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

Riccardo Gavioli\textsuperscript{a,*}, Silvia Cellini\textsuperscript{a}, Arianna Castaldello\textsuperscript{b}, Rebecca Voltan\textsuperscript{b}, Eleonora Gallerani\textsuperscript{a}, Francesca Gagliardoni\textsuperscript{a}, Cinzia Fortini\textsuperscript{a}, Egidio Brocca Cofano\textsuperscript{b}, Chiara Triulzi\textsuperscript{b}, Aurelio Cafaro\textsuperscript{c}, Indresh Srivastava\textsuperscript{d}, Susan Barnett\textsuperscript{d}, Antonella Caputo\textsuperscript{b}, Barbara Ensoli\textsuperscript{c}

\textsuperscript{a} Department of Biochemistry and Molecular Biology, Via L. Borsari 46, University of Ferrara, 44100 Ferrara, Italy
\textsuperscript{b} Department of Histology, Microbiology and Medical Biotechnology, Via A. Gabelli 63, University of Padova, 35122 Padova, Italy
\textsuperscript{c} National AIDS Center, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Roma, Italy
\textsuperscript{d} Novartis Vaccines and Diagnostics, 4560 Horton Street, 4.3, Emeryville, CA 94608, United States

Received 24 May 2007; received in revised form 12 November 2007; accepted 16 November 2007
Available online 4 December 2007

**KEYWORDS**
AIDS; Vaccine; Tat

**Summary** We have previously shown that the biologically active Tat protein targets and efficiently enters dendritic cells, and increases the proteolytic activities of the immunoproteasome, thereby favoring the generation and presentation of the subdominant MHC-I binding CTL epitopes of heterologous antigens. In the present study, we demonstrate that Tat broadens \textit{in vivo} epitope-specific T cell responses directed to heterologous antigens including HIV structural proteins. Specifically, co-immunization of mice with OVA and Tat proteins induces CTL responses against subdominant and cryptic OVA-derived epitopes, which are not detected in mice vaccinated with OVA alone. Similarly, mice vaccinated with the HIV-1 Gag, Env or V2-deleted Env antigens in combination with Tat show Th1-type and CTL responses directed to a larger number of T cell epitopes, as compared to mice vaccinated with these proteins in absence of Tat. In contrast, Tat did not affect Th2-type responses to these structural HIV proteins. These results indicate that Tat is not only an antigen but also a novel Th1-type adjuvant capable of broadening \textit{in vivo} the spectrum of epitopes recognized by T cells, and suggest that Tat can be considered an optimal co-antigen in the development of novel vaccination strategies against AIDS.

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* Corresponding author. Tel.: +39 0532974407; fax: +39 0532974484.
E-mail address: r.gavioli@unife.it (R. Gavioli).

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doi:10.1016/j.vaccine.2007.11.040
Introduction

Identification of the immune mechanisms controlling HIV infection is critical for the rational development of preventive and therapeutic vaccine strategies against HIV/AIDS. Several studies indicate that both HIV-specific CD4- and CD8-mediated T cell responses play a key role during acute and chronic infection [38], and that long-term nonprogressors have consistently higher and broader levels of HIV-specific T cell responses than progressors [16,31]. Therefore, the ability of candidate HIV vaccines to elicit strong, broad and long-lasting T cell responses is likely to be crucial. However, immunodominance represents a major obstacle to developing vaccines that generate effective T cell responses. In particular, narrow T cell responses favor the emergence of viral escape mutants [1,14] in contrast to T cell responses directed to subdominant T cell epitopes, which have also been shown to correlate to the in vivo control of viral infections such as HIV [7,12].

In previous studies we have demonstrated that vaccination with the Tat protein or tat DNA is safe in mice, monkeys and humans [2—5,18], and that it induces long-term protection in monkeys challenged with a pathogenic simian-human immunodeficiency virus [2,3,27].

We have also shown that biologically active Tat protein displays immunomodulatory features, which make it an attractive co-antigen with other HIV proteins and for the design of new combined subunit vaccines. In particular, native Tat protein is very efficiently taken up by the design of new combined subunit vaccines. In particular, native Tat protein is very efficiently taken up by monocyte-derived dendritic cells (MDDC), and, upon cellular entry, promotes MDDC maturation and activation, leading to more efficient presentation of both allogeneic and exogenous soluble antigens and resulting in increased T cell responses against heterologous antigens [11]. In addition, the Tat protein modulates the CTL epitope hierarchy of heterologous antigens in vitro by modifying the catalytic subunit composition of the immunoproteasome [13].

This body of evidence indicates that native Tat may function as both an antigen and a novel adjuvant, thereby prompting us to evaluate its in vivo effects on the induction of T cell responses against OVA as well as HIV-1 Gag and Env proteins. OVA was first chosen as model antigen since its dominant, subdominant and cryptic MHC-I epitopes have been well characterized and OVA-specific CTL responses have been extensively documented [6,26]. HIV-1 Gag and Env proteins were selected for these studies as they are relevant antigens for the development of an anti-HIV/AIDS prophylactic and/or therapeutic vaccine [8,22,23,25,34,36,37,39,42].

The results of the present study show that Tat broadens the epitope-specific T cell responses directed against OVA as well as HIV-1 Gag, Env and V2-deleted Env antigens in vivo. This evidence thus suggests that Tat may represent a useful tool to drive and to increase Th1-like and CTL responses against heterologous antigens, and could therefore be considered an optimal co-antigen in the development of novel vaccination strategies against AIDS, as well as other diseases.

Materials and methods

HIV-1 proteins and peptides

Endotoxin-free HIV-1 Tat protein from the human immune deficiency virus type 1 (HIV-1) IIIB isolate (BH10 clone) was expressed in E. coli, purified by heparin-affinity chromatography and HPLC as previously described [11], and provided by Diateva (Fano, Italy). To prevent oxidation and loss of biological activity, the Tat protein was stored lyophilised at −80 °C, reconstituted in sterile degassed PBS before use, and handled as described [9]. The HIV-1SF162 Gag (502 aa) and the HIV-1SF162 V2-deleted Env gp140 (820 aa) proteins were obtained from Chiron (Emeryville, USA) and HIV-1SF162 Env gp120 (483 aa) was provided by the NIH AIDS Reference and Reagent Program.

Tat and OVA peptides were synthesized by the solid phase method, purified by HPLC to >98% purity [30] and provided by UFPeptides (Ferrara, Italy). The VCF (VCFITKALGISYGRK, aa 36—50) Tat peptide contains a Kd-restricted CTL epitope and a CD4 T cell epitope [40]. Gag and Env peptides, 15 amino acids long and overlapping by 10—11 amino acids, spanning the entire Gag (HIV-1 consensus subtype B) or Env sequence (HIV-1 subtype B consensus and SHIV SF162P3), were provided by the NIH AIDS Repository Reagents and References Program. Peptides were dissolved in DMSO at 10−3 M, kept at −80 °C, and diluted in PBS before use.

Mice immunization

C57BL/6 mice (Charles River Laboratories) were immunized with 25 μg of ovalbumin (Sigma—Aldrich) alone or in combination with 5 μg of native monomeric biologically active Tat protein (Sigma—Aldrich). Complete Freund’s adjuvant was used for the first immunization, whereas incomplete Freund’s adjuvant was used for boosting. BALB/c mice (Charles River Laboratories) were immunized with 5 μg of HIV-1 Gag, Env gp120 or V2-deleted Env gp140 protein alone or in combination with 5 μg of the Tat protein in Freund’s adjuvant or in Alum. In some experiments, groups of mice were also immunized with 5 μg of Tat alone in Alum adjuvant. Each group was composed of six animals. Immunogens (100 μl) were given subcutaneously at a single site in the back at days 1, 14 and 28, and mice were sacrificed at day 38. No signs of adverse local or systemic reactions were ever observed in mice receiving the immunogens, as compared to control or untreated mice. Animal use was according to European and Institutional guidelines. Each experiment was repeated three times.

Splenocyte purification

Splenocytes were purified from spleens squeezed on filters (Becton Dickinson). Following red blood cell lysis with RBC lysing buffer (Sigma), cells were washed with RPMI 1640 containing 10% FBS (Hyclone), spun for 10 min at 300 × g, resuspended in RPMI 1640 containing 10% FBS. Pools of spleens were used for each experimental group. Cells were immediately used for the analysis of antigen-specific
cellular immune responses or stimulated in vitro with peptides and tested after 5 days.

Cytotoxic assay

Fresh or peptide-stimulated splenocytes were tested against 51Cr-labeled target cells pulsed or not with the relevant peptide [30]. Percent specific lysis was calculated as $100 \times \frac{cpm\ sample - cpm\ medium}{cpm\ Triton\ X-100 - cpm\ medium}$. Spontaneous release was always less than 20%. Data are expressed as % specific lysis calculated by subtracting the lysis of unpulsed target cells, which was always ≤5%.

Elispot assay

Enzyme-linked immunospot (Elispot) assays were performed for Th1 (INF-$\gamma$) and Th2 (IL-4) cytokines, before (fresh) and after in vitro restimulation, using commercially available murine INF-$\gamma$ and IL-4 Elispot kits (BD, Pharmingen) according to the manufacturer’s instructions. Briefly, for analysis of immune responses on fresh cells, total splenocytes ($5 \times 10^5$ cells/well) or purified CD8+ T cells ($1 \times 10^5$ cells/well) were added to 96-well Elispot plates pre-coated with the cytokine-specific capture antibody, and incubated at 37°C for 24 h in the absence (untreated) or presence of the peptide ($10^{-6}$ M). For analysis of immune responses after in vitro restimulation, splenocytes ($3 \times 10^6$ ml$^{-1}$) were cultured with the peptide or with pools of peptides ($3 \mu$g/ml) for 5 days, extensively washed with RPMI 1640 containing 10% FBS and placed on (4—5 $\times 10^4$ cells/well) Elispot plates. Controls were represented by cells incubated with 5 μg/ml of Concanavaline A (positive control) or with medium alone (negative control). Spots were quantified using an Elispot reader (Aelvis). Responses at least three-fold higher than the mean number of spots in the control wells and ≥20 spots/well/10$^6$ cells were defined as positive when fresh splenocytes were used in the assay, and ≥300 for in vitro restimulated splenocytes. Results are expressed as number of spot-forming cells (SFC)/10$^6$ cells.

Statistical analysis

The two-way ANOVA test was performed.

Results

In vivo modulation of epitope-specific CTL responses against OVA by the native HIV-1 Tat protein

We have previously demonstrated in vitro that Tat increases proteasome activity, thereby favoring the generation and presentation of subdominant MHC-I binding CTL epitopes of heterologous antigens [13]. To determine the in vivo relevance of these findings, the effect of Tat on the induction of epitope-specific CTL responses was investigated in mice immunized with OVA, chosen as a model antigen since OVA-specific CTL responses against subdominant and cryptic epitopes are not usually detected [26].

The generation of K$^b$-restricted CTL responses directed to the immunodominant SIINFEKL (SII), subdominant KVVRFDKL (KVV) and cryptic CFDVFKEL (CFD) epitopes [6,26] was evaluated in C57BL/6 mice vaccinated with OVA alone or with OVA in combination with Tat using the Freund’s adjuvant. The presence of epitope-specific CTLs was tested in cytotoxicity and IFN-$\gamma$ Elispot assays using fresh ex vivo splenocytes.

Figure 1  OVA-specific T cell responses in mice vaccinated with OVA protein alone or combined with the Tat protein. After three immunizations, fresh splenocytes isolated from control mice (−), from mice immunized with OVA alone or with OVA in combination with Tat, were pooled and tested by cytotoxicity (panel A) against EL4 cells pulsed with the immunodominant SII, subdominant KVV, or cryptic CFD peptides or by IFN-$\gamma$ Elispot (panel B) after stimulation with the indicated peptides. Data are expressed as percent specific lysis, calculated by subtracting lysis of untreated EL4 cells (always below 5%) or as SFC/million cells, calculated by subtracting SFC/10$^6$ cells of unstimulated splenocytes (always below 10). The mean ± S.D. of the results from three independent experiments, performed in triplicate, is shown.
Splenocytes isolated from mice immunized with OVA alone lysed target cells pulsed with the immunodominant SII epitope, as did splenocytes from mice immunized with the OVA/Tat combination, albeit to a lesser degree (Fig. 1A). However, immunization with the OVA/Tat combination generated clear CTL responses also to the subdominant KVV and cryptic CFD epitopes, which were not detected after immunization with OVA alone (Fig. 1A), in agreement with the results of previous studies [6,26].

**Figure 2** Peptide-specific IFN-γ release in mice vaccinated with Gag protein alone or with the combination Gag and Tat. Mice were immunized with Gag alone or with the combination Gag and Tat in Freund’s adjuvant. After three immunizations, fresh splenocytes were pooled and stimulated with the Gag peptide pools P1–P22 by the Gag peptide matrix (panels A and B) or with the indicated single Gag peptides (panel C), and tested by IFN-γ Elispot assay. Positive responses to the peptide pools or the single peptides tested are highlighted in black. Results are expressed as SFC/10^6 cells. The mean ± S.D. of three independent experiments, performed in duplicate, is shown.
Fresh splenocytes from immunized animals were also tested by IFN-γ Elispot assay after stimulation with SIINFEKL, KKV, or CFD peptides. Mice immunized with OVA alone presented responses only to the immunodominant SIINFEKL peptide, whereas mice immunized with the combination OVA and Tat showed responses to the subdominant KKV and cryptic CFD peptides but very poor responses to the immunodominant SIINFEKL peptide (Fig. 1B). These findings demonstrate that co-immunization with OVA and Tat induces CTL responses against subdominant and cryptic OVA-derived CTL epitopes, which are not elicited by vaccination with OVA alone.

In vivo modulation of peptide-specific Th1-type T cell responses against HIV-1 Gag by the native HIV-1 Tat protein

To evaluate the effect of the native Tat protein on epitope-specific T cell responses directed to a relevant vaccine antigen, BALB/c mice were immunized with the HIV-1 Gag protein alone or HIV-1 Gag protein in combination with Tat using the Freund’s adjuvant. After three immunizations, fresh splenocytes purified from immunized mice were assayed by IFN-γ Elispot using a peptide-based matrix approach, since few Gag-derived epitopes have been characterized thus far [28,29,43]. Fifteen amino acid-long peptides, overlapping by 10 amino acids and covering the entire Gag sequence, were divided into 22 pools; each peptide was present in 2 independent pools, thereby providing positive internal controls (Fig. 2A).

As shown in Fig. 2A and B, mice immunized with Gag alone responded to seven pools (P5, P6, P9, P10, P16, P17 and P18), whereas mice immunized with the Gag/Tat combination not only responded to these same seven pools but also to an additional four (P3, P13, P15 and P19). None of the pools tested elicited a response in splenocytes from control mice (data not shown).

To identify which peptide/s within each positive pool were recognized by T cells, fresh splenocytes were then stimulated with the 30 individual 15mers identified as potential targets by the matrix approach and assayed by IFN-γ Elispot.

As shown in Fig. 2A and C, splenocytes from mice immunized with Gag alone responded to seven different peptide pools (42, 49, 50, 53, 65, 75 and 76), two of which (49, 50) contain the previously identified AMQ CTL epitope [29], indicating that T cell responses induced by Gag vaccination are directed to six different T cell epitopes. It should be noted that a positive response to peptide 42 was detected, even though responses to pool 15 were always found to be negative.

In contrast, mice immunized with Gag and Tat responded to 11 different T cell epitopes (20, 21, 39, 42, 49/50, 53, 65, 69, 75, 76 and 80), 5 more than those detected in mice vaccinated with Gag alone (Fig. 2A and C). The sequence of Gag-derived peptides inducing a positive response is reported in Table 1. In order to characterize the T cell populations which responded to the identified peptides, CD8+ T cells were purified from fresh whole splenocytes then stimulated with the peptides which elicited a positive response and assayed by IFN-γ Elispot. Most of the peptides were found to be targets of CD8+ T cells (data not shown), except peptides 39 and 75, thereby confirming previous results which suggested that peptides 39 and 75 contain two CD4 T cell epitopes [28,43]. The minimal CD8 target epitopes were then identified using the peptide-binding motif for Kd class I molecules. By this approach, five different potential epitopes within the sequences of the responding 15mers (peptides 20, 21, 42, 65, 69) were identified and synthesized. The 8–10 amino acid long peptides were then assayed on purified CD8+ cells by IFN-γ Elispot (data not shown). By this approach, four (LYCVHQR, MFSALSEGA, IYKRWIILGL and MYSPTSLDI) out of the five predicted CTL epitopes-induced IFN-γ release, thereby demonstrating that these peptides correspond to CD8+ epitopes contained in peptides 21, 42, 65, 69.

These results demonstrate that Tat broadens T cell responses directed against the HIV-1 Gag antigen.

### Table 1: Gag- and Env-derived peptides

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<td>SLYNTVATLYCVHQKR</td>
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<tr>
<td>21</td>
<td>TVATLYCVHQRIEVK</td>
<td>81–95</td>
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<tr>
<td>39</td>
<td>NAWVKKVEEAKFSPE</td>
<td>153–167</td>
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<td>42</td>
<td>SPEVIPMFSALSEGA</td>
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<td>49</td>
<td>GHQAMQLKETINE</td>
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</tr>
<tr>
<td>53</td>
<td>AMQMLKETINEAAE</td>
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</tr>
<tr>
<td>65</td>
<td>PVGEYIKR威尔GN</td>
<td>257–271</td>
</tr>
<tr>
<td>69</td>
<td>IVMYSPTSLDIQR</td>
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<td>75</td>
<td>VDRFYKTLRAEQASQ</td>
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<td>76</td>
<td>YKTLRAEQASQEVKN</td>
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<tr>
<td>80</td>
<td>MTETLTVQANAPDCK</td>
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<table>
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</tr>
<tr>
<td>5</td>
<td>GLLLLLGMLMSCAVE</td>
<td>17–31</td>
</tr>
<tr>
<td>6</td>
<td>LGMLMSCAVEKLLW</td>
<td>21–35</td>
</tr>
<tr>
<td>8</td>
<td>AVEKLWTVYGGVPA</td>
<td>29–43</td>
</tr>
<tr>
<td>10</td>
<td>VYGGVPWKTEATTTL</td>
<td>37–51</td>
</tr>
<tr>
<td>11</td>
<td>VPWKEATTTLFCAS</td>
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<td>12</td>
<td>KETTLFCASDAKA</td>
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<td>14</td>
<td>CASDAAKYTEVHN</td>
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<td>16</td>
<td>DTEVHNWATHACVP</td>
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<td>62</td>
<td>VTCGHGRPVSTQL</td>
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<td>73</td>
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<td>78</td>
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<tr>
<td>82</td>
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<td>323–337</td>
</tr>
<tr>
<td>86</td>
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<td>88</td>
<td>LQAQFENKTVIFQOS</td>
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<td>116</td>
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**In vivo modulation of peptide-specific Th1-type T cell responses against HIV-1 Env and V2-deleted Env by the native HIV-1 Tat protein**

To evaluate the *in vivo* effect of Tat on the induction of T cell responses against another relevant vaccine antigen, BALB/c...
Figure 3  Peptide-specific IFN-γ release in mice vaccinated with wild-type Env protein alone or with the combination Env and Tat. Mice were immunized with Env alone or with the combination Env and Tat in Alum adjuvant. After three immunizations, the splenocytes were pooled, re-stimulated in vitro with peptide pools for 5 days and assayed by IFN-γ Elispot after stimulation with the indicated Env peptide pools P1—P26 by the Env peptide matrix (panels A and B) or single Env peptides (panel C). Positive responses to the peptide pools or the single peptides tested are highlighted in black. Results are expressed as SFC/10⁶ cells. The mean ± S.D. of three independent experiments, performed in duplicate, is shown.
mice were immunized with the HIV-1 Env or V2-deleted Env proteins alone or in combination with Tat. In fact, both wild-type and V2-deleted trimeric Env are being exploited for vaccine strategies.

To exclude any additional effect caused by the use of Freund’s adjuvant and to be on line with trials development, in this set of experiments all vaccinations were performed in Alum, which is the only adjuvant approved for human use worldwide. Parallel experiments with Env alone or in combination with Tat were also carried out using Freund’s adjuvant.

After three vaccinations, fresh splenocytes purified from immunized mice were assayed by IFN-γ Elispot using a peptide-based matrix approach similar to that described above. Fifteen amino acid-long peptides, overlapping by 10 amino acids and covering the entire Env sequence, were divided into 26 pools (Fig. 3A). Since fresh splenocytes did not respond to any of the Env peptides (data not shown), splenocytes were first re-stimulated in vitro for 5 days with peptide pools and then assayed by IFN-γ Elispot.

As shown in Fig. 3A and B, mice immunized with Env alone responded to seven pools (P1, P4, P5, P12, P17, P18 and P23), whereas mice immunized with the Env/Tat combination not only responded to these seven pools but also to an additional six (P7, P9, P13, P15, P19 and P21). None of the pools tested elicited a response with splenocytes from control mice (data not shown).

To identify which peptide/s within each positive pool were recognized by T cells, splenocytes were then stimulated with the 36 individual 15mers identified as potential targets by the matrix approach and assayed by IFN-γ Elispot.

As shown in Fig. 3A and C, splenocytes from mice immunized with Env alone responded to five different peptides (11, 16, 62, 73 and 86). In contrast, mice immunized with Env and Tat responded to 17 peptides (2, 5, 6, 8, 10, 11, 12, 14, 16, 62, 73, 78, 82, 86, 87, 88 and 116), 12 more than those detected in mice vaccinated with Env alone.

As shown in Fig. 4, mice immunized with V2-deleted Env responded only to 4 peptides (11, 16, 62 and 86), in contrast to mice immunized with V2-deleted Env and Tat which responded to 17 peptides (2, 5, 6, 8, 10, 11, 12, 14, 16, 62, 73, 78, 82, 86, 87, 88 and 116), 13 more than those detected in mice vaccinated with V2-deleted Env alone. The sequence of Env-derived peptides inducing a positive response is reported in Table 1.

These results demonstrate that Tat increases the number of Env or V2-deleted Env epitopes recognized by T cells.

Similar results were observed in mice immunized with Env alone or in combination with Tat using Freund’s adjuvant (data not shown), thus excluding immunomodulatory effects of the co-administered adjuvant.

As shown in Fig. 4, mice immunized with V2-deleted Env responded only to 4 peptides (11, 16, 62 and 86), in contrast to mice immunized with V2-deleted Env and Tat which responded to 17 peptides (2, 5, 6, 8, 10, 11, 12, 14, 16, 62, 73, 78, 82, 86, 87, 88 and 116), 13 more than those detected in mice vaccinated with V2-deleted Env alone. The sequence of Env-derived peptides inducing a positive response is reported in Table 1.

These results demonstrate that Tat increases the number of Env or V2-deleted Env epitopes recognized by T cells.

The native HIV-1 Tat protein does not affect peptide-specific Th2-type T cell responses against a co-administered heterologous antigen

To assess whether co-immunization with Tat affects the release of IL-4, a typical marker of Th2-type immune responses, splenocytes from mice immunized with Env or V2-deleted Env alone or in combination with Tat were also tested for IL-4 production. To this end, cells were re-stimulated in vitro with the peptide pools and then assayed by IL-4 Elispot using the peptide-based matrix described above. As shown in Fig. 5A and B, no differences in IL-4 responses were observed between the groups. In fact, mice immunized with Env alone or with the Env/Tat formulation responded to the same seven peptide pools (P1, P4, P5, P12, P17, P18 and P23). None of the peptide pools tested elicited an IL-4 response in splenocytes from control mice (data not shown). In addition, to identify which peptides within each positive pool induced IL-4 release, splenocytes were stimulated with 12 individual 15mer peptides identified as potential targets by the matrix approach. As shown in Fig. 5A and C, splenocytes from mice immunized with Env alone responded to four peptides (11, 16, 62 and 86)
Figure 5  Peptide-specific IL-4 release in mice vaccinated with Env alone or with the combination Env and Tat. Mice were immunized with Env alone or with the Env and Tat combination in Alum adjuvant. After three immunizations, splenocytes were pooled, re-stimulated in vitro with peptide pools for 5 days and assayed by IL-4 Elispot assay after stimulation with the indicated Env peptide pools P1—P26 by the Env peptide matrix (panels A and B) or single Env peptides (panel C). Positive responses to the peptide pools or the single peptides tested are highlighted in black. Results are expressed as SFC/10^6 cells. The mean ± S.D. of three independent experiments, performed in duplicate, is shown.

Co-immunization with Env and Tat proteins does not affect cellular responses against Tat

The results of the experiments described above clearly indicate an adjuvant effect of Tat on Th1-type T cell responses against HIV-1 structural antigens and suggest that Tat may be considered an optimal co-antigen in
and then tested by IFN-γCFITKALGISYGRK (VCF) T at-derived T cell epitope for 5 days of three immunizations, splenocytes were stimulated with the alone or in combination with V2-deleted Env protein. After experiments, groups of mice were also vaccinated with T at using the Alum adjuvant. In parallel proteins, mice were immunized with T at alone or in combination with Env using the Alum adjuvant. In parallel experiments, performed in duplicate, is shown.

The results of these experiments showed that the number of IFN-γ and IL-4 secreting cells were similar in mice vaccinated with Tat alone and with the Env/Tat formulation (p > 0.05) (Fig. 6A). Similar results were obtained in mice immunized with Tat alone and with the V2-deleted Env/Tat vaccine formulation (p > 0.05) (Fig. 6B), thereby indicating that both Th1- and Th2-type cellular responses against Tat are not significantly affected by co-immunization with Env proteins.

Discussion
In this study, we demonstrated that the native HIV-1 Tat protein broadens in vivo epitope-specific CTL and Th1-type immune responses directed to heterologous antigens, which are relevant for new HIV vaccine design.

In particular, co-immunization with OVA and Tat induces CTL responses and IFN-γ release against subdominant and cryptic OVA-derived CTL epitopes which were not elicited by vaccination with OVA alone. It should be noted that the lack of subdominant and cryptic OVA-specific CTL responses has previously been ascribed to poor generation of target epitopes by the proteasome [32, 35]. This, together with our previous results showing that Tat modulates the activity of the immunoproteasome and antigen presentation of heterologous antigens in vitro [13], suggests that Tat affects antigen processing and presentation of OVA also in vivo, resulting in the activation of CTL responses against subdominant and cryptic CTL epitopes of the co-administered OVA protein.

Tat also expands in vivo epitope-specific IFN-γ T cell responses directed to the HIV-1 Gag, Env and V2-deleted Env antigens, which are all relevant candidate components in HIV vaccine development.

Specifically, we demonstrated here that mice immunized with Gag alone respond to six different Gag-derived T cell epitopes. These include the AMQ peptide, which represents the major Kd-restricted CTL epitope [29, 43] and is often used as a marker of vaccine-induced cellular responses in mice vaccinated with Gag. In contrast, mice immunized with Gag and Tat responded to 11 different T cell epitopes, 5 more than those detected in mice vaccinated with Gag alone. We also demonstrated that mice vaccinated with Env in combination with Tat responded to 17 peptides, 12 more than mice vaccinated with Env alone, and similar results were also obtained when mice were immunized with the V2-deleted Env. Of note, co-immunization with Tat did not affect Th2-type immune responses to the co-administered antigen, thereby demonstrating the capacity of Tat to specifically drive Th1-type responses and to increase the generation and presentation of class I restricted peptides in vivo [11, 13].

These findings demonstrate that Tat is not only an antigen [2, 3], but also a novel and potent adjuvant capable of broadening the spectrum of epitopes recognized by T cells. This in vivo adjuvant effect may be due to a combination of the numerous immunomodulatory properties of the Tat protein which have been described in previous studies. Indeed, it is known that the Tat protein (i) promotes MDDC maturation and activation, thereby leading to more efficient T cell responses against heterologous antigens [11], (ii) increases the major proteolytic activities of the immunoproteasome, thereby favoring the generation and presentation of the subdominant MHC-I binding CTL epitopes of heterologous antigens [13, 41], (iii) contains a basic domain able to increase the surface expression
of MHC class I binding peptides [24], and (iv) possesses auto-adjuvanticity connected to its ability to dimerize [21].

These results indicate that Tat should be exploited as a component in the development of subunit-based vaccines against HIV/AIDS [10,17,33,44]. To this respect the observation that co-immunization with Env proteins and Tat did not result in immunodominance hindrance of T cell responses against Tat is also noteworthy, since it implies that a vaccine formulation containing both Tat and Env proteins may have the advantage of controlling the replication of the virus both at early and late stages of the infection. Based on this rational, the combined vaccine Tat and V2-deleted Env is being developed and will be tested in human trials within the AIDS vaccine integrated project (AVIP). On the other hand, induction of T cell responses to new epitopes may indeed be particularly relevant in vaccination strategies against other viral infections or tumors. In fact, it is widely held that increasing the size and broadening the diversity of T cell responses to a given antigen would increase the efficacy of a vaccine and also reduce epitope mutation and CTL escape [15,19,20].

Acknowledgments

The authors wish to thank M. Magnani, E. Laguardia, E. Fanales, F. Nappi, S. Moretti for testing the Tat protein lots, the NIH AIDS Repository Reagents and References Program for reagents, and Anna Forster for editorial assistance. This work was supported by grants from the Istituto Superiore di Sanità [National AIDS Project and the Italian Concerted Action on HIV-AIDS Vaccine Development (ICAV)], the Italian Association for Cancer Research [AIRC], and the Ministero dell’Istruzione, dell’Università e della Ricerca Scientifica (MIUR).

References


Tat broadens T cell responses to HIV antigens


