Human Immunodeficiency Virus-1 Tat Protein: Immunological Facets of a Transcriptional Activator

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Received 22 May 2007; revised 20 September 2007

Human immunodeficiency virus-1 (HIV-1) infection is characterized by chronic immune activation and progressive loss of CD4+ T cells, leading to a wide array of immune dysfunction, particularly involving immune response directed against viral antigens. HIV-1 encodes for fifteen proteins, which might serve as a target for immune recognition. Immune response to the envelope proteins have been studied more due to their presence on the surface of the virus. Recent studies on HIV vaccine development have focused on the Gag and Pol proteins. The transactivator Tat and Rev proteins have also been the focus of immunization studies due to their potent regulatory activity. The Tat (transactivator of transcription) protein although being nuclear in localization is also released from infected cells and acts on uninfected cells. Extracellular Tat seems to play an important role in AIDS pathogenesis. Furthermore, a correlation has been found between anti-Tat immune response and slow progression of the disease. Although several studies have shown Tat as a potential vaccine candidate with encouraging results, there are also reports raising doubt about its efficacy in multi-component HIV vaccine strategy. Here, we have addressed the issue of immune response to the most indispensable HIV-1 regulatory protein Tat.

Keywords: HIV-1, Tat, Vaccine, Cytokine, Transcription Activator, Immunosuppression

Introduction

The epidemic of acquired immunodeficiency syndrome (AIDS) has taken a tremendous toll in respect to decreased quality and loss of life worldwide especially in Africa, where more than 70% of deaths are from HIV infection. The HIV-1, a retrovirus belonging to lentivirus family having RNA as its genome has been clearly implicated as the primary cause of AIDS. The HIV genome of about 9.2 kb codes not only for gag, pol, and env which are common to all retroviruses, but also comprises genes involved in virion maturation, infectivity and morphogenesis (vpu, nef and vif), in regulation of viral replication (Tat and Rev) and for nuclear transport (vpr). Viral regulatory proteins are encoded by multiply spliced 2.0 kb mRNA species, while the structural and enzymatic viral proteins are translated products of unspliced or singly spliced transcript.

The Tat Protein

Tat (transactivator of transcription) is a small nuclear protein of 86-101 amino acids, which is encoded from two separate exons. The first exon encodes amino acids 1-72 and in most strains of HIV-1, the second exon encodes amino acids 73-101. Tat has been divided into several distinct domains, based on their amino acid composition. N-terminal activation domain (amino acids 1-20) has 13 amino acids with amphipathic characteristics. The function of this acidic domain remains to be clearly elucidated. The second domain comprises of amino acids 22-37 and contains seven cysteine residues that are highly conserved between different isolates of HIV. This region seems to be important for intramolecular disulfide bond formation and Tat function, as mutation of most of the individual cysteines abolishes Tat function. The third domain comprises of amino acids 40-47 containing RKGLGI motif conserved between different HIV and simian immunodeficiency virus (SIV) Tat. This region along with domains 1 and 2 is suggested to be the minimal activation domain of HIV-1 Tat. Finally, the fourth domain of first exon comprises of amino acids 49-72, which contains the basic PKKRRQRRR motif. This arginine-rich motif seems to be responsible for transactivator response region (TAR) RNA binding property of Tat and is
also important for nuclear localization of the protein\(^3\)\(^4\). The second coding exon of Tat seems to be functionally less significant and has two short motifs. The first one is RGD motif, probably used as a cell adhesion signal for binding of cellular integrins, although recent data suggest that the basic domain of Tat is probably more important for cellular uptake. The other domain of second exon has an ESKKKVE motif, conserved in most HIV-1 Tat proteins, but its functional significance remains to be understood\(^5\).

**Regulation of Viral Replication by Tat**

The life cycle of HIV involves six steps: (i) binding of virus to the target cell, (ii) fusion with the cell and uncoating of the virus, (iii) reverse transcription of the RNA genome, (iv) integration of DNA into the host genome, (v) transcription and translation of the viral genes, and (vi) finally replication and budding of new virions. Immediately after HIV infects the cell, virion RNA is copied into DNA and the viral genome is transported into the nucleus and integrated into host cell genome. Once integrated into the host chromosome, HIV-1 provirus becomes subject to regulation by cellular transcription machinery, as well as its own regulatory proteins.

The Tat primarily controls the HIV transcription. It dramatically increases the expression of all genes linked to the viral long terminal repeat (LTR) promoter\(^6\). Tat protein is an obligatory requirement for viral replication. It binds to the TAR RNA that forms a stable RNA stem loop at the 5’ end of all the viral transcripts and recruits positive transcription elongation factor B complex (pTEFB) comprising of cyclin T1 and CDK9. Hyperphosphorylation of carboxy terminus domain of RNA polymerase II by CDK9 leads to enhanced elongation of the transcription from the viral promoter\(^7\). Tat provides one of the first examples of regulation of a viral gene expression through control of elongation by host RNA polymerase II. In the absence of Tat, initiation from the LTR is efficient, but transcription is impaired because the promoter engages poorly processive polymerase that falls off from the DNA template prematurely. Recent study also suggests that HIV-1 Tat interacts with Nef and induces HIV-1 gene expression\(^8\).

**Extracellular Tat Protein**

In addition to its major role in transcriptional activation of viral gene expression, Tat seems to be involved in a number of functions in HIV-1 infection. Several studies suggest that Tat has a role in viral infectivity and contributes to pathogenesis\(^9\)\(^10\). It seems to be actively released by HIV-infected cells and is detected in the serum of HIV-1 infected individuals. Extracellular Tat is able to enter latent infected cells and activate the transcription of viral genome. In HIV infection, reactivation of HIV-1 by extracellular Tat may explain the burst of replication associated with early phase of HIV infection. Moreover, extracellular Tat upregulates expression of CXCR4, CCR5 and CCR3, co-receptors for HIV-1 in

![Fig. 1—Schematic diagram showing biological role of Tat in HIV-1 pathogenesis and immune response](image-url)
infected peripheral blood mononuclear cells (PBMCs). This correlates with enhanced infectivity of macrophage and T cell tropic HIV-1 strains.

Extracellular Tat also acts on different types of uninfected cells by binding with integrins, vascular endothelial growth factor (VEGF); low density lipoprotein receptor related (LRP) protein and heparin sulfate proteoglycans (HSPGs). The binding of Tat with LPR and HSPGs mediates its internalization inside the cell and this internalized Tat retains the capacity of transactivating viral or cellular gene expression. This property of HIV-1 Tat is used in transporting proteins inside the cell. Tat is also shown to be a potent chemoattractant and promotes migration of several cell types including monocytes, endothelial cells and dendritic cells (DCs), thereby favoring the spread of HIV-1. It has sequence homology with β chemokines and mimics their functional feature by signaling through chemokine receptors, which plays a role in chemotaxis. This helps in recruiting monocytes and macrophages towards HIV producing cells, which express these receptors. This chemotactic property requires both RGD and the basic domains. These domains are also involved in the migration of DCs. Tat in its native form selectively binds to and is taken up by DCs, and induces their maturation. DCs exposed to Tat upregulate key co-stimulatory molecules, such as CD40, CD80, CD86, LFA, MHC class I and II antigen, lymphotixin, MIP-1α, MIP-1β, RANTES, IL-12 and TNFα.

Role of Tat in HIV Associated Dementia

HIV associated dementia (HAD) is a severe from of neurological disorder observed in about 30% of the patients with AIDS. It is characterized by infiltration of inflammatory cells and abnormalities of dendritic and neuronal cells, leading to the death. It is suggested that Tat may play an important role as a secreted, soluble neurotoxin in HAD. It can interact with astrocytes, microglia, and brain endothelial cells to increase expression of inducible nitric oxide synthase (iNOS) and release nitric oxide (NO). Other mechanisms for Tat neurotoxicity include modulation of calcium homeostasis and stimulation of TNF-α, NF-κB and glutamate receptors.

HIV-1 Tat protein also seems to play a role in apoptosis. It mediates dysregulation of caspase 8 pathway, which induces apoptosis in neuron and T cells. It also seems to increase expression of TNF-related apoptosis-inducing ligand (TRAIL) and Fas ligand (FasL), thus promoting induction of apoptosis. Tat can also act as an anti-apoptotic molecule, thus helping in cell survival. It has been shown to upregulate cell survival growth factors, including anti-apoptotic genes in infected cells and hence may protect them against apoptosis. The different effects of Tat on apoptosis seem to depend upon the differential activation of pro- and anti-apoptotic genes, cell types involved and growth factor released in the cellular microenvironment. Interestingly, in certain neuronal cell lines, endogenous expression of Tat gene by transfection does not cause cell death, but when the same cells are exposed to extracellular Tat, it shows toxic effects. However, Tat-induced neurotoxicity is not observed in all neuronal cell culture.

Immunological Aspects of Tat

For many years, Tat was not considered a candidate for an AIDS vaccine because of its intracellular localization. However, the discovery of an extracellular role for Tat has enabled it for use in HIV vaccine strategy. Several findings suggest Tat as a potential candidate for an anti-HIV vaccine. First, during immune response to HIV infection, immune reaction against Tat is quickest among the viral proteins. Secondly, anti-Tat immune response has been shown to be directly proportional with the delayed progression of disease in several clinical studies. Furthermore, it increases the permissiveness for HIV infection, thus suggesting that neutralization with anti-Tat humoral response may lead to protection from HIV infection. Immunization with Tat may also prevent AIDS-associated pathologies in which extracellular Tat plays a pathogenic role. Finally, Tat is the most conserved protein of HIV-1, making the development of a viral clone resistant to Tat vaccination very difficult.

Two regions of Tat (amino acids 1-9 and 70-83) have been found to be potent in eliciting humoral immune response and three other regions have been described to elicit cytotoxic T-lymphocytes (CTLs). Interestingly, uninfected individuals have shown to have natural IgM antibodies against two regions of Tat, suggesting that these might be the basis for natural immunity against viruses in some individuals. Natural IgM antibodies reacting with Tat may influence the course of AIDS progression.
and provide an initial defense against the pathological effects of Tat after HIV infection. The mucosal delivery of Tat in nonprimates elicits mucosal IgA and neutralizing antibody. The neutralizing antibody against Tat is also reported in primates. Although neutralizing antibodies against Tat inhibit Tat-mediated trans-activation, but they are unable to give protection against virus challenge.

Tat is essential for productive viral infection and expressed early in viral life cycle. An immune response disrupting its function or targeting its CTL epitopes could effectively diminish acute phase viral load. Immunization studies using Tat protein, tat DNA, or tat expression vectors protecting against challenge with pathogenic viruses have been reported in animal models. Several studies have also promoted the use of a chemically modified form of Tat named as Tat toxoid. The use of native Tat is based on the possibility that the structural modification introduced in toxoid would destroy the main immunogenic epitope of the protein. Although Tat toxoid is demonstrated to be safe in mice, seronegative humans and immunocompromised HIV infected patients, macaques immunized with Tat toxoid and challenged with SHIV 89.6 have shown attenuation of SHIV replication and disease, but no protection against infection. Thus, despite its potential therapeutic implication, a Tat vaccine might not be sufficient to cure AIDS.

More likely, Tat should be considered as a constituent of a composite vaccine. Recent studies have shown that immune response generated with viral proteins (i.e. env, gag, pol, nef, and rev) is not sufficient to protect the host. Some reports also indicate that codon optimized Tat or Tat with adjuvants induces strong immune response. Studies have been also conducted with combination of Nef, Tat and Rev showing strong immune response. However, the response is weaker when they are co-immunized with structural proteins like gp160 and gag. HIV-1 gp120 is variable among different subtypes, while Tat is relatively conserved. The role of Tat in immune response when co-immunized with gp120 has been a subject of controversy. Some reports suggest that it enhances the immune response when co-immunized with gp120 and gag, while in another report it has been found to diminish the immune response towards gp120. Tat also suppresses the immune response to p24, when co-immunized with p24 protein. Extracellular Tat has been shown to inhibit proliferation of naïve and memory B cells. This may explain the inhibition of anti-gag antibody response, following co-administration with gag protein as immunogen. These mixed outcomes probably reflect species differences, route or strain of challenge, or differences in preparation of Tat immunogens or immunization regimens. However, immunosuppressive role of Tat, particularly towards the immune response of a co-immunized antigen remains to be clearly elucidated.

The immunosuppressive effect of extracellular Tat is thought to contribute to AIDS pathogenesis. Immunosuppression might be because Tat suppresses mitogen-, alloantigen, and antigen-induced lymphocyte proliferation in vitro by stimulating suppressive levels of α-interferon or inducing apoptosis in activated lymphocytes. The use of native Tat is a cell-surface glycoprotein with dipeptidyl peptidase IV (DPPIV) activity in its extracellular domain exhibits co-stimulatory function and plays an important role in immune response via its ability to bind adenosine deaminase (ADA) and association with CD45. The N-terminal portion of Tat binds to CD26 with high affinity and is believed to be responsible for CD26-mediated immunosuppressive activity.

Immunosuppression of cells seems to be an important cause for progression of AIDS. Tat also blocks L type calcium channels, which contribute to progressive immunosuppression during HIV-1 infection. This leads to inhibition of natural immunity mediated by natural killer cells. Tat possesses a unique biological activity that it alters the function of monocytes, DCs, CD4+ T cells and CD8+ T cells in vitro. In addition, HIV-1 Tat regulates the expression of several cellular genes, thereby modulating cell behavior. The expression of MMP-9, lymphotoxin, TNFα, IL-2, IL-6, IL-8 and IL-10 is upregulated by Tat treatment. There could be possibility that Tat diminishes immune response through alteration of different cytokines secreted from host cells. Tat induces IL-10 through PKR pathway involving p38 MAPK downstream through Ets and Sp1. IL-10, a cytokine secreted from macrophages, DCs and T cells and possessing immunosuppressive activity is upregulated in HIV-1 infected individuals. Thus, Tat-induced upregulation of IL-10 could be one of the mechanism of Tat-mediated suppression of immune system, in addition to its possible role in inhibiting viral replication.
In contrast, Tat is also reported to enhance immune response by inducing secretion of IFNγ and expression of Tbet in Th cells. In HIV-1 infected individuals, CTL precursor (CTLp) against Tat correlates inversely with rapid progression to AIDS. Studies indicate that Tat-specific CTL, induced after primary Simian immunodeficiency virus (SIV) infection in rhesus macaques are able to control primary infection. Thereafter, subsequent replacement occurs in Tat CTL epitopes that might prevent CTL killing of Tat expressing targets by the escape variants. Viral escape from Tat specific CTL occurs with kinetics similar to those seen during the emergence of drug-resistant mutants. This may happen due to the fact that Tat is expressed very early, even before Nef could downregulate MHC class I expression.

Concluding Remarks

Numerous studies have convincingly established that HIV-1 Tat plays a pivotal role both in HIV-1 replication cycle and pathogenesis of infection. Tat regulates HIV-1 replication by increasing the provirus transcription rate several hundred-fold and acting on the elongation of viral transcripts. It is a powerful transactivator of gene expression, and acts by inducing chromatin remodeling and recruiting elongation-competent transcriptional complexes on to the viral promoter. Tat recruits various histone acetyl transferases (HATs) to the HIV-1 promoter region and can also be acetylated at the same time. Unmodified Tat is involved in binding to the CBP/p300 and cdk9/cyclin T complexes and facilitates transcription initiation. Acetylated Tat dissociates from the TAR RNA structure and recruits bromodomain-containing chromatin modifying complexes such as p/CAF and SWI/SNF to facilitate transcription elongation.

In addition to these transcriptional activities, Tat is released from the cells and enters neighboring cells, when present in the extracellular environment, a process that is possibly involved in HIV disease pathogenesis. Given its pleiotropic functions (Fig. 1), Tat represents a highly appealing target for both vaccine and drug development. In particular, Tat modulates the expression of several cellular genes and triggers the activation of some signal transduction pathways and transcription factors, suggesting a complex role in the scenario of HIV-1 infection. Finally, HIV-1 Tat is more conserved than envelope proteins, is essential in virus life cycle and is expressed very early upon virus entry. In addition, both humoral and cellular responses to Tat have been reported to correlate with a delayed progression to disease in both humans and monkeys. This suggests that Tat could be an optimal target for vaccine development. Although several studies have shown promising results with Tat as a vaccine candidate, recent reports have also shown Tat-mediated immunosuppression of co-immunized antigens, suggesting that further elucidation of the role of Tat in immune response may be required, prior to its use as a HIV vaccine candidate.

Acknowledgements

The authors are thankful to Dr. G C Mishra, Director, NCCS for his constant encouragement and Department of Biotechnology, Govt. of India for financial support. SG is a Senior Research Fellow of NCCS.

References

1. World Health Organization (WHO) AIDS Epidemic Update, December 2006
64 Li J C & Lau A S (2007) *Immunology* 121, 337-348