Induction of gp120-specific protective immune responses by genetic vaccination with linear polyethylenimine–plasmid complex

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Abstract

The induction of IFN-γ-secreting CD8+ T cells and neutralizing antibodies to HIV-1 are both key requirements for prevention of viral transmission and clearance of pathogenic HIV. Although DNA vaccination has been shown to induce both humoral and cellular immune responses against HIV antigens, the magnitude of the immune responses has always been disappointing. In this report, we analyze the ability of polyethylenimine (PEI)–DNA complex expressing an HIV–glycoprotein 120 (gp120) antigen (PEI–pgp120) to induce systemic CD8+ T cell and humoral responses to the gp120 antigen. The administration of PEI–plasmid complex resulted in rapid elevation of serum levels of IL-12 and IFN-γ. Furthermore, a single administration of PEI–pgp120 complex elicits a number of gp120-specific CD8+ T cells 20 times higher than that elicited by three intramuscular injections of naked DNA. Interestingly, we found that systemic vaccination with PEI–pgp120 induced protective immune responses against both systemic and mucosal challenges with a recombinant vaccinia virus expressing a gp120 antigen. The data also demonstrated that the depletion of macrophages with liposome-encapsulated clodronate completely abolished gp120-specific cellular response. Overall, our results showed that a single administration of PEI–pgp120 complexes, eliciting strong immune responses, is an effective vaccination approach to generate protection against systemic and mucosal viral infections.

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1. Introduction

An optimal acquired immunodeficiency syndrome (AIDS) vaccine should be able to induce both envelope glycoprotein 120 (gp120)-specific neutralizing antibodies, as well as systemic and mucosal cellular immune responses to human immunodeficiency virus (HIV)-infected cells [1–7]. Vaccine strategies which elicit potent cytotoxic T cell (CTL) responses lead to reduced virus loads and long-term protection against immunodeficiency disease in macaque challenge studies using simian immunodeficiency virus (SIV) or pathogenic simian-human immunodeficiency virus chimeras (SHIV) [8,9]. It has been recently reported that viral escape from CTL recognition can result in the eventual failure of the immune protection induced by candidate AIDS vaccines [10] and rhesus macaques infected with SIV/SHIV show significantly higher viral loads if CD8+ T cells are eliminated [11,12]. Glycoprotein 120 by itself is weakly immunogenic and immunizations with plasmid DNA encoding gp120 alone

Abbreviations: PEI, polyethylenimine; gp120, glycoprotein 120; HGH, human growth hormone
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are not efficient. DNA immunizations with gp120 of HIV usually require boosting with viral vaccines, co-administration of various cytokines or expression as a fusion protein with proinflammatory chemotaxant to achieve optimal efficacy [13–17].

The ability of plasmid DNA to induce both humoral and cellular immune responses against a variety of infectious agents has been well documented [18]. Numerous scientific publications have reported the effectiveness of DNA vaccines in providing potent immune responses or protective immunity against viruses, bacteria and parasites in several species including human volunteers [19–21]. DNA vaccination has become an accepted method in the research community, and is now being tested on humans. Although safety and efficacy have been demonstrated, the potency of the immune response against the antigens induced by plasmid immunization compared with the use of viral vectors has been disappointing [22]. The main problem associated with DNA administration is the low efficiency of cellular uptake and expression in vivo. A number of different strategies are being developed to increase the efficacy of DNA vaccines. The development of appropriate vehicles that increase plasmid transfection and protect from degradation in the biological environment is one of the strategies being examined. Production of DNA complexes with polycations is being widely studied as a strategy to improve DNA immunization protocols [23].

The cationic polymer, polyethylenimine (PEI), is known to be an efficient carrier of DNA that promotes uptake by and transfection of cells in vitro [24]. It appears to work by compacting DNA into particles by way of electrostatic interactions, thereby protecting the DNA from enzymatic degradation and providing a global positive charge, which facilitates uptake by cells [25]. The ability of PEI to act as an efficient vector for DNA transfer resides also in its capacity to permeabilize the endosomal membranes, thus providing the DNA with access to the cytoplasm. Although the mechanism for PEI-mediated transfection is not fully understood, there is evidence that PEI and PEI–DNA complexes undergo nuclear localization more effective than cationic lipids [25]. A number of PEI molecules have been described with varying molecular sizes and structures—among them, branched PEI with an average molecular weight of 800 kDa (PEI800) or 25 kDa (PEI25), and linear forms with an average molecular weight of 22 kDa (PEI22)—have been widely used and characterized both in vivo and in vitro [26]. In vivo, systemically delivered PEI22–DNA complexes resulted in the transduction of the lung, spleen, liver, heart and kidney, with no histological change observed in these organs [27]. PEI22–DNA complexes can repeatedly be administered to animals without eliciting an immune response against the polymer, although the development of an immune response against the exogenously expressed protein has been reported to reduce the level of protein expression [28]. In fact, administration of lung-targeted macroaggregated polyethylenimine–albumin conjugates expressing human growth hormone (HGH) has been shown to elicit a humoral immune response against the HGH [29].

In this report, we seek to develop a simple AIDS vaccine strategy using a particulate gene delivery system formed through non-covalent complexation of PEI and a DNA plasmid expressing HIV–gp120 antigen. We demonstrate that administration of these complexes generates significantly more IFN-γ-secreting gp120-specific CD8+ T cells than naked DNA vaccines expressing gp120. Although the PEI–DNA vaccine was systemically delivered, protection was observed against both systemic and mucosal challenges with a recombinant vaccinia virus. We also show that PEI–DNA-induced immune response depends on the activation of macrophages.

2. Materials and methods

2.1. Experimental animals

Female, 5–6-week-old, BALB/c mice were obtained from Taconic Laboratories and maintained according to the regulations of our institution. All mice were acclimatized for 1 week before immunization. Prior to immunization, mice were bled orbitally and sera were collected for subsequent enzyme-linked immunosorbent assay (ELISA).

2.2. Plasmid DNA

The gp120 gene under the control of the human cytomegalovirus (CMV) promoter/enhancer was a gift from Dr. M. Esteban and Dr. D. Rodríguez. The plasmids were produced and purified using reagents and columns for endotoxin-free DNA (Quiagen, Valencia, CA) and then dissolved in an endotoxin-free 5% glucose solution to the desired concentration. The plasmids were predominantly supercoiled and were quantitated using UV absorbance. The recombinant VVgp120 employed in this study was provided by Dr. M. Esteban and Dr. D. Rodríguez. The virus was grown in human HeLa cells, and sucrose cushion purified virus was titrated in African green monkey kidney BSC–40 cell monolayers by plaque assays.

2.3. Preparation of PEI/DNA complexes

The desired amount of DNA in a solution of 5% glucose (water containing 5% glucose) was complexed with PEI 22 kDa by adding the DNA to the PEI while vigorously vortexing the solution. The solution was then allowed to incubate at room temperature for 15 min prior to animal administration. PEI/DNA complexes were formed at a molar ratio of PEI nitrogen to DNA phosphate (N/P) = 4.

2.4. Immunization protocol

PEI–DNA complexes were injected intravenously as a single 400 µl volume via the tail vein. Animals were kept under...
Fig. 1. Effect of i.v. administration of PEI–plasmid complexes on the IL-12 and IFN-γ production in serum and in different organs. Groups of BALB/c mice (n = 6) were injected via tail vein with 100 μg of PEI–luciferase complexes at an amine over phosphate ratio of 4 in 400 μl 5% glucose. (A) From a group of five mice, serial blood samples were obtained and the total amounts of IL-12 and IFN-γ in the serum were assayed by ELISA. Results displayed are means of total cytokine quantity. (B) A second group of five mice were sacrificed 6 h after injection and the lungs, liver, kidney, heart, spleen from each mouse were excised, the organs were homogenized in RIPA buffer and assessed individually for the expression of IL-12 and IFN-γ. Results displayed are means of cytokine quantity per mg of organ or ml of serum.

Fig. 2. gp120-specific cellular and humoral immune response in BALB/c mice using different immunization protocols. (A) Groups of three BALB/c mice were immunized i.v. with different doses of PEI–pgp120 complexes (N/P = 4) from 5 to 150 μg in a volume of 400 μl, and 2 weeks later, splenic lymphocytes were isolated and the number of IFN-γ-secreting p18-specific CD8+ T cells was determined by an ELISPOT assay. (B) Groups of three BALB/c mice were immunized with a single dose of 100 μg of PEI–pgp120 complexes in a volume of 400 μl, 100 μg of pgp120 administered i.v. in a volume of 400 μl of 5% glucose, i.p. with 5 × 10⁸ PFU of V acc-gp120, or three doses of 100 μg of pGp120 i.m. administered. Two weeks later, splenic lymphocytes were isolated and the number of IFN-γ-secreting p18-specific CD8+ T cells was determined by an ELISPOT assay. (C) Serum samples obtained from the groups of mice in (A) 2 weeks after last immunization and the presence of anti-gp120 antibodies was measured by antigen-specific ELISA. Mean optical densities (±S.D.) for each group are shown for a serum dilution of 1:100, with the background OD of wells with serum from non-immunized mice subtracted.

2.5. Quantification of epitope specific CD8+ T cells by ELISPOT assay

We determined the number of CD8+ T cells that recognized HIV antigen in immunized mice by obtaining splenocytes from these mice, and stimulating them with the MHC-compatible target cells P815 incubated with 1 μM of p18 peptide (RQGSPGPRAPVTGK) for 1 h at 37 °C. This peptide contains the H-2 d restricted CD8+ T cell epitope corresponding to the V3 loop of HIV strain IIIB. Two weeks after immunization, mice were sacrificed and single-cell suspensions were prepared from the spleen. Ninety-six-well nitrocellulose plates (Multiscreen HA, Millipore Corp., Bedford, MA) were coated with anti-mouse IFN-γ mAb, R4. After overnight incubation at room temperature, the wells were washed and incubated with the medium for 3 h at 37 °C. Targets cells not pulsed with the peptide or pulsed with an irrelevant CD8+ epitope from hepatitis B core antigen were used as negative controls. Serially diluted lymphocytes, starting at 1 × 10⁶ cells per well, were co-cultured with 1 × 10⁵ target cells in the ELISPOT wells. After incubating the plates for 24 h at 37 °C and 5% CO₂, the plates were treated as previously described [30] and the number of spots corresponding to IFN-γ secreting cells determined. The background level, namely the number of cells which secrete IFN-γ in the absence of antigen, was subtracted in every experimental group.
High-binding polystyrene microtiter plates (MaxisorpTM; Nunc A/S, Roskilde, Denmark) were coated with 100 μl of the peptide 2 x RIQRGPRGAVFTGK at 20 ng/ml in 0.05 M carbonate-bicarbonate buffer pH 9.6 overnight at 4°C. The wells were washed twice with 0.05% Tween-20 in PBS (PBS-T) and blocked with PBST containing 1% no-fat dry milk for 1 h at room temperature. Serum samples diluted in blocking solution were added in a volume of 100 μl/well and incubated for 1 h at RT. Plates were washed six times before the detection antibody was added: peroxidase-conjugated rabbit antimouse immunoglobulin G (IgG) antibody (Sigma, St. Louis, MO) diluted 1:5000 in blocking solution and incubated for 1 h at RT. After being washed six times with PBS-T and three times with PBS, the plates were developed with 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) peroxidase substrate. After 10 min incubation, plates were read at 405 nm on a plate reader. Mean optical densities (±S.D.) for each group were obtained at a serum dilution of 1:100, with the background OD of wells with serum from non-immunized mice subtracted.

2.7. IL-12 and IFN-γ ELISA

Levels of IL-12 and IFN-γ were determined in serum and in the different organ samples of mice by using a cytokine enzyme-linked immunosorbent assay kit (PharMingen, San Diego, California) according to the manufacturer’s instructions. Organ samples for IL-12 and IFN-γ assay were disintegrated in 500 μl of ice-cold Ripa buffer. After incubation on ice for 30 min, samples were centrifuged twice at 20,000 × g for 15 min at 4°C and the resulting supernatants were used for assay. The threshold of detection of the IFN-γ and the IL-12 assay is 14 and 4 pg/ml, respectively.

2.8. In vivo protection against infection with a recombinant vaccinia virus expressing gp120

Two weeks after immunization with PEI-ppg120 complexes, mice were challenged intraperitoneally with 5 × 10^7 PFU of the recombinant vaccinia virus expressing gp120. Five days after vaccinia challenge, mice were sacrificed and ovaries and lungs removed, homogenized, sonicated and assayed for virus titer by plating serial 10-fold dilutions of samples on a plate of BSC-1 indicator cells. After 2 days of culture, cells were stained with crystal violet to detect PFU at each serial dilution. For quantitative real time PCR titering, viral DNA from the homogenized ovaries and lungs were extracted using the High Pure Viral Nucleic Acid Kit (Boehringer Mannheim), according to manufacturer’s instructions and then subjected to real time PCR. Primers and TaqMan probes (Applied Biosystems, Foster City, CA) for vaccinia virus hemaglutinin gene (GenBank: AF375124) (sense: 5′-TGACTACGTGGTTATGAGTGGCTGG-3′, anti-sense: 5′-TTATCAAAAATAAAGACGTGCATT-3′, probes: 5′-AGGGAGCACAATCCCAATATCTTCTTTAG-3′) designed using the Primer-Express software were used for the PCR. The reaction was performed in 20 μl with 2 μl of sample, MgCl₂ 5 mM, 0.3 μM of each primer, 0.1 μM of probe and 2 μl of LightCycler-FastStart DNA Master Hybridization Probes (Roche Diagnostic GmbH, Mannheim, Germany). PCR was performed according to the following parameters: initial denaturation for 10 min at 95°C, and 40 cycles of 10 s at 95°C and hybridization/elongation of 30 s at 60°C with a temperature transition rate of 20°C/s. The fluorescence signal delivered during PCR amplification was monitored using the LightCycler System (Roche Diagnostics, Basel, Switzerland). The copy number of vaccinia DNA in each sample was determined by interpolation, using an external standard consisting of serial dilutions (10⁶–10⁻²) of a plasmid containing the amplified fragment.

2.9. Plasma AST and ALT

The concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured in plasma samples with kits obtained from Sigma (St. Louis, MO) according to the manufacturer’s protocol.

2.10. Macrophage depletion

Clodronate was a gift from Roche Diagnostics GmbH (Mannheim, Germany). Phosphatidylcholine (Lipoid E PC) (Lipoid GmbH, Ludwigshafen, Germany) and cholesterol (Sigma, St. Louis, MO) were used to prepare the liposomes as described earlier [31,32]. Mice were intravenously injected with 20 μl per 10 g body weight of the standard suspension of clodronate liposomes diluted in PBS in a final volume of 50 μl.

3. Results

3.1. Induction of immunostimulatory cytokine expression after intravenous administration of PEI-DNA complexes

We first asked whether the administration of PEI-DNA complexes is able to induce an innate immune response as has been described for naked DNA [33]. For this purpose, two groups of five mice were injected intravenously with PEI-DNA complexes generated with 100 μg of luciferase- or GFP-expressing plasmids together with PEI-22 kDa at NP ratio of 4 in 5% glucose solution. The same amount of PEI or DNA dissolved in 5% glucose solution was injected into two groups of five mice as controls. Serum IL-12 levels were measured 6, 12, 24 and 48 h after the injection by ELISA. High levels of IL-12 (180–200 pg/ml) were detected 6 h after i.v. injection of PEI–luciferase, decreasing to 2–3 ng/ml 24 h post injection, and finally disappearing at 48 h (Fig. 1A, data...
not shown). The analysis of IFN-γ expression in the serum showed that in the case of IL-12, the maximum level was obtained during the first 12 h after injection, with the level decreasing 100 times after 24 h, although low levels of IFN-γ were maintained in the serum for a longer period of time (215 pg/ml, 72 h) (Fig. 1A). Similar results were obtained in the group of mice that received PEI–pGFP complex, and no IL-12 or IFN-γ was detected in the serum of mice that received PEI or DNA alone (data not shown).

We next analyzed IL-12 and IFN-γ expression levels in different organs 6 h after i.v. injection of 100 μg of PEI–luciferase complex. As shown in Fig. 1B, the expression of both cytokines was detected in all the organs tested, including lung, kidney, liver, spleen and heart. We found high levels of IL-12 in the liver and kidney, whereas IFN-γ has a different organ distribution with high levels of IFN-γ found in the spleen (Fig. 1B). Surprisingly, low levels of IL-12 and IFN-γ were detected in the organ that showed the highest transgene expression 6 h after PEI–luciferase administration, the lung [34].

3.2 gp120-specific CD8+ T cell response elicited by i.v. administration of PEI–DNA complexes expressing HIV–gp120 antigen

To test whether PEI–DNA complexes could induce specific immune responses against a given antigen, increasing amounts (from 5 to 150 μg of DNA) of PEI–pgp120 complexes at a N/P ratio of 4 in 400 μl 5% glucose were injected i.v. into five BALB/c mice per group (Fig. 2A). For comparative purposes, mice were also immunized i.m. with three doses of 100 μg of pgp120 naked DNA, a single dose of 100 μg of pgp120 naked DNA injected i.v. and a single i.p. dose of 5 × 107 of a recombinant vaccinia virus expressing gp120 (Fig. 2B). Spleen cells from the mice were obtained 2 weeks after immunization and the numbers of gp120-specific IFN-γ-secreting CD8+ T cells were determined by an ELISPOT assay. Mice carrying H-2b haplotype, such as BALB/c mice, generate CD8 T cell responses specific for an epitope (p18 epitope) within V3 loop of gp120, and the amino acid sequence is RIQRGPGRFVITQK. Therefore, the p18 peptide was incubated with P815 plasmacytoma cells, which were used for in vitro stimulation of splenocytes obtained from gp120-immunized mice. As shown in Fig. 2A, there was a dose-dependent, gp120-specific CD8+ T cell response, when the dose of the plasmid used was below 100 μg. However, when higher amounts of DNA were administered to the mice, the CD8+ T cell response was diminished. No gp120-specific response was observed in the spleen of mice immunized with PEI–luciferase, a control immunogen (data not shown).

As shown in Fig. 2B, the relative number of gp120-specific, IFN-γ-secreting CD8+ T cells obtained after a single i.p. immunization with a recombinant vaccinia virus expressing gp120 was 20 times higher than that elicited after three i.m. immunizations with 100 μg of pgp120 and similar to that elicited after a single i.p. immunization with a recombinant vaccinia virus expressing gp120. The differences found in the number of gp120-specific, IFN-γ-secreting cells among the different vaccination strategies varied from one experiment to the other. However, the immunization with PEI–pgp120 complexes consistently resulted in a 20-fold increase in gp120-specific IFN-γ-secreting CD8+ T cells compared to the number of the T cells elicited after three i.m. immunizations with a naked DNA. The analysis of IL-12 and IFN-γ in the serum of mice, immunized from Fig. 2A, 6, 12, 24 and 48 h after the injection showed a dose-dependent expression of both cytokines (data not shown), with the same pattern of expression as described for PEI–luc (Fig. 1A).

3.3 Anti-gp120 antibody response elicited by i.v. administration of PEI–DNA complexes expressing HIV–gp120 antigen

Sera from mice employed in the experiment described above were used to measure the titers of IgG antibodies specific for the V3 region of gp120 antigen. As shown in Fig. 2C, the antibody response was dose-dependent, with no antibody response being detected in the group of mice that received the lower doses (5–25 μg), whereas the optimal antibody response was obtained in the group of mice that received 100 μg of PEI–pgp120. Similarly to cellular response, the administration of the higher doses resulted in a lower antibody response.

The level of antibody response after a single i.v. administration of 100 μg PEI–pgp120 was similar to that observed in the group of mice immunized i.m. with three doses of 100 μg of naked DNA.

3.4 Administration of PEI–gp120 complex protects animals against a systemic and mucosal challenge with recombinant vaccinia expressing gp120

We have shown that PEI–DNA immunization induces both cellular and humoral immune responses against the antigen of interest. However, our ultimate goal is to induce a protective response against a viral challenge. Therefore, to test whether the PEI–DNA vaccination could induce a protective immune response against a viral infection, mice were immunized with 100 μg of PEI–pgp120, followed by an intraperitoneal or intranasal challenge with 5 × 107 PFU of a recombinant vaccinia virus expressing gp120 antigen (Vac-gp120). Ten control mice received 100 μg of PEI–luc. Five days after challenge, ovaries and lungs were removed from the mice, and the virus titers were measured by a sensitive quantitative PCR assay, as well as by conventional plaque assay method. Challenge experiments were replicated three times; Fig. 3 shows the results obtained in one representative experiment. In the ovaries of mice immunized with PEI–luc, i.p. challenge showed viral titers higher than 106 PFU. In contrast, no viral replication in the ovaries was observed in 8 out of 10 PEI–pgp120 immunized mice. Thus, 80% of immu-
Fig. 3. Vaccinia virus titers in ovaries and lungs after intraperitoneal and intranasal challenge with vaccinia virus encoding gp120. Ten BALB/c mice per group were immunized with 100 μg of PEI–pgp120 i.v.; 2 weeks later, immunized mice were challenged with 5 × 10^7 PFU each of vaccinia virus encoding gp120 i.p. or i.n. As controls, mock-immunized mice were challenged with the same virus. The ovaries and lungs were harvested 5 days after challenge, and vaccinia virus titers were determined by quantitative PCR. Data are shown for each mouse, and the mean vaccinia titer is indicated.

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3.5. Toxicity

The toxicity associated with i.v. administration of PEI–DNA complexes has been described as lung embolism and liver injury [34]. In order to assess the toxicity of our PEI–pgp120 complexes, we looked at the morbidity and mortality of injected mice, and measured biochemical parameters that indicate liver injury. More specifically, groups of five BALB/c mice received different doses of PEI–pgp120 complexes intravenously, with control mice receiving the highest dose of PEI–DNA (150 μg) dissolved in 5% glucose solution. As a positive control, a group of mice received a single injection of Concanavalin A (ConA), a compound that, in mice, rapidly leads to massive hepatocyte apoptosis [35].

Overall, we did not observe a lethal effect with our PEI–pgp120 complexes even at the highest dosage used in our experiments. The results of the serum levels of ALT and AST determined 6 h after vaccine administration are shown in Fig. 4. Intravenous administration of 125 and 150 μg of PEI–pgp120, as well as ConA, resulted in significantly increased plasma levels of the transaminases, indicating a liver injury. No significant increases in transaminase levels were observed in the rest of the groups, suggesting that lower doses of the PEI–pgp120 complexes are non-toxic. Interestingly, the toxicity associated with the highest doses of PEI–pgp120 complexes correlates with the lower immune responses induced by these doses (Fig. 2A).

3.6. Role of macrophages in the immune response induced by PEI–DNA complexes

In mice, a single i.v. injection of clodronate liposomes results in the depletion of splenic and bone marrow macrophages and liver Kupffer cells 24 h after injection [31,32]. To test the influence of macrophages on the immune response induced by PEI–DNA complexes, clodronate liposomes were i.v. injected into BALB/c mice, and 24 h later, these as well as non-treated control mice received a single i.v. dose of 100 μg PEI–pGp120 complexes. Six hours after DNA immunization, sera were collected from the mice, and the level of IL-12 measured. As shown in Fig. 5A, the depletion of macrophages prior to PEI–DNA immunization resulted in decreased serum levels of IL-12. We then measured the levels of gp120-specific T cell response by an ELISPOT assay in macrophage-depleted mice and non-depleted mice 2 weeks after DNA immunization. As shown in Fig. 5B, macrophage-depleted mice failed to mount a gp120-specific CD8+ T cell response. These results indicate that macrophages mediate the production of IL-12 in the sera and also the generation of an optimal antigen-specific CD8+ T cell response following PEI–DNA vaccination.

Furthermore, the analysis of transaminase levels (ALT, AST) 6 h after the PEI–DNA complex immunization revealed that non-depleted mice showed normal transaminase levels, whereas macrophage-depleted mice showed an increase in transaminase levels (Fig. 5C). Thus, macrophages may act as a first-line barrier in the liver, by capturing PEI–DNA complexes and preventing PEI–DNA-induced death of hepatocytes.
IFN-γ have received a single dose of PEI–DNA complexes showed expression of both cytokines in different organs of the mice that administered the dose that could induce an optimal level of both CD8+ T cell response and protection against viral challenge. Only when the amounts of the administered plasmid were very high, i.e. 125–150 µg, did we detect a toxic effect on the

4. Discussion

The primary observation from this study is that intravenous administration of PEI-DNA complexes induces a strong innate immune response, with high levels of IL-12 and IFN-γ expression within 6 h of administration. Furthermore, an administration of PEI-DNA complex expressing a viral antigen induces a generation of a strong antigen-specific cellular immune response and systemic and mucosal protection against a viral challenge.

DNA vaccination that can induce both cellular and humoral immune responses has become an attractive strategy for combating infectious diseases that require such responses for clearance, such as HIV, malaria, viral hepatitis or tuberculosis. Unfortunately, clinical trials have shown the limited efficacy of DNA vaccination in human volunteers, which necessitates the identification of technologies that can enhance DNA vaccines [20,21]. Among the strategies being evaluated to increase the efficiency of DNA vaccination is the use of non-viral vectors to deliver the DNA. One such vector, PEI 22, has been previously described to efficiently deliver reporter genes in vivo, with particularly high expression in the lungs and lower expression in other organs like the spleen, kidney and liver [34]. Here, we show that PEI22-DNA complex shortly—6 h—after intravenous injection, induces high levels of IL-12 and IFN-γ in the serum. Analysis of the expression of both cytokines in different organs of the mice that received a single dose of PEI-DNA complexes showed high levels of IL-12 in the liver and kidney and high levels of IFN-γ in the spleen, but low levels of these cytokines in the lungs. These cytokine expression patterns do not correlate with the pattern of in vivo transgene expression.

Intravenous administration of PEI-ggp120 induced dose dependently a gp120-specific CD8+ T cell response, and the optimal response was obtained after immunization with 100 µg of the plasmid. When we determined the relative number of gp120-specific CD8+ T cells that secrete IFN-γ by an ELISPOT assay, we found that a single dose of intravenous immunization with 100 µg of PEI-gp120 could elicit a 20-times higher number of gp120-specific CD8+ T cells than that elicited by three doses of intramuscular immunization with 100 µg of pgp120 DNA vaccine. The number of gp120-specific CD8+ T cells elicited by a single immunizing dose of PEI-ggp120 was comparable to that induced in mice vaccinated intraperitoneally with the vaccinia-gp120 vector, a well known immunizing agent [36].

Vaccines capable of protecting against sexually transmitted infections, such as human immunodeficiency virus, will depend on the induction of potent systemic and mucosal responses [13]. Therefore, we evaluated the level of protective mucosal and systemic response elicited in PEI-DNA immunized mice by challenging the mice intranasally and intraperitoneally with a sublethal dose of a recombinant vaccinia virus expressing gp120 antigen and monitoring viral loads. A single dose of PEI-ggp120 vaccine vector afforded a significant degree of protection against a vaccinia-gp120 challenge regardless of the route the virus was administered. These results indicate that a systemic administration of PEI-DNA complex can elicit strong mucosal and systemic protective responses.

Although side-effects associated with PEI-DNA complex administration have been reported, the evidence is still contradictory [34]. This has been mainly due to the use of PEIs with different molecular weights, structures and chemical modifications, and to the use of different nitrogen/phosphate ratios. In our study, the PEI used has a molecular weight of 22 kDa, is linear with no modifications and has a N/P ratio of 4. We did not observe any morbidity or mortality when we administered the dose that could induce an optimal level of both CD8+ T cell response and protection against viral challenge. Only when the amounts of the administered plasmid were very high, i.e. 125–150 µg, did we detect a toxic effect on the liver, as indicated by the increase in the serum levels of ALT and AST, and in fact when PEI-DNA-associated toxicity did occur, the result was the inhibition of the immune response.

The main sources of IL-12 are macrophages and dendritic cells. High serum levels of IL-12 observed in mice 6 h after PEI-DNA immunization suggest that PEI-DNA complex may target macrophages and/or dendritic cells soon after i.v. administration. Here, we show that selective in vivo depletion of macrophages using liposome-encapsulated clo-
dronate, a widely accepted approach to deplete macrophages, completely abolished IL-12 production, as well as gp120-specific CD8+ T cell response, after PEI–DNA administration. Our data indicate that macrophages mediate IL-12 production following PEI–DNA complex immunization, resulting in enhanced systemic and mucosal immune responses.

In this paper, we show that PEI is likely to act as a powerful adjuvant to generate high levels of antigen-specific immune response. Most of the currently available adjuvants approved for humans fail to enhance cellular immune responses against an antigen, while others that do enhance cellular immune response have associated side-effects that make them unsuitable for human use [37]. We have demonstrated that a single immunizing dose of PEI–DNA complex is able to induce a strong cellular immune response capable of providing both systemic and mucosal protections against a viral infection. We also show that an optimal immune response can be induced by PEI–DNA vaccination without causing any toxicity. A strong advantage of PEI–DNA-based vaccine over other immunization agents, such as recombinant viruses, is that PEI–DNA immunization does not generate neutralizing antibodies to the vector, and therefore, it can be administered repeatedly. Overall, our results indicate that PEI–DNA complex is a highly efficient vector for generating a strong antigen-specific CD8+ T cell response and systemic and mucosal protective immunity. This PEI–DNA-based vaccine is, therefore, potentially an attractive candidate for the development of a successful vaccine against human pathogens.

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