Free Transmission of HIV to T-cells and Hut-102

Cells	Treatment	Infectivity	
		T-cells	Hut-102
Patients # 12 & 19			
Monocytes	None	-	-
Monocytes	Con A	-	-
T-cells	Con A	+	+
Monocytes	Con A supernatant	-	-
Monocytes	Con A T cells	+	+
Monocytes	HUT-102	-	-
Monocytes	LPS	-	-
Monocytes	CSF-1, GM-CSF, TNF, IL6	-	-
Monocytes	Con A T membranes	+	ND
T cells	Con A T membranes	-	ND

Viral transmission to Hut-102 and PHA activated T cells as a measure of viral production was performed using cell-free supernatants was assessed as previously described in the Methods. Infectivity was determined by the presence of HIV nucleic acids detected by PCR as described and HIV p24 detected by Elisa. Cytokines were used at the following concentrations CSF-1, GM-CSF, IL-6: 20-200 ng/ml and TNF: 10-1000 u/ml.

and placed into culture wells previously seeded with adherent patient monocytes failed to activate virus. In addition, supernatants from Con A-activated T cells could not stimulate HIV expression (Figure 32,Table 10). Viral transmission experiments using supernatants from these cocultures were performed using the cell line HUT 102. The presence of infectious virus was detected from media from activated T cell plasma membranes treated monocytes but not in the media of cocultures with unactivated T cells, Con A, cytokine-rich T cell supernatant or media controls (Table 10). These results suggest that cell contact is required to activate factor(s) in the monocyte responsible for overcoming HIV latency.

DISCUSSION

Lentiviruses, including HIV, cause slowly progressive, chronic and in some instances, fatal diseases in their hosts. The time from initial HIV infection to clinically observed symptoms is usually measured in years (161). Two-six weeks following infection with HIV, an individual may remain asymptomatic or have an acute flu-like illness. During this time, cell free virus can be isolated from the blood and cerebrospinal fluid (CSF). Symptoms of the acute illness may include fever, headache, malaise, sore throat and rash. During the acute illness, antibodies to the virus are generally not detectable, while p24 antigen is detected. The first antibodies detected in the serum are usually anti-gp120 antibodies which usually remain elevated throughout the disease. Antibodies directed against the core protein p24 appear in the serum coincident with the declines in p24 antigen levels. In the second phase of infection, which can last 2 to >10 years HIV can be detected in the DNA. However, individuals usually remain seropositive and virus may or may not be cultured from the blood or CSF (162). Late in this phase of infection, symptoms develop which comprise the condition known as AIDS related complex (163). These can include: persistent fever, night sweats, chronic diarrhea, weight loss, generalized lymphadenopathy, herpes zoster, oral candidiasis and oral hairy leukoplakia. CDC criteria for diagnosis of AIDS consist of certain

opportunistic infections such as pneumocystis carinii pneumonia, disseminated cytomegalovirus infection, as well as cancers such as kaposi's sarcoma and B cell lymphomas (164). In addition, HIV-related encephalopathy and wasting syndrome are now recognized among the criteria of AIDS.

Elucidation of the viral life cycle during the long clinical latency is critical to the understanding of the pathophysiology of AIDS. In particular, it is not known if there are cells in the patient that are truly latent i.e. cells that contain integrated provirus but are producing no virus. Such cells could contribute to the onset of the disease state if certain events in the patient induced activation of latent virus to a productive state. To examine the question of viral latency in patients, a cell line model of latency which could be used to address these issues was developed.

Likely related to these questions concerning viral latency and persistence, is the tropism which all animal lentiviruses, including HIV, show for cells of the mono-cyte/macrophage lineage, non-dividing cells in which differentiation signals lead to increased viral expression (73,165,166). Similarly differentiation signals and cytokines such as GM-CSF, TNFα, IL-1, and IL-6, produced as a result of monocyte activation by polyclonal activators such lipopolysaccharide (LPS) have been shown to modulate HIV expression from monocyte cell lines in vitro (62).

Restriction of virus expression has also been characterized in monocyte/macrophages. For example, in visna-maedi infection, in vivo, relatively few monocyte/macrophages are infected. Additionally, immature macrophages in visna infected sheep had only a few copies of viral RNA compared to many copies seen in mature tissue macrophages. Interestingly, differentiation signals could not activate visna viral expression from sheep brain macrophages suggesting different levels of restriction exist. Further, infection of Kupffer macrophages of the liver could not be demonstrated suggesting different viral tropism in various subsets of macrophages (166). CAEV shares many biological properties with visna, such as the ability to replicate primarily in monocyte/macrophages of both sheep and goats (7,167). However, in contrast to visna-maedi, CAEV does not replicate in sheep choroid plexus cell lines (fibroblasts) but in epithelial cell lines developed from synovial membranes of goats. This cellular tropism distinct to each lentivirus, is reflected in the disease complex. In visna-maedi, the central nervous system and the lungs are the main symptomatic organ systems, while the main disease in goats is synovitis and progression to crippling arthritis. These ruminant lentivirus provide model systems for lentivirus persistence and latency in monocyte/macrophages.

Several studies have been reported which show the monocyte/macrophage as a major site of virus infection and

persistence during the subclinical phase of HIV infection. HIV can be cultured from monocytes obtained from blood (103,168), lungs (106) and lymph nodes of patients with AIDS. (169). Similarly, HIV infection of monocytes derived from blood, bone marrow, brain and lung could be demonstrated in vitro (170,171,172). Importantly, in contrast to the CD4+ lymphocyte, the monocyte/macrophage is generally resistent to the cytopathic effects of HIV. Further, monocyte functions such as phagocytosis and IL-1 production are relatively normal in vitro (105).

Several early studies detected HIV specific RNA and DNA in the brains or CSF of AIDS patients with subacute encephalitis (80,79,173). These data taken together with the role of the monocyte/macrophage in the neurologic syndromes of other lentiviruses such as visna and CEAV support a direct role for the monocyte/macrophage in the neurological abnormalities seen in AIDS patients. Subsequently, it was shown that the macrophage is the major cell type infected in the brain (81,174). While the mechanisms involved in the macrophage mediated pathogenesis are unclear, a recent study suggests HIV infected macrophages/microglia but not T cells, produce specific factors which act through NMDA receptors producing neurologic disease through chronic secretion of neurotoxic factors (83,84).

Thus, the monocyte serves as a reservoir of HIV.

However, because of the difficulties inherent in long term growth of normal monocytes in vitro, it is not possible to generate enough cells to study the biochemical mechanisms of HIV latency. Therefore, a model was developed in a monocyte rather than a T cell line. THP-1, a cell line derived from a patient with acute monocytic leukemia was chosen for the model because it possesses morphological, biochemical, phenotypic and functional characteristics of normal mature monocytes including: phagocytosis, IL-1 production and accessory cell function (142,143). These characteristics make THP-1 the best available cell line to mimic the effects of HIV infection in normal monocytes.

An acute productive infection of THP-1 cells was established using HIV-1 and HIV-2. Between day 14 and 21, 20-40% of the cells contain antigens recognized by anti-p24 and anti-gp 160:41. These data are consistent with published reports using $CD34^+$ hematopoietic stem cells (175), fresh monocytes (103,170,172) and U937 (62,111). Several weeks after infection of THP-1 cells by HIV, entire cultures spontaneously became restricted in viral expression while in the producer cultures >90% of the cells were viral antigen positive. In some cases, spontaneous nonproducers have been reported for T cells (176,108) but not for monocytes. Two distinct types of infected cultures with altered expression were identified in THP-1: (a) cells with restricted HIV expression, and (b) cells with complete latency.

Characterization of these two types of infected monocytoid cultures has shown that they are clearly different at the molecular and cellular level.

It is unlikely that these cultures arose due to selection of clonal variants since with infection at low MOI no THP-1 cytotoxicity or loss of viability, growth, or function was seen in infected cultures. However, it is not possible to rule out that the cells with restricted or latent infection were present from the first day of infection and eventually overgrew the other cells in the culture. While it is not possible to determine how these cultures arose, their existence has important implications for HIV viral persistence and pathology. Furthermore, in both types of restricted cultures, cells could be induced to produce virus after 10 months in continuous culture. Thus, the phenotype and genotype of these viral cultures was stable.

Regulation of viral transcription in infected cells is affected by a variety of stimuli such as antigen activation (108,176), cytokine stimulation (147,148), hypomethylation (157), DNA viral infection (68,69), and phorbol ester treatment (177). 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. The ability of these agents to induce or augment virus production in these cells with latent and restricted HIV expression allows the

distinction between these two mechanisms of viral suppression.

These treatments that positively regulate HIV transcription can be mediated by cellular factors such as NFκB (21), SP-1(127,23), AP-1 (61) that bind to specific sequences in the HIV LTR. These factors are present in THP-1 cells as well as monocytes and T cells (70). In addition, Tat has been postulated to mediate its effects through cellular factors which bind to the sequences responsive to tar (178). Viral factors such as the nef gene product which can repress HIV transcription (45,46) and vpu which affects viral release (51) could be important in establishing these states of viral suppression. In addition to the cytokines that upregulate viral expression, α IFN has been shown to restrict viral production in human monocytes (65) and U-937 cells (153,154). It is clear that multiple pathways regulate HIV transcription, and that negative regulation of viral expression in THP-1 cells probably involves viral and cellular factors.

Characterization of THP-1 with restricted expression (RTHP-1) revealed several similarities to other models of chronic low level expression, infection of fresh monocytes with HIV as well as animal lentivirus infection in monocytes. First, a lower number of cells in the culture are infected, 20-30% compared to 95% in productively infected cultures. This is likely the result of decreased

spread of virus throughout the culture because of intracellular sequestration of the virus. Electron microscopy of HIV infected monocytes reveals intracellular budding into vacuoles with little budding from plasma membranes. While the mechanisms involved are unknown, it is possible that this is in part due to differential regulation in monocytes of HIV regulatory proteins such as viral infectivity factor, Vif (53), and viral protein U (Vpu) believed to be involved in viral release (51). A second level of restriction of viral expression was revealed by northern and PCR analysis as substantially reduced accumulation of viral RNA. In addition, production of genomic viral RNA is being reduced in these restricted cells with a concomitant appearance of a novel subgenomic 7.5 kb RNA (Figure 4). This RNA is not seen when the virus is then reinfected into T cells. HIV-LTR-directed in vitro transcription analysis demonstrates that this 7.5kb species is not the result of incorrect initiation of transcription (Figure 10) in RTHP-1. The origin and any function of this unique transcript in viral expression is not known. Nuclear run-on experiments indicate the lower accumulation of viral mRNA is the result of a reduced rate of transcription (Figure 11).

Furthermore, mixing studies suggest HIV-specific factors present in the nucleus of restrictedly infected cells that can negatively regulate transcription of

productively infected cells. That this modulation of HIV expression was mediated at the level of DNA binding was shown using gel mobility shift analysis (Figure 12). The formation of DNA binding complexes associated with Tat are specifically eliminated by nuclear extracts from restrictedly infected cells suggesting that initiation of transcription is being regulated. Furthermore, gel shift analysis of NFκB, SP-1 and TATA, sequences in the enhancer region of the LTR demonstrated the modulation of DNA binding by extracts from THP-1 with restricted expression (RTHP-1) (Figure 13) to NFκB and Sp-1 sites. This is in agreement with several studies which have shown that stimulation of HIV infected cells with agents, such as phorbol esters which are known to activate NFκB translocation from the cytosol to the nucleus, significantly increase HIV expression (21).

Further research on the specific cellular factors in RTHP-1 involved in this modulation of NFκB binding and subsequent decrease in HIV-1 expression was done in collaboration with Raziuddin (145). These studies revealed that THP-1 cells with restricted infection contain factors in the nucleus which specifically inhibit the interaction of the NFκB 65+50 heterodimer with the consensuses sequences in the HIV-1 LTR. Thus, one mechanism by which differentiation signals and polyclonal activators can mediate viral expression through transcription proteins is blocked in RTHP-1 by the presence of a specific inhibitor(s) to this

transcription factor.

In cells with latent virus, viral expression and the ability to negatively regulate transcription of productively infected cells were not observed. By PCR analysis, no viral RNA expression was seen in these cells. However, these cells could be induced by 5 azacytidine treatment to produce infectious virus. This is the first demonstration that natural infection of monocytoid cells can lead to classical latency at the molecular level. This shows the involvement of at least two distinct levels of negative regulation of HIV expression in monocytes.

Many cytokines are produced by monocytes in response to T cell stimulation during the primary immune response. Studies examining the ability of cytokines and other agents to induce or upregulate HIV expression from THP-1 cells with restricted infection and THP-1 cells with latent infection revealed that the ability of various agents to reactivate viral expression in these two infected cell types is distinct. In agreement with several others (147,148,64,62,110,155), we find various cytokine treatments, LPS, PMA, TNF-α, and GM-CSF, can stimulate expression in restricted cells such that viral levels approach that of a productive cell. However, none of these treatments stimulate detectable production from cells with latent virus (Table 6). Latently infected THP-1 could be stimulated to produce infectious virus by 5-azacytidine but

not by other treatments. However, the combination of LPS and 5-azacytidine augmented activation and production of HIV from latently infected cells suggesting these activation could aid in overcoming latency. Therefore, each of these states provides at least one mechanism of the establishment of HIV viral persistence. Understanding these diverse interactions between HIV and monocytes is important in understanding the nature of viral persistence and its relationship to disease.

The biological consequences of these types of restricted HIV expression may also be important in the pathogenesis of the disease. In the cells with restricted expression, most if not all infectious virus produced is sequestered intracellularly. The cells eventually store sufficient virus to kill T cells as efficiently as productively infected T cells (Figure 8), probably through cell-cell contact with the uninfected target. Suppression of extracellular virus production and cell surface viral antigen expression allows the monocyte with restricted expression to escape recognition and subsequent lysis by the immune system (Table 5), which is the fate of productively infected cells (76). Furthermore, while some have reported abnormalities in functions of monocytes isolated from HIV-infected individuals as well as from in vitro infection (88-88-89,133), the majority of monocyte/macrophage functions studied in vitro appear to be normal (105). Similarly, the

functional abilities of THP-1 cells were essentially normal regardless of the state of viral expression (Table 4). In addition, in the cells with latent infection, no virus is seen. Infectious virus can be activated and can efficiently kill T cells even after being quiescent for long periods of time. From molecular and biological aspects, these two states are mechanistically and functionally distinct but respresent two ways of establishing HIV persistence in the host.

The ability of 5-azacytidine to reactivate virus production suggests that methylation is involved in the regulation of HIV expression in these latently infected cells. It has been previously shown that methylation of HIV-LTR sequences could occur (146) using enzymatically methylated CpG sites in the HIV LTR, transcription of both reporter genes and infectious provirus was silenced. Several models can be envisioned by which CpG methylation causes transcriptional repression. Direct: essential transcription factors see methylated CpG as a mutation in their binding site and are unable to bind. Indirect: methylated DNA is bound by a nuclear proteins which secondarily prevents transcription factor binding or methylation of the DNA causes a conformational change which prevents transcription factors from recognizing their binding site (157,179). Several transcription factors, such as the cAMP CREB factor have been shown to be sensitive to

methylation. Latently infected THP-1 were initially examined for LTR methylation. No difference was seen in Southern analysis using the methylation specifice restriction endonucleases, Msp-1 and HpaII and probing with the HIV-1 LTR.

However, not all transcription factors are methyl sensitive. Sp-1 can bind and activate transcription equally well regardless of methylation (180). Strong transactivating proteins such as the adenovirus transactivator and HIV-1 Tat have also been shown to mediate their effects regardless of methylation (157,179). Because Tat had been shown to transactivate in the presence of methylated CpG sites in the LTR, expression of the transactivating proteins tat and tev in latently infected THP-1 induced expression of HIV-1. However, Tat is not produced in the latent THP-1 cells. This suggests that other regions of the HIV-1 genome may be methylated thus blocking the production of tat message perhaps by inhibiting splicing.

The ability of 5-azacytidine treatment and expression of tat protein to overcome latency stimulated attempts to define physiological mechanisms which could also induce HIV-1 expression. Because of the interdependence of the T cells and monocytes in the primary immune response, concanavalin A (con A)-activated T cells were co-cultured with latently infected THP-1 cells resulting in the induction of

infectious virus (Figure 24). This result suggested immune activation could overcome HIV-1 latency and enabled the determination of the relevance of this model in HIV infected individuals. PCR analysis of RNA isolated from monocyte monolayers cultured from HIV-1 seropositive asymptomatic individuals showed only 3 of 21 samples analyses were positive for HIV specific RNA. HIV-specific DNA could only be detected in 9 of 21 patients. Six of these had no detectable HIV-RNA (Table 8). However, co-culture of these same patient's monocytes with con A-activated T cells now resulted in HIV specific RNA in 17 of 21 as detected by PCR analysis using primer pairs to both tat and gag regions of the viral genome. Thus, infectious HIV can remain completely latent in monocytes from asymptomatic HIV-infected patients. In agreement with Schnittman et. al. (107), HIV-1 specific DNA could be detected in only 821 samples no HIV specific DNA by PCR in monocytes. However, in 8/12 of those samples, co-culture with Con A-activated T cells induced expression of infectious HIV. This supports the idea that PCR, like any technique, has defined limits of sensitivity and that biological amplification of virus can be as useful as biochemical amplification.

Despite the low levels of latently infected monocytes in these cultures, the cells could still be induced by uninfected Con A-activated T cells to produce infectious virus. These results in addition to the ability of virus

from L-THP-1 to be activated by the same mechanism strongly suggests that HIV can be latent in patient monocytes. However, three alternatives were considered and investigated: HIV is passively adsorbed to monocytes; contaminating latent T cells (181,67) are being activated; and that contaminating viral producing cells either T cells or monocytes are responsible for viral spread. First, Hut-102, an extremely sensitive permissive cell for HIV which can be infected by co-culture with the low level producer cell line, U1, does not become infected by co-culture with either adherent patient monocytes or LTHP-1. However, after virus has been reactivated from these cells, it can be transmitted to Hut-102 where it causes a cytopathic infection. Second, Con A and PHA, which have been shown to activate HIV from latently infected T cells (181,67), will activate virus from T cells and not from monocytes of these donors even when sufficient Hut-102 cells, primary monocytes or THP-1 are cocultured to amplify any viral production (Table 9). The experimental data presented for first two possibilities also makes the third unlikely. Furthermore, in cocultures using serial dilutions of U1 and ACH2, chronically infected cell lines producing HIV at a low level (110,156), with monolayers of normal monocytes for 7 days as in the patient samples, we could consistently detect 10 infected cells per $1x10^7$ normal monocytes (Figure 31). Using RNA from 8E5 which contains a single HIV proviral copy

per cell, we could detect 10-100 femptograms of RNA which a log greater sensitivity than for 8E5 DNA, indicating it is much more difficult to get a false negative for HIV RNA than DNA. In addition, LPS and cytokines known to increase HIV production (147-149,155,170) did not stimulate viral production in these monocytes. These results provide strong evidence that neither contaminating T cells, virus producing cells nor virus passively adsorbed to monocytes are responsible for the HIV expression induced in these monocytes.

It is interesting that in both THP-1 and patient monocytes, Con A-activated T cells can stimulate expression of latent virus suggesting that immune activation can play a role in HIV pathogenesis. These studies agree with those of Schrier et. al. (182), who showed that Con A-activated autologous cells or autologous plus allogeneic cells could stimulate HIV production from macrophages, although no clear conclusions could be drawn from those studies about the cellular origin of the virus or its state of latency. The intimate relationship between monocytes and T cells suggests that these cells could be infecting each other during the immune response by activating latent virus. The recent finding of McElrath et. al. (183) showing the presence of HIV DNA in monocytes of AIDS patients in samples obtained up to nine months apart suggests that continuous infection of monocytes is occurring; however, in the absence of RNA data

no conclusion can be made concerning viral latency. Thus, these data show that HIV was truly latent in these monocytes and suggests that activated T cells stimulate monocytes to produce a factors which induces expression of HIV.

The cellular requirements of activation of latent HIV from monocytes were also studied. In LTHP-1, activated CD4+ T cells but not CD8+ cells, resting T cells, Hut 102 (a continually proliferating T cell line) or T cell supernatants couls activate viurs. Surprisingly, in patient monocytes, activated T cells containing at least 50% CD8+ shown by Levy and co-workers to suppress HIV replication (184,185) were able to activate and stimulate the spread of latent virus through a cell contact requirement. It is interesting that only a few latently infected monocytes either in patient monolayers or in uncloned in L-THP-1 can lead to a productive cytopathic infection. The ability of activated T cell plasma membranes treated monocytes to produce infectious virus suggest that cell contact is required to activate factors in the monocyte responsible for overcoming HIV latency.

The question of which monocyte derived factors are responsible for overcoming HIV latency awaits further study. Since the monocyte/macrophage is an active secretory cell with the capacity to elaborate approximately 100 different substances affecting the inflammatory response, it is unclear which class of molecules is involved. However, some

candidates could be products of the arachidonic acid pathway such as leukotrienes, prostaglandins and platelet activating factor (PAF). In particular, leukotriene B_4 production is greatly enhanced in response to LPS stimulation and T cell stimulation. The E series prostaglandins mediate the inhibition arm of immune regulation, in part by modulating T cell production of IFNγ and IL-2 (186). Other possibilities include the class of molecules which activate the macrophage, such as macrophage activating factor (MCAF). Although individual macrophage produced cytokines did not overcome latency, it is possible that combinations of cytokines or other inflammatory molecules mediate this response.

Considerable controversy exists concerning the existence and role of latency on the pathogenesis of AIDS. The finding that HIV replication occurs in the lymph nodes of asymptomatic patients has been interpreted as proving latency does not exist (187). However, HIV infected individuals can harbor HIV for up to five years without seroverting and with no evidence of viral expression (188,189). These clinical observations and data presented here clearly demonstrate that true latency at the molecular level does exist both in a tissue culture model and in HIV infected individuals.

The presence of silently infected monocytes capable of producing virus during an immune response also has some

important therapeutic implications. Therapies based on preventing viral replication need to be continually present during the immune response. Since these latently infected monocytes may become long-lived tissue macrophages, it would be difficult to eliminate these cells. In particular, it may be necessary to determine if latently infected macrophages could still produce the neurological effects recently observed in productively infected THP-1 and patient monocytes (83,84). Further, it is important to determine whether an appropriate combination of immune stimulation plus antiviral therapy is capable of eliminating latent HIV from human monocytes in vitro. Such an approach could make currently available antiviral agents more effective in vivo by eliminating an important reservoir of virus that may intermittently reactivate and infect uninfected T cells.

Taken together these results suggest that the monocyte is an important reservoir, in which HIV expression can be regulated in several ways and that viral latency plays a role in the pathogenesis of AIDS.

REFERENCES

1. Varmus, H. 1988. Retroviruses. Science 240, 1427-1435.
2. Cooper, G.M. 1990. Oncogenes. Jones and Bartlett publishers, Boston, MA.
3. Poiesz, B.J., F.W. Ruscetti, A.F. Gazdar, P.A. Bunn, J.D. Minna, and R.C. Gallo. 1980. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cel lymphoma. Proc. Natl. Acad. Sci. USA 77, 415-7419.
4. Gonda, M.A. 1988. Molecular genetics and structure of the human immunodeficiency virus. J. Electron Microsc. 8, 17.
5. Narayan, O., and L.C. Cork. 1985. Lentiviral disease of sheep and goats: chronic leukoencephalomyelitis and arthritis. Rev. Infect. Dis. 7, 89.
6. Stephens, R.M., J.W. Casey, N.R. Rice. 1986. Equine infectious anemia virus gag and pol genes: relatedness to visna and AIDS virus. Science 231, 589-594.
7. Crawford, T.B., D.S. Adams, W.P. Cheevers, and L.C. Cork. 1980. Chronic arthritis in goats caused by a retrovirus. Science 207, 997-999.
8. Daniel, M.D., N.L. Letvin, N.W. King, M. Kannagi, P.K. Sehgal, R.D. Hunt, P.J. Kanki, M. Essex, and R.C. Desrosiers. 1985. Isolation of T-cell lymphotropic HTLV-III-like virus from macaques. Science, 228, 1201-

1204.

9. Kanki, P.J., J. Alroy, and M. Essex. 1985b. Isolation of T-lymphotropic retrovirus related to HTLV-III/LAV from wild caught African green monkeys. Science 230, 951-954.

10. Benveniste, R.E., L.O. Arthur, C.-C. Tsai, R. Sowder, T.D. Copeland, L.E. Henderson, and S. Oroszlan. 1986. Isolation of a lentivirus from a macaque with lymphoma: Comparison with HTLV-III/LAV and other lentiviruses. J. Virol. 60, 483-490.

11. Pedersen, N.C., E.W. Ho, M.L. Brown, and J.K. Yamamoto. 1987. Isolation of a T lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. Science 235, 790-793.

12. Levy, J.A., A.D. Hoffman, S.M. Kramer, J.A. Landis, J.M. Shimabukuro, and L.S. Oshiro. 1984. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. Science 225, 840-842.

13. Gallo, R.C., S.Z. Salahuddin, M. Popovic, G.M. Shearer, M. Kaplan, B.F. Haynes, T.J. Parker, and P.D. Markham. 1984. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science 224, 500-503.

15. Haase, A.T. 1986. Pathogenesis of lentivirus infections. Nature 322, 130-136.

16. Maddon, J.S., A.G. Dalgleish, J.S. McDougal, P.R.

Clapham, R.A. Weiss, and R. Axel. 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and in the brain. Cell 47, 333-348.

17. McDougal, J.S., M.S. Kennedy, J.M. Slingh, S.P. Cort, A. Mawle, and J.K.A. Nicholson. 1986. Binding of HTLV-III/LAV to T4+ T cells by a complex of a 110k viral protein and the T4 molecule. Science 231, 382-385.

18. Sattentau, Q.J., and R.A. Weiss. 1988. The CD4 Antigen: Physiological Ligand and receptor. Cell 52, 631-633.

19. Rosen, C.A., and G.N. Pavlakis. 1990. Tat and rev: Positive regulators of HIV expression. AIDS 4, 499-509.

20. Rabson, A.B., and M.A. Martin. 1985. Molecular organization of the AIDS retrovirus. Cell 40, 477-480.

21. Nabel, G., and D. Baltimore. 1987. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. Nature (Lond.). 326, 711.

22. Rosen, C.A., J.G. Sodroski, and W.A. Hazeltine. 1985. The location of cis-acting regulatory sequences in the human T cell lymphotropic virus type III (HTLV-III/LAV) long terminal repeat. Cell 41, 813-823.

23. Jones, K.A. 1990. HIV Transactivation and

transcriptional control mechanisms. New Biologist 1, 127-135.

24. Pavlakis, G.N., and B.K. Felber. 1990. Regulation of Expression of Human Immunodeficiency Virus. New Biologist 2, 20-31.

25. Ratneswaren, R., M. Sheldon, L. Johal, and N. Hernandez. 1990. The HIV-1 long terminal repeat contains an unusual element that induces the synthesis of short RNAs from various mRNA and snRNA promoters. Genes & Development 4, 2061-2074.

26. Dayton, A.I., J.G. Sodrowski, C.A. Rosen, W.C. Goh, W.A. Hazeltine. 1986. The transactivator gene of the human T-cell lymphotropic virus type III is required for replication. Cell 44, 941-947.

27. Fisher, A.G., B. Ensoli, L. Ivanoff, S. Petteway, M. Chamberlain, L. Ratner, R. Gallo, and F. Wong-Staal. 1987. The sor gene of HIV is essential for efficient transmission in vitro. Science 237, 888-893.

28. Wright C.M., B.K. Felber, H. Paslakis, and G.N. Pavlakis. 1986. Expression and characterization of the trans-activator of HTLV-III/LAV virus. Science 234, 988-992.

29. Garcia, J.A., D. Harrich, E. Soultanakis, F. Wu, R. Mitsuyasu, and R.B. Gaynor. 1989. Human immunodeficiency virus type 1 LTR TATA and tar region sequences required for transcriptional regulation. EMBO

J. 8, 765-778.

30. Rosen, C.A., E. Terwilliger, A. Dayton, J.G. Sodroski, and W.A. Hazeltine. 1988. Intragemic cis-acting art gene-responsive sequences of the human immunodeficiency virus. Proc. Natl. Acad. Sci. USA. 85, 9224-9228.

31. Calnan, B.J., S. Biancalana, D. Hudson, and A.D.Frankel. 1990. Analysis of arginine-rich peptides from HIV tat protein reveals unusual features of RNA-protein recognition. Genes Dev. 5, 201-210.

32. Weeks, K.M., C. Ampe, S.C. Schultz, T.A. Steitz, and D.M. Crothers. 1990. Fragments of the HIV-1 tat Protein specifically bind tar RNA. Science 249, 1281-1285.

33. Berkhout, B., A., R.H. Silverman, K-T, Jeang. 1989b. Tat trans activates the human immunodeficiency virus through a nascent RNA target. Cell 59, 273-282.

34. Braddock, M.A., A. Chambers, W. Wilson, W. Esnouf, M.P. Adams, A.J. Kingsman, and S.M. Kingsman. 1989. HIV-1 tat "activates" presynthesized RNA in the Nucleus. Cell 58, 269-279.

35. Southgate C., M.L. Zapp, and M.R. Green. 1990. Activation of transcription by HIV-1 tat protein tethered to nascent RNA through another protein. Nature 345, 640-643.

36. Berkhout, B., A. Gatignol, A.B. Rabson, and K.-T. Jeang. 1990. Tar-independent activation of the HIV-1

LTR: evidence that tat requires specific regions of the promoter. Cell 62, 757-767.

37. Feinberg, M.B., D. Baltimore, and A.D. Frankel. 1991. The role of tat in the human immunodeficiency virus life cycle indicates a primary effecct on transcriptional elongation. Proc. Natl. Acad. Sci. USA. 88, 4045-4049.

38. Gatignol, A., A. Buckler-White, B. Berkhout, and K.-T. Jeang. 1991. Characterization of a human tar RNA-binding protein that activates the HIV-1 LTR. Science 251, 1597-1600.

39. Feinberg, M.B., R.F. Jarrett, A. Aldovini, R.C. Gallo, and F. Wong-Staal. 1986. HTLV-III expression and production involve complex regulation at the levels of splicing and translation of viral RNA. Cell 46, 807-817.

40. Sodroski, J., W.C. Goh, C. Rosen, A. Dayton, E. Terwilliger, and W.A. Hazeltine. 1986. A second post transcriptional transactivator gene required for HTLV-III replication. Nature 321, 412-417.

41. Dayton, A.I., E.F. Terwilliger, J. Potz, M. Kowalski, J.G. Sodroski, and W.A. Hazeltine. 1988. Cis-acting sequences responsive to the rev gene product of the human immunodeficiency virus. J. AIDS 1, 441-452.

42. Malim, M.H., J. Hauber, S.-Y. Lee, J.V. Maizel, and B.R. Cullen. 1989. The HIV-1 rev trans-activator

acts through a structured target sequence to activate nuclear export of unspliced viral mRNA. Nature 338, 254-257.

43. Felber, B.K., M. Hadzopoulou-Cladaras, C. Cladaras, T. Copeland, and G.N. Pavlakis. 1989b. The rev protein of HIV-1 affects the stability and transport of the viral mRNA. Proc. Natl. Acad. Sci. USA. 86, 1495-1499.

44. Daefler, S., M.E. Klotman, and F. Wong-Staal. 1990. Trans-activating rev protein of the human immunodeficiency virus 1 interacts directly and specifically with its target RNA. Proc. Natl. Acad. Sci. USA. 87, 4571-4575.

45. Luciw, P., C. Cheng Mayer, and J. A. Levy. 1987. Mutational analysis of the human immunodeficiency virus: the orf-B region downregulates virus replication. Proc. Natl. Acad. Sci. USA. 84, 1434.

46. Ahmad, N., and S. Venkatesan. 1988. nef Protein of HIV-1 is a transcriptional repressor of HIV-1 LTR. Science (Wash. DC). 241, 1481.

47. Franchini, G., G.M. Robert, J. Ghrayeb, N.T. Chang, and S.F. Wong. 1986. Cytoplasmic localization of the HTLV-III 3' orf protein in cultured T cells. Virology 155, 593-599.

48. Venkatesan, S., R.K. Maitra, A. Nebreda, P. Dickie, N. Ahmad, and S.M. Santos. 1991. Functional studies of HIV-1 nef protein. Presented at the VII International

Conference on AIDS.

49. Niederman, M.J., B.J. Thielan, and L. Ratner. 1989. Human immunodeficiency virus type 1 negative factor is a transcriptional silencer. Proc. Natl. Acad. Sci. USA. 86, 1128-1132.

50. Kestler, H.W., D.J. Ringler, K. Mori, D.L. Panicali, P.K. Dehgal, M.D. Daniel, and R.C. Derosiers. 1991. Importance of the nef gene for maintenance of high virus loads and for development of AIDS. Cell 65, 651-662.

51. Strebel, K., T. Klimkait, and M. Martin. 1988. A novel gene of HIV-1, vpu, and its 16 kilodalton product. Science (Wash. DC). 241, 1221.

52. Lee, T.H., J.E. Coligan, J.S. Allan, M.F. McLane, and J.E. Groopman. 1986. A new HTLV-III/LAV protein encoded by a gene found in cytopathic retroviruses. Science 231, 1546-1549.

53. Sodroski, J., W.C. Goh, C. Rosen, A. Dayton, E. Terwilleger, W.A. Hazeltine. 1988. A second post transcriptional transactivator gene required for the HTLV-III replication. Nature 321, 412-417.

54. Cohen, E.A., E.F. Terwilliger, Y. Jalinoos, J. Proulx, J.G. Sodroski, and W.A. Hazeltine. 1990. Identification of HIV-1 vpr Product and Function. J. Acquired Immun. Def. Syn. 3, 11-18.

55. Sonigo, P., M. Alizon, K. Staskus, D. Klatzman, S.

Cole, O. Danos, E. Retzel, P. Tiollais, A. Haase, and S. Wain-Hobson. 1985. Nucleotide Sequence of the Visna Lentivirus: Relationship to the AIDS Virus. Cell 42, 369-382.

56. SenGupta, S.N., and R.H. Silverman. 1989. Activation of interferon-regulated, dsRNA-dependent enzymes by human immunodeficiency virus leader RNA. Nucleic Acids Res. 3, 969-977.

57. Edary, I., R. Petryshyn, and N. Sonenberg. 1989. Activation of Double stranded RNA-dependent kinase (dSl) by the tar region of HIV-1 mRNA: A novel translational control mechanism. Cell 56, 303-312.

58. Dasgupta, P., P. Saikumar, C.D. Reddy, and E.P. Reddy. 1990. Myb protein binds to human immunodeficiency virus 1 long terminal repeat (LTR) sequences and transactivates LTR-mediated transcription. Proc. Natl. Acad. Sci. USA. 87, 8090-8094.

59. Patarca, R., J. Schwartz, R.P. Singh, Q.-T. Kong, E. Murphy, Y. Anderson, F.-Y. Sheng, P. Singh, K.A. Johnson, S.M. Guarnagia, T. Durfee, F. Blattner, and H. Cantor. 1988. rpt-1, an intracellular protein from helper/inducer T cells that regulates gene expression of interleukin 2 receptor and human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. USA. 85, 2733-2737.

60. Smith, M.R., and W.C. Greene. 1989. The same 50-kDa cellular protein binds to the negative regulatory elements of the interleukin 2 receptor α-chain gene and the human immunodeficiency virus type 1 long terminal repeat. Proc. Natl. Acad. Sci. USA. 86, 8526-8530.

61. Kato, H., M. Horikoshi, and R.G. Roeder. 1991. Repression of HIV-1 Transcription by a cellular protein. Science 251, 1476-1479.

62. Folks, T.M., J. Justement, A. Kinter, C.A. Dinarello, and A.S. Fauci. 1987. Cytokine-induced expression of HIV-1 in a chronically infected promonocyte cell line. Science (Wash. DC). 238, 800.

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

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

65. Kornbluth, R.S., P.S. Oh, J.R. Munis, P.H. Cleveland, and D.D. Richman. 1989. Interferons and bacterial

lipopolysaccharide protect macrophages from productive infection by human immunodeficiency virus in vitro. J. Exp. Med. 169, 1137.

66. McDougal, J.S., A. Mawle, S.P. Cort, J.K.A. Nicholson, G.D. Cross, J.A. Scheppler-Campbell, D. Hicks, and J. Sligh. 1986. Cellular tropism of the Human retrovirus HTLV-III/LAV. J. Immunol. 135, 3151.

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

68. Mosca, J.D., D.P. Bednarik, N.B.K. Raj, C.A. Rosen, J.G. Sodroski, W.A. Haseltine, and P.M. Pitha. 1987. Herpes simplex virus type-1 can reactivate transcription of latent human immunodeficiency virus. Nature (Lond.). 325, 67.

69. Davis, M.G., S.C. Kenney, J. Kamine, J.S. Pagano, and E.-S. Huang. 1987. Immediate-early region of human cytomegalovirus trans-activates the promoter of human immunodeficiency virus. Proc. Natl. Acad. Sci. USA. 84, 8642.

70. Griffin, G.E., K. Leung, T.M. Folks, S. Kunkel, and G.J. Nabel. 1989. Activation of HIV gene expression during monocyte differentiation by induction of NF-κβ. Nature (Lond.). 339, 70.

71. Dinter, H., R. Chiu, M. Imagawa, M. Karin, and K. A. Jones. 1987. In vitro activation of the HIV-1 enhancer in extracts from cells treated with a phorbol ester tumor promoter. EMBO (Eur. Mol. Biol. Organ.). 6, 4067.

72. Gimble, J.M., E. Duh, J.M. Ostrove, H.E. Gendleman, E.E. Max, and A.B. Rabson. 1988. Activation of human immunodeficiency virus long terminal repeat by herpes simplex virus type 1 is associated with a nuclear factor that binds to the NFλB/core enhancer sequence. J. Virol. 62, 4104-4112.

73. Gendleman, H.E., O. Narayan, S. Kennedy-Stoskopf, P.G.E. Kennedy, Z. Ghotbi, J.E. Clements, J. Stanley, and G. Pezeshkpour. 1986. Tropism of sheep lentiviruses for monocytes: susceptibilty to infection and virus gene expression increases during maturation of monocytes to macrophages. J. Virol. 58, 67-76.

74. Narayan, O., and J.E. Clements. 1989. Biology and pathogenesis of Lentiviruses. J. Gen. Virol. 70, 1617-1639.

75. Ojo-Amaize, E.A., P. Nishanian, D.E. Keith Jr., R.L. Houghton, D.F. Heitjan, J.L. Fahey, and J.V. Giorgi. 1987. Antibodies to human immunodeficiency virus in human sera induce cell-mediated lysis of human immunodeficiency virus-infected cells. J. Immunol. 139, 2458-2463.

76. Ruscetti, F.W., V.S. Kalyanaraman, R. Overton, J. Mikovits, H. Stevenson, K. Stromberg, R.B. Herbermnan, W.L. Farrar, and J.R. Ortaldo. 1986. Analysis of effector mechanisms against HTLV-I and HTLV-III/LAV infected lymphoid cells. J. Immunol. 136, 3619.

77. Plata, F., B. Autran, L.P. Martins, S. Wain-Hobson, M. Raphael, C. Mayaud, M. Denis, J.M. Guillon, and P. Debre. 1987. AIDS virus-specific cytotoxic T lymphocytes in lung disorders. Nature 328, 348-351.

78. Walker, B.D., S. Chakrabarti, B. Moss, T.J. Paradis, T. Flynn, A.G. Durna, R.D. Blumberg, J.C. Kaplan, M.S. Hirsch, and R.T. Schooley. 1987. HIV-specific cytotoxic T lymphocytes in seropositive individuals. Nature 328, 348-351.

79. McElrath, M.J., J.E. Pruett, and Z.A. Cohn. 1989. Mononuclear phagocytes of blood and bone marrow: comparative roles as viral reservoirs in human immunodeficiency virus type 1 infections.

80. Shaw, G.M., M.E. Harper, and B.H. Hahn. 1985. HTLV-III infection in brains of children and adults with AIDS encephalopathy. Science 227, 177-182.

81. Koenig, S., H.E. Gendelman, J.M. Orenstein, M.C. DalCanto, G.H. Pezeshkpour, M. Yungbluth, F. Janotta, A. Aksamit, M.A. Martin, and A.S. Fauci. 1986. Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. Science (Wash.