Requirement for the Second Coding Exon of Tat in the Optimal Replication of Macrophage-Tropic HIV-1

Christine Neuveut a Robert M. Scoggins b David Camerini b Richard B. Markham c Kuan-Teh Jeang a

a Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, Bethesda, Md.; b Myles H. Thaler Center for AIDS and Human Retrovirus Research, University of Virginia Health Science Center, Charlottesville, Va.; c Department of Molecular Microbiology and Immunology, Johns Hopkins School of Public Health, Baltimore, Md., USA

Key Words
HIV • Macrophage tropism • Tat • AIDS pathogenesis • SCID mouse

Abstract
HIV-1 Tat is essential for virus replication and is a potent transactivator of viral gene expression. Evidence suggests that Tat also influences virus infectivity and cytopathicity. Here, we find that the second coding exon of Tat contributes a novel function for the replication/infectivity of macrophage-tropic HIV-1. We show that macrophage-tropic HIV-1 which expresses the full-length two-exon form of Tat replicates better in monocyte-derived macrophages (MDM) than an otherwise isogenic virus which expresses only the one-exon form of Tat. Similarly, two-exon Tat expressing HIV-1 also replicates better than one-exon Tat expressing HIV-1 in two different models of human cells/tissue reconstituted SCID mice.

Introduction
Acquired immunodeficiency syndrome (AIDS), the disease induced by HIV-1, is a complex process that involves both multiple viral and host determinants [8, 20, 26, 36]. AIDS development can be viewed as occurring in three phases: a brief primary infection, followed by a long asymptomatic period, with finally disease manifestation and death. The detailed mechanisms underlying the initiation and development of AIDS remain incompletely understood. However, even at its asymptomatic stage, HIV-1 replicates actively in the host. Hence, even at time of low initial viremia, HIV-1 RNA, nevertheless, can be found in both the circulating peripheral blood as well as in lymphoid organs of infected individuals. Later, very high levels of viral replication with daily production of $10^7$ to $10^9$ virions accompanied by the demise of about $2 \times 10^9$ CD4+ T cells/day have been estimated [26].

There is a strong correlation between virus load and disease progression in AIDS [18]. However, the precise sequence of molecular events governing disease development in vivo remains to be fully elucidated. Indeed, the respective roles of macrophage-tropic (M- or CCR5-tropic) and T-cell-tropic (T- or CXCR4-tropic) HIV-1 in
AIDS is still incompletely understood [5]. CCR5-utilizing viruses dominate exclusively in virtually all newly HIV-1-infected individuals. By contrast, more than 50% of individuals infected with HIV-1 for 7 years or greater will be found to have mostly CXCR4-tropic HIV-1 [5]. One thought is that the transition from a CCR5-using (i.e. macrophage infecting) to a CXCR4-using (i.e. T-cell infecting) HIV-1 reflects the progression to a more rapidly replicating and disease-potent form of virus [2, 23, 62]. Nevertheless, the fact that individuals homozygous for genetic deletion of CCR5, a coreceptor necessary for infection of CD4+ cells by M-tropic HIV-1, are resistant to naturally transmitted HIV-1 infections argues for a critical role of macrophages (and macrophage-tropic HIV-1) in new infections [5].

Tat is essential for HIV-1 replication and viability. Tat is a potent transcriptional activator of the HIV-1 LTR [47] that acts through binding to a nascent TAR leader RNA [6, 7] and functions to enhance the initiation and processive elongation of RNA polymerase II at the viral promoter [21, 30–32, 35, 49, 50, 60]. For its transcriptional function, Tat interacts with a host of cellular factors including Sp1 [17, 63], p300/CBP [4, 40], PKR [12], TBP [34], cyclin T1/CDK9 [9, 16, 25, 58], Tip60 [33], granulin [27, 57], mRNA capping factors [15], and RNA polymerase II [22, 41] amongst others. There is evidence that Tat aids HIV-1 replication through mechanisms in addition to its transcriptional activation of the LTR. Hence, Tat can modulate the expression of cellular genes such as TGF-β [37], IL2 [59]; IL6 [3]; Bcl-2 [65], and TNF β and α [11]. Moreover Tat is secreted into the extracellular milieu [10, 23, 62] and can activate quiescent T lymphocytes [46].

Tat is encoded by two exons. The first coding exon of Tat spans amino acids 1–72 while the second coding exon spans amino acids 73–101. Findings from subgenomic transfection experiments have demonstrated that the first coding exon of Tat is sufficient for transcriptional activation of the HIV-1 LTR [29, 32], and that deletion of the second coding exon of Tat appears to minimally alter the transactivation of LTR reporter plasmids. However, the second coding exon of Tat is conserved amongst all HIV-1 strains, as well as in HIV-2 and SIV, arguing that this exon contains a function important for the virus. Indeed, we have previously shown that the second coding exon of Tat is necessary for optimal virus replication in T cells [45] and for the chronic in vivo replication of SIV in macaques [56a]. Work from our laboratory has also shown that the second coding exon of Tat interacts with the translation elongation factor EF-1α, an interaction that affects the translation of cellular mRNA in HIV-1 infected cells [61].

Here, we have investigated the contribution of the second coding exon of Tat to replication of HIV-1 in macrophages. We have constructed otherwise isogenic macrophage-tropic HIV-1s which express either the first exon of Tat or the two-exon full-length Tat protein. We have compared the replication of these viruses in MDM and in human cells/tissue-reconstituted SCID (hu-SCID) mice. Our results support that the second coding exon of Tat contributes to optimal virus replication in macrophages. Deletion of the second exon of tat was significantly deleterious for viral replication in vivo in reconstituted hu-PBL-SCID mice and hu-SCID (thymus/liver) mice.

Materials and Methods

Plasmid Constructions

All molecular viral clones were derived from pNL4-3 [1] engineered by E. Freed to contain an AD8 macrophage-tropic Env (pNLAD8). We have previously described the conditions for infection with pNLAD8-derived macrophage-tropic viruses [51]. Using pNLAD8, we created pNLAD8/101 which expresses only the first coding exon of tat. pNLAD8 was constructed by introducing a stop codon at amino acid 72. pNLAD8/101 was constructed by removing a premature stop codon [32] in pNL4-3 after amino acid 86 of Tat. Here, a single nucleotide change extends the Tat protein in NL4-3 from its 86 amino acids to the 104 amino acid form found in most primary HIV-1 isolates [32]. pNLAD8/m54 and pNLAD8/m60 contain an engineered stop codon at amino acid 54 and 60, respectively; these two molecular clones encode highly abbreviated forms of Tat.

Cells, Transfection and Viral Infections

U38 cells were cultured in RPMI-1640 with 10% fetal calf serum. HeLa cells were propagated in Dulbecco's modified Eagle's medium (DMEM with 10% fetal bovine serum). Transfection of HeLa cells was performed using calcium phosphate. Virus production was monitored and normalized by measuring supernatant reverse transcriptase (RT) activity [45, 56]. U38 cells were electroporated with the various molecular clones using (300 V and 960 gF; Bio-Rad electroporator). MDM were obtained from elutriated monocytes which were allowed to differentiate over a 14-day period in 100-mm2 bacteriological petri plates and then were transferred to a 24-well tissue culture plates (1 x 106 cells) and allowed to adhere overnight prior to infection [19]. MDM were cultured in DMEM 10% human serum. Infection of MDM was performed using viruses normalized for RT activity (105 cpm RT). Virus was first absorbed in a small volume (100 μl) for 3 h at 37°C. TCID50 was determined after infection of human peripheral blood monocytes (PBMC) for 14 days. PBMC were stimulated with phytohemagglutinin (PHA, 250 ng/ml final concentration) for 3 days prior to infection and were maintained in RPMI-1640 with 20% fetal bovine calf serum and 10% interleukin 2 (Pharmacia). Usually 4 x 106 PBMCs in a 0.1-ml volume were infected with virus supernatants.