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# Efficient systemic and mucosal responses against the HIV-1 Tat protein by prime/boost vaccination using the lipopeptide MALP-2 as adjuvant

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**Abstract**

A major goal of HIV-1 vaccine development is the induction of mucosal immune responses able to stop or reduce viral infection directly at the portal of entry. We established a heterologous prime/boost vaccination protocol based on intradermal priming with the HIV-1 Tat protein and intranasal boosting with the Tat protein co-administered with the mucosal adjuvant MALP-2. Strong Tat-specific humoral responses were elicited in vaccinated mice at both systemic and mucosal levels. The cellular responses were characterized by a Th1 dominant helper pattern. The heterologous prime/boost regimen was also able to induce Tat-specific CTL, which were absent in animals receiving the homologous prime boost scheme. Thus, the heterologous prime/boost protocol was the only regimen able to evoke both CTL and sIgA responses. This suggests that a similar approach can be exploited to develop multi-component vaccines against HIV-1 infections able to induce both systemic and mucosal immune responses.


*Keywords:* AIDS; Prime; Boost vaccination; Mucosal adjuvant; Tat

## 1. Introduction

HIV/AIDS is the most important infection leading to 3.1 million (2.8–3.5 million) deaths in 2004, particularly in developing countries (UNAIDS, 2004; http://www.unaids.org) [1–3]. Therefore, the development of a vaccine against HIV/AIDS constitutes an urgent need and the only realistic hope to stop the spread of HIV infections and AIDS. Most of the vaccine candidates tested so far have approached this challenge by the induction of neutralizing antibodies against envelope proteins. However, none of these prototypes induced efficient protection against homologous challenge [4,5]. In fact, the first phase III trial performed using the gp120-based vaccine candidate AIDSVAX from VaxGen was a failure [6–9]. Vaccines based on HIV/SIV structural products alone, such as Env or Gag, have failed to prevent infection but in some cases have contained viral replication. Moreover, the high variability of the envelope antigens between different HIV clades prevents their use as universal vaccine candidates. Novel vaccine strategies, which focus on the capability to control viral infection and disease progression (non-sterilizing immunity) are currently being developed. More recently, vaccine approaches based on early regulatory gene products (Tat/Rev/Nef) have shown to be safe, immunogenic (in mice, monkeys and humans) and to contain virus replication at its early phases (monkey models).

*Abbreviations:* i.n., intranasal; i.d., intradermal; MALP-2, macrophage activating lipopeptide-2

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To approach this goal, the HIV-1 Tat protein has been identified as an attractive candidate antigen, because it is expressed very early in the virus life cycle [10], it plays an essential role for viral replication [11,12], and its functional and immunogenic domains are highly conserved among different HIV clades [13,14]. Importantly, Tat specific antibodies, which spontaneously develop in about 20% of individuals infected with HIV from different clades, cross-recognize a Tat protein derived from a clade B virus [14] and their presence correlates with non-progression in infected individuals [15]. Extracellular Tat also has important effects on immunoregulatory functions [16,17]. In particular, bioactive soluble Tat selectively binds to and enters immature and mature dendritic cells (iDC and mDC, respectively), drives iDC maturation and activation toward a T helper 1 (Th-1) inducing phenotype [18,19], facilitates major histocompatibility complex (MHC) class I antigen presentation [20,21], and modulates proteasome catalytic subunit composition, modifying the hierarchy of epitopes presented in favor of subdominant and cryptic ones [22]. Of note, all these properties belong to the native protein, and are lost when Tat is oxidized or chemically modified (Tat toxoid). Pre-clinical studies also demonstrated that parenteral immunization of macaques with biologically active Tat protein or *tat* DNA protects them from systemic challenge with the highly pathogenic virus SHIV89.6P blocking virus replication and preventing disease onset [18,23–25]. Based on these data, preventive and therapeutic phase I trials with biological active Tat protein are being completed in Italy and preliminary results indicated lack of toxicity and development of both humoral and cellular immune responses (unpublished data).

Since most HIV infections occur either via sexual transmission or by breastfeeding, mucosal immunity is considered to be important for the protection against HIV infections [26]. In fact, analysis of the immune responses in highly exposed seronegative persons revealed strong mucosal IgA, as well as CTL responses [27,28]. Recently, we have reported the efficient induction of mucosal immune responses against the Tat protein by intranasal (i.n.) co-administration of biologically active Tat protein with a synthetic derivative of the macrophage-activating lipopeptide of 2 kDa (MALP-2) from *Mycoplasma fermentans* [29]. The mucosal immunization of mice with MALP-2 promoted the elicitation of strong humoral immune responses against the co-administered Tat protein at the systemic level. In addition, secretory IgA were detected in the bronchial as well as in the vaginal lavages of vaccinated animals [29]. Antibodies from both sera and mucosal lavages targeted functional domains of the Tat protein, thereby neutralizing its *trans*-activating activity ([29], unpublished data). Moreover, Tat specific T-cell responses after co-administration with MALP-2 were characterized by strong proliferation and a Th1 profile. However, we were unable to promote the elicitation of CTL responses in vaccinated mice.

In the present study, we implemented a prime-boost vaccination protocol based on intradermal (i.d.) priming with the biologically active Tat protein and i.n. boosting with Tat protein co-administered with MALP-2 in an attempt to modulate and broaden the immune response elicited. We show here that by this approach both secretory IgA, as well as systemic CTL responses were efficiently induced.

## 2. Materials and methods

### 2.1. Animals and cell cultures

Female BALB/c (H-$2^d$) mice of 6 weeks of age were purchased from Harlan-Winkelmann (Germany). Cells were grown in complete medium [RPMI 1640 supplemented with 10% FCS, 100 U/ml of penicillin, 50 μg/ml of streptomycin and 1 mM L-glutamine (GIBCO BRL, Germany)].

### 2.2. Antigen production

The Tat protein (aa 1–86) from the HTLV-IIIB isolate, BH10 clone (clade B), was expressed in *Escherichia coli*, purified by heparin-affinity chromatography and high-performance liquid chromatography and stored at −80 °C. The purified Tat protein was fully monomeric [23,30,31], as determined by PAGE, Western blotting and high performance liquid chromatography analysis, and had full biological activity as assessed by virus *trans*-activation assay and by dendritic cells uptake [23,30–33]. MALP-2 was synthesized according to established protocols [34].

### 2.3. Immunization protocols

Groups of five mice were immunized on days 0, 14, 28 and 42 with 10 μg of Tat protein either intradermally (in 50 μl volume, injection into the femoral part of the right hind leg, homologous systemic immunization), or co-administered with MALP-2 (0.5 μg) intranasally (10 μl per nostril, homologous mucosal immunization). The group vaccinated according to the heterologous prime/boost protocol was primed intradermally with the Tat protein (10 μg/50 μl) on days 0 and 14, and then boosted intranasally with Tat and MALP-2 (10 μl per nostril), on days 28 and 42. Negative controls received only PBS.

### 2.4. Sample collection

Serum samples were collected on days 0, 13, 27, 41 and 52. Mice were sacrificed on day 52 and vaginal and lung lavages were obtained by flushing the organs with a final volume of 0.5 and 1 ml of PBS supplemented with 5% FCS and 40 μM phenylmethanesulfonyl fluouride (PMSF), respectively. Lavages were then centrifuged to remove debris (10 min at 13,000 × *g*), and supernatants were stored at −20 °C. Sub-mandibular lymph nodes and spleens were removed and pooled for the analysis of cellular immune responses.

### 2.5. Detection of anti-Tat IgG in serum

Antibody titers were determined by ELISA using microtitre plates coated with 100 μl/well of Tat (1 μg/ml in 0.05 M carbonate buffer, pH 9.6), as previously described [29]. Endpoint titers were expressed as the reciprocal log of the last dilution, which gave an optical density at 405 nm of 0.1 units above the values of the negative controls after 15 min of incubation. The amount of Tat-specific IgG subclasses present in serum samples were determined using an isotype-specific ELISA, as previously described [29].

### 2.6. Determination of total and anti-Tat IgA

The amount of total and Tat-specific IgA present in lung and vaginal lavages was determined by ELISA, as previously described [29]. To compensate for variations in the efficiency of recovery of secretory antibodies among animals, the results were normalized and expressed as percentage of Tat-specific IgA with respect to the total amount of IgA present in the sample.

### 2.7. T-cell proliferation assays

Proliferation assays were performed in triplicates, as previously described [29]. The results are expressed as the ratio of the mean [$^3$H]-thymidine uptake between stimulated and unstimulated samples.

### 2.8. ELISPOT assay

To determine the concentration of IFN-$\gamma$ and IL-4 secreting cells, the murine IFN-gamma and IL-4 (BD-Pharmingen, Germany) ELISPOT kits were used. Cells ($1 \times 10^6$/well for IFN-gamma and $5 \times 10^5$/well for IL-4) were incubated for 16 h in the absence or in the presence of a mixture of Tat peptides (10 μM each), which were known to contain H2$^d$ restricted CTL epitopes (ACTNCYCKKCCFHCQVCFIT aa 21–40, WKHPGSQPKTACTNC aa 11–25, SQPKTACTNCYCKKC aa 16–30, KQPTSQSRGDPTGPK aa 71–85, and QPKTACTNC aa 17–25; [35,36]). Then, cells were removed and the plates processed according to manufacturer's instructions. Colored spots were counted under a light microscope.

### 2.9. CTL assay

To measure Tat-specific cytolytic activity splenocytes were incubated with the same mixture of Tat peptides used for the ELISPOT assay. After 24 h, 20 U/ml of rIL-2 were added. Five days later, cells were washed and their concentration was adjusted to $5 \times 10^6$ cells per ml. In parallel, $1 \times 10^7$ P815 target cells were incubated in 500 μl of either RPMI medium without FCS (negative control) or medium containing a mixture of the Tat peptides. After 2 h, 100 μl of RPMI medium without FCS containing 100 μCi of $^{51}$Chromium were added. After 1–2 h, target cells were extensively washed with RPMI medium containing FCS (binding of free $^{51}$Chromium) and co-incubated in triplicates with splenic effector cells at effector:target (E:T) ratios of 6.25:1; 12.5:1; 25:1 and 50:1. After 4 h, cells were centrifuged and the radioactivity in supernatants was measured by scintillation counting. Maximal lysis was detected in supernatants after Triton X-100 lysis, spontaneous lysis was measured in supernatants of untreated target cells. Results are expressed as percentage of lysed cells: (sample − spontaneous lysis)/(maximal lysis − spontaneous lysis) × 100.

### 2.10. Statistical analysis

Comparisons between experimental groups were made by using the Student's *t*-test. $P < 0.05$ was considered as significant.

## 3. Results

### 3.1. Efficient humoral immune responses are stimulated at both systemic and mucosal levels by combining an intradermal priming with the HIV-1 Tat protein alone with an intranasal boost with Tat co-administered with MALP-2

To optimise the immune responses induced by vaccination, we immunized mice with the native Tat protein (10 μg/dose) by i.d. route on day 1 and 14, and boosted them with Tat ad-mixed with MALP-2 (0.5 μg/dose) on day 28 and 42. This immunization protocol was expected to efficiently elicit both cellular responses, due to the intradermal priming, and mucosal responses, as a result of the intranasal boost. Control animals received on days 0, 14, 28 and 42 either Tat + MALP-2 or Tat alone by i.n. or i.d. route, respectively.

Firstly, we assessed the stimulation of humoral immune response at the systemic level. As shown in Fig. 1A, all three vaccination strategies resulted in the stimulation of high Tat-specific serum IgG titers. The highest titers (1:1,200,000) were observed in animals which received Tat + MALP-2 by the i.n. route, followed by those vaccinated with Tat i.d. (1:410,000) or with the heterologous prime/boost regimen (1:180,000) (Fig. 1A). Thus, mucosal immunization was (by far) the best strategy to induce IgG responses in mice, and the difference with respect to the other two experimental groups reached statistical significance ($p < 0.05$).

Then, we investigated the capacity of the three different vaccination protocols to induce mucosal immune responses. To this end, Tat-specific IgA was measured in lung and vaginal lavages from vaccinated animals (Fig. 1B). As expected, intranasal immunization with Tat + MALP-2 induced strong antigen-specific IgA responses both in lungs and in the genital tract (10–11% of the total IgA). Efficient local responses were also observed in mice receiving the prime/boost protocol (6 and 5% of the total IgA, respectively). In contrast, Tat-specific

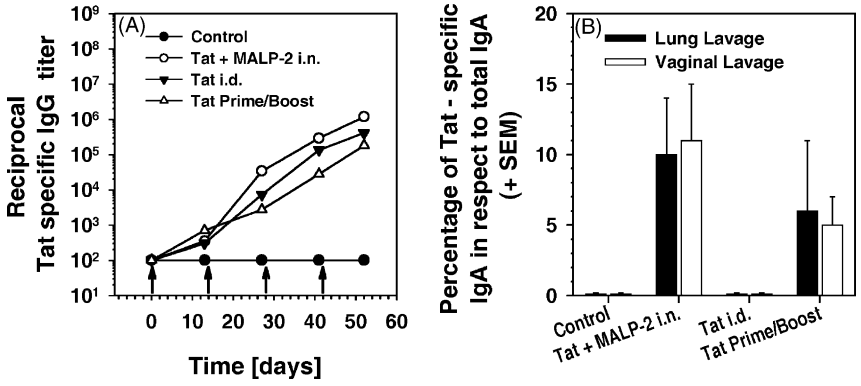


Fig. 1. Humoral immune responses stimulated in vaccinated animals. (A) Kinetics of anti-Tat IgG responses in sera from mice immunized with PBS, Tat + MALP-2 (i.n.), Tat (i.d.) or Tat prime/boost. Immunizations (day 0, 14, 28 and 42) are indicated by arrows. Results are expressed as the reciprocal log of the geometric mean endpoint titer. ((*) Statistical significance at $P < 0.05$). (B) Tat-specific IgA in lung and vaginal lavages of immunized mice. Results are expressed as the percentage of Tat-specific IgA with respect to the total IgA. The total mean IgA values for the groups immunized with PBS, Tat + MALP-2 (i.n.), Tat (i.d.) and Tat Prime/Boost were 0.137, 4.88, 0.101 and 0.588 μg/ml in the lung washes, and 0.254, 1.327, 0.523 and 0.31 μg/ml in the vaginal lavages, respectively. IgG and IgA titers represent the mean of five animals per experimental group. One representative out of two independent experiments is shown. SEM is indicated by vertical lines.

IgA were below the detection level in mice receiving Tat alone by the intradermal route.

### *3.2. The implementation of a prime/boost regime results in the elicitation of Tat-specific cellular immune response*

Lympho-proliferative assays were then carried out to evaluate and to compare the efficacy of the three different vaccination protocols at inducing cellular immune responses. Immune cells isolated from spleens or mandibular lymph nodes on day 52 after the first immunization were re-stimulated in vitro with the biologically active Tat protein. The strongest proliferative response was observed in mice vaccinated with Tat + MALP-2 by i.n. route, both in spleens and draining lymph nodes (Fig. 2). Only weak responses were detected in the rodents that received the Tat protein alone by the i.d. route, whereas intermediate levels of response were observed in mice immunized with the prime/boost protocol, both at systemic and regional levels.

### *3.3. Prime/boost immunization with the native HIV-1 Tat protein induces Th1 responses*

To evaluate the effect of the immunization route on the T helper response pattern, the subclass distribution of Tat-specific serum IgG was analyzed and expressed as IgG1:2a ratio, since these isotypes are considered to result from stimulation by Th2 and Th1 cells, respectively [37]. As recently shown [29], i.n. co-administration of Tat protein and MALP-2 led to similar levels of IgG1 and IgG2a. In contrast,

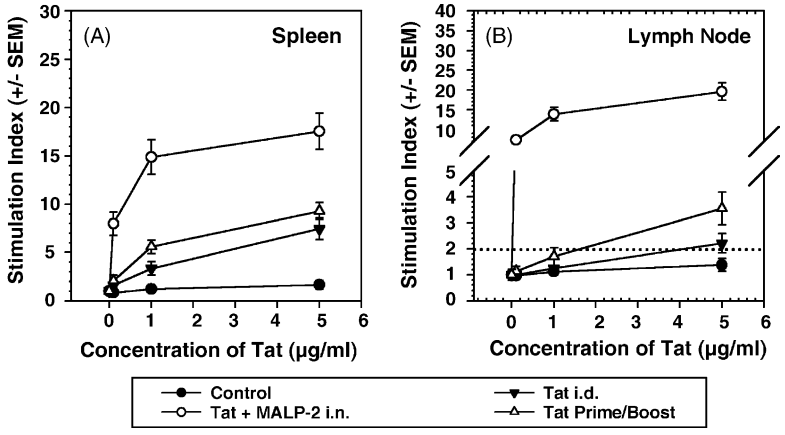


Fig. 2. Proliferative responses stimulated in vaccinated animals. Cells from (A) spleen and (B) lymph nodes were restimulated with different concentrations of soluble Tat for 4 days. Results are expressed as the ratio between values (average of triplicates) from stimulated and non-stimulated samples (stimulation index). For the cellular assays: (A) spleen and (B) lymph node cells of the different experimental groups were pooled. Data show triplicates of one representative experiment out of three. SEM is indicated by the vertical lines.

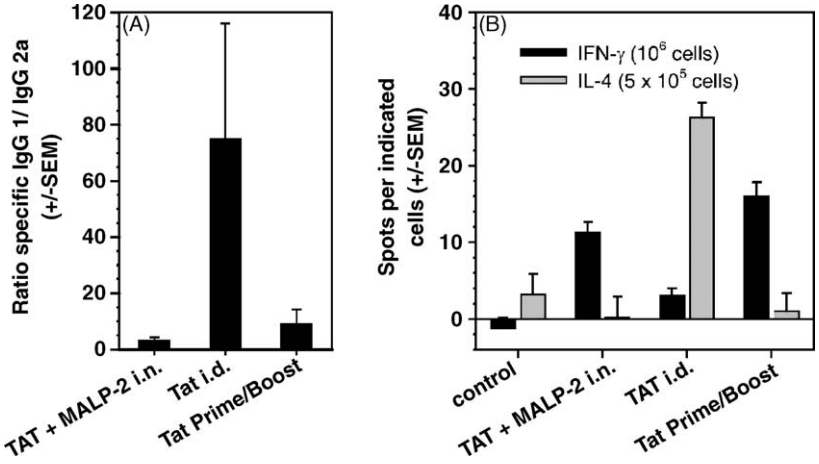


Fig. 3. Analysis of IgG isotypes and cytokine secretion in vaccinated animals. (A) Anti-Tat IgG isotypes were determined by ELISA. Results are expressed as ratio between IgG1 and IgG2a end point titers. For values of total anti-Tat IgG see Fig. 1A. (B) To detect IFN-γ and IL-4-secreting spleen cells $1 \times 10^6$ and $5 \times 10^5$ cells/well were incubated for 16 h in the presence or absence of a mixture of Tat peptides. The numbers of IFN-γ- or IL-4-producing cells were determined by ELISPOT. The values shown were subtracted from those obtained from non-stimulated cells. As mentioned in Section 2, for cellular assays, cells of the different experimental groups were pooled. Data show triplicates of one representative experiment out of three.

i.d. immunization induced a dominant Th2 response. Of note, intranasal boosting with Tat + MALP-2 of mice primed i.d. shifted the pattern towards a Th1-type response (Fig. 3A and B).

To further characterize the T helper response, the presence of Tat-specific IFN-γ and IL-4 secreting cells was assessed by ELISPOT after in vitro re-stimulation of splenocytes with the Tat peptide pool for 16 h. In agreement with what observed for the IgG isotypes, IFN-γ but no IL-4 secreting cells were detected in mice vaccinated with Tat + MALP-2. In contrast, i.d. vaccination promoted mainly the expansion of IL-4 producing cells. In mice receiving the prime/boost regimen, splenocytes predominantly secreted IFN-γ in response to Tat, whereas very few IL-4 producing cells were detected. These data demonstrated that i.n. boosting with Tat plus MALP-2 had modified the T helper pattern towards a dominant Th1, which seems to correlate with protection against HIV-1 in pre-clinical models.

### *3.4. The prime/boost regimen results in the stimulation of Tat-specific CTL*

We then evaluated in a standard chromium release assay whether the prime/boost regimen was able to stimulate the production of Tat-specific CTL. To this end, splenocytes from immunized animals were re-stimulated in vitro over 5 days with a pool of Tat-peptides representing H-$2^d$ restricted CTL epitopes (see Section 2), which were also used to load the target cells. While both homologous immunization approaches were unable to induce detectable Tat-specific CTL, splenocytes from mice immunized with the prime/boost regimen were able to lyse Tat-labeled target cells in a dose-dependent manner (Fig. 4A). Lysis of unlabeled target cells after co-incubation with any of the effectors was negligible (<4%) at all E:T ratios (Fig. 4B).

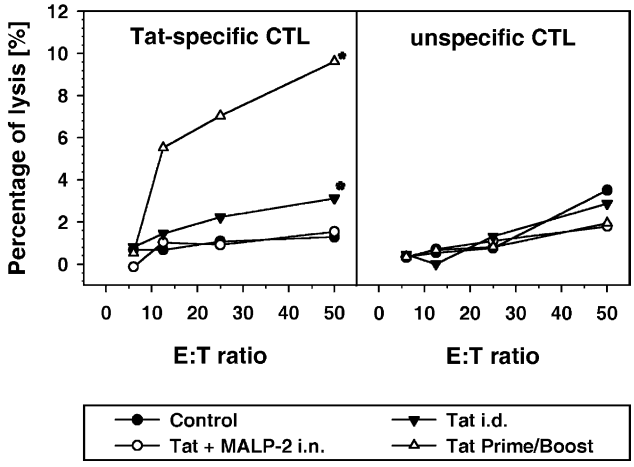


Fig. 4. Cytolytic activity of spleen cells from vaccinated animals. Cytolysis was determined by chromium release assay. Target cells were labeled with (Tat-specific) or without (unspecific) a mixture of Tat peptides and $Cr^{51}$. The $10^4$ target cells were co-incubated for 4 h in different ratios (Effector:Target (E:T)) with splenic effector cells (pool of five mice per group), which were stimulated before with the same mixture of peptides for five days. Then, radioactivity in supernatants was quantified by scintillation counting. Maximal lysis was determined after incubation with TX-100 (4500 cpm), spontaneous lysis represents the radioactivity in supernatants of unlysed cells (300 cpm). Results are presented as percentage of lysed cells: (sample − spontaneous lysis)/(maximal lysis − spontaneous lysis) × 100. For cellular assays, spleen cells of the different experimental groups were pooled. Data show triplicates of one representative experiment out of three. The statistic analysis showed that the differences in CTL activation between control and immunized animals were significant at $p < 0.05$ (*).

## 4. Discussion

A major goal in the development of vaccines against HIV/AIDS is the induction of broad humoral and cellular immune responses at both systemic and mucosal levels, capable of controlling viral replication and disease onset

[27,28]. In this context, we have recently demonstrated that MALP-2 is an efficient mucosal adjuvant when co-administered intranasally with biologically active Tat protein [24]. In that study vaccinated animals developed strong Tat-specific humoral responses at both mucosal and systemic levels. Moreover, strong antigen-specific proliferation was observed when cells from either spleens or regional lymph nodes of vaccinated animals were tested. Although IFN-γ secretion was significantly increased in vaccinated mice, CTL responses remained undetectable [29]. We have recently shown that the increased production of secretory IgA and IgG2b observed using MALP-2 as adjuvant is, at least in part, mediated by TGF-ß signaling [38]. TGF-ß has been reported to be an immune suppressive and anti-inflammatory cytokine, which contributes to mucosal homeostasis [39]. Thus, we conclude that the MALP-2-mediated induction of suppressive cytokines might explain the absence of antigen-specific cytolytic responses.

In the present study, we investigated whether the responses elicited after vaccination with biologically active Tat protein could be modulated and broadened by implementing a prime/boost vaccination protocol. Animals were primed with the Tat protein on days 1 and 14, a protocol known to stimulate Th and CTL responses. Then, they were boosted with Tat + MALP-2 by the i.n. route on days 28 and 42, in an attempt to stimulate also the production of mucosal IgA. As controls, animals received four doses of either Tat (alone) by the i.d. route (homologous systemic immunization) or Tat + MALP-2 by the i.n. route (homologous mucosal immunization).

The immune responses obtained demonstrated that i.n. vaccination with Tat + MALP-2 promotes strong humoral immune responses characterized by high titers of Tat-specific antibodies both in serum and in mucosal lavages (Fig. 1). Moreover, strong proliferative responses were induced in both regional lymph nodes and spleen (Fig. 2). Despite the induction of IFN-γ secreting cells and the low IgG1:IgG2a ratio, which indicates a dominant Th1 profile (Fig. 3), no CTL activity was detectable (Fig. 4). This was not completely unexpected, since the observation that IFN-γ secreting cells are not always cytolytic has already been reported by others, especially in vaccine studies in human and non-human primates [27,40–42]. An explanation for the discrepancy between the presence of IFN-γ secreting cells and the lack of CTL is that the 15 and 20mer peptides used for ELISPOT assays are able to stimulate both CTL activity and IFN-γ and/or IL-4 producing CD4+ T cells after Tat + MALP-2 vaccination [43]. Moreover, the special cytokine environment induced by mucosal vaccination [44] and/or the properties of the used adjuvant (i.e. MALP-2) may result in the induction of effector cells that specifically localized in mucosal sites, thereby being undetected in peripheral lymphoid tissues.

The immune responses induced by i.d. vaccination with the Tat protein were characterized by high titers of Tat-specific IgG in the serum. However, secretory IgA were not detected (Fig. 1B). Proliferative responses were weaker than in mice receiving Tat + MALP-2, despite the fact that cytokine secretion studies and IgG isotype analysis indicated a pronounced stimulation of a Th2 response (Figs. 2 and 3). Interestingly, no CTL response was detected (Fig. 4). This is in contrast with the results obtained using this route in the non-human primate model [44]. However, differences were also observed with regard to the humoral response in that, while in the mouse anti-Tat Ab were readily induced and were mostly of the IgG1 subclass upon i.d. vaccination, in the monkey model the anti-Tat Ab response induced by the i.d. route was delayed, weak, transient and associated with a Th1-like response pattern [37]. Taken together these data indicate that the pattern of T helper response may account for the differences observed. In this respect, BALB/c mice have been reported as being more prone to mount Th2 responses, and this feature has recently been correlated to the pattern of Toll-like receptor expression on DCs, which display a more immature phenotype as compared to other mouse strains, and to the high number of CD25+ T regulatory cells present in this strain [45,46]. Thus, it is conceivable that native Tat, which inherently drives Th1 responses by selectively targeting DCs and promoting a Th1 inducing DC phenotype [19], is unable to do so when given i.d. in an animal with a strong genetic and multi-factorial background favoring Th2 responses. This underscores the importance to further evaluate and select HIV-1/AIDS vaccine candidates in outbred non-human primates prior to proceeding to human studies. In this respect, the increasing number of phase I/II clinical trials that are being conducted after or concurrently with studies in monkeys appear to validate non-human primates as a good model to accurately predict immunogenicity in humans (reviewed in [47]). However, due to logistic, ethical and cost-related considerations, it is not feasible to perform in this model the initial screening and priorisation studies to select the most promising candidates and formulations for evaluation in humans.

The systemic prime/mucosal boost approach induced both systemic and mucosal responses. In particular, following the i.d. priming, serum IgG were induced, whereas two mucosal booster immunizations with Tat + MALP-2 by the i.n. route were sufficient to consistently increase the levels of antigen-specific secretory IgA in mucosal lavages (Fig. 1). However, sIgA and to a lesser extent serum IgG titers were lower than those observed in the homologous mucosal or systemic vaccination protocol, respectively. More striking, though, was the shift toward a Th1 pattern observed in the heterologous systemic prime/mucosal boosting group. In fact, while the homologous systemic i.d. vaccination induced a pronounced Th2 response and, conversely, the mucosal (i.n.) immunization stimulated a strong Th1 response, the mixed systemic/mucosal protocol induced a pronounced shift towards a Th1 response, as indicated by the reduction in the IgG1:IgG2a ratio, the drop in the number of IL-4 producing cells and the increment in the number of IFN-γ secreting cells (Fig. 3). Although the number of Tat-specific IFN-γ secreting cells in spleens from prime/boost vaccinated mice was

even higher than that observed for splenocytes from those animals vaccinated i.n. with Tat + MALP-2, the results were not significant. More importantly, the prime/boost regimen was the only vaccination protocol that elicited Tat-specific CTL responses (Fig. 4). There is a general consensus on the fact, that the induction of mucosal and systemic immunity to HIV-1 is critical to confer protection against HIV/AIDS. We showed here, that the desired immune responses can be elicited by an experimental prime-boost regimen consisting of two parenteral (intradermal) immunizations with recombinant HIV-1 Tat protein alone, followed by mucosal application of the HIV-1 Tat protein co-administered together with the new mucosal adjuvant MALP-2. The two booster immunizations with the HIV-1 Tat protein together with the mucosal adjuvant MALP-2 allowed the induction of mucosal as well as systemic immunity to HIV-1, as shown by the secretion of IFN-γ, phenotype of Tat-specific CTL, stimulation of enhanced secretory IgA responses and the production of IgG.

The induction of both strong Tat-specific cellular responses and IgA is highly desirable, because it may sensibly contribute to contain HIV-1 infection directly at the portal of entry [26]. The present study shows that a systemic prime/mucosal boost approach is superior to either the homologous systemic or mucosal regimen, in that it induced mucosal IgA, a strong Th1 response, and CTL. Thus, this type of mucosal targeted prime-boost vaccination approach should be considered for the development of more efficient vaccines against HIV-1/AIDS.

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