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Immunogenicity of a novel engineered HIV-1 clade C synthetic consensus-based envelope DNA vaccine

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Abstract

DNA vaccines require significant engineering in order to generate strong CTL responses in both non-human primates and humans. In this study, we designed a clade C *env* gene (*EY3E1-C*) to decrease the genetic distances of virus isolates within clade C and focus the induced T cell responses to conserved clade C epitopes. After generating a consensus sequence by analyzing full-length clade C *env* early transmitter sequences, several modifications were performed to increase the expression of the *EY3E1-C*, including codon/RNA optimization, addition of Kozak sequence and addition of an IgE leader sequence. We also shortened the V1 and V2 loops to approximate early transmitter isolate sequences and the cytoplasmic tail was truncated to prevent envelope recycling. When studied as a DNA vaccine in Balb/c mice, compared to a primary codon-optimized clade C envelope DNA vaccine (p96ZM651gp140-CD5), this novel construct is up to three times more potent in driving CTL responses. Importantly this construct not only induces stronger cross-reactive cellular responses within clade C, it also induces stronger immune responses against clade B and group M envelope peptide pools than p96ZM651gp140-CD5. Epitope mapping demonstrated that *EY3E1-C* was able to induce clade C envelope-specific immune responses against 15 peptide pools, clade B envelope-specific immune responses against 19 peptide pools and group M envelope-specific immune responses against 16 peptide pools out of 29, respectively, indicating that a significant increase in the breadth of induced immune responses. The analysis of antibody responses suggested that vaccination of p*EY3E1-C* could induce a clade C envelope-specific antibody response. The cellular immune responses of p*EY3E1-C* could be further enhanced when the DNA was delivered by using electroporation (EP). Thus, the synthetic engineered consensus *EY3E1-C* gene is capable of eliciting stronger and broader CTL responses

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than primary clade C envelopes. This finding suggests that such synthetic immunogens could be important for examination of their potential as part of an efficient HIV DNA vaccine.

Keywords

HIV-1; Clade C; Envelope; Consensus; Cellular immune responses

1. Introduction

The global prevalence of human immunodeficiency virus type 1 (HIV-1) subtype C has been increasing. In Southern Africa, the Horn of Africa, India and China, HIV-1 subtype C represents more than half of all circulating group M infections with more than 94% of HIV infections being caused by subtype C in Southern Africa alone [1]. Moreover, subtype C is the only subtype that accounts for prevalence rates as high as 20 to 40% in the adult population of the southern countries of Africa [2]. These extremely high prevalence rates underscore the desperate need for an effective vaccine that could impact on the rapid spread of a clade C virus.

A major obstacle for AIDS DNA vaccine development is the genetic diversity, rapid mutation and recombination rates of HIV-1 [3, 4]. Previous studies have shown a relatively high level of HIV-1 subtype C intrasubtype diversity [5–7], which increases the magnitude of the challenge. It is unlikely that a candidate DNA vaccine derived from individual isolates will elicit sufficient cross immune reactivity to protect against diverse circulating viruses. To overcome the challenge caused by the genetic variation of HIV-1, “centralized” gene sequences have been suggested to use in HIV-1 immunogen design. One of strategies generating “centralized” sequences is to use consensus sequences [2, 8–10]. Haynes *et al* have demonstrated that the consensus HIV-1 group M envelope genes are able to express envelope glycoproteins that retain the structure, functional, and immunogenic properties of wild-type HIV-1 envelopes [11]. Phylogenetic analysis have also shown that the consensus sequences may provide an appropriate sequence for a DNA vaccine and is largely representative of the major circulating viral strains [8, 12].

DNA immunization has been a useful technology for the development and analysis of immunogens. Direct injection of naked DNA either intramuscularly or intradermally can induce protective CD8⁺ cytotoxic lymphocyte (CTL) responses in specific model systems [13]. Studies have shown the importance of HIV-1 specific CTL responses in controlling viral load during acute and asymptomatic infection and preventing the development of AIDS [14, 15]. However, DNA vaccines expressing the HIV-1 envelope glycoprotein have been reported to be weakly immunogenic [16, 17]. It is very important to produce high expressing envelope antigens to induce potent immune responses. Several methods have been used to increase the expression levels of DNA immunogens, including codon optimization [18, 19], RNA optimization [20, 21] and addition of immunoglobulin leader sequences that have weak RNA secondary structure [22].

Derdeyn *et al.* analyzed HIV-1 Envelope glycoprotein sequences in eight African heterosexual transmission pairs and found that shorter V1-V4 length, fewer glycans are the

common features shared by the sequences obtained from early viral transmitters [23]. Here we report development of a novel engineered consensus HIV-1 clade C envelope immunogen based on analysis of sequences from such clade C early transmitters. We show that this novel engineered consensus envelope construct could elicit much stronger and broader CTL immune responses, indicating that this approach could be beneficial for possible strategy as a component of an HIV-1 DNA vaccine cocktail.

2. Materials and Methods

2.1. Envelope immunogens

Plasmids expressing the HIV-1 clade C consensus Envelope glycoprotein was made synthetically using nucleotide sequences designed to disrupt viral RNA structures that limit protein expression by using codons typically found in Human cells. Briefly, a consensus sequence of clade C was generated based on the sequences retrieved from HIV databases (<http://www.hiv.lanl.gov>). To produce a CCR5-tropic version of the HIV-1 envelope, six important amino acids in V3 loop were designed according to the sequences of early transmitter isolates. Six amino acids in V1 loop and three amino acids in V2 loop were deleted. The cytoplasmic tail region was removed to promote higher expression of Env protein. The gp120/41 Env cleavage site was incorporated to promote proper folding of the synthetic Env protein. A more efficient leader sequence was added to the N-terminal of the gene. The synthesized EnvC gene (EY3E1-C) was digested with *Bam*HI and *Not*I, cloned into the expression vector pVAX (Invitrogen) under the control of the cytomegalovirus immediate-early promoter and the construct was designated as pEY3E1-C.

Another envelope immunogen, p96ZM651gp140-CD5 was obtained from NIH AIDS Research & Reference Reagent Program (Catalog number: 8660). The env gene in this construct was reconstructed by substituting original viral codons with those of highly expressed human genes. The original signal peptide was replaced with the signal peptide from the human CD5 protein. The size of the translated env protein is 707 aa since a stop codon was introduced right after the transmembrane domain. The modified env gene (GenBank accession number: AY181197) was subcloned into the pcDNA3.1(-) expression vector.

2.2. In vitro transcription and translation

The expression of EY3E1-C in vitro was detected by utilizing TNT[®] Quick Coupled Transcription/Translation System (Promega). The gene product was immunoprecipitated using an envelope-specific monoclonal antibody 2G12 (NIH AIDS Research & Reference Reagent Program, MD). After immunoprecipitation, the immunoprecipitated protein was run on a SDS-PAGE gel (12%) for analysis. The gel was fixed, dried and the synthesized protein with incorporation of radioactive ³⁵S was detected by autoradiography.

2.3. Indirect immunofluorescent assay

An indirect immunofluorescent assay for confirming EY3E1-C gene expression was performed as described previously [12]. The anti-mouse HIV-1 env monoclonal F105 (NIH AIDS Research and Reference Reagent Program, Rockville, MD, USA) was used as a

primary antibody. The images were analyzed using the Phase 3 Pro program for fluorescent microscopy (Media Cybernetics).

2.4. Immunization of mice

Female 4–6-week-old BALB/c mice were purchased from The Jackson Laboratory, Bar Harbor, ME. Their care was in accordance with the guidelines of the National Institutes of Health and the University of Pennsylvania Institutional Care and Use Committee (IACUC). Each mouse was immunized intramuscularly three times with 50 ug of DNA at biweekly intervals. There were four mice in each group and mice in control group were immunized with control pVAX DNA. For the vaccination delivered by EP, each mouse was immunized with 10 ug of DNA by intramuscular injection into the quad muscle followed by *in vivo* electroporation using the CELLECTRA[®] adaptive constant current electroporation device (Inovio Pharmaceuticals, Blue Bell, PA). Two 0.1 Amp constant current square-wave pulses were delivered through a triangular 3-electrode array consisting of 26-gauge solid stainless steel electrodes. Each pulse was 52 milliseconds in length with a 0.2 second delay between pulses. The mice received a total of 3 immunizations administered 2 weeks apart. Mice were sacrificed one week after the third immunization and their spleens were removed aseptically. Spleen cells were collected and resuspended in RBC lysis buffer to remove erythrocytes. After lysis, the splenocytes within each group were pooled and resuspended in RPMI 1640 medium with 10% FBS. Cells were counted and utilized for the immunology studies.

2.5. IFN- γ ELISpot assay

Mouse IFN- γ ELISpot assay was performed as described previously [12]. Five sets of peptides, each containing 15 amino acid residues overlapping by 11 amino acids representing the entire protein consensus sequences of HIV-1 clade B, C and group M, and the entire protein sequences of HIV-1 C. UY. 01.TRA3011 and C. ZA. 01. J54Ma (two clade C isolates) envelope, were obtained from NIH AIDS Research and Reference Reagent Program. Each set of env peptides were pooled at a concentration of 2 ug/ml/peptide into 4 pools as antigens for specific stimulation of the IFN- γ release. Concavalin A (Sigma–Aldrich, St. Louis, MO), at 5 g/ml, and complete culture medium were used as positive and negative control, respectively. The average number of spot forming cells (SFC) was adjusted to 1×10^6 splenocytes.

2.6. HIV-1 consensus clade B, C and group M Env (15-mer) peptides mapping

As mentioned above, three sets of peptides each containing 15 amino acid residues overlapping by 11 amino acids representing the envelope protein of HIV-1 consensus Clade B, C and group M, respectively, were obtained from NIH AIDS Research and Reference Reagent Program. In order to map the immuno-dominant epitopes, all three sets of peptides were pooled into 29 pools and IFN- γ ELISpot assay was performed as described above. These different sets of 29 pooled stimulators were used in a matrix assay which facilitates epitope mapping.

2.7. Clade C envelope-specific antibody determination

The measurement of IgG antibodies specific for envelope was performed by ELISA (enzyme linked immunosorbent assay) in both immunized and control mice. The plates were coated with 1 µg/ml of subtype C primary envelope protein HIV-1 96ZM651 gp120 (NIH AIDS Research and Reference Reagent Program) and ELISA was performed as described previously [12]

2.8. Statistical Analysis

Student paired t-test was used for comparison of cellular immune response. In this study, $p < 0.05$ has been considered statistically significant.

3. Results

3.1. Construction and design of novel clade C envelope gene

The consensus sequence of HIV-1 clade C was generated from thirty-six clade C sequences retrieved from the Los Alamos HIV sequence database. The phylogenetic analysis of the clade C envelope proteins was performed and the relevant placement of the consensus sequence was indicated in Fig. 1A.

As shown in Fig. 1B, several modifications were carried out after obtaining the consensus envelope sequence of clade C. Briefly, in order to get higher level of expression, the codon usage of the gene was adapted to the codon bias of *Homo Sapiens* genes. In addition, RNA optimization was also performed: regions of very high (>80%) or very low (<30%) GC content and the cis-acting sequence motifs such as internal TATA boxes *et al* have been avoided. The Kozak and IgE leader sequence were fused into the upstream of the start codon to further increase the expression level. Two hypervariable regions, V1 and V2, were shortened based on reported early transmitter isolate sequences [23]. The trans-membrane domain of envelope was kept intact while the cytoplasmic tail was truncated. The synthetic engineered *EY3E1-C* gene was synthesized and its full length was 2140 bp. The *EY3E1-C* gene was subcloned into pVAX at *Bam*HI and *Not*I sites for future study (Fig. 1C).

3.2. Expression of pEY3E1-C

In vitro translation of pEY3E1-C was carried out by the T7 coupled transcription and translation reaction. After immunoprecipitation, the expression of EY3E1-C gene was assessed by SDS-PAGE gel (12%). The immunoprecipitated protein migrated at a rate corresponding to a molecular weight of approximately 80 kDa as expected. No protein band could be observed in the pVAX group (Fig. 2A).

To further confirm the expression of the *EY3E1-C* construct, an Indirect Immunofluorescent Assay was performed. Stained cells were observed under the fluorescent microscope in the pEY3E1-C transfected cells. As indicated in Fig. 2B, the transfected cells expressing envelope protein showed typical rhodamine fluorescence. Again, the EY3E1-C protein was absent in control pVAX transfected RD cells.

3.3. Envelope-specific antibody responses induced by pEY3E1-C

To determine whether the clade C consensus envelope immunogen could elicit antigen-specific antibody responses, an ELISA was performed using the sera collected from BalB/C mice immunized with pVAX, pEY3E1-C or p96ZM651gp140-CD5. As shown in Fig. 3, higher-titer clade C envelope-specific antibody responses were observed in pEY3E1-C immunized mice compared to these in p96ZM651gp140-CD5 immunized mice. In contrast, the mice immunized with pVAX didn't develop antigen-specific antibody responses. This result indicates that the synthetic consensus-based immunogen exhibits a relatively native conformation and preserves some native antigenic epitopes.

3.4. Strong and broad clade C envelope-specific cellular responses induced by pEY3E1-C

Clade C envelope-specific IFN- γ secreting cells were analyzed in response to four pools of peptides from HIV-1 consensus clade C protein by a qualitative ELISpot assay (Fig. 4A). The additive frequency of SFU/per million splenocytes for all four pools in pEY3E1-C and p96ZM651gp140-CD5 immunized mice were 726 and 198, respectively. Cells from mice immunized with pVAX vector were used as a negative control, showing only background levels of 22 SFU/per million splenocytes for four peptide pools ($p < 0.05$). These results indicate that pEY3E1-C construct is up to three times more potent in driving cellular immune responses compared to the primary optimized p96ZM651gp140-CD5 construct.

In addition, we were interested in characterizing the cellular immune responses that were observed in the previous ELISpot assay. Accordingly, the ELISpot assay was performed against a library of peptides spanning consensus subtype C. Two-hundred and eleven 15-mer peptides with 11 amino acid overlaps between them, which span the entire HIV-1 consensus envelope C protein, were used. IFN- γ ELISpot analysis of splenocytes derived from the pEY3E1-C vaccinated mice revealed that the consensus immunogen reacted to about 15 peptide pools out of 29 pools (Fig. 4B). In comparison, there were only 4 reactive peptide pools induced in the p96ZM651gp140-CD5 immunized mice. These results suggested that there is a significant increase in the breadth and magnitude of cellular immune responses induced by the EY3E1-C immunogen.

3.5. Strong Cross-reactive cellular immune responses induced by pEY3E1-C

To determine whether the EY3E1-C immunogen could induce cross-reactive cellular immune responses within clade C and cross-clade cellular responses, IFN- γ ELISpot was performed using HIV-1 C.UY.01.TRA3011 and C.ZA.01.J54Ma (two clade C isolates), HIV-1 group M, consensus clade B envelope peptides as targets.

HIV-1 C.UY.01.TRA3011 and C.ZA.01.J54Ma are two clade C-specific isolate peptide sets that can serve as a stringent control for evaluating breadth and cross-reactivity obtained by other peptide sets and approaches. Here we used these two peptide sets to evaluate the cross reactivity of cellular immune responses within clade C induced by vaccination. As shown in Fig 5A and B, the additive number of SFU/per million splenocytes against HIV-1 C.UY.01.TRA3011 and C.ZA.01.J54Ma peptides in pEY3E1-C immunized mice were 390 and 498, respectively. Compared to the number of spots induced by the primary envelope C vaccine (163 and 198, respectively), the consensus clade C immunogen could enhance the

cross-reactive cellular immune responses within clade C. Moreover, EY3E1-C could also elicit the stronger cross-clade immune responses. The additive number of SFU/per million splenocytes against four pools of consensus subtype B peptides in pEY3E1-C-vaccinated mice was 375 (Fig. 5C). The number of IFN- γ spots in response to stimulation with four HIV-1 group M peptides pools was 425 (Fig. 5D). Compared to the immune responses elicited by p96ZM651gp140-CD5 vaccine, pEY3E1-C was about three times more potent in driving cross-clade cellular immune responses.

In order to characterize the cross-clade immune responses observed above, peptides mapping studies were carried out as well. The results indicated that the EY3E1-C immunogen reacted to about 19 consensus clade B envelope peptide pools (Fig. 6A). In addition, there were 16 group M envelope peptide pools showing the immune responses elicited by pEY3E1-C (Fig. 6B). By comparison, there were only about two or three either clade B or group M envelope peptide pools showing the immune responses in the mice immunized with the primary envelope C vaccine. Clearly, there was a significant enhancement in the breadth of cellular responses elicited by vaccination using pEY3E1-C.

3.6. Cellular immune responses induced by pEY3E1-C were further enhanced by electroporation

Prior studies from our lab and others have shown that electroporation as a DNA delivery method has great potential for DNA vaccination applications since a more efficient level of plasmid DNA transfer and transgene expression has been reported. Here, we investigated whether the strong cellular immune responses induced by pEY3E1-C could be further increased by electroporation. We again compared the cellular immune responses induced by pEY3E1-C vs p96ZM651gp140-CD5 delivered by EP. As show in Fig. 7, not only the magnitude of immune responses, but also the breadth of immune responses (cross-clade) was significantly increased by EP immunization with pEY3E1-C. Compared to 726 SFU/per million splenocytes for the clade C consensus peptides, the number of IFN- γ secreting cells per million splenocytes went up to 2262 when only one-fifth amount of DNA was used for immunization (Fig. 7A). The additive frequency of SFU/per million splenocytes for the clade B consensus and group M consensus envelope peptides in pEY3E1-C immunized mice were 950, and 1063, respectively (Fig. 7B and C). Compared to the cross-clade immune responses by intramuscularly infection (375 and 425, respectively), EP was very efficient at increasing the cross-reactive cellular immune responses.

4. Discussion

Worldwide HIV-1 DNA vaccine efforts have been guided by the principle that HIV-specific T cell responses may provide protection from infection. In primate models in which a rather avirulent virus challenge was used, DNA vaccination has been shown to induce protective immunity [24, 25]. However, in more pathogenic animal models, only live attenuated virus was successful [26, 27]. These results suggest that a potential vaccine meeting safety requirements for humans can only be successful if strategies to increase immunogenicity are developed. In this study, in order to achieve a stable HIV envelope construct with high-level expression, we modified the envelope sequence extensively. First, the codon usage was

adapted to the codon bias of *Homo sapiens* genes. Second, RNA optimization was performed to avoid RNA secondary structure and remove Rev dependence. Thirdly, an immunoglobulin leader sequence with a Kozak sequence upstream the start codon was added to further increase the expression level. Studies also suggested that the cytoplasmic tail region of envelope gene is responsible for ER retention and down modulation of envelope protein surface expression, so we also eliminated this region to promote more stable and higher expression [28, 29]. Expression data clearly showed that this engineered new envelope could be efficiently expressed. Most importantly, the IFN- γ ELISpot results demonstrated that EY3E1-C was highly immune potent, exhibiting between 4–5X increases in cellular responses compared to an optimized primary envelope immunogen p96ZM651gp140-CD5. The cellular immune responses induced by pEY3E1-C are likely to provide additional information following additional cytokine analysis.

Sequence homology between the vaccine candidate and the infecting or challenge virus may be an important consideration in HIV-1 vaccine design. An effective way to minimize the degree of sequence dissimilarity between a vaccine strain and contemporary circulating viruses is to create artificial sequences that are “central” to these viruses, such as consensus sequences. Previous phylogenetic analysis of more than 70 HIV-1 subtype C genomes has suggested that the amino acid distances to the consensus sequence were significantly lower than distances between samples within all HIV-1 subtype C isolates [2]. Only three to eight percent amino acid differences were observed when comparing the subtype C consensus sequence with the sets of primary subtype C sequences. The high probability that a new sequence will be within the identified consensus sequence, and the consensus sequence homology to the introduction of a new isolate strongly suggest that a consensus-based vaccine could be a superior immunogen to any particular viral isolate [30]. Since the use of consensus would enhance the cross-reactive potential of a vaccine, we thought pEY3E1 could elicit higher cross-clade reactivity as well. Our result showed EY3E1 could elicit strong CTL responses within clade C as well as to subtype B and group M consensus envelopes. Peptide mapping results also indicated that EY3E1 broadened the immune response. Broader cross-reactive CTL response appears an additional benefit of this strategy.

As a consensus sequence is generated based on contemporary isolates, it may more likely reflect escape variants relevant to the host population than an ancestral sequence would. If most viruses in the circulating population had already lost original epitopes because of immune escape, and if the epitopes induced a dominant response upon vaccination with a strain that carried it, then this may be an additional advantage of using consensus sequences. However, if the wild-type form of the epitope was still infrequently circulating, and if the epitope was very potent, the ancestral sequences may have an advantage. To minimize the disadvantages of the consensus sequences, we took early transmitter sequences into account when designed the pEY3E1-C vaccine. Studies have indicated that viruses encoding clade C Envelope glycoproteins with shorter V1, V2 and V4 regions are transmitted in recipients with a frequency significantly greater than would be expected by chance [23]. Therefore, in this paper, we shortened the V1 and V2 regions when we generated consensus sequence. As for the protective benefit of this vaccine on early transmitters, however, more experimental data needs to be collected.

A differential coreceptor usage of HIV variants has been demonstrated for different subtypes [31, 32]. Subtype C viruses appear to be different from most other subtypes because an underrepresentation of CXCR4 using HIV variants in subtype C has frequently been reported [33]. Therefore, CCR5 utilization should be a very crucial consideration for a vaccine design, especially for subtype C. Previous reports showed that the V3 region of gp120 plays an important role in coreceptor utilization. Six residues in V3 loop have been identified to be critical for the CCR5 interaction: arginine³⁰⁷, lysine³¹⁴, isoleucine³¹⁶, arginine³²², phenylalanine³²⁴ and alanine³³⁷ [34]. However, based on the sequences of early transmitters, the residue at position 322 should be glutamine instead of arginine. These constructs would likely benefit not solely the induced cellular immune response but the induced humoral responses as well. Future studies focusing on the induced neutralizing antibodies are important.

Taken together, our results suggest that EY3E1 serves as an improved immunogen that could increase both the magnitude and breadth of CTL responses compared to native immunogens. In more general terms, the data presented here may help to develop improved DNA vaccines against diverse strains of HIV.

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Figure 1.

HIV-1 subtype C consensus envelope DNA construct (pEY3E1-C). (A) Phylogenetic analysis based on a neighbor-joining evaluation of HIV-1 clade C envelope alignment. The star indicates the consensus sequence, relative to its component viruses. (B) Schematic representation of functional domains in EY3E1-C. (C) EY3E1-C was cloned into pVAX at 5'-BamHI and 3'-NotI.

Figure 2.

Expression of pEY3E1-C. (A) In vitro translation of the *EY3E1-C* gene. The coupled transcription/translation reaction product was immunoprecipitated with HIV-1 envelope-specific monoclonal antibody 2G12 and subjected to a SDS-PAGE gel. (B) Immunofluorescence assay of the EY3E1-C gene. The transfected RD cells expressing EY3E1-C protein showed typical red fluorescence. HIV-1 envelope-specific monoclonal antibody F105 served as the source of primary antibody. The vector pVAX was used as a negative control.

Figure 3.

Total clade C envelope-specific IgG antibody titers in the sera of the immunized mice. Humoral immune responses after immunization with DNA constructs pEY3E1-C or p96ZM651gp140-CD5 were detected by enzyme-linked immunosorbent assay (ELISA). Each BalB/C mouse was immunized intramuscularly with three times, each of 50 μ g of DNA at bi-weekly intervals. Mice from each group (n=4) were bled one week after the third immunization and equally pooled sera were diluted in blocking buffer and analyzed as described in Materials and Methods. Pooled sera collected from mice immunized with pVAX were used as a control. Absorbance (OD) was measured at 450 nm. Each data point represents averaged three OD values from five mice sera per group and values represent the mean of ELISA obtained in three separate assays.

Figure 4.

Induction of strong cell-mediated immune responses by pEY3E1-C in Balb/c mice. (A) Frequencies of clade C envelope-specific IFN- γ spot forming cells (SFC) per million splenocytes after DNA vaccination with pEY3E1-C and p96ZM651gp140-CD5. The splenocytes were isolated from individual immunized mice and stimulated in vitro with four overlapping consensus clade C envelope peptide pools. (B) Characterization of clade C envelope-specific dominant epitopes. The splenocytes were cultured with 29 individual HIV-1 subtype C consensus envelope peptide pools for 24 hours. IFN- γ secreting cells were determined by ELISpot assay. Backbone pVAX immunized mice were included as a negative control. The values are the means + standard deviations of the means of IFN- γ SFCs.

Figure 5.

Cross reactivity induced by pEY3E1-C. Cellular immune responses against HIV-1 C.UY.01.TRA3011 envelope peptides (A), HIV-1 C.ZA.01.J54Ma (B), HIV-1 subtype B consensus envelope peptides (C) and HIV-1 group M consensus envelope peptides (D) induced by vaccination with pEY3E1-C or p96ZM651gp140-CD5 were measured by ELISpot assay as described above. Backbone pVAX immunized mice were included as a negative control.

Figure 6.

Characterization of cross-clade immune responses induced by pEY3E1-C. (A) Characterization of the immune responses against 29 HIV-1 subtype B consensus envelope peptide pools induced by pEY3E1-C. (B) Characterization of the immune responses against 29 individual HIV-1 group M consensus envelope peptide pools induced by pEY3E1-C. The splenocytes were cultured with the HIV-1 subtype B and group M consensus envelope peptide pools for 24 hours, respectively. IFN- γ secreting cells were determined by ELISpot assay as described above.

Figure 7.

Enhanced cellular immune responses induced by pEY3E1-C delivered by electroporation. (A) Frequencies of clade C envelope-specific IFN- γ spot forming cells (SFC) per million splenocytes after DNA vaccination with pEY3E1-C or p96ZM651gp140-CD5. (A) Frequencies of clade B envelope-specific IFN- γ spot forming cells (SFC) per million splenocytes after DNA vaccination with pEY3E1-C or p96ZM651gp140-CD5. (A) Frequencies of group M envelope-specific IFN- γ spot forming cells (SFC) per million splenocytes after DNA vaccination with pEY3E1-C or p96ZM651gp140-CD5.