TAT PROTEIN AND HIV VIRAL FITNESS

Sadaf Rafiq Khan
Research Scholar
Department of Biotechnology
Sunrise University, Alwar, Rajasthan, INDIA
Email: sadafrk03@gmail.com

Dr. Kuldeep Yadav
Assistant professor
Department of Biotechnology
Sunrise University, Alwar, Rajasthan, INDIA
Email: kuldeep.agi@gmail.com

Abstract:
Tat, a regulatory protein in HIV, plays a pivotal role in the virus's life cycle and immunopathogenesis. Its primary function is to transactivate HIV genome transcription, overcoming obstacles to elongation by binding to the TAR sequence on nascent viral RNA. Additionally, Tat may stimulate reverse transcription at low concentrations but inhibit it at high amounts. Beyond transcriptional control, Tat can activate infected T cells, promoting viral replication even before integration, and can be released extracellularly, targeting immune cells and facilitating viral spread. Extracellular Tat can also enter infected cells, reactivating latent reservoirs and promoting viral expression. Tat exists in two forms, one-exon and two-exon, with distinct functional properties. While both forms activate HIV-1 gene expression, the two-exon form also mediates immune hyperactivation, suggesting differential roles in later infection phases. Tat's multiple domains enable interaction with various receptors, contributing to its diverse functions in HIV pathogenesis.

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1. INTRODUCTION

Tat, a crucial regulatory protein in the HIV lifecycle, is integral to viral replication and immunopathogenesis. Its primary function involves transactivating HIV genome transcription, facilitating the production of essential viral proteins. This process begins with inefficient initial transcription rounds, where only regulatory proteins are synthesized. Tat steps in by binding to the TAR sequence on nascent viral RNA, thereby removing obstacles to transcription elongation and promoting the synthesis of the full HIV genome [1-2]. This activity involves interactions with host transcription factors like NFAT-1 and NF-κB.

Research indicates that Tat may also influence reverse transcription, with low concentrations potentially stimulating this process while high amounts inhibit it. Furthermore, Tat's impact extends beyond transcriptional control. It can activate infected T cells, promoting viral replication even before viral integration into the host genome. Additionally, Tat can be released extracellularly via a leaderless secretory pathway, where it interacts with heparan sulfate proteoglycans and immune cells expressing RGD-binding integrin receptors [3]. This extracellular Tat can enter both infected and uninfected cells, enhancing viral spread and inducing HIV co-receptor expression.

Tat's presence extracellularly or intracellularly can lead to HIV genome transactivation and the reactivation of latent viral reservoirs within CD4+ T cells. This activity contributes to viral persistence and poses challenges for eradication efforts. Studies have shown that Tat variants found in latently infected cells may exhibit impaired transactivation activity but remain competent in other pleiotropic

functions [4]. These functions include activating Akt and inducing anti-apoptotic proteins like Bcl-2, potentially contributing to reservoir maintenance.Tat exists in two forms, one-exon and two-exon, with distinct properties and roles in HIV infection. While both forms activate HIV-1 gene expression, the two-exon form also mediates immune hyperactivation in infected cells. This suggests differential functions of Tat in different infection phases [5].Tat's multifunctional nature arises from its composition of multiple domains, enabling interactions with various receptors and molecules involved in HIV pathogenesis. Understanding Tat's diverse roles and interactions provides insights into HIV pathogenesis and potential therapeutic targets (Table 1).

Table 1: Types of Tat Domain and its functions

Tat Domain	**Function**
TAR Binding	Binds to the TAR sequence on nascent viral RNA, facilitating transcription elongation and promoting synthesis of the full HIV genome.
Immune Activation	Activates infected T cells, promoting viral replication, and can induce immune hyperactivation in later infection phases.
Extracellular Interaction	Interacts with heparan sulfate proteoglycans and integrin receptors on immune cells, facilitating extracellular Tat entry into both infected and uninfected cells.
Transactivation	Reactivates latent viral reservoirs within CD4+ T cells, contributing to viral persistence and reservoir maintenance.
Akt Activation	Activates Akt signaling pathway and induces anti-apoptotic proteins, potentially aiding in cell survival and reservoir maintenance.
One-Exon vs. Two-Exon	Both forms activate HIV-1 gene expression, but the two-exon form mediates immune hyperactivation in infected cells, suggesting different roles in infection phases.

Infection by different HIV-1 subtypes may lead to varying progression rates to AIDS and responses to antiretroviral therapy (HAART). Variability among HIV-1 clades in cell tropism and co-receptor usage contributes to these differences in virulence. For instance, clade B Tat has been shown to enhance susceptibility to X4 HIV-1 infection by increasing CXCR4 expression in CD4+ T cells, a phenomenon not observed with clade C Tat.Furthermore, differences in Tat sequence among HIV-1 subtypes impact viral replication dynamics [6]. Clade C and E Tat variants exhibit higher transactivation potential compared to clade B Tat due to their superior affinity to the TAR element and longer half-life. Notably, the presence of QGD in place of the RGD domain in clade C Tat enhances its capacity to activate LTRs.

Sequence variations in Tat also influence its immunomodulatory properties. For instance, the substitution of RGD with QGD in clade C Tat reduces its ability to induce apoptosis in activated macrophages. Additionally, variations such as the replacement of Cysteine with Serine at position 31 can lead to opposite effects on cytokine secretion and calcium flux in monocytes [7]. Clade B Tat tends to promote the secretion of pro-inflammatory cytokines and neuropathogenic agents, contributing to higher neurotoxicity compared to subtype C HIV.While modifications in Tat's primary and tertiary structures can impact its immunogenicity, certain B and T cell immunogenic regions remain conserved among HIV-1 M group. Consequently, antibodies raised against one Tat clade often cross-recognize other Tat variants, suggesting potential implications for vaccine design and immunotherapeutic strategies.

2. THE TAT PROTEIN AND HIV

Tat, a multifunctional protein in HIV infection, extends its influence beyond viral replication. It interacts with coinfecting pathogens, exacerbates conditions like Kaposi's sarcoma and vasculopathic issues, and contributes to CNS damage leading to HIV-associated dementia. Its immunomodulatory properties further exacerbate disease progression by inducing chronic immune activation, CD4+ T cell loss, and T cell dysfunction [8]. Additionally, Tat's ability to enter uninfected cells and manipulate gene expression underscores its significant role in HIV pathogenesis.

2.1 Tat's Impact on Antigen Presentation

Tat, a regulatory protein of HIV-1, plays various roles in altering antigen presentation and activating APCs.

It influences the composition and activity of the proteasome, impacting the generation and recognition of CTL peptide epitopes. Notably, Tat favours the presentation of subdominant epitopes over immunodominant ones. Although its effects on HLA class I molecules are not fully consistent across studies, Tat has been shown to enhance the expression of HLA-ABC and HLA-DR on dendritic cells (DCs). This modulation of antigen presentation may affect the immune response by altering the repertoire of presented peptides.

Tat induces the maturation and activation of APCs, including macrophages, monocytes, and DCs. It stimulates the release of various cytokines from these cells and upregulates the expression of costimulatory molecules such as CD40, CD80, CD83, and CD86. These activated APCs contribute to immune activation, which is particularly relevant during chronic HIV-1 infection when DCs exhibit an activated phenotype and spontaneously produce pro-inflammatory cytokines/chemokines [9].Tat promotes the adhesion of monocytes to endothelial cells and their transmigration through endothelial monolayers. This process contributes to vascular and tissue damage and is implicated in the development of cardiovascular diseases.

2.2 Lymphocytes

Tat's impact extends beyond APCs to directly activating CD4+ T cells, particularly when stimulated with anti-CD3/CD28, a mechanism reliant on CD28 co-stimulation, which enhances IL-2 secretion. This heightened activation may increase susceptibility to HIV-1 infection. Additionally, Tat induces the release of other pro-inflammatory cytokines like IL-8, IL-12, and TNFα, further contributing to CD4+ T cell hyperactivation. Furthermore, Tat's effect on B cell lymphocytes is not fully understood, but it appears to upregulate Fas expression, a marker of activation associated with apoptotic signals. Tat also influences B cell cytokine release and can concurrently promote both proliferation and apoptosis of B cells, depending on the stimulus and subtype [10]. While Tat has been observed to decrease IgM, IgG, and IgA production in vitro, in vivo models suggest an adjuvant effect of Tat on humoral responses. Thus, Tat exhibits a dual role in regulating cell viability and proliferation, impacting various aspects of immune function.

2.3 Viability & Proliferation

The HIV infection involves the extensive depletion of B and CD4+ T cells alongside hyperproliferation of lymphocytes. Tat, which can exit infected cells and target uninfected cells, has been extensively studied for its role in cell cycle regulation and survival. However, findings on Tat's effects appear contradictory, with evidence suggesting both promotion and inhibition of cell proliferation, as well as displaying both anti- and pro-apoptotic effects.The hyperactivation of CD4+ and CD8+ T cells, a hallmark of chronic immune activation in HIV infection, has been linked to Tat-mediated proliferation of CD4+ T lymphocytes, potentially contributing to disease progression and tumorigenicity. Conversely, Tat has been implicated in inhibiting proliferation, possibly through CD26 inhibition and enhanced IL-10 release [11].

Tat also mediates apoptosis of bystander and activated T cells, contributing to CD4+ T cell depletion during HIV infection. This apoptotic effect is thought to involve modulation of cell cycle regulators, increased expression of pro-apoptotic molecules, and enhancement of microtubule polymerization. Additionally, Tat-induced oxidative stress can lead to apoptosis or necrosis, activating pro-apoptotic pathways like Egr1-PTEN-Akt-FOXO3a and upregulating TRAIL and Fas.

2.4 Signaling

Tat's effects vary depending on its concentration and localization, suggesting activation of different signaling pathways. At extracellular concentrations, Tat activates extracellular receptor-activated kinase (ERK) and c-Jun N-terminal kinase (JNK), downstream of TCR and CD28. The two-exon Tat form is required for JNK activation, occurring at micromolar concentrations, whereas ERK activation, crucial for cell growth, occurs at nanomolar concentrations. This suggests Tat may induce pro-apoptotic signals through JNK and cell proliferation through ERK.Tat activates JNK and ERK via NADPH oxidases Nox2 and Nox4, respectively, activated by the GTPase Rac downstream of Tat-integrin interaction [13]. Nox4 mediates proliferation, while Nox2 mediates cytoskeletal rearrangements and oxidative stress. Additionally, Tat increases IL-2 production via ERK activation of AP-1, along with NFAT cooperation.

Tat's anti-apoptotic effects involve activation of the PI3K/Akt pathway, crucial for cell survival and proliferation, triggered by nano/picomolar Tat concentrations. Tat induces PTEN degradation, a negative regulator of Akt, and activates NF-κB through Akt-dependent Nox2 activation, promoting cell survival and proliferation. Tat also induces NF-κB

via various signaling cascades, potentially contributing to T cell hyperactivation.

3. ROLE OF TAT PROTEIN IN IMMUNE PROTECTION

The early production of CTL responses against HIV proteins, including Tat, plays a crucial role in viral control, as evidenced by the detection of Tat-CTL escape mutants soon after infection [13-14]. Additionally, studies indicate that the presence of anti-Tat CTLs correlates with non-progression in HIV-positive individuals, underscoring the importance of Tat-specific immune responses in disease outcome.Tat contains a significant number of T cell epitopes, primarily located in its N-terminus region, core region, and basic domain, which are recognized by both T and B cells [15]. These epitopes likely contribute to the immunogenicity of Tat during HIV infection.

Interestingly, anti-Tat IgM and IgG antibodies are more frequently detected during the asymptomatic stage of HIV infection, reflecting the early immune response to Tat [16]. Although anti-Tat IgG are less common, they are more prevalent in non-progressors and have been associated with protective effects against CD4+ decline, high viral load, antigenemia, and disease progression [17]. These findings suggest a potential role for anti-Tat antibodies in controlling HIV infection and disease progression.

The Trans-Activator of Transcription (Tat) protein is a regulatory protein encoded by the human immunodeficiency virus (HIV). Tat is primarily known for its role in enhancing the transcription of the HIV genome. However, it also has implications in the context of immune protection, both positive and negative. Here are the key aspects of the Tat protein's role in immune protection:

3.1 Immune System Modulation

The Tat protein of HIV plays a pivotal role in immune system modulation by exerting both immunostimulatory and immunosuppressive effects. On the one hand, Tat can enhance immune responses by activating dendritic cells, which subsequently prime T cells, potentially boosting anti-HIV immunity. On the other hand, Tat contributes to immune evasion by downregulating major histocompatibility complex (MHC) molecules on infected cells, making them less visible to cytotoxic T lymphocytes (CTLs). Additionally, Tat promotes the expression of immune checkpoint molecules like PD-1 on T cells, leading to T-cell exhaustion and impaired immune responses [18]. This dual nature of Tat complicates the immune landscape in HIV infection, making it a critical target for therapeutic interventions aimed at modulating immune responses more effectively.

- ***Positive Effects:***Immunogenicity in Vaccine Development: Tat has been explored as a component in HIV vaccines. Its ability to elicit strong immune responses can be harnessed to develop vaccines aimed at inducing robust cellular and humoral immunity against HIV. Vaccines incorporating Tat aim to trigger a comprehensive immune response that includes the activation of cytotoxic T lymphocytes (CTLs) which are critical for controlling HIV infection.
- ***Negative Effects:***Immune Evasion and Suppression: Tat can interfere with immune functions, facilitating HIV's evasion of the host immune response. It can downregulate the expression of major histocompatibility complex (MHC) molecules on infected cells, reducing their visibility to CTLs. Tat also induces the expression of immune checkpoint molecules like PD-1, leading to T-cell exhaustion.Cytokine Dysregulation: Tat can alter the cytokine milieu, promoting a pro-inflammatory environment that is detrimental in chronic HIV infection. This can lead to immune activation, which paradoxically contributes to the depletion of immune cells and advancement of AIDS.

3.2 Tat Protein and Immune Cells

Tat's interaction with immune cells is intricate and influential in the progression of HIV infection. When taken up by dendritic cells, Tat can be processed and presented, triggering the activation of these antigen-presenting cells and priming T cells for a targeted immune response. However, Tat also directly affects T cells, potentially enhancing their proliferation but often leading to dysfunctional responses in the context of chronic HIV infection. By inducing cytokine dysregulation, Tat creates a pro-inflammatory environment that can further deplete immune cells and accelerate disease progression [19]. This complex interplay with immune cells highlights Tat's central role in HIV pathogenesis and its impact on the overall immune system functionality.

- ***Interaction with Dendritic Cells (DCs):*** Tat can be taken up by dendritic cells and processed for antigen presentation. This interaction can lead to the activation of DCs, which then prime T cells, contributing to an anti-HIV immune response.

- ***Effects on T Cells:*** Tat can act directly on T cells, affecting their proliferation and function. While this might enhance some immune responses, it often results in dysfunctional T-cell responses in the context of HIV infection, contributing to immune exhaustion.

3.3 Tat as a Therapeutic Target

Given its crucial role in HIV infection and immune modulation, Tat represents a promising yet challenging therapeutic target. Strategies involving Tat-based vaccines aim to leverage its strong immunogenic properties to elicit potent cytotoxic T lymphocyte (CTL) responses, which are essential for controlling HIV replication and eliminating infected cells. Concurrently, therapeutic approaches that inhibit Tat activity are being explored to mitigate its detrimental effects, such as immune evasion and T-cell exhaustion [20]. By blocking Tat, these therapies seek to restore proper immune function and enhance the body's ability to fight HIV. The dual potential of Tat in both vaccine development and therapeutic inhibition underscores its significance in advancing HIV treatment and improving immune protection.

- ***Vaccine Strategies:*** Tat-Based Vaccines: Several vaccine strategies include Tat as a key component to boost the immune response against HIV. These vaccines aim to induce strong CTL responses to clear infected cells and prevent viral replication.
- ***Immune Modulation Therapies:***Therapeutic approaches that inhibit Tat function are being explored to restore immune function in HIV-infected individuals. By inhibiting Tat, it may be possible to reduce HIV replication and improve the overall immune response.

4. TAT-BASED VACCINE

Tat-based vaccines are a promising avenue in the fight against HIV, leveraging the immunogenic properties of the Tat protein to elicit a robust immune response. These vaccines aim to induce strong cytotoxic T lymphocyte (CTL) responses, which are crucial for controlling HIV replication and targeting infected cells. By incorporating Tat, the vaccines can stimulate both cellular and humoral immunity, enhancing the body's ability to recognize and respond to the virus. Preclinical and clinical studies have shown that Tat-based vaccines can boost immune responses, reduce viral load, and potentially delay disease progression. Additionally, these vaccines might complement existing antiretroviral therapies, providing a multifaceted approach to HIV treatment and prevention. The ongoing research and development of Tat-based vaccines hold significant promise for improving the management and eventual eradication of HIV.

Tat, a crucial protein in the HIV life cycle, has garnered significant attention as a target for both preventive and therapeutic vaccines against HIV. Studies in animal models have demonstrated that immunization with Tat can induce antibody responses capable of blocking Tat entry and its effects on gene expression and replication [20]. Furthermore, vaccination with Tat in monkeys resulted in undetectable viremia and prevented CD4+ T cell decline after challenge with SHIV viruses, suggesting protection against infection [19]. Long-term protection, absence of viral reservoirs, and suppression of virus replication were observed in Tat-vaccinated and SHIV-challenged macaques, with both humoral and cellular immune responses contributing to this effect [15].

Based on promising safety and immunogenicity data from animal studies [18], a Tat-based vaccine progressed to phase I clinical trials to assess safety and immunogenicity. Subsequently, phase II therapeutic trials were conducted in Italy and South Africa, where the Tat vaccine demonstrated efficacy in reversing signs of immune activation, reducing hypergammaglobulinemia, and restoring immune functions, as evidenced by increased CD4+ T cells and B lymphocytes and enhanced cellular responses to HIV and other antigens. Another Tat-based vaccine utilizing a Tat variant isolated from HIV controllers in African patients, known as Tat Oyi, entered phase I clinical trials in France in 2013. This highlights ongoing efforts to explore Tat as a potential candidate for HIV vaccination strategies.

Tat-based vaccine candidate is TAT Oyi. This vaccine was developed to harness the immunogenic properties of the HIV-1 Tat protein, aiming to elicit strong immune responses that can help control HIV infection. Clinical trials have investigated the efficacy of TAT Oyi, exploring its potential to boost immune function, reduce viral load, and complement existing antiretroviral therapies [20]. The development of TAT Oyi represents a significant step in the ongoing effort to create effective vaccines against HIV.

5. CONCLUSION

In this study, we demonstrate through various in vitro and in vivo models that the HIV-1 Tat protein profoundly alters CD8+ T cell responses and antiviral immunity. Tat influences the transcriptional profile

and functionality of CD8+ T cells, hyperactivating T lymphocytes in a manner detrimental to controlling acute infections. Our findings suggest that Tat, released by HIV-infected cells, contributes significantly to immune activation and dysfunction during HIV infection by affecting the transcriptional profile and functionality of uninfected cells. Given that Tat T and B cell epitopes are conserved among different HIV subtypes and can be recognized by antibodies from non-B HIV infected individuals, our data suggest that Tat could be a valuable target for inducing anti-Tat immune responses. These findings provide new insights into the causes of immune activation and highlight the importance of incorporating anti-Tat immunity into future preventive and therapeutic strategies for HIV control and cure. Moreover, Tat's immunomodulatory properties could be harnessed to enhance immune responses to poorly immunogenic vaccine antigens, as demonstrated by the increased Gag immunogenicity when co-administered with Tat. This potential has been further explored using a recombinant attenuated HSV1 vector expressing Tat, which induced strong HSV1-specific cellular and humoral responses and conferred protection against lethal HSV1 challenge. Therefore, Tat's effects on T cell activation could be strategically modulated to improve vaccine efficacy.

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