A Tat subunit vaccine confers protective immunity against the immune-modulating activity of the human immunodeficiency virus type-1 Tat protein in mice

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The rational design of new therapies against HIV-1 necessitates an improved understanding of the mechanisms underlying the production of ineffective immune responses to HIV-1 in most infected individuals. This report shows that the CD8⁺ T cell responses to gp120 were greatly diminished in mice vaccinated with a bicistronic gp120-Tat DNA vaccine, compared with those induced by a DNA vaccine encoding gp120 alone. The CD8⁺ T cell responses induced by the latter included strong gp120-specific IFN- γ secretion and protective antiviral immunity against challenge by a vaccinia-*env* pseudotype. The degree to which Tat influenced CD8⁺ T cell responses depended on the bioactivity of Tat. Thus, a bicistronic DNA vaccine that expresses gp120 and a truncated Tat defective for LTR activation elicited strong IFN- γ -secreting CD8⁺ T cell responses to gp120 but conferred only marginal protection against the vaccinia-*env* challenge. The effect of Tat was completely blocked, however, by immunization with inactivated Tat protein before vaccination with the bicistronic gp120-Tat DNA vaccine.

HIV-1 | immune response | CD8⁺ T cell | regulation

The HIV type-1 (HIV-1) Tat protein has long been implicated as an important factor in the manifestation of immune dysfunction in many HIV-1-infected individuals before substantial loss of CD4⁺ T cells (1, 2). In fact, a preponderance of reports has unequivocally established that Tat possesses a unique biological activity that alters the function of monocytes, dendritic cells (DCs), and CD4⁺ and CD8⁺ T cells *in vitro* (1–19). In addition, detectable levels of extracellular Tat are found in culture supernatants from cells infected with HIV-1 (20–22), and Tat is efficiently taken up by a variety of cells (21–25). These evidentiary links suggest that foci of HIV-1 infected cells, similar to those observed in simian immunodeficiency virus-infected macaques (26), release sufficient levels of Tat to alter the function of immune cells associated with, or in close proximity to, the infection foci.

The hypothesis that extracellular Tat plays a role in the immunopathogenesis of HIV-1 predicts that high-affinity neutralizing antibodies against this viral protein will improve the clinical prognosis of HIV-1-infected patients (reviewed in ref. 27). In support, a limited number of epidemiological studies have documented an inverse relationship between the level of Tat-specific serum antibodies and the rate of disease progression (28–30). Also, a recent Tat vaccine study in nonhuman primates generated partial restoration of the immune response to simian/HIV antigens after challenge (31). However, the protection conferred by the Tat vaccine in this study was generally modest (31). One interpretation of this finding is that extracellular Tat only plays a minor role in the immunopathogenesis of HIV-1 and related viruses. Alternatively, we believe that this Tat vaccine study represents an important milestone and that the key to the development of a highly effective Tat vaccine will depend on the

identification of an appropriate Tat vaccine formulation that induces immune responses that completely block the immune-modulating activity of Tat. To accelerate the development of improved Tat vaccine formulations, we have developed an affordable animal model that enables the evaluation of novel Tat vaccine candidates *in vivo*. We show that this model can be used to assess both the safety and effectiveness of HIV-1 Tat vaccines.

Materials and Methods

Construction of DNA Vaccines. The construction of gp120 DNA vaccine, designated pcDNA::120, which carries a synthetic HIV-1_{MN} gp120 gene (syngp120), is described elsewhere (32). A Tat DNA vaccine, designated pTat_{syn}, was constructed by annealing a synthetic *tat* gene (*tat*_{syn}), which carries codons optimized for expression in mammalian cells and was designed by using the full-length 102-aa sequence of the HIV-1_{MN} *tat* gene (GenBank accession no. AR034234) as the blueprint as described by Haas *et al.* (33), into *Bam*HI-/*Eco*RI-digested pcDNA3.1_{ZEO} by using T4 DNA ligase. The bicistronic DNA vaccine p120-Tat was constructed by three-way ligation of pcDNA::120, a PCR-generated DNA fragment encoding the internal ribosome entry site in pCITE4a and *tat*_{syn}. A second bicistronic DNA vaccine, designated p120- Δ Tat, that expresses gp120 and a mutant derivative of Tat (Δ *tat*_{31–50}) defective for LTR activation, was constructed by replacing the *tat*_{syn} with Δ *tat*_{31–50}, which lacks a 66-bp region of *tat*_{syn} that encodes amino acids 30–51, by conventional techniques. Plasmids pcDNA::120, pTat_{syn}, p120-Tat and p120- Δ Tat were further characterized by restriction endonuclease analysis, PCR amplification, and sequencing to authenticate the DNA sequences.

Cell Line Culture and Transfection Procedures. The BALB/c cell line, P815 (H-2d; ATCC no. TIB-64), was obtained from the American Type Culture Collection, and cultured as described (32). Plasmids pcDNA::120, pTat_{syn}, p120-Tat, and p120- Δ Tat were introduced into P815 cells with FuGENE (Roche Molecular Biochemicals). A stable derivative of the P815 cell line, PH1001, which harbors pcDNA::120 and expresses HIV-1_{MN} gp120, was isolated by selecting for zeomycin-resistant cells 7 days after the transfection. HeLa-CD4-LTR- β -gal cells (34) were obtained from the AIDS Research and Reference Program, National Institutes of Health, Bethesda. Expression of β -galactosidase by HeLa-CD4-LTR- β -gal cells was determined by using a fluorochromogenic β -galactosidase assay system (Promega; catalog no. E2000); β -galactosidase activities were expressed in terms of the net increase of relative light units per mg of protein per hour above background levels.

Abbreviations: DC, dendritic cell; IFN- γ -ELISPOT, IFN- γ -specific enzyme-linked immunospot.

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Table 1. DNA vaccine characteristics

Vaccine	Gene cassette	Gp120 level*	LTR- <i>lacZ</i> [†]
p120	P _{CMV} - <i>syngp120</i> _{MN}	278 ± 55	NS
pTat _{syn}	P _{CMV} - <i>tat</i> _{syn}	<10	2.78 ± 0.7
p120-wT	P _{CMV} - <i>syngp120</i> -IRES- <i>tat</i> _{syn}	356 ± 63	0.97 ± 0.3
p120-ΔT	P _{CMV} - <i>syngp120</i> -IRES-Δ <i>tat</i> _{syn}	198 ± 34	NS

NS denotes that the levels of LacZ were not significantly elevated above background levels.

*Expressed as the amount of gp120 (pg) per 10⁶ P815 cells.

[†]Expressed as relative light units × 10⁵ per 10⁶ HeLa-CD4-LTR-β-gal cells.

Vaccination of Mice. Female specific-pathogen free BALB/c, C57BL/6, and C57BL/6-β-microglobulin-null mice aged 6–8 weeks (Charles River Breeding Laboratories) were maintained in a microisolator environment. Groups of 3–6 mice were vaccinated intramuscularly with an average of 25 μg of endotoxin-free plasmid DNA (<5 endotoxin units per mg of DNA; purchased from Aldevron, Fargo, ND), as described (35). Booster vaccinations were injected by using the same vaccination protocol 2 and 6 weeks after the primary vaccination.

Anti-Tat IgG. Tat-specific IgG in sera obtained before and at regular intervals after vaccination were measured by ELISA (36), using 96-well Immobulon-1 plates (Dynex Technologies, Chantilly, VA) coated with purified HIV-1_{IIIB} recombinant Tat (rTat; 1 μg/ml; Advanced BioScience) and alkaline phosphatase-labeled goat anti-mouse IgG (Sigma) as a secondary antibody.

Cell-Mediated Immune Responses. CD8⁺ T cell responses to gp120 were evaluated by using an IFN-γ-specific enzyme-linked immunospot (IFN-γ-ELISPOT) assay, as described (37, 38), and adapted by our group (32). Briefly, splenocytes were harvested 2 weeks after the final vaccination and restimulated *in vitro* for 6 days with the immunodominant peptide of HIV-1 Env, P18_{MN} (RIHIGPGRAFYTTKN; ref. 39). After restimulation, CD4⁺ and CD8⁺ cells were depleted by negative selection with CD4⁺- and CD8⁺-specific Dynabeads (DynaL Biotech, Lake Success, NY), respectively, according to the manufacturer's protocols.

Vaccinia-env Challenge. The level of antiviral protection induced by each DNA vaccine modality was determined by using a vaccinia-env challenge model, as described (40) and adapted by our group (32).

Results

Expression of gp120 and Tat. To develop DNA vaccines that induced cell-mediated immune response to gp120 and an early antigen of HIV-1, Tat, two bicistronic DNA vaccines were constructed. The first, p120-Tat, expresses both the gp120 and 102 aa Tat protein of HIV-1 MN, and the second, p120-ΔTat, expresses both gp120 and a truncated derivative of Tat, ΔTat_{31–50}. Semiquantitative gp120-capture assays (41) verified that the bicistronic DNA vaccines, p120-Tat and p120-ΔTat, expressed similar levels of gp120 in BALB/c P815 cells as parent plasmid pcDNA::120 (Table 1).

The activity of Tat expressed by pTat_{syn}, p120-Tat and p120-ΔTat was assessed by transient transfection of HeLa-LTR-*lacZ* cells. Plasmids pTat_{syn} and p120-Tat significantly elevated long terminal repeat (LTR)-*lacZ* reported gene expression, whereas p120-ΔTat did not (Table 1). Whole cell extracts of P815 cells transiently transfected with pTat_{syn}, p120-Tat, and p120-ΔTat were fractionated by PAGE and analyzed by Immunoblot; each of the plasmids expressed Tat molecules of the anticipated molecular weights (data not shown). Thus, pTat_{syn} and p120-Tat express bioactive Tat proteins, whereas p120-ΔTat produces a truncated Tat molecule that is incapable of LTR activation.

Fig. 1. Enumeration of gp120-specific IFN-γ-ELISPOTs. Individual groups of BALB/c mice were vaccinated intramuscularly three times with 100 μg of pTat_{syn} (A), pcDNA::120 (B), p120-Tat (C), or p120-ΔTat (D). Suspensions of splenocytes were prepared 2 weeks after the final vaccination and cultured in the presence of the immunodominant peptide of HIV-1_{MN} Env (RIHIG-PGRAFYTTKN_{COOH}; ref. 39). After 6 days of *in vitro* stimulation, Env-specific IFN-γ-secreting T cells were measured by IFN-γ-specific ELISPOT assay (37, 38), in unfractionated (dark bars), CD4-depleted (white bars), and CD8-depleted (gray bars) splenocytes. The results are expressed as the mean values of triplicate samples ± SD.

Induction of gp120-Specific Cell-Mediated Responses. As an initial gauge of the relative immunogenicities of the mono- and bicistronic DNA vaccines in mice, we measured CD8⁺ T cell responses to the encoded antigens. BALB/c mice were vaccinated intramuscularly with three 100-μg doses of one of the DNA vaccines on days 0, 14, and 42. This vaccination protocol was based on our prior experience with the gp120 DNA vaccine, pcDNA::120 (32). Two weeks after the third vaccination, CD8⁺ T cell responses to gp120 were inferred from the numbers of IFN-γ-ELISPOTs produced by unfractionated, CD4-depleted and CD8-depleted spleen cells (Fig. 1). Spleen cells from mice vaccinated with p120-Tat only produced low numbers of gp120-specific IFN-γ-ELISPOTs (Fig. 1). In contrast, substantial numbers of gp120-specific spot-forming cells were present in mice vaccinated with either pcDNA::120 and p120-ΔTat (Fig. 1). Consistent with previous findings with pcDNA::120 (32), depletion of CD8⁺ cells reduced the number of spot-forming cells to background levels, whereas depletion of CD4⁺ cells only marginally affected the response (Fig. 1).

The above data indicated that expression of full-length Tat by the gp120-Tat bicistronic DNA vaccine significantly diminished the IFN-γ-secreting CD8⁺ T cell response to the immunodominant epitope of gp120. Reinforcing this conclusion, a similar low number of gp120-specific, IFN-γ-ELISPOT-forming cells (160 ± 25 spot-forming cells) were observed in mice vaccinated with the gp120-Tat DNA vaccine, when PH1001 cells were used as stimulators *in vitro*. Mice vaccinated with three doses of the gp120 DNA vaccine, on the other hand, displayed similar numbers of gp120-specific spot-forming cells when PH1001 cells were used as stimulators (1,150 ± 230 spot-forming cells), compared with those detected when P815 cells pulsed with gp120 peptide P18 were used as stimulators (1,430 ± 110 spot-forming cells). It is unlikely therefore, that the low-level CD8⁺ cell responses to gp120 in mice vaccinated with the bicistronic gp120-Tat DNA vaccine were caused by an altered immunodominance pattern of major histocompatibility complex class I-restricted gp120 epitopes.

It was possible, however, that the poor gp120-specific responses in mice vaccinated with the bicistronic gp120-Tat DNA vaccine were caused by redirection of this response to Tat. To assess this possibility, mice were vaccinated intramuscularly three times with either p120-Tat, as above; negative-control mice

Fig. 2. Antiviral immunity in the vaccinated mice. Mice were vaccinated three times with pTat_{syn} (A), pcDNA::120 (B), p120-Tat (C), and p120-ΔTat (D), or not vaccinated (E). The level of antiviral immunity was assessed by inoculating these mice i.p. with 3×10^7 plaque-forming units (PFU) of vaccinia-env vector vP1174, 9 days after the third vaccination. A further 6 days after inoculation with vP1174, the number of viable vP1174 virions was enumerated by a direct plaque assay. The results are expressed as the mean values of two independent experiments $\pm$ SD.

were vaccinated three times with pcDNA3.1_{NEO}, also as above. IFN- γ -ELISPOTs were enumerated by using overlapping Tat peptides spanning amino acids 22–48 and 38–72 to stimulate spleen cells from the vaccinated mice. These peptides span the domain absent in Δ Tat_{31–50}, the deletion of which restored the IFN- γ -ELISPOT response to gp120. Thus, if an H-2D^d-restricted immunodominant epitope were present in Tat, the IFN- γ -ELISPOT data suggested it might reside within or overlap amino acids 31–50 of Tat. However, only low numbers of Tat_{22–48}- and Tat_{38–72}-specific IFN- γ -ELISPOTs (110 ± 50 and 65 ± 15 IFN- γ -ELISPOTs per 10^6 splenocytes, respectively) were present, in mice vaccinated with p120-Tat. Thus, the region spanned by these peptides only induced a modest number of IFN- γ -secreting cells, which concurs with others that Tat DNA vaccines do not induce a measurable cytotoxic T lymphocyte response to Tat in mice (42). The low-level response to Tat, therefore, suggests that the diminished CD8⁺ T cell responses to gp120 in mice vaccinated with the bicistronic DNA vaccine were not caused by a redirection of this response to Tat.

Tat Diminishes Antiviral Immunity to gp120. The strong antigen-specific IFN- γ -secreting CD8⁺ cell response to gp120 in mice vaccinated with pcDNA::120 and p120-ΔTat suggested that these mice might display protective antiviral immunity. To address this hypothesis, a vaccinia-env challenge model (40) was used to measure the level of antiviral activity in mice 9 days after the third vaccination. Surprisingly, only vaccination with pcDNA::120 conferred strong antiviral immunity against the challenge virus, vaccinia-env_{MN} vP1174 (Fig. 2), whereas neither p120-Tat nor p120-ΔTat induced a significant degree of antiviral immunity. This result supports our evidence that Tat does not skew the immunodominance pattern of CD8⁺ T cell responses to gp120. Also, vaccination of C57BL/6J mice with three doses of the bicistronic gp120-Tat DNA vaccine only induced a low-level antiviral protection against vaccinia-env, whereas vaccination with three doses of pcDNA::120 induced strong protection (data not shown). Thus, the capacity of Tat to influence antiviral immunity to gp120 is not confined to BALB/c H-2D^d mice. Moreover, three doses of pcDNA::120 did not induce antiviral protection in C57BL/6 β 2-microglobulin-null mice (ref. 43 and data not shown), supporting the idea that the antiviral protection depends on an effector CD8⁺ T cell response to gp120 and that vaccination with p120-Tat and p120-ΔTat fails to generate such T cells. This conclusion is in agreement with data reported by

Fig. 3. Recall of CD8 T cell responses after a viral challenge. Mice were vaccinated three times with pTat_{syn} (A), pcDNA::120 (B), p120-Tat (C), and p120-ΔTat (D) then challenged with vaccinia-env. Ten days after challenge, Env-specific IFN- γ -secreting CD8⁺ T cells were enumerated by an IFN- γ -specific ELISPOT assay, using unfractionated (dark bars), CD4-depleted (open bars), and CD8-depleted (striped bars) cell populations (see Materials and Methods). The results are expressed as the mean values of triplicate samples $\pm$ SD.

others (40) showing that elimination of CD8⁺ cells significantly decreases antiviral protection against vaccinia in mice.

The discrepancy between IFN- γ -ELISPOT numbers in mice vaccinated with p120-ΔTat and the level of antiviral immunity in these mice may be caused by poor reactivation of gp120-specific CD8⁺ T cells by the challenge virus. To assess this possibility, we measured the magnitude of the CD8⁺ T cell responses to gp120 10 days after the vaccinia-env challenge. Consistent with the prechallenge IFN- γ -ELISPOT numbers, mice vaccinated with either pcDNA::120 or p120-ΔTat displayed robust responses to gp120 after the vaccinia-env challenge (Fig. 3). In contrast, splenocytes from mice vaccinated with p120-Tat displayed comparatively low numbers of IFN- γ -ELISPOTs to gp120 after challenge (Fig. 3). Therefore, the inability of p120-ΔTat to confer protection against the challenge virus could not be attributed to poor recall of gp120-specific CD8⁺ T cells.

A Tat Subunit Vaccine Confers Protection. The above findings suggested that this system could be exploited to determine whether Tat vaccines confer protection against the immunomodulating properties of Tat. To assess this possibility, mice were vaccinated with three 10- μ g doses of oxidized HIV-1_{IIIB} rTat (rTat_{OX}; Advanced BioScience Laboratories) at 2-week intervals. The rTat_{OX} was formulated with 25 μ g of bacterial lipopolysaccharide from *Escherichia coli* strain MLK986, which produces a nonpyrogenic lipid A (44), which possesses a similar adjuvant activity to that of monophosphoryl-lipid A (Shata and Hone, in preparation). A second group of mice was vaccinated three times with 100- μ g doses of pTat_{syn}, using the same dose spacing. Control groups of mice remained unvaccinated or were vaccinated intramuscularly three times with 100 μ g of pcDNA3.1_{NEO}, also as above.

To assess the relative immunogenicity of the two Tat vaccine modalities, the levels of IgG to Tat were measured by ELISA in sera collected before and at regular intervals after vaccination. Mice vaccinated with rTat_{OX} produced a strong serum IgG response to Tat (reciprocal end-point titers ranging from 51,200 to 102,400 in individual mice), whereas vaccination with three doses of pTat_{syn} did not significantly increase the level of Tat-specific serum IgG (reciprocal end-point titer <100 in all mice). The contrasting immunogenicity of the two Tat vaccine modalities, therefore, provided an excellent setting to evaluate the anti-Tat protective properties of these vaccines.

To assess anti-Tat protection, unvaccinated control mice and

Fig. 4. Protection against the immune modulating activity of Tat. Groups of BALB/c mice were prevaccinated with three 100- μ g doses of purified rTat (A), or three 100- μ g doses of pTat_{syn} DNA (B) intramuscularly on days 0, 14, and 28. Control groups of mice were vaccinated intramuscularly three times with 100 μ g of pcDNA3.1_{NEO} (C), using the same vaccination schedule used for A and B, or were not vaccinated (D). These mice were given a second series of vaccinations, consisting of three doses of p120-Tat (A and B) injected intramuscularly 2, 4, and 8 weeks after the last dose in the first series of vaccinations. The control groups of mice were revaccinated three times intramuscularly with 100 μ g of pcDNA::120 (C), using the same vaccination schedule used for groups 1 and 2, or remained unvaccinated (D). Antiviral immunity was assessed 6 days after the third vaccination by inoculating the mice i.p. with 3×10^7 plaque-forming units (PFU) of vaccinia-env vector vP1174. Six days after inoculation with vP1174, the number of viable vP1174 virions was enumerated by a direct plaque assay (see *Materials and Methods*). Results are expressed as the mean values of two independent experiments $\pm$ SD.

those prevaccinated with either rTat_{OX} or pTat_{syn} were given a second series of vaccinations with three 100- μ g doses of p120-Tat injected two, four and eight weeks after the final dose of either rTat_{OX} or pTat_{syn} (see *Materials and Methods*). At the same time, mice prevaccinated with pcDNA3.1_{NEO} were vaccinated three times with 100 μ g of pcDNA::120, injected at the same intervals used for p120-Tat. Nine days after the second series of vaccinations, the mice were challenged with vaccinia-env vector vT26 and the numbers of infectious vaccinia-env viral particles present in the mice 6 days after the challenge were determined by direct plaque assay (Fig. 4). Only prior vaccination with rTat_{OX} conferred strong protection against the immune-modulating activity of Tat (Fig. 4), indicating that prior vaccination with rTat_{OX} enabled mice to generate gp120-specific antiviral T cells in response to vaccination with the bicistronic gp120-Tat DNA vaccine. In contrast, mice immunized with the Tat DNA vaccine did not develop significant levels of protection against the immune-modulating activity of Tat in p120-Tat (Fig. 4).

Discussion

The studies in this report show that Tat modulates antigen-specific CD8⁺ T cell responses. As DCs play a central role in the induction of CD8⁺ T cell responses to DNA vaccine-encoded antigens (45), it is likely that DCs were instrumental in the generation of CD8⁺ T cell responses in mice vaccinated with the various DNA vaccines used in our studies. Moreover, DCs are often in close proximity to infection foci in lentivirus-infected macaques (26). The release of Tat by HIV-1 infected cells (20–22), therefore, may alter the phenotype of proximal DCs and thereby modulate HIV-1-specific T cell responses that originate in such locales. In agreement, bioactive rTat has been shown to alter the phenotype of dendritic cells *in vitro* (8, 10, 19). It remains to be determined, however, whether the modulation of CD8⁺ T cells by Tat-stimulated DCs occurs through direct interactions, indirectly through the modulation of type 1 CD4⁺ T helper cells, which are central to the development of functional cytotoxic CD8⁺ T cells (47), or a combination of both pathways. Although the distinct immune-modulating properties of full-length Tat and Δ Tat_{31–50} suggest that the modulating activity may affect two immune functions, further characterization of host

CD4⁺ and CD8⁺ T cell responses is warranted before such a conclusion can be definitively drawn.

Atypical functional phenotypes have been noted in the HIV-1-specific CD8⁺ T cell responses that develop in infected individuals (48, 49). Furthermore, although a substantive number of Tat-specific CD8⁺ T cells develop during acute-phase simian immunodeficiency virus infections (50, 51), the majority of these CD8⁺ T cells failed to produce IFN- γ after restimulation with immunodominant Tat peptides *in vitro* (50). Such perturbations in CD8⁺ T cell responses to other HIV-1 antigens, which appear to be more evident during the chronic phase of infection (48, 49), are not caused by a generalized systemic immune dysfunction, as CD8⁺ T cell responses to other viral antigens, such as cytomegalovirus, display functional effector phenotypes (48, 49). In this vein, the immune responses induced by the bicistronic gp120- Δ Tat DNA vaccine mimicked events that occur in individuals chronically infected with HIV-1, who develop subsets of HIV-1-specific CD8⁺ T cells that express IFN- γ but lack cytotoxic effector functions (48, 49). The immune-modulating activity of the bicistronic gp120-Tat DNA vaccine resembles events that occur in chronically infected individuals who undergo a change in clinical status concomitantly with the loss of HIV-1-specific CD8⁺ T cells (52, 53). Furthermore, the gradation of potencies displayed by the Tat DNA vaccines used in our study suggests that the degree to which CD8⁺ T cell responses are altered by Tat depends on the bioactivity of Tat present in immune inductive sites.

An encouraging aspect of the studies described above is that an appropriately formulated Tat vaccine confers complete protection against Tat-mediated immune modulation. This report shows that immunization against Tat is capable of completely blocking Tat-mediated immune modulation in an animal model. As antibody responses to Tat only occurred in mice vaccinated with rTat_{OX}, humoral immunity may be the pivotal factor in the anti-Tat immunity that produced the difference in efficacy between the rTat_{OX} subunit vaccine and the Tat DNA vaccine. However, because it is likely that the cellular responses induced by the two Tat vaccine modalities were distinct, further experimentation is required to resolve the relative contribution of humoral and cell-mediated immune responses to the anti-Tat protective immunity. It is important to note that other immune-evasion tactics used by HIV-1 also contribute to the establishment of chronicity and disease progression, and that this tactical redundancy allows for the loss of one activity, as in Δ nef quasi-species (54–56), without sacrificing the survival of the resultant virus and in some individuals without complete loss of virulence (56). The impact of immunity to Tat, therefore, will depend on these other parameters, and it is not surprising that the efficacy of Tat vaccines in animal models is influenced by the potency of the lentivirus challenge stock (31, 57, 58).

In summary, data are presented demonstrating that Tat modulates CD8⁺ T cell responses. The degree to which Tat modulated CD8⁺ T cells depended on the bioactivity of Tat in immune inductive sites. This finding raises doubts about the safety of full-length Tat vaccines (46, 59). On the other hand, this report demonstrates that the key to the development of an effective vaccine against Tat will be the identification of Tat vaccine formulations that completely block the immune-modulating activity of this protein *in vivo*. We believe that the availability of a small animal model of Tat-mediated immune modulation will provide a rapid and inexpensive means to improve the safety and effectiveness of Tat DNA vaccines.

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