Review

HIV Tat Regulates the Immune Responses Induced by Vaccines

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Abstract | As the crucial transcription activator of HIV, Tat has been considered as an important immunogen for HIV vaccine candidates for a long time. However, HIV Tat also plays immunoregulation roles in improving immune responses of vaccines. Herein, we review recent developments of HIV Tat as immunoregulator using for optimizing the efficacy of vaccines. By highlighting preclinical/clinical applications of Tat and its working mechanisms, this review exhibits the superior potential of Tat as immunoregulator on optimizing immune responses mediated by vaccines. These examples of selected studies will provide researchers some implications and inspirations for using HIV Tat to optimize immune responses of vaccines.

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Introduction

The early expression protein, Tat (trans-activator of transcription), plays the crucial roles in activating *in vivo* transcription of HIV (human immunodeficiency virus), mediating cell-to-cell virus transmission and controlling disease progression (Arya et al., 1985; Fisher et al., 1986; Li et al., 1997; Ott et al., 1997). Although the whole crystal structure of Tat has not been characterized in details, native Tat protein is usually divided into two separated domains based on its amino acids sequence. The large domain (amino acids 1–72) which is encoded by the first exon possesses most immunological epitopes and the major biology functions, such as binding the TAR (transactivation responsive region) and activating virus transcription (Feng and Holland, 1988; Jones and Peterlin, 1994; Van Lint et al., 2013; Wei et al., 1998). The small domain contains 15-30 amino acids (depending on the viral strain), and its bio-effects have not been explored clearly (Eberle and Gurtler, 2012). The relatively conserved gene sequences and the crucial roles on regulating HIV transcription makes Tat become an ideal target for HIV vaccines. Some early studies have mapped the main vaccine-targeted epitopes of Tat, including B-cell immunodominant epitopes (Krone et al., 1988), cytotoxic T lymphocyte epitopes (Blazevic et al., 1993) and naturally occurring IgM epitopes (Rodman et al., 1993). Since the 1990s, most researchers devoted to use Tat as an immunogen of HIV vaccines, and Tat-based vaccine candidates have also been pushed into the clinical stage. Recent Phase II clinical investigation demonstrated that therapeutic immunization with Tat induced safe and durable immune responses, as well as effectively improved HAART (Highly Active Anti-Retroviral Therapy) efficacy and restored immune homeostasis (Ensoli et al., 2010). However, few people focus on investigating the effects of Tat on regulating immune responses induced by vaccines until now.

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Tat regulates the enzymatic activity of immunoproteasome

During the process of antigen-adapted immune responses, one crucial step is to product multi-amino acids epitopes peptides (Margulies, 2009). Briefly, heterologous antigens would be captured by APCs (antigen presenting cells) once they enters into the body. Subsequently, these foreign proteins are transported to immunoproteasomes (one type of professional antigen-degradation unit) and cut into small piecess of peptides, which can combinated with MHC (major histocompatibility complex) molecules and are presented onto the cell surface eventually (Chen et al., 2001; Ferrington and Gregerson, 2012).

The immunoproteasome contains three important functionality subunits, LMP2 (low molecular mass polypeptide 2, or latent membrane protein 2 (Areste and Blackbourn, 2006), LMP7 and MECL-1 (multicatalytic endopeptidase complex like-1). HIV Tat can affect the peptide-cutting characters of immunoproteasome via regulating the composition ratio among above three subunits. In practical, *in vivo* STAT1-IRF1 complex (signal transducer and activator of transcription 1 and interferon-regulatory factor 1) occupies the promote element of LMP which locates the overlapping parts between ICS-2 (interferon consensus sequence-2) and GAS (interferon-gamma-activated sequence) and controls the gene transcription of three subunits (Chatterjee-Kishore et al., 2000; Taniguchi et al., 2001). Once entering into the APCs, Tat protein can compete with STAT1 to bind to IRF-1 and affect the gene expressions of three subunits. By up-regulating production of LMP7 and MECL-1 and down-regulating the expressions of LMP-2, Tat breaks the balance of components of immunoproteasome (Remoli et al., 2006). Such modifications would change the enzymatic activity of immunoproteasome, and then reshape the T cell epitopes cutting characters, eventually modulate the profile of TCR-recognized (T cell receptor) epitope peptides pool (Figure 1) (Areste and Blackbourn, 2006; Gavioli et al., 2004). It is also considered as the important reason why Tat can modify dominant T cell epitopes and effectively broaden epitope-specific T cell responses against other HIV antigens *in vivo* (Gavioli et al., 2008).

The Auto-adjuvant Property of Tat

Vaccinations with protein antigens alone are hard to induce robust immune responses *in vivo*. It usually requires adjuvant to help to raise the immunity.

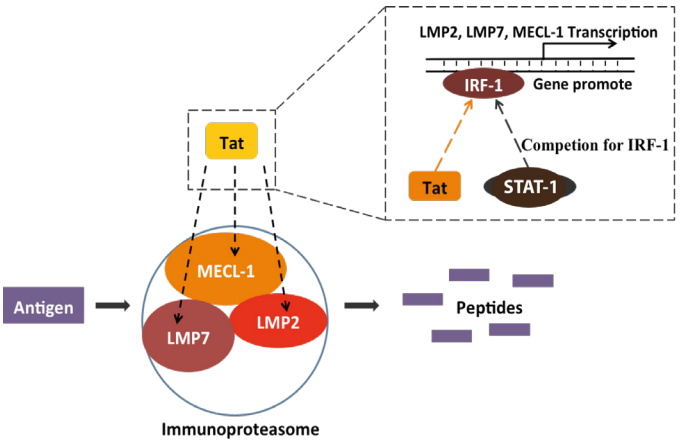

Figure 1: *Tat regulates the expressions of LMP2, LMP7 and MECL-1. Top right area shows the working mechnism of Tat on controling the gene transcription. Normally, the complex of STAT-1 and IRF-1 occupies the regulated element of promotor and start the gene expression. Once Tat enters into the nucleus, it competes with STAT-1 to bind IRF-1, and alter the gene transcription. Bottom left area shows the course of antigen degradation via immunoproteasome.*

However, HIV Tat exhibits the capability to trigger strong anti-itself immune response, which is called auto-adjuvant property (Cafaro et al., 1999). Cysteine-rich region of Tat located on the N-terminal is the core element and controls the auto-adjuvant function of Tat. Moreover, such adjuvant-free humoral response against Tat itself is dependent on oligomerization of Tat protein (dimer) mediated by two position-34 cysteines, but independent of B-cell recognition and T-cell stimulation, suggesting that an appropriate space conformation of Tat oligomers is crucial for maintaining the autoadjuvant activity (Campbell and Loret, 2009; Kittiworakarn et al., 2006).

The adjuvant property of Tat can also transfer to the unrelated antigens by a T cell-dependent way. By mapping the functional region, amino acid 1-57 of Tat is in charge of the adjuvant property, and Tat37-57 plays a core role in controlling its adjuvant effects (Gadzinski et al., 2012). These results might be valuable for the designs of adjuvant-free Tat-based vaccines, or using Tat as an adjuvant to improve the efficacies of other protein vaccines.

Protein Transduction Domains of Tat

PTD (protein transduction domains) is one sort of special element which can improve the efficacy of exogenous proteins on entering into their targeted cells when it is fused onto the terminal of protein. HIV Tat possess such element, and related recombinant antigens containing Tat PTD element have been reported in some published papers (Shibagaki and Udey, 2002, 2003).

HBcAg (hepatitis B core antigen) fused by Tat PTD (47-57 amino acids) possesses higher efficacy on entering into the cytoplasm of dendritic cells. Further in vivo results from mice model showed that PTD-HBcAg not only induced stronger humoral responses to effectively reduce HBV DNA and the expression of HBsAg in liver tissue, but also increased the percentages of IFN-gamma $CD8^+$ subtype T cells and the productions of multiple other cytokines, such as IL-2, IL-4 and IL-10 (Chen et al., 2010).

Similar enhanced immunogenicity caused by Tat PTD was also demonstrated in the animal test for cancer vaccine. Compared with DCs pulsed with unconjugated antigen, DCs (dendritic cells) loaded with Tat-conjugated antigen triggered stronger type 1 T cell responses and cytotoxic T lymphocyte responses, causing the delay of tumour growth in the transgenic tumour model as well as in the tumour injection model. Tat fused antigen was also helpful to break the tolerance in the transgenic breast tumour mouse and inhibit the growth of breast tumor (Yang et al., 2009). Moreover, PTD element is able to promote the accumulation of protein in the cytosol of cells which is independent of endocytosis (Wadia and Dowdy, 2005). Such PTD-fusion-antigens are more efficient to be internalized by DCs, resulting in a stronger epitope presenting process to cytotoxic T lymphocytes mediated by proteasomes (Kim et al., 2008; Shibagaki and Udey, 2002, 2003). However, researchers in another group obtain reverse results. The data shows that PTD from de novo synthesized Tat protein not only fails to enhance the antigen-specific immune responses, but also does not cause the translocation of an intracellular protein to the cell surface, but instead merely to increases binding to the cell surface (Leifert et al., 2002). This phenomenon might explain why Tat PTD effects can be observed in the tests using fixed cells or denatured proteins, and hardly success to use for gene therapy and vaccination to data.

Tat Promotes the Maturation of DCs

DCs (Dendritic cells) are the most important antigen presenting cells *in vivo*. The improvement of activation, migration or maturation of DCs would significantly promote antigen-specific immune response (Mercier et al., 2013; Molino et al., 2013; Muto et al., 2014). Biologically active HIV Tat protein, rather than oxidative Tat protein, can selectively target and effectively enter into the CD1a-expressed monocyte-derived DCs in a short time and by a dose-dependent way. The uptake of Tat by monocyte-derived DCs is mediated by a receptor-dependent endocytosis, and can be effectively stopped in a cold condition. Endocellular Tat is able to promote the maturation of monocyte-derived DCs and enhance the efficacy of antigen presentation (Fanales-Belasio et al., 2002). Such bio-effects of Tat have been applied in the therapeutic vaccine. In particular Tat acted as a collaborated factor was fused to the terminal of the Leishmania antigens and pulsed into the DCs. Subsequently, these DCs were delivered into the Leishmania major-infected mice for treating pathogen. Compared with the mice vaccinated with DCs plused by Leishmania antigens alone, the proliferation of *in vivo* CD8+ T cell was enhanced and also exhibited a better treatment effects (Kronenberg et al., 2010). By scanning the mutation of single amino acid, the cysteine22 located in Tat was determined to be the crucial site for maintaining the function on promoting the maturation of monocyte-derived DCs. It suggested that oligo-Tat which was formed via the disulfide bonds between cysteines of Tat might play the core roles in regulating DCs maturation (Fanales-Belasio et al., 2009).

Tat Inhibits NKs

Natural killer (NK) cells are a type of cytotoxic lymphocyte which provide the first defense system *in vivo* and kill the extraneous pathogens in the very early infection stage (Marcus et al., 2014). HIV Tat was found to be able to inhibit the activities of NKs, especially lowering the abilities of NK on releasing IFN-gamma and splitting DCs via a direct NK-DC contact. It is considered as one of mechanisms that HIV evades the attacks from immune responses in early phase of virus infection (Poggi et al., 2002). The detailed molecular mechanism indicates that Tat protein impairs CAMK-II (calcium-calmodulin kinase-II) activation on NKs via regulating the expression of crucial factor, LFA-1, which is responsible for activating CAMK-II. The inhibition of CAMK-II activation leads to the

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block of calcium influx in NK cells, therefore decreasing the secretion of perforin, granzymes and IFN- γ , eventually reducing the extension of NK/DC contacts. By region-mapping, the C-terminal domain of Tat protein is found to be responsible for such inhibition.

Moreover, exogenous Tat protein can block the L-type calcium channels expressed on NKs and inhibit NK cell-mediated cytotoxicity of tumor targets (Zocchi MR et al., 1998). Tat also impairs the secretion of IL-12 by dendritic cells, which is one kind cytokine that up-regulates NK cell function (Poggi et al., 1998; Rubartelli et al., 1998). These details involving how Tat inhibits the bio-function of NK cells and which part of Tat plays the crucial inhibition roles provide us clues to avoid side effects of Tat during the vaccine design.

Tat and Hyperactivation

In HIV infection individuals, T cell hyperactivation is linked to the exhaustion of T cell pool *in vivo* and might promote the progress of disease, which therefore is viewed as a typical marker for predicting the AIDS progression. Although some data showed that Tat was able to induce T cell hyperactivation *in vivo*, suggesting Tat might be harmful for health via regulating the excessively activated immune responses (Kwon et al., 2008; Secchiero et al., 2000), the capacity of Tat which activates CD8+ T cell responses can still be applied by us as adjuvant for improving the immunity of vaccines which only can induce relatively modest immune responses *in vivo* (Nicoli et al., 2013), such as DNA vaccines.

Moreover, the uncovered working mechanism makes us understand the roles of Tat on regulating T cell hyperactivation better (Figure 2). In practical, T cell hyperactivation in HIV infections is a consequence which is synthetically impacted by Tat, NF-κB (nucleus factor-Kappa B), histone acetyltransferase p300 and nicotinamide adenine dinucleotide (NAD+)-dependent deacetylase (SIRT1 in short). During HIV resting stage, the activity of NF-κB which activates the gene transcription is suppressed by its inhibitor, IκB (Inhibitor Kappa B). Once the complex of NF-κB and IκB is phosphorylated which can be indirectly mediated by Tat (Milani et al., 1996), IκB will depart from the complex and NF-κB translocates into the uncleus. Subsequently, histone acetyltransferase p300 acetylates the lysines 218, 221 and 310 in p65 subunit of NF-κB enhances the capacity of DNA binding and transcriptional activity of NF-κB (Chen et al., 2002; Kiernan et al., 2003). In contrast, SIRT1 specifically deacetylates p65 subunit, suppresses the function of NF-κB (Yeung et al., 2004) and negatively regulates T cell activation. Tat is a substrate for the deacetylase activity of SIRT1 (Pagans et al., 2005) and can directly bind to the deacetylase domain of SIRT1. Such adhesion between Tat and SIRT1 will impair the effects of SIRT1 on deacetylating p65 subunit of NF-κB, leading to an increasing NF-κB activity (Biswas et al., 1995). Therefore, Tat is considered to be able to positive-regulate the hyperactivation of T cells indirectly (Kwon et al., 2008). Such probable side-effects caused by Tat should also be considered in the design of HIV vaccines in future.

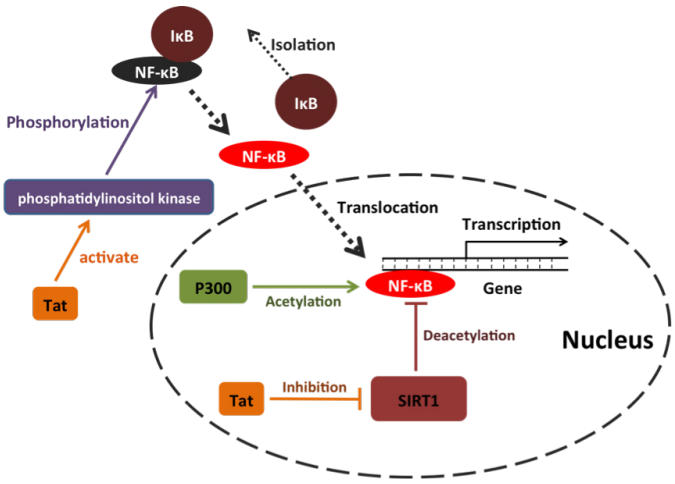

Figure 2: Tat regulates T cell hyperactivation. *Extranuclear Tat activate phosphatidylinositol kinase to make the phosphorylation of complex of NF-κB and IκB, leading to NK-κB translocate into uncleus. Acetylation of NF-κB by P300 starts the activation of NF-κB and promots the gene transcription. In contrast, SIRT1 deacetylates NF-κB and inhibit its activation. Tat acted as the inhibitor of SIRT1 can indirectly improve the acetylation level of NF-κB and activate its function, resulting in T cell hyperactivation.*

Conclusions

In this mini-review, we summarized the research results on using HIV Tat as a helper to regulate the immune responses induced by vaccines. Tat exhibited

its versatility on modulating the physiological behaviors of multiple types of cells and functional units involving DCs, NKs, T cells and immunoproteasomes. Considering the good biosafety of Tat which has been demonstrated in multiple clinical trials (Caputo et al., 2004; Ensoli et al., 2008; Longo et al., 2009) and its immunoregulation effects, Tat is potential to become one adjuvant to optimize the vaccine efficacy.

Competing interests

The authors declare that they have no competing interests

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