Immunization with low doses of HIV-1 tat DNA delivered by novel cationic block copolymers induces CTL responses against Tat

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Abstract

Cytotoxic T cell responses are key to the control of intracellular pathogens including HIV-1. In particular, HIV-1 vaccines based on regulatory proteins, such as Tat, are aimed at controlling HIV-1 replication and at blocking disease development by inducing cytotoxic T cell responses. Naked DNA is capable of inducing such responses but it requires several inoculations of high amounts of DNA, and/or prime-boost regimens. Here, we show that a novel class of cationic block copolymers protect the DNA from DNAse I digestion, and improve DNA delivery to antigen-presenting cells (APCs) after intramuscular (i.m.) vaccination. In particular, three cationic block copolymers (K1, K2 and K5) were used to deliver the HIV-1 pCV-tat DNA vaccine in BALB/c mice. The results indicate that vaccination with a very low dose (1 μg) of pCV-tat delivered by the cationic block copolymer K2 is safe and, as compared to naked DNA (up to 30 μg), greatly increases the CTL response against Tat, which was detected in all animals in the absence or in the presence of re-stimulation.

Keywords: Cationic block copolymers; HIV-1 tat gene; Mice immunization

1. Introduction

Synthetic non-viral vectors based on polymeric systems are very promising systems to deliver plasmid DNA since they are safe and capable of improving the potency of DNA vaccines [1]. A number of cationic polymers have been tested in transfection experiments [e.g. poly(l-lysine), polyethylenimine, derivatized chitosan and poly(dimethylamino ethyl methacrylate) (PDMAEMA)] [2–6]. One of the most promising approaches of plasmid DNA delivery with synthetic polymers is the use of block or graft copolymers made of a cationic block, able to interact with DNA, and a hydrophilic non-ionic block, capable of sterically stabilizing the complex [7,8]. So far, several polycations have been used in combination with hydrophilic non-ionic polymers to produce complexes with DNA. They are able to protect the DNA from enzymatic degradation and to deliver it to the desired location at predetermined rates and duration to generate an optimal immune response. Another advantage of these synthetic delivery systems is that the combination of slow release and depot effect may reduce the amount of DNA used in the vaccine, and eliminate or reduce the number of booster shots needed to induce proper immunization. Furthermore, a controlled and particulate delivery system can efficiently direct DNA into antigen-presenting cells (APCs) [9] improving antigen DNA expression, subsequent presentation onto MHC class I molecules and induction of cytotoxic T lymphocytes (CTL) [10].

Recent studies indicate that an early CTL response against the HIV-1 Tat protein correlates with non-progression to AIDS in humans [11,12] and in non-human primates [13]. Furthermore, anti-Tat CTL immune response correlates with protection with the HIV-1 Tat protein vaccine [14,15], and...
with the SIV tat-rev vaccine [16]. The immunogenicity and safety of the HIV-1 tat DNA has been previously demonstrated in mice [17–20] and in humans [21] and, more importantly, recent evidence indicate that in monkeys DNA immunization with a HIV-1 tat expressing vector (pCV-tat) elicits a CTL response against Tat, with absent or barely detectable anti-Tat antibodies, that protects against SHIV89.6P primary infection by blocking virus replication at its early stage [22].

Within this frame and to improve the potency of the HIV-1 pCV-tat DNA vaccination approach to induce CTL responses, we designed and prepared a new class of cationic block copolymers [23], able to prevent opsonization of the macromolecular carrier [24]. Previous studies, using five of these copolymers (K1–K5) and the pCV-tat plasmid DNA, showed that the cationic block copolymers self-assemble by electrostatic interactions with plasmid DNA in aqueous solution and condense around DNA forming micellar-type particles of 100–300 nm in diameter. Formation of the DNA/copolymer complex resulted in DNA-nuclease resistance increase, no apparent toxicity and efficient intracellular delivery of DNA both in vitro and in vivo. However, only K1, K2 and K5 copolymers released pCV-tat from the complexes and improved pCV-tat expression as compared to naked DNA [23,25].

The first aim of the present study was to determine whether pCV-tat DNA complexed with the block copolymers K1, K2 or K5 could elicit an immune response to Tat, and to compare the strength of this response versus naked DNA vaccination. The second aim was to evaluate whether doses of tat DNA that are lower (1–30 μg) than those previously used (50–100 μg) by us and others [17–20,26,27] could induce or improve the anti-Tat immune response when associated with the block copolymers. The last aim of the study was to determine the safety of the DNA/copolymer complexes.

The results show that this delivery system is safe and highly efficient in inducing anti-Tat CTL response, which are elicited by very low doses (1 μg) of tat DNA, particularly when associated with the K2 block copolymer, as compared to naked DNA, both inoculated intramuscular (i.m.).

2. Materials and methods

2.1. Cationic block copolymers

Cationic block copolymers, constituted by a positively charged block derived from poly(dimethylamino ethyl methacrylate) (PDMEMA), either partly or fully alkylated, capable of interacting with negatively charged DNA, and by a neutral and highly hydrophilic poly(ethylene glycol) (PEG) block, were synthesized and characterized as described previously [23,25]. Three copolymers (K1, K2 and K5) were examined in this study. K1 (Mn 28.000) and K2 (Mn 91.000) copolymers are fully methylated with methyl iodide and possess 90 and 300 positive charged groups per molecule, respectively. K5 (Mn 48.200) is partly methylated (30%) and possess 70 positive charged groups per molecule. For calculation of the charge ratio an average mass per charge of 330 Da was used for DNA. The mass per charge for all the cationic copolymers was calculated from the degree of polymerization obtained by 1H-NMR spectra [23]. Particle size and ξ-potential measurements were determined as described previously [25]. Block copolymers were resuspended (10 mg/ml) in sterile phosphate-buffered saline (PBS) without calcium and magnesium.

2.2. DNA/copolymer complex assembly

The plasmids pCV-0 and pCV-tat, were described previously [28–30]. Plasmid DNAs were purified onto two CsCl gradients, resuspended in PBS and controlled as previously described [22,23,31,32]. DNA/copolymer complexes were prepared immediately before use as described [23,25]. In this study, the DNA/K1 and DNA/K2 complexes were prepared at defined molar ratio of copolymer to plasmid ammumion positive groups to DNA phosphate negative groups (N-to-P ratio) of 1.0, whereas DNA/K5 complexes of 5.0.

2.3. Mice immunization

Animal use was according to national guidelines and institutional policies. Six–eight-week-old female BALB/c mice (Nossan, Milan, Italy) were immunized with plasmid pCV-tat (1, 10 or 30 μg) complexed with K1, K2 or K5 copolymer, or with pCV-tat alone. Control animals included mice injected with pCV-0 (30 μg) associated with K1, K2 or K5, or with pCV-0 without the copolymers. Each experimental group vaccinated with the DNA/copolymer complexes was composed of seven mice, and each animal was identified with a serial number (mice ID 1–7). Groups vaccinated with naked pCV-tat or pCV-0 DNA consisted of 10 mice (mice ID 1–10). Immunogens were given by intramuscular (i.m.) injections in the quadriceps muscles of the posterior legs. Boosts were given at weeks 2, 5, 9, 13, 17 after the first immunization. The long-term vaccination protocol was specifically chosen to analyze the safety of the immunogens. During the course of immunization, three blood samplings were taken at weeks 7, 11, and 15 after the 3rd, 4th and 5th boost, respectively, for analysis of humoral responses. Blood samples were withdrawn by endocardic puncture. For this procedure mice were anesthetized intraperitoneally with 100 μl of isotonic solution containing 1 mg of Zoletan (Virbac, Milan, Italy), and 200 μg Rompun (Bayer, Milan, Italy); however, the endocardic puncture caused the accidental loss of few animals in the groups. Mice were sacrificed 2 weeks after the last boost (week 19) to collect blood and organs for analysis of humoral and cellular responses, and for histological, histochemical and immunohistochemical studies. Five days before immunizations, 100 μl of 0.3% bupivacaine hydrochloride (Sigma,
tion was determined by [3 H]thymidine incorporation [17]. Cells were cultured in the presence of Tat

2.5. Tat-specific T cell proliferation

Monolayer cultures of murine BALB/c 3T3 fibroblasts (H2d haplotype) were grown in Dulbecco’s minimal essential medium (Sigma) supplemented with 10% FBS and transfected with pRPneo-c/Tat vector or pRPneo-c control plasmid [33]. Stable clones of BALB/c 3T3-Tat expressing cells and BALB/c 3T3-pRPneo-c (referred to as BALB/c-control cells) were selected with G418 (350 μg/ml, Sigma). Tat-expression was monitored following transfection of cells with pU3RCA T where transcription of the reporter chloramphenicol acetyl transferase (CAT) gene is driven by the HIV-1 LTR promoter [32,33]. Mononuclear cells were purified from spleen of mice as described above, and seeded (2 × 10^5 per well) in 96 U-bottomed well plates (Nunc). Tat-specific CTL responses were assayed on fresh unstimulated lymphocytes, and on lymphocytes stimulated for 5 days by co-cultivation at 20:1 ratio with BALB/c 3T3-Tat expressing cells, previously irradiated with 30 Gy (137Cs). Cytotoxic activity of the cultures was tested in standard 5 h 51 Cr-release assays at an E/T ratio of 20:1 [34]. Percent specific lysis was calculated as 100 × (cpm sample − cpm medium)/(cpm Triton-X 100 − cpm medium). Spontaneous release was always below 20%. Lysis of BALB/c-control cells (non-specific lysis) by effector cells ranged between 0 and 2%. Per-centage of Tat-specific lysis were subtracted of values of non-specific lysis, and net values ≥10% were considered positive.

2.7. Histological, histochemical and immunohistochemical procedures

At sacrifice animals were subjected to autopsy. Sample of cuts, subcuts and skeletal muscles at the sites of injection and other organs (lungs, heart, intestine, kidneys, spleen and liver) were taken and processed for histologic examination, after fixation in 10% formalin for 12-24 h and embedding in paraffin. The 3-5 μm paraffin-embedded sections were stained with hematoxylin and eosin, subjected to periodic acid Shiff (PAS) reaction without or with diastase (Sigma) treatment, and to Perl’s reaction for ferric iron. The avidin–biotin–peroxidase complex technique was used for the immunohistochemical studies performed on paraffin sections. The panel of antibodies included S-100 (DAKO, Denmark), BH-F 35 (DAKO) for detection of α-actin, CD68 and Mac387 (DAKO) for detection of macrophages. Briefly, after deparaflinization and rehydration, endogenous peroxidase was blocked with 0.3% H2O2 in methanol; samples were then incubated with primary antibodies for 10-12 h at 4 °C. Biotinated-anti-mouse and anti-rabbit immunoglobulins (Sigma) were utilized as secondary antibodies. Specific reactions were detected following incubation with avidin–biotin-peroxidase-conjugated and development in diaminobenzidine (Sigma).
2.8. Statistical analysis

χ²-test was performed as described [35].

3. Results

3.1. Analysis of the humoral response to Tat

Anti-Tat-specific IgG were never detectable in all mice immunized with pCV-tat/K1, pCV-tat/K2, and pCV-tat/K5, independently of the dose of immunogen that was administered, and of the block copolymer forming the micellar-type particles. Similarly, a lack of detectable anti-Tat IgG was observed in control mice immunized with pCV-tat alone (data not shown). These results are in agreement with the previous pCV-tat DNA monkeys’ study showing that anti-Tat antibodies were absent or barely detectable in animals vaccinated intramuscularly with the pCV-tat plasmid DNA [22].

3.2. Analysis of Tat-specific T cell proliferation

The presence of cell-mediated immune responses to Tat was evaluated on splenocytes of immunized mice. Tat-specific T cell proliferation was detected only in mice immunized with pCV-tat/K1 and pCV-tat alone. Specifically, as shown in Table 1, Tat-specific T cell proliferation was detectable in 4/4 (100%), in 3/5 (60%) and in 2/6 (33 %) mice immunized, respectively, with 1, 10 and 30 μg of pCV-tat/K1, and in 1/5 (20%), in 7/7 (100%) and in 1/5 (20%) mice immunized, respectively, with 1, 10 or 30 μg of naked pCV-tat DNA. From estimates of χ², the percentage of the responders in the group of mice immunized with 1 μg of pCV-tat/K1 was significantly different from that of mice immunized with 1 μg of naked pCV-tat (P < 0.05). In contrast, the difference in the percentage of the responders was not significant between groups immunized with 10 or 30 μg of pCV-tat/K1 or pCV-tat alone. All animals vaccinated with pCV-tat/K2 or with pCV-tat/K5 did not show detectable Tat-specific T cell proliferation, at any dose of the immunogen (data not shown).

3.3. Analysis of anti-Tat CTL responses

Specific anti-Tat CTL activity was detected after immunization with pCV-tat/K2, pCV-tat/K5, or with naked pCV-tat DNA (Table 2), but not with pCV-tat/K1 (data not shown). A substantial CTL response to Tat was induced in 5/5 (100%) mice vaccinated with 1 μg of pCV-tat/K2, both with fresh splenocytes or with splenocytes co-cultivated with BALB/c 3T3-Tat expressing cells. In some animals, the magnitude of response was not significantly increased after re-stimulation. A possible reason may be that anti-Tat CTL responses derive from cells that in vivo are highly activated, as suggested by the results on fresh unstimulated splenocytes. Thus, in vitro re-stimulation may lead to super-activation, and death, of antigen-specific CTLs that, consequently, are not detectable after 5 days of co-cultivation. In contrast, no CTL response to Tat was observed in mice immunized with 10 or 30 μg of pCV-tat/K2, as in control mice injected with pCV-0/K2. Similarly, in the groups of mice immunized with pCV-tat/K5, only vaccination with 1 μg of pCV-tat/K5 generated specific CTL responses in 4/6 (66%) mice, whereas anti-Tat CTLs were not detectable in animals immunized with 10 μg and 30 μg of pCV-tat/K5, as in control mice injected with pCV-0/K5. CTL responses to Tat were also detectable in splenocytes, fresh or co-cultivated, from 2/5 (40%), 4/9 (44%) and 1/5 (20%) mice, respectively, after immunization with 1, 10 or

<table>
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<th>ConA</th>
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<td>17.6</td>
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<td>2/2</td>
<td>0.9</td>
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<td>2/2</td>
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*Values represent the SI of murine splenocytes after Tat (1 μg/ml) or ConA (10 μg/ml) addition. SI higher than cut-off (1.6 and 2.75 for mice immunized with pCV-tat/K1 or with pCV-tat alone, respectively) were considered positive. All mice inoculated with pCV-tat/K2 and pCV-tat/K5 responded to ConA (SI between 10 and 89) but not to Tat.

The difference in T cell proliferation between the groups vaccinated with Tat 1 μg of pCV-tat/K1 and naked pCV-tat was statistically significant (P < 0.05).
As shown in Fig. 1, small foci of necrosis involving muscle fibers with a poor cellular inflammatory reaction were observed at the site of injection in mice injected with the DNA/copolymer complexes, as compared to control mice injected with naked pCV-0 or pCV-tar DNA. In particular, macrophages, showing good reactivity to CDE68 and Mac387 antibodies, were constantly present in the muscular fascia, in the surrounding adipose tissue and, to a lesser extent, in the subcutaneous tissue. The number of macrophages was related to the dose of copolymer injected, since it was higher in the mice inoculated with the highest dose of DNA/copolymer complexes and inconspicuous in control animals injected with naked pCV-0 or pCV-tar DNA. The intensity of the macrophages reaction was, instead, not related to the dose of pCV-0 or pCV-tar DNA in the complexes. The cytoplasm of the macrophages contained PAS-positive microspherules, corresponding to the DNA/copolymer complexes. T and B lymphocytes were not found in the inflammatory reaction. Macrophages containing PAS-reactive microspherules were observed in dilated sinususes of some regional lymph nodes of mice immunized with the DNA/copolymer complexes, but not in control mice immunized with naked DNA. Liver presented normal lobular architecture with increased number of Kupffer cells. In the portal tract, some Kupffer cells and macrophages contained PAS-reactive microspherules in the cytoplasm, whereas hepatocytes appeared normal. These cells were not observed either in the sinusoid or in the portal tracts of control mice immunized with naked DNA. No specific alterations that may be related to injection of DNA/copolymer complexes were reported in the other organs examined, as compared to mice injected with naked DNA.

3.4. Histological, histochemical and immunohistochemical analyses of mice tissues

As shown in Table 2, CTL response to Tat was significant (P < 0.05). 

3.5. Evaluation of the immunogenicity of cationic block copolymers

Since one of the most important characteristics required for a DNA delivery system is the lack of immunogenicity, we determined whether K1, K2 and K5 were capable of eliciting antibody responses. The presence of antibody to K1, K2 and K5 was determined in mice sera after the 3rd, 4th and 5th immunization and at sacrifice (bleeding IV). The results are reported in Table 3. Data indicated that the cationic block copolymers were scarcely immunogenic. In fact, an antibody response to K1, K2 and K5 was found only in a few mice, and was proportional to the dose and the number of boosts of block copolymers. Indeed, specific antibodies anti-cation block copolymers were generally absent or at very low titres in mice injected with the highest doses of K1, K2 and K5 developed antibodies. Moreover, after the injection of 10 or 30 μg of K1 or K2, and of 200 or 600 μg of K5, antibodies were detectable earlier, after the 4th immunization (bleeding II), as compared to mice receiving 1 μg of K1 or K2, or 20 μg of K5 in which antibodies were barely detectable even after six immunizations (bleeding IV).
Fig. 1. Histologic examination of mice tissues after injection of DNA/copolymer complexes by the i.m. route. One representative mouse is shown. Inflammatory reaction consisting exclusively of macrophages infiltrates in the muscular fibers at the site of inoculation (a), and the contiguous interstitial tissue (b); macrophages around to the muscle fibers show large cytoplasm containing PAS-reactive granules corresponding to the DNA/copolymer complexes (c) and strong reactivity to anti-CD68 antibody (d). Kupffer cells and macrophages containing granules are found in the liver (e) and in the marginal sinus of regional lymph nodes (f). No other differences were reported between mice injected with the DNA/copolymer complexes and naked DNA. PAS reaction: (a) 160×, (c) 400×. Hematoxylin–eosin staining: (b) 63×, (e) 250×. Anti-CD68 staining: (d) 160×. Perl’s reaction: (f) 63×.

4. Discussion

A DNA vaccine is easy and cheap to produce in large amount, stable and safe, as suggested by studies in mice and monkeys [17–20,22,27] and by many independent DNA vaccination studies in small and large animal models [26,36], in non-human primates [37], and in human volunteers [21,38,39]. In vivo synthesized antigens elicit broad immune responses, including CTLs [27,40]. Furthermore, due to the presence of unmethylated CpG sequences, a DNA vaccine has the advantage to boost the innate immunity that potentiate the adaptive immunity [22,41]. However, DNA vaccination generally requires high dose of DNA (100 μg in mice up to 2–4 mg in non-human primates and in humans) and many boosters [17,19,22,27,37,39] which raise the costs of production and, most importantly, may reduce the success of vaccination especially in developing countries where infrastructures and logistic may represent a major obstacle. The high dose and multiple boosts requirements are likely due to massive enzymatic degradation, both extra- and intracellularly, of the majority of the DNA molecules injected, and to the low efficiency of transfection of naked DNA, so that only few intact DNA molecules may reach the nucleus, be expressed and available for antigen presentation. To improve the DNA vaccination approach, we have developed a novel class of cationic block copolymers capable of protecting DNA from DNase I degradation and of enhancing delivery of DNA to APCs after i.m. injection. To use a vaccine relevant antigen, which is a relatively poor immunogen and for which CTL responses are required for protection [11–16,22], we chose the HIV-1 tat gene. As concerned tat DNA vaccination, studies in which high doses of different tat expressing plasmids and multiple boosts were used have been already published by us (in
mice and monkeys) and by others (in mice and humans) [17–22]. Anti-Tat-specific CTL responses were detected in two of these studies. One is our monkeys study, where 0.5–1 mg of pCV-tat DNA was given intramuscularly for seven immunizations (22); the other is a mice study (20) in which animals were immunized by gene gun with 6 μg of a different tat expressing plasmid for six times. Of note, in the same study anti-Tat CTL responses were not detected in mice immunized six times i.m. with 150 μg of tat DNA.

This study demonstrates an enhancement of the antigen-specific CTL response with 1 μg of tat DNA associated with K2 and with K5, as compared to 1 μg of naked pCV-tat, even though the degree of CTL induction was higher and significantly different only with the tat/K2 complex. In contrast, vaccination with 10 and 30 μg of tat/K2 or tat/K5 was totally ineffective at inducing an immune response to Tat. In addition, the results show a significant enhancement of antigen-specific T cell proliferation again with the lowest dose of tat DNA complexed with K1, as compared to 1 μg of naked tat DNA, whereas at the higher doses differences were not statistically significant.

The enhancing effect of the lowest dose of DNA/copolymer complexes on anti-Tat immune response, as compared to immunization with naked tat, may depend on several reasons. First, the block copolymers protect DNA from enzymatic degradation [23]. In this respect, we have observed in previous studies that the expression of 1 μg of pCV-tat DNA in vivo is detectable by RT-PCR only when the DNA is complexed with the copolymer, and even when mice are injected with 10 μg of naked pCV-tat DNA the expression of tat remains undetectable or is barely detectable [25]. Second, at the higher doses the dissociation of the DNA/copolymer complexes, and/or the DNA topology, may be inadequate in vivo, whereas the dose of 1 μg may be optimal for slow and continuous release and expression of DNA from the polycationic DNA complex, determining a prolonged antigen expression and presentation [42]. Third, the micellar-type particles induce a more intense macrophage recruitment at the site of inoculation, as compared to naked DNA. This situation may favor antigen capture and processing by macrophages and by immature circulating dendritic cells (DCs), which are the most effective antigen-capturing cells and key for initiation and regulation (Th1 or Th2) of the immune response, as shown by others [24,42–48], leading to a pure Th1 response, with enhancement of antigen-specific proliferation and CTL responses. In this respect, although the reason for the differential induction of antigen-specific proliferation and CTL responses by K1, and K2–K5, respectively, is unclear, it is possible that upon APC uptake the K1 and the K2–K5/DNA complexes are differently processed and the antigen-derived epitopes are presented to different subsets of antigen-presenting cells [42].

As concerns naked DNA vaccination, the results show that the strongest immune response against Tat was elicited by the dose of 10 μg. The reason why immunization with 30 μg of naked tat was less effective is unclear. However, since the antigen concentration is critical for induction of immune responses and each antigen has its optimal immunogenic dose, it is conceivable that co-immunization with both tat/copolymers complexes may broaden the immune response to Tat.

As concerned naked DNA vaccination, the results show that the strongest immune response against Tat was elicited by the dose of 10 μg. The reason why immunization with 30 μg of naked tat was less effective is unclear. However, since the antigen concentration is critical for induction of immune responses and each antigen has its optimal immunogenic dose, it is conceivable that 10 μg is the optimal dose for pCV-tat DNA vaccination in this system.

An important concern of a delivery system for use in humans is toxicity and immune reactivity [40,49]. To this goal, a long-term vaccination protocol, with repeated boosts, was used and serological responses to the copolymers were analyzed. With regard to the safety issue, the results indicate that this novel class of cationic block copolymers associated with DNA is safe, even after six administrations with high doses. No local adverse reactions nor difference in health
conditions and behavior, such as liveliness, vitality, weight, motility, sheen of hair, were observed during the 5 months of observation in mice vaccinated with all the DNA/copolymer complexes, as compared to mice treated with naked pCV-tat or pCV-0 DNA, or to untreated animals. Moreover, no specific histological alterations that may be related to injection of the DNA/copolymer complexes were observed, as indicated by similar histological pictures reported for all mice, vaccinated both with naked and copolymer-condensed DNA. As regards to immune reactivity, the results suggest that the copolymers are scarcely immunogenic. An antibody response was detected only after four or five injections of the highest doses of the DNA/copolymer complexes, whereas it was absent or barely detectable after six administrations of the lowest dose. Thus, it is conceivable that if fewer immunizations with the lower dose of the DNA/copolymer complexes can elicit an effective immune response to Tat, the immune response to the delivery system is likely to be insignificant.

In conclusion, the results presented here indicate that these new synthetic cationic block copolymers (in particular K2) represent a novel approach for gene delivery for vaccination purpose. They are safe, cheap and easy to produce, to store and to transport. In addition, the tat DNA/copolymer complexes are easy and fast to prepare, and no purification steps are required. They protect DNA from DNase I degradation, and enhance the anti-Tat proliferation and, importantly, the antigen-specific CTL response with a very low dose of DNA. Studies are ongoing to determine the effectiveness of these polymers after fewer immunizations. Of note, our new synthetic strategy allows a fine control of the copolymer structure and, consequently, a fine modulation of the inherent complexing propensity of the polycation block towards DNA [23]. Finally, even though the concept of cationic block copolymers that assemble and condense of cationic block copolymers that assemble and condense DNA has also been described by other groups [46], this is the first report, to our knowledge, of their application to DNA vaccination purpose.

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