HIV Tat regulates the immune responses induced by vaccines

Ye liu[2], Yulong Cong[2], Yiming Shao[1].

1 State Key Laboratory for Infectious Disease Prevention and Control, National Center for AIDS/STD Control and Prevention, Chinese Center for Disease Control and Prevention, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, 155 Changbai Road Changping District, Beijing 102206, China.

2 Department of Clinical Laboratory, Chinese P. L. A. General Hospital, No. 28 Fuxing Road, Beijing 100853, China.

Correspondence: yshao08@gmail.com; yulongc@263.net.

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.[1-4] Although the whole crystal structure of Tat has not been characterized in details, native Tat protein is usually divided into two separate 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. [5-8] The small domain contains 15-30 amino acids (depending on the viral strain), and its bio-effects have not been explored clearly. [9]

The relatively conserved gene sequences and the crucial roles on regulating HIV transcription makes Tat become the ideal target for HIV

vaccines. Some early studies mapped the main vaccine-targeted epitopes of Tat, including the B-cell immunodominant epitopes,[10] cytotoxic T lymphocyte epitopes,[11] and naturally occurring IgM epitopes.[12] 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.[13] However, few people focus on investigating the effects of Tat on regulating immune responses until now.

Tat regulates the enzymatic activity of immunoproteasome

During the antigen-adapted immune responses, one crucial step is to product multi-amino acids epitopes peptides.[14] 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 short peptides, which are able to combinated with MHC (major histocompatibility complex) molecules and are presented onto the cell surface eventually.[15, 16]

The immunoproteasome contains three important functionality subunits, LMP2 (low molecular mass polypeptide 2, or latent membrane protein 2 [17]), 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 these 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 (γ-interferon-activated sequence),[18, 19] and controls the gene transcription of three subunits. After Tat protein enters into the APCs, it can compete with STAT1 to bind to IRF-1 and affect the gene expressions of three subunits. By up-regulating the production of LMP7 and MECL-1 and down-regulating the expression of LMP-2,[20] Tat breaks the balance of components of immunoproteasome. Such modifications would change the enzymatic activity of immunoproteasome, and then shape the T cell epitopes cutting characters, eventually modulate the profile of TCR-recongnized (T cell receptor) epitope peptides pool (Figure 1).[17, 21] It is also considered as the important reason that Tat can modify the dominant T cell epitopes and effectively broaden the epitope-specific T cell responses against other HIV antigens *in vivo.*[22]

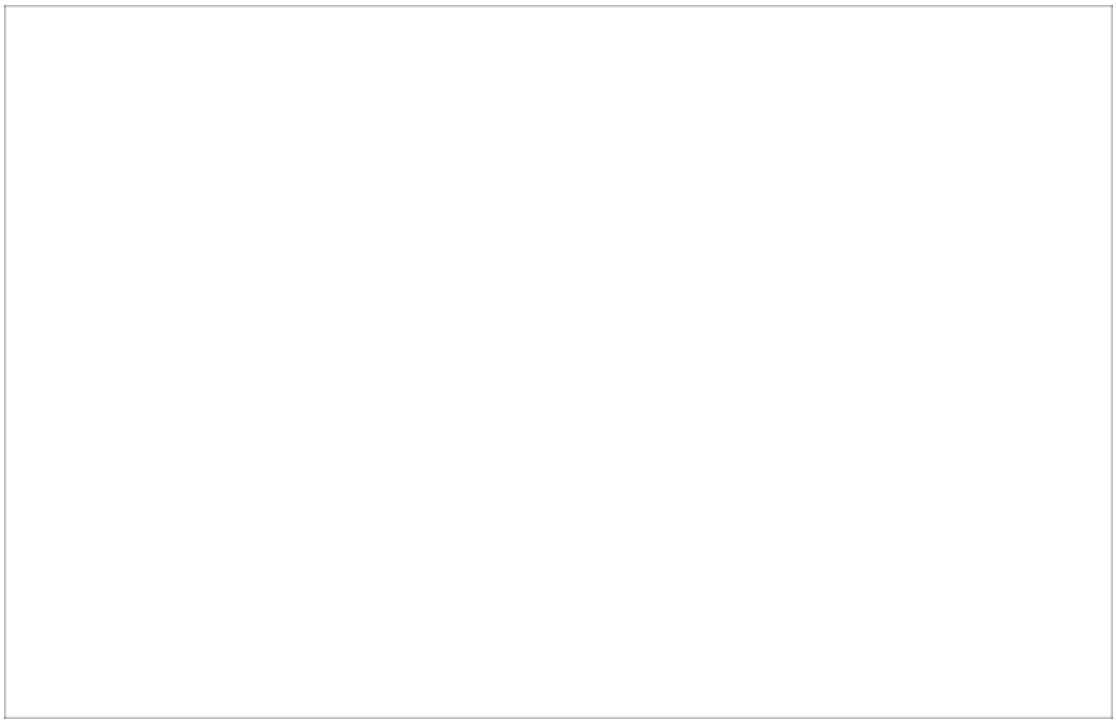

Figure 1: Tat regulates the expressions of LMP2, LMP7 and MECL-1.

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.

The auto-adjuvant property of Tat

Vaccinations with protein antigens alone do not induce the robust immune responses *in vivo*. It usually requires adjuvant to help to raise their immunity. However, HIV Tat exhibits the capability to trigger strong

anti-itself immune response, which is called auto-adjuvant property.[23] The 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 the B-cell recognition and T-cell stimulation, suggesting that an appropriate space conformation of Tat oligomers is crucial for maintaining the autoadjuvant activity.[24, 25]

The adjuvant property of Tat can also be transferred to the unrelated antigens by a T cell-dependent way. By mapping the functional region, amino acid 1-57 of Tat is sufficient to transfer the adjuvant property, and Tat37-57 plays a core role in controlling its adjuvant effects.[26] These results might be valuable for the designs of the 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 enters into their target cells when it is fused onto the terminal of protein. HIV Tat also is found to possess such element, and related recombinant antigens containing Tat PTD element have been constructed.[27, 28]

In HBV vaccines, Tat PTD (47-57 amino acids) was fused to the terminal of HBcAg (hepatitis B core antigen). It was able to improve the entering efficacy of antigen into the cytoplasm of dendritic cells, and enhance the HBcAg-specific T cell responses. The results from mice

model showed that PTD-HBcAg fusion protein not only induced stronger humoral responses which reduced effectively 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.[29]

Similar enhanced immunogenicity caused by Tat PTD was also demonstrated in cancer vaccine animal test. Compared with DCs pulsed with unconjugated antigen, DCs 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 model, where positive breast cancers grew spontaneously.[30] Moreover, PTD element is able to promote the accumulation of protein in the cytosol of cells which is independent of endocytosis [31]. Such PTD-fusion-antigens are more efficiently transduced DCs, resulting in a stronger epitope presenting process to cytotoxic T lymphocytes mediated by proteasomes.[27, 28, 32] In contrast, 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.[33] 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 the antigen-specific immune response.[34-36] 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.[37] Such bio-effects of Tat have been applied in the therapeutic vaccine. Firstly, 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.[38] 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. [39]

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.[40] HIV Tat was found to be able to inhibit the activities of NKs, especially lowering the abilities of NK on releasing IFN-γand 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.[41] 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 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 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.[42] Tat also impairs the secretion of IL-12 by dendritic cells, which is one kind cytokine that up-regulates NK cell function.[43, 44] T 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 the 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,[45, 46] 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 induce relatively modest immune responses *in vivo,*[47] 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,[48] 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,[49, 50] enhances the capacity of DNA binding and transcriptional activity of NF-κB. In contrast, SIRT1 specifically deacetylates p65 subunit, suppresses the function of NF-κB,[51] and negatively regulates T cell activation. Tat is a substrate for the deacetylase activity of SIRT1 [52] 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, [53] Therefore, Tat is considered to be able to positive-regulate the hyperactivation of T cells indirectly. [46]

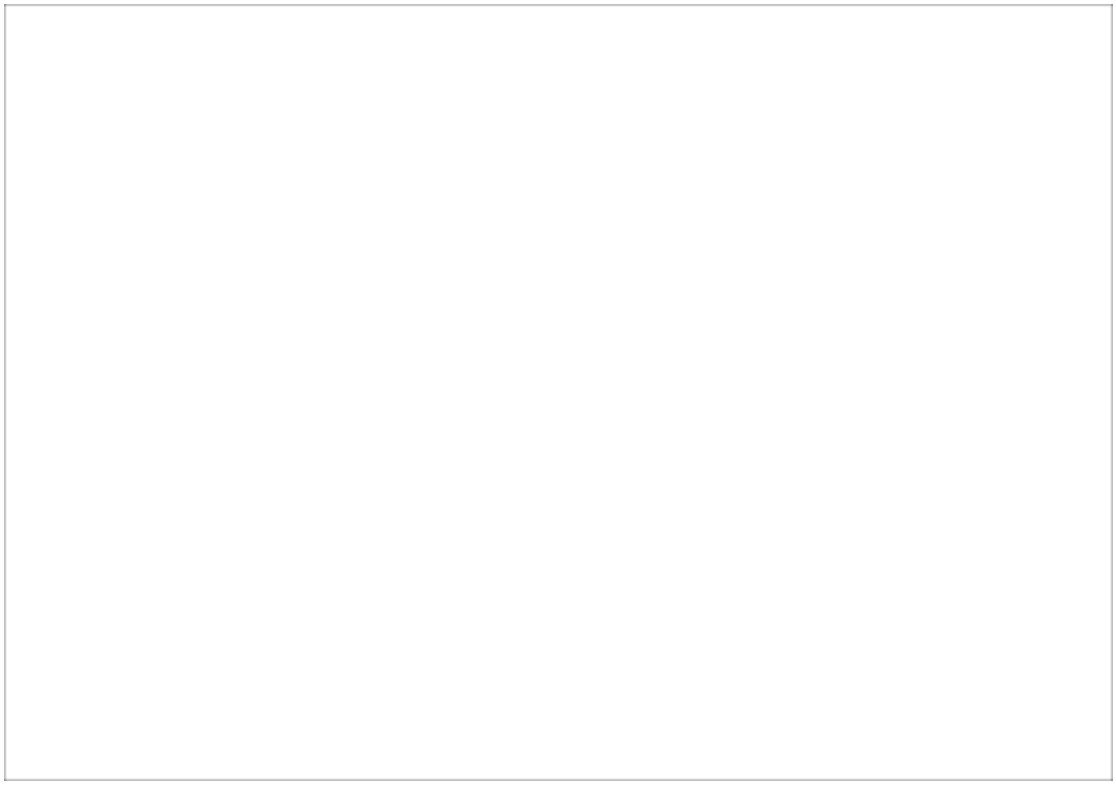

Figure 2: Tat regulates T cell hyperactivation.

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

Conclusion

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 [54-56] 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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References

1. Fisher AG, Feinberg MB, Josephs SF, Harper ME, Marselle LM, Reyes G, Gonda MA, Aldovini A, Debouk C, Gallo RC, et al.: **The trans-activator gene of HTLV-III is essential for virus replication.** *Nature* 1986, **320:**367-371.
2. Ott M, Emiliani S, Van Lint C, Herbein G, Lovett J, Chirmule N, McCloskey T, Pahwa S, Verdin E: **Immune hyperactivation of HIV-1-infected T cells**

mediated by Tat and the CD28 pathway. *Science* 1997, **275:**1481-1485.

3. Li CJ, Ueda Y, Shi B, Borodyansky L, Huang L, Li YZ, Pardee AB: **Tat protein induces self-perpetuating permissivity for productive HIV-1 infection.** *Proceedings of the National Academy of Sciences of the United States of America* 1997, **94:**8116-8120.
4. Arya SK, Guo C, Josephs SF, Wong-Staal F: **Trans-activator gene of human T-lymphotropic virus type III (HTLV-III).** *Science* 1985, **229:**69-73.
5. Feng S, Holland EC: **HIV-1 tat trans-activation requires the loop sequence within tar.** *Nature* 1988, **334:**165-167.
6. Jones KA, Peterlin BM: **Control of RNA initiation and elongation at the HIV-1 promoter.** *Annual review of biochemistry* 1994, **63:**717-743.
7. Wei P, Garber ME, Fang SM, Fischer WH, Jones KA: **A novel CDK9-associated C-type cyclin interacts directly with HIV-1 Tat and mediates its high-affinity, loop-specific binding to TAR RNA.** *Cell* 1998, **92:**451-462.
8. Van Lint C, Bouchat S, Marcello A: **HIV-1 transcription and latency: an update.** *Retrovirology* 2013, **10:**67.
9. Eberle J, Gurtler L: **HIV types, groups, subtypes and recombinant forms: errors in replication, selection pressure and quasispecies.** *Intervirology* 2012, **55:**79-83.
10. Krone WJ, Debouck C, Epstein LG, Heutink P, Meloen R, Goudsmit J: **Natural antibodies to HIV-tat epitopes and expression of HIV-1 genes in vivo.** *Journal of medical virology* 1988, **26:**261-270.
11. Blazevic V, Ranki A, Mattinen S, Valle SL, Koskimies S, Jung G, Krohn KJ: **Helper T-cell recognition of HIV-1 Tat synthetic peptides.** *Journal of acquired immune deficiency syndromes* 1993, **6:**881-890.
12. Rodman TC, To SE, Hashish H, Manchester K: **Epitopes for natural antibodies of human immunodeficiency virus (HIV)-negative (normal) and HIV-positive sera are coincident with two key functional sequences of HIV Tat protein.** *Proceedings of the National Academy of Sciences of the United States of America* 1993, **90:**7719-7723.
13. Ensoli B, Bellino S, Tripiciano A, Longo O, Francavilla V, Marcotullio S, Cafaro

A, Picconi O, Paniccia G, Scoglio A, et al: **Therapeutic immunization with HIV-1 Tat reduces immune activation and loss of regulatory T-cells and improves immune function in subjects on HAART.** *PloS one* 2010, **5:**e13540.

14. Margulies DH: **Antigen-processing and presentation pathways select antigenic HIV peptides in the fight against viral evolution.** *Nature immunology* 2009, **10:**566-568.
15. Ferrington DA, Gregerson DS: **Immunoproteasomes: structure, function, and antigen presentation.** *Progress in molecular biology and translational science* 2012, **109:**75-112.
16. Chen W, Norbury CC, Cho Y, Yewdell JW, Bennink JR: **Immunoproteasomes shape immunodominance hierarchies of antiviral CD8(+) T cells at the levels of T cell repertoire and presentation of viral antigens.** *The Journal of experimental medicine* 2001, **193:**1319-1326.
17. Areste C, Blackbourn DJ: **HIV Tat-mediated transcriptional regulation of proteasome protein cleavage specificity.** *The Biochemical journal* 2006, **396:**e13-15.
18. Chatterjee-Kishore M, Wright KL, Ting JP, Stark GR: **How Stat1 mediates constitutive gene expression: a complex of unphosphorylated Stat1 and IRF1 supports transcription of the LMP2 gene.** *The EMBO journal* 2000, **19:**4111-4122.
19. Taniguchi T, Ogasawara K, Takaoka A, Tanaka N: **IRF family of transcription factors as regulators of host defense.** *Annual review of immunology* 2001, **19:**623-655.
20. Remoli AL, Marsili G, Perrotti E, Gallerani E, Ilari R, Nappi F, Cafaro A, Ensoli B, Gavioli R, Battistini A: **Intracellular HIV-1 Tat protein represses constitutive LMP2 transcription increasing proteasome activity by interfering with the binding of IRF-1 to STAT1.** *The Biochemical journal* 2006, **396:**371-380.
21. Gavioli R, Gallerani E, Fortini C, Fabris M, Bottoni A, Canella A, Bonaccorsi A, Marastoni M, Micheletti F, Cafaro A, et al: **HIV-1 tat protein modulates the generation of cytotoxic T cell epitopes by modifying proteasome**

composition and enzymatic activity. *J Immunol* 2004, **173:**3838-3843.

22. Gavioli R, Cellini S, Castaldello A, Voltan R, Gallerani E, Gagliardoni F, Fortini C, Cofano EB, Triulzi C, Cafaro A, et al: **The Tat protein broadens T cell responses directed to the HIV-1 antigens Gag and Env: implications for the design of new vaccination strategies against AIDS.** *Vaccine* 2008, **26:**727-737.
23. Cafaro A, Caputo A, Fracasso C, Maggiorella MT, Goletti D, Baroncelli S, Pace M, Sernicola L, Koanga-Mogtomo ML, Betti M, et al: **Control of SHIV-89.6P-infection of cynomolgus monkeys by HIV-1 Tat protein vaccine.** *Nature medicine* 1999, **5:**643-650.
24. Kittiworakarn J, Lecoq A, Moine G, Thai R, Lajeunesse E, Drevet P, Vidaud C, Menez A, Leonetti M: **HIV-1 Tat raises an adjuvant-free humoral immune response controlled by its core region and its ability to form cysteine-mediated oligomers.** *The Journal of biological chemistry* 2006, **281:**3105-3115.
25. Campbell GR, Loret EP: **What does the structure-function relationship of the HIV-1 Tat protein teach us about developing an AIDS vaccine?** *Retrovirology* 2009, **6:**50.
26. Gadzinski A, Matz D, Favre E, Leonetti M: **Transfer of the ability of HIV-1 Tat to raise an adjuvant-free humoral immune response to unrelated antigens.** *Vaccine* 2012, **30:**2859-2868.
27. Shibagaki N, Udey MC: **Dendritic cells transduced with protein antigens induce cytotoxic lymphocytes and elicit antitumor immunity.** *J Immunol* 2002, **168:**2393-2401.
28. Shibagaki N, Udey MC: **Dendritic cells transduced with TAT protein transduction domain-containing tyrosinase-related protein 2 vaccinate against murine melanoma.** *European journal of immunology* 2003, **33:**850-860.
29. Chen X, Lai J, Pan Q, Tang Z, Yu Y, Zang G: **The delivery of HBcAg via Tat-PTD enhances specific immune response and inhibits Hepatitis B virus replication in transgenic mice.** *Vaccine* 2010, **28:**3913-3919.

30. Yang H, Cho NH, Seong SY: **The Tat-conjugated N-terminal region of mucin antigen 1 (MUC1) induces protective immunity against MUC1-expressing tumours.** *Clinical and experimental immunology* 2009, **158:**174-185.

31. Wadia JS, Dowdy SF: **Transmembrane delivery of protein and peptide drugs by TAT-mediated transduction in the treatment of cancer.** *Advanced drug delivery reviews* 2005, **57:**579-596.

32. Kim SG, Park MY, Kim CH, Sohn HJ, Kim HS, Park JS, Kim HJ, Oh ST, Kim TG: **Modification of CEA with both CRT and TAT PTD induces potent anti-tumor immune responses in RNA-pulsed DC vaccination.** *Vaccine* 2008, **26:**6433-6440.

33. Leifert JA, Harkins S, Whitton JL: **Full-length proteins attached to the HIV tat protein transduction domain are neither transduced between cells, nor exhibit enhanced immunogenicity.** *Gene therapy* 2002, **9:**1422-1428.

34. Molino NM, Anderson AK, Nelson EL, Wang SW: **Biomimetic protein nanoparticles facilitate enhanced dendritic cell activation and cross-presentation.** *ACS nano* 2013, **7:**9743-9752.

35. Muto J, Morioka Y, Yamasaki K, Kim M, Garcia A, Carlin AF, Varki A, Gallo RL: **Hyaluronan digestion controls DC migration from the skin.** *The Journal of clinical investigation* 2014.

36. Mercier SK, Donaghy H, Botting RA, Turville SG, Harman AN, Nasr N, Ji H, Kusebauch U, Mendoza L, Shteynberg D, et al: **The microvesicle component of HIV-1 inocula modulates dendritic cell infection and maturation and enhances adhesion to and activation of T lymphocytes.** *PLoS pathogens* 2013, **9:**e1003700.

37. Fanales-Belasio E, Moretti S, Nappi F, Barillari G, Micheletti F, Cafaro A, Ensoli B: **Native HIV-1 Tat protein targets monocyte-derived dendritic cells and enhances their maturation, function, and antigen-specific T cell responses.** *J Immunol* 2002, **168:**197-206.

38. Kronenberg K, Brosch S, Butsch F, Tada Y, Shibagaki N, Udey MC, von Stebut E: **Vaccination with TAT-antigen fusion protein induces protective, CD8(+) T cell-mediated immunity against Leishmania major.** *The Journal of*

investigative dermatology 2010, **130:**2602-2610.

39. Fanales-Belasio E, Moretti S, Fiorelli V, Tripiciano A, Pavone Cossut MR, Scoglio A, Collacchi B, Nappi F, Macchia I, Bellino S, et al: **HIV-1 Tat addresses dendritic cells to induce a predominant Th1-type adaptive immune response that appears prevalent in the asymptomatic stage of infection.** *J Immunol* 2009, **182:**2888-2897.
40. Marcus A, Gowen BG, Thompson TW, Iannello A, Ardolino M, Deng W, Wang L, Shifrin N, Raulet DH: **Recognition of tumors by the innate immune system and natural killer cells.** *Advances in immunology* 2014, **122:**91-128.
41. Poggi A, Carosio R, Spaggiari GM, Fortis C, Tambussi G, Dell'Antonio G, Dal Cin E, Rubartelli A, Zocchi MR: **NK cell activation by dendritic cells is dependent on LFA-1-mediated induction of calcium-calmodulin kinase II: inhibition by HIV-1 Tat C-terminal domain.** *J Immunol* 2002, **168:**95-101.
42. Zocchi MR, Rubartelli A, Morgavi P, Poggi A: **HIV-1 Tat inhibits human natural killer cell function by blocking L-type calcium channels.** *J Immunol* 1998, **161:**2938-2943.
43. Rubartelli A, Poggi A, Sitia R, Zocchi MR: **HIV-I Tat: a polypeptide for all seasons.** *Immunology today* 1998, **19:**543-545.
44. Poggi A, Rubartelli A, Zocchi MR: **Involvement of dihydropyridine-sensitive calcium channels in human dendritic cell function. Competition by HIV-1 Tat.** *The Journal of biological chemistry* 1998, **273:**7205-7209.
45. Secchiero P, Zella D, Curreli S, Mirandola P, Capitani S, Gallo RC, Zauli G: **Pivotal role of cyclic nucleoside phosphodiesterase 4 in Tat-mediated CD4+ T cell hyperactivation and HIV type 1 replication.** *Proceedings of the National Academy of Sciences of the United States of America* 2000, **97:**14620-14625.
46. Kwon HS, Brent MM, Getachew R, Jayakumar P, Chen LF, Schnolzer M, McBurney MW, Marmorstein R, Greene WC, Ott M: **Human immunodeficiency virus type 1 Tat protein inhibits the SIRT1 deacetylase and induces T cell hyperactivation.** *Cell host & microbe* 2008, **3:**158-167.
47. Nicoli F, Finessi V, Sicurella M, Rizzotto L, Gallerani E, Destro F, Cafaro A,

Marconi P, Caputo A, Ensoli B, Gavioli R: **The HIV-1 Tat protein induces the activation of CD8+ T cells and affects in vivo the magnitude and kinetics of antiviral responses.** *PloS one* 2013, **8:**e77746.

48. Milani D, Mazzoni M, Borgatti P, Zauli G, Cantley L, Capitani S: **Extracellular human immunodeficiency virus type-1 Tat protein activates phosphatidylinositol 3-kinase in PC12 neuronal cells.** *The Journal of biological chemistry* 1996, **271:**22961-22964.
49. Chen LF, Mu Y, Greene WC: **Acetylation of RelA at discrete sites regulates distinct nuclear functions of NF-kappaB.** *The EMBO journal* 2002, **21:**6539-6548.
50. Kiernan R, Bres V, Ng RW, Coudart MP, El Messaoudi S, Sardet C, Jin DY, Emiliani S, Benkirane M: **Post-activation turn-off of NF-kappa B-dependent transcription is regulated by acetylation of p65.** *The Journal of biological chemistry* 2003, **278:**2758-2766.
51. Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA, Mayo MW: **Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase.** *The EMBO journal* 2004, **23:**2369-2380.
52. Pagans S, Pedal A, North BJ, Kaehlcke K, Marshall BL, Dorr A, Hetzer-Egger C, Henklein P, Frye R, McBurney MW, et al: **SIRT1 regulates HIV transcription via Tat deacetylation.** *PLoS biology* 2005, **3:**e41.
53. Biswas DK, Salas TR, Wang F, Ahlers CM, Dezube BJ, Pardee AB: **A Tat-induced auto-up-regulatory loop for superactivation of the human immunodeficiency virus type 1 promoter.** *Journal of virology* 1995, **69:**7437-7444.
54. Ensoli B, Fiorelli V, Ensoli F, Lazzarin A, Visintini R, Narciso P, Di Carlo A, Monini P, Magnani M, Garaci E: **The therapeutic phase I trial of the recombinant native HIV-1 Tat protein.** *AIDS* 2008, **22:**2207-2209.
55. Longo O, Tripiciano A, Fiorelli V, Bellino S, Scoglio A, Collacchi B, Alvarez MJ, Francavilla V, Arancio A, Paniccia G, et al: **Phase I therapeutic trial of the HIV-1 Tat protein and long term follow-up.** *Vaccine* 2009, **27:**3306-3312.
56. Caputo A, Gavioli R, Ensoli B: **Recent advances in the development of HIV-1**

Tat-based vaccines. *Current HIV research* 2004, **2:**357-376.