Longitudinal Analysis of Simian Immunodeficiency Virus (SIV) Replication in the Lungs: Compartmentalized Regulation of SIV

Sheila A. Barber,¹ Lucio Gama,¹ Ming Li,¹ Tauni Voelker,¹ John E. Anderson,¹ M. Christine Zink,¹ Patrick M. Tarwater,³ Lucy M. Carruth,² and Janice E. Clements¹

¹Department of Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine, Baltimore, and ²Cylex, Inc., Columbia, Maryland; ³University of Texas Health Science Center at Houston, School of Public Health, El Paso Regional Campus, El Paso, Texas;

Background. Before the onset of AIDS, replication of human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) in the lungs is considered to be latent. When and how virus replication is controlled in the lungs is unclear. In the present study, we examine virus replication in the lungs and in cells recovered from bronchoalveolar lavage (BAL) samples in a comprehensive, longitudinal analysis of an SIV/macaque model.

Methods. Gene-specific RNA and DNA were quantitated by polymerase chain reaction (PCR) and by real-time reverse-transcription PCR (RT-PCR). Alveolar macrophages were isolated using Dynabeads CD14 (Invitrogen). Expression of CCAAT/enhancer-binding protein β (C/EBPβ) isoforms was examined by Western blot analysis.

Results. SIV replication occurred in the lungs during acute infection and correlated with plasma viral load. Innate immune responses involving interferon-β and the dominant-negative isoform of C/EBPβ were induced at this time. SIV RNA expression was suppressed in the lungs during asymptomatic infection, when no correlation existed with plasma viral load until SIV RNA levels rebounded again during late-stage disease. Modulation of viral RNA levels in BAL cells reflect RNA levels in lung tissue throughout each phase of infection.

Conclusion. Quantitation of SIV RNA in BAL cells provides a consistent surrogate assessment of virus replication in lung tissue. Innate immune responses contribute to compartmentalized suppression of acute SIV replication in the lungs.

Pulmonary complications of AIDS are major causes of morbidity and mortality in HIV-infected patients, and, despite the advent of highly active antiretroviral therapy (HAART), the lungs continue to be the most frequently involved organ in AIDS autopsy cases [1, 2]. Productive HIV/simian immunodeficiency virus (SIV) replication in lung tissue has predominantly been associated with cells of monocyte/macrophage lineage [3–7]. Before the onset of AIDS, it is thought that infection of alveolar macrophages is largely latent, perhaps because the alveolar microenvironment contains low levels of HIV/SIV (and thus alveolar macrophages escape infection locally) [8] or because innate immune responses in the lungs [9] and/or bioactive materials in the lung epithelial lining fluid (such as IgG, IgA, or surfactant proteins) [10] suppress virus replication (reviewed in [11]). Existing reports describe widely variable frequencies and numbers of virus particles and infected cells recovered from bronchoalveolar lavage (BAL) samples from HIV-infected individuals without opportunistic infection or pneumonia, even from those with advanced AIDS [12–14].

Studies of HIV-infected individuals coinfected with Mycobacterium tuberculosis have clearly demonstrated that latent HIV is present in lung macrophages and that this latent state is maintained in uninvolved lobes by innate immune responses involving interferon (IFN)–β [9, 15]. IFN-β, the most potent nonpharmacologic
inhibitor of HIV replication in macrophages [16], suppresses HIV replication through the induction of liver-enriched transcriptional inhibitory protein (LIP) [17], which is the dominant-negative isoform of the liver-enriched transcriptional activator protein (LAP) [17] that is commonly referred to as “CCAAT/enhancer-binding protein β” (C/EBPβ) [15, 18]. This truncated LIP isoform inhibits HIV-1 long terminal repeat (LTR)–dependent transcription in macrophages and in alveolar macrophages from HIV-infected individuals coinfectcd with M. tuberculosis [9, 15]. The elegance of this inhibitory mechanism can be appreciated through studies demonstrating that LAP (C/EBPβ) is a crucial activator of the HIV-1 LTR and that active replication of HIV in primary macrophages and differentiated promonocytic cell lines in vitro (but not in lymphocytes/cell lines) requires C/EBP transcription factors and at least 1 C/EBP binding site in the LTR [19–21]. Loss of LIP expression and reactivation of latent HIV in alveolar macrophages occurs during pulmonary tuberculosis and infiltration of T lymphocytes [9].

What remains unclear is when and how latent reservoirs are seeded in the lungs after HIV infection. In the few studies of acute SIV infection in the lungs, no or rare productively infected macrophages are detected by in situ hybridization 2 weeks after inoculation [3, 22], although SIV DNA is detectable by semiquantitative polymerase chain reaction (PCR) 7–14 days after inoculation [23, 24]. There are no longitudinal studies incorporating more-sensitive techniques to detect transcriptional activation in the lungs during various stages of HIV/SIV disease [11]. In the present study, we provide the first to our knowledge, comprehensive and longitudinal analysis of viral load and comparison of gene expression. We inoculated using the same protocol, and CD14+ cells were isolated from BAL samples at 10 days after inoculation as described below.

**Positive selection of alveolar macrophages from BAL samples.** CD14+ cells were positively selected from BAL cells collected at necropsy from macaques infected with SIV for 10 days by use of Dynabeads CD14 (Invitrogen), in accordance with the manufacturer’s protocol. The efficacy of CD14+ selection was determined by flow-cytometry (FACS) analysis, as described elsewhere [27], using phycoerythrin-conjugated anti-CD14 antibodies (Beckman Coulter).

**Real-time RT-PCR analysis of SIV, IFN-β, and myxovirus A (MxA) RNA.** RNA was extracted from punches of lung tissue (snap-frozen) by use of the RNeasy fibrous tissue minikit and from BAL cells by use of the RNeasy minikit (both from Qiagen), in accordance with the manufacturer’s protocol. The real-time RT-PCR assay used to quantitate SIV RNA as well as macaque IFN-β and MxA mRNA has been described elsewhere [25, 28]. To test for significant differences in continuous variables between 2 time points, we performed the nonparametric Mann-Whitney test of population central tendency. Spearman’s rank correlation test was used to determine the degree of correlation between viral load in plasma and in lung tissue.

**Real-time PCR analysis of SIV DNA.** DNA was extracted from punches of lung tissue (snap-frozen) by use of the DNeasy tissue kit (Qiagen), in accordance with the manufacturer’s protocol. Real-time PCR for SIV DNA has been described elsewhere [29]. Copy numbers of SIV DNA were normalized to copy numbers of a single-copy cellular gene (the IFN-β gene).

**Western blot.** Western blot analysis of C/EBPβ has been described elsewhere [30] and was performed on homogenized punches of lung tissue (snap-frozen).

**Immunohistochemistry.** For antigen retrieval, sections of Streck-fixed paraffin-embedd lung tissue from SIV-infected macaques were heated in 0.01 mol/L Na citrate (pH 6.0) in a microwave for 8 min. Tissues were immunohistochemically stained, first with antibody to SIV gp41 (kk41; National Institutes of Health AIDS Research and Reference Reagent Program), as described elsewhere [31]; immunohistochemical staining was then repeated with antibody to CD68 (Dako). Tissues were not counterstained.

**RESULTS**

**Real-time RT-PCR analysis of SIV replication in the lungs and in cells recovered from BAL samples.** To provide a comprehensive analysis of virus replication in the lung and in cells recovered from BAL samples, 4 groups of 6 macaques were inoculated using our accelerated and consistent SIV model of HIV lung disease. In this model, all infected macaques develop AIDS, and essentially all develop some degree of lung disease.
(without pathological evidence of opportunistic infection) by 84 days after inoculation [26]. SIV RNA was quantitated in lung tissue obtained postmortem from uninfected macaques; in lung tissue from macaques infected with SIV for 10, 21, 56, and 84 days; and in cells from BAL samples collected longitudinally from macaques infected with SIV for 84 days. SIV replication in lung tissue was biphasic (figure 1A), peaking (average, 5.9 × 10^5 copies of SIV RNA/μg of lung RNA) during acute infection at 10 days after inoculation, declining significantly (>1 log SIV RNA/μg of lung RNA) by 21 days after inoculation, and increasing again during late-stage disease (between 56 and 84 days after inoculation). SIV replication in BAL cells was detected as early as 7 days after inoculation (figure 1B) and also exhibited a biphasic pattern, peaking (average, 1.0 × 10^5 copies of SIV RNA/μg of lung RNA) during acute infection in most macaques between 7 and 10 days after inoculation, declining (>1 log SIV RNA/μg of lung RNA) between 14 and 28 days after inoculation, and increasing again during late-stage disease. Note that, despite frequent flushin of cells during acute infection through BAL, SIV RNA was readily detected in BAL cells, suggesting that the predominance of this SIV RNA arises from newly recruited infected alveolar macrophages and/or T lymphocytes. We conclude the following from these studies. First, active SIV replication can occur in lung and BAL cells quite soon after infection, and, thus, SIV-infected cells seed the lungs during acute infection. Second, although active replication predominates during acute and late-stage disease, SIV RNA can be detected at all times, indicating that all infected cells are not eliminated after acute infection and all virus is not in a latent state. Finally and importantly, monitoring SIV replication in BAL cells by real-time RT-PCR provides a consistent surrogate assessment of replication events occurring in lung tissue.

**SIV replication in alveolar macrophages during acute infection.** Studies using SIV/macaque models have led to the conclusion that virus replication in macrophages in the lungs is nonexistent or extremely low during acute infection [3, 22]. Because the composition of BAL cells is on average >90% alveolar macrophages [32] and because SIV RNA was readily detectable in BAL cells during acute infection in our SIV/macaque model (figure 1B), it seemed highly likely that alveolar macrophages would contribute substantially to viral load in the BAL cells at this time. Immunohistochemical staining of lung tissue confirmed that alveolar macrophages were infected at 84 days after inoculation (figure 1C). To examine acute SIV replication in alveolar macrophages, an additional 5 macaques were inoculated, and SIV RNA was quantitated in CD14^+ cells isolated from BAL samples 10 days after inoculation by use of Dynabead technology, as described in Materials and Methods. The efficac of CD14^+ cell selection was evaluated by FACS analysis, which demonstrated efficiency recovery (>85%) of
CD14+ cells from BAL samples (data not shown). CD14+ BAL cells from each macaque expressed 927,500–3,775,677 copy equivalents (eq.) of SIV RNA/μg of RNA (average, \(2.4 \times 10^8\) copy eq. of SIV RNA/μg of RNA), indicating that alveolar macrophages are productively infected and support high levels of virus replication during acute infection.

**Induction of IFN-β and MxA in the lungs during acute SIV infection.** Thus far our data have indicated that productive virus replication occurs in lung macrophages during acute infection and that virus replication in the lungs is suppressed by 21 days after inoculation. This replication pattern is strikingly similar to that observed in the brain, where acute virus replication is also suppressed in macrophages/microglia (the predominant sources of SIV/HIV replication in the brain) by 21 days after inoculation [29, 30]. Because suppression of acute virus replication in the brain correlates with induction of IFN-β and because IFN-β can suppress ongoing SIV replication in macrophages [30], we next quantitated expression of IFN-β and MxA (a sensitive marker for type I IFN activity [33]), in the lungs (figure 2). IFN-β expression increased substantially (∼10-fold) over basal levels (those in uninfected macaques) during acute infection at 10 days after inoculation, declined by 21 days after inoculation, and increased again (∼5-fold) during late-stage disease. Because increased expression of type I IFN is amplified through the expression of IFN-inducible genes such as MxA, it is not surprising that MxA mRNA expression in the lungs was significantly higher (∼20-fold) than basal levels at 10 days after inoculation, declined to basal levels at 21 days after inoculation and increased again (∼5-fold) by 84 days.

These data demonstrate that type I IFN responses are induced in the lungs as an innate immune response to acute SIV infection in temporal concert with the expression of both of these innate immune genes in the brain [28].

**Induction of LIP (the dominant-negative isoform of LAP) in the lungs during acute SIV infection.** IFN-β–dependent suppression of HIV and SIV transcription is mediated through induction of LIP, the dominant-negative isoform of LAP (C/EBPβ). This, along with 2 additional observations, prompted us to examine expression of LAP and LIP in the lungs. First, increased expression of LIP relative to LAP occurs in the brains of SIV-infected macaques coordinately with increased expression of IFN-β and MxA and with suppression of acute SIV replication [28–30]. Second, LIP maintains the latent state of HIV in alveolar macrophages in uninvolved lobes of the lungs in HIV-infected individuals coinfected with M. tuberculosis [9, 15]. Quantitative Western blot analysis of lung homogenates demonstrates a significant decline in the ratio of LAP to LIP between 10 and 21 days after inoculation in favor of LIP expression, which continues throughout infection (figure 3). These data provide another parallel to our observations in the brain and demonstrate that increased expression of LIP relative to LAP is established very soon after infection. Therefore, we conclude that innate immune responses involving type I IFN immune responses and LIP likely contribute to suppression of acute SIV replication in lung macrophages, although this mechanism ultimately fails in macaques with high viral loads in the lungs during late-stage disease.

**Figure 2.** Quantitation of interferon (IFN)-β and myxovirus A (MxA) mRNA in the lungs by real-time reverse-transcription polymerase chain reaction (RT-PCR). Shown are copy nos. of IFN-β (A) and MxA (B) mRNA present in lung tissue obtained at necropsy from 1 group of 6 uninfected macaques (0 days after inoculation) and 4 groups of 6 simian immunodeficiency virus (SIV)–infected macaques (10, 21, 56, or 84 days after inoculation). Medians (black bars) for each experimental group are indicated. Data for each macaque are the mean of triplicate real-time RT-PCR analyses. IFN-β mRNA expression increased significantly over basal levels (those in uninfected macaques) during acute infection at 10 days after inoculation (\(P = .004\)), declined by 21 days after inoculation (\(P = .004\)), and increased again during late-stage disease (\(P = .004\) for 56 vs. 84 days after inoculation). The presence of IFN-β protein was confirmed by Western blot analysis of lung homogenates (data not shown). MxA mRNA expression in the lungs increased significantly over basal levels at 10 days after inoculation (\(P = .004\)), declined at 21 days after inoculation (\(P = .004\) for 10 vs. 21 days), and increased again during late-stage disease (\(P = .01\), for 56 vs. 84 days after inoculation). Eq., equivalents.
SIV Replication in the Lungs

Figure 3. Ratio of liver-enriched transcriptional activator protein (LAP) to liver-enriched transcriptional inhibitory protein (LIP) expression in the lungs. Homogenates were prepared from lungs obtained at necropsy from uninfected macaques (0 days after inoculation) and simian immunodeficiency virus (SIV)-infected macaques (7, 10, 21, 56, or 84 days after inoculation). Quantitative Western blot analysis was performed as described elsewhere [30]. LAP (37 kDa) expression was divided by expression of LIP (16 kDa) to generate a ratio (LAP:LIP) for each macaque. Medians (black bars) for each experimental group are indicated. Results demonstrate a significant decline in the ratio of LAP to LIP between 10 and 21 days after inoculation ( and , for uninfected vs. 21 days respectively) in favor of LIP expression, which continued throughout infection. Inset, Representative Western blot of LAP and LIP expression in lung homogenates from macaques infected with SIV for the indicated times.

Figure 4. Quantitation of simian immunodeficiency virus (SIV) DNA by real-time polymerase chain reaction (PCR). SIV DNA copy nos. in lungs obtained at necropsy from 1 group of 6 uninfected macaques (0 days after inoculation) and 4 groups of 6 SIV-infected macaques (10, 21, 56, or 84 days after inoculation). Data for each macaque are the mean of triplicate real-time PCR analyses. Medians (black bars) for each experimental group are indicated. SIV DNA levels declined after acute infection ( , for 10 days vs. 21 days), persisted throughout infection, and increased again during late-stage disease (84 days after inoculation; , for 56 days vs. 84 days). Eq., equivalents.

Compartmentalized regulation of SIV replication in the lung. One feature of our accelerated and consistent SIV/macaque model is the induction and maintenance of high plasma viral load. SIV RNA levels peak (10^5–10^6 copy eq./mL of plasma) during acute infection, decrease transiently (0.3–1 log) over the next 2 weeks, and either stabilize or increase thereafter, sustaining high viral loads (10^7–10^8 copy eq./mL of plasma) throughout the course of disease [25]. Therefore, we reasoned that, if innate immune responses to SIV infection mediate compartmentalized regulation of acute SIV replication in the lungs, there would be no correlation between viral load in plasma and in the lungs during the asymptomatic phase of infection. Spearman’s rank correlation test was used to determine the degree of correlation between plasma viral load and viral load in the lung. The plasma viral loads for each macaque in this study have been published elsewhere [25, 29]. The results indicate that a significant correlation exists between plasma viral load and viral load in the lungs only during acute (10 days after inoculation; correlation value, 0.83; ) and late-stage (84 day after inoculation; correlation value, 0.89; ) infection. No correlation exists between these 2 compartments during asymptomatic infection (21–56 days after inoculation; correlation values, -0.49 and -0.03, respectively; ). Thus, we conclude that, as in the brain, immune responses involving IFN-β and LIP contribute to compartmentalized suppression of acute SIV replication in the lungs, resulting in low levels of virus replication during the asymptomatic stage.

Real-time PCR analysis of SIV DNA in the lung. The present data and previous studies in this model [28] demonstrate that the kinetics of SIV replication and the induction of innate immune responses in the lungs and brain appear to be in temporal concordance. In the brain, although SIV RNA levels are suppressed after acute infection, there is no concomitant loss of SIV DNA, indicating that there is no significant component of cell death (potentially mediated by the cytopathic effects [CPE] or cytotoxic T lymphocyte [CTL]–mediated killing) involved in the suppression; rather, virus replication is suppressed at a transcriptional level [28]. Unlike the brain, however, which is protected by the blood-brain barrier, the lungs are readily accessible to traffick immune cells, some of which may be infected and either expelled from the airway or lost, perhaps due to CPE and/or CTL-mediated killing. To further explore the parallels between SIV replication in the lungs and brain in this model, we next quantitated SIV DNA in the lungs (fig. 4). SIV DNA levels declined to some extent (<1 log SIV DNA/μg of lung DNA) after acute infection, persisted throughout infection, and increased again during late-stage disease (84 days after inoculation), indicating that some but not all infected cells are lost after acute infection. Thus,
mechanisms in addition to innate immune responses and LIP contribute to suppression of acute SIV replication in the lungs.

**DISCUSSION**

In this study, we provide the first to our knowledge, comprehensive and longitudinal analysis of viral load and innate immune responses to SIV infection in the lungs, using quantitative and highly sensitive real-time RT-PCR. The primary goals of this study were to determine when the lung become infected after intravenous SIV exposure, to determine when innate immune mechanisms known to suppress HIV/SIV replication become established in the lungs, and to compare and contrast the kinetics of SIV replication and induction of innate immune responses in lung and brain tissues, 2 tissues commonly associated with HIV/SIV-related diseases in which macrophages are the primary cell type productively infected with HIV/SIV.

The data from our macaque model demonstrate unequivocally that monitoring SIV replication in BAL samples by real-time RT-PCR provides a consistent surrogate assessment of replication events occurring in lung tissue without the need for more-invasive biopsies. Furthermore, these studies demonstrate that SIV infection can be established during acute infection in the lungs and in BAL cells, including alveolar macrophages. These later finding are in apparent contrast to those of 2 other studies in SIV models that did not detect acute SIV replication in macrophages from the lungs [3, 22]. At least 2 explanations exist that would reconcile these conflicting reports. First is the inherent difference in the sensitivities of the experimental techniques used (in situ hybridization vs. real-time RT-PCR). A second significant factor is the particular SIV inoculum and species of macaque studied. In both earlier studies, rhesus macaques (Macaca mulatta) were inoculated with the immunosuppressive viral swarm SIV/DeltaB670. In our studies, pigtailed macaques (Macaca nemestrina) were inoculated with a combination of SIV/DeltaB670 and a macrophage-tropic virus, SIV/17Efr, which specifically replicates better than other macrophage-tropic SIV isolates in the lungs [26]. The plasma viral loads observed during acute infection in all of these studies overlap (5.4 × 10^5–1.0 × 10^6 vs. 5.0 × 10^5–1.0 × 10^6 copies/mL [22, 29]) and, thus, are likely not a major differentiating factor; however, it is highly conceivable that the presence of specific viral genotypes contributes to the discrepancy between these studies. In our model, SIV/17Efr, but not macrophage-tropic clones of SIV/DeltaB670, has been found to have a selective advantage for replication in the lungs and BAL cells, compared with peripheral-blood mononuclear cells (PBMCs) [26]. In short, specific viral genotypes and host factors may influence the ability to detect SIV/HIV replication in the lungs before the onset of AIDS and perhaps by extension account for the variable isolation of HIV from BAL samples from infected individuals.

In light of our data demonstrating significant suppression of acute SIV replication in lungs and BAL cells by 21–28 days after inoculation, it is not surprising that innate immune responses that control virus replication in the lungs are activated soon after infection. In our model, induction of IFN-β and MxA occurs 10 days after inoculation, and the decreased ratio of LAP to LIP favoring LIP is established by 21 days after inoculation and maintained throughout infection. It appears that the type I IFN response initiates the increased expression of LIP [15, 28], which is maintained thereafter either by the constitutive expression of IFN-β or, perhaps, by a yet-undetermined mechanism present in the lungs. These data support other reports implicating LIP in suppression of HIV replication in alveolar macrophages from uninfamed lung from HIV-infected individuals coinfected with M. tuberculosis [9, 15] and further indicate that this innate mechanism is established in the lungs very soon after infection, as it is in the brain [28, 30]. Unlike the brain, which is protected by the blood-brain barrier, the lungs are a highly vascular tissue in which PBMCs (some of which may be infected) traffic through its capillaries at high volume. Thus, although SIV RNA levels become undetectable in the brain after acute infection and rebound with a tight correlation to severity of central nervous system (CNS) disease, the same would not necessarily be expected in the lungs. In fact, our data demonstrate that, although SIV RNA levels in the lungs drop after acute infection and no longer correlate with plasma viral loads, SIV RNA levels are readily detectable throughout infection and do not correlate with severity of lung disease [4, 26]. Moreover, the loss of SIV DNA (interpreted as a loss of infected cells) after acute infection in the lungs, but not in the brain, indicates that mechanisms in addition to LIP contribute to regulation of SIV replication in the lungs. The continued presence of both SIV RNA and DNA indicates that all infected cells are not eliminated in the lungs and that all virus is not in a latent state when evaluated by highly sensitive real-time RT-PCR.

The present data in conjunction with other independent observations (cited below) reveal several strong parallels between the lung and brain with respect to SIV replication and associated diseases. First, the prevalence of noninfectious pulmonary complications and HIV-associated CNS disease has increased in the HAART era [34–37]. Second, macrophages are the primary cells productively infected in the lungs and brain [3–7, 38, 39]. Third, the biphasic patterns of virus replication are temporally coordinate in the lungs and brain [29]. Fourth, suppression of acute virus replication occurs coordinately with the induction of IFN-β and MxA as well as with increased expression of LIP in the lungs and brain [28, 30]. Fifth and importantly, because all infected cells are not eliminated after acute infection, brain and lung macrophages provide a reservoir of SIV/HIV [9, 15, 28, 29]. Sixth, resurgent high-level virus
replication observed in the lungs and brain after asymptomatic infection (i.e., after the onset of AIDS) is temporally parallel in this model [25, 29], suggesting that SIV/HIV replication in nonlymphoid tissues is coordinately regulated. Seventh, active viral replication in the lungs and brain is associated with local inflammation (acute infection) and infiltration of CTLs (later in infection) [8, 9, 14, 29]. Finally, compartmentalization of viral genotypes occurs both in the lungs and in the brain, some of which are shared in both tissues [12, 26, 40–43]. In fact, in our macaque model, the 3 predominant viruses found in the lungs and brain are SIV/17EFr and the macrophage-tropic clones of DeltaB670, Cl-2, and Cl-12 [26, 41]. Moreover, the virus strains that predominate in PBMCs are rarely the strains that predominate in the lung and are frequently different from the strains that predominate in the brain. Collectively, these observations suggest that common mechanisms that regulate HIV/SIV replication exist in tissues in which macrophages are the primary cell type that is productively infected and present a different cellular environment to be considered in pharmacologic and/or immunomodulatory therapies designed to suppress HIV replication in these tissues.

**Acknowledgments**

We thank Drs. Robert J. Adams and Joseph L. Mankowski, for their invaluable contributions to the animal studies; and Suzanne Queen, Brandon Bullock, April Hargrove, and Chante Austin, for superb technical assistance. We also thank Justyna M. Dudaronek, for development of the press HIV replication in these tissues. We also thank Justyna M. Dudaronek, for development of the

**References**

17. Descombes P, Schibler U. A liver-enriched transcriptional activator protein, LAP, and a transcriptional inhibitory protein, LIP, are translated from the same mRNA. Cell 1991; 67:569–79.


43. Itescu S, Simonelli PF, Winchester RJ, Ginsberg HS. Human immunodeficiency virus type 1 strains in the lungs of infected individuals evolve independently from those in peripheral blood and are highly conserved in the C-terminal region of the envelope V3 loop. Proc Natl Acad Sci USA 1994;91:11378–82.