Characterization of Humoral Immune Responses Elicited by a DNA Prime and Envelope Protein Boost Vaccine Regimen in Rabbits and Nonhuman Primates

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Introduction

Recent preclinical studies have demonstrated the use of properly folded trimeric HIV-1 envelope proteins in eliciting broadly neutralizing antibodies (bNAbs). These bNAbs target highly conserved sites on the envelope glycoprotein of HIV which consists of trimeric units of non-covalently associated surface subunit, gp120, and transmembrane subunit, gp41. Understanding the type of antibody responses generated by such envelope proteins may be important for the development of an effective HIV-1 vaccine.

Objectives

Current vaccine designs for developing an effective HIV vaccine are based on envelope protein formulations capable of eliciting broadly neutralizing antibody response in the immunized host. Several preclinical studies have demonstrated that oligomeric HIV-1 envelope elicits broader neutralizing antibody compared to the monomeric gp120. Since the ability to generate broader neutralizing antibody responses is one of the major considerations for HIV vaccine development, we analyzed humoral responses induced by SIVmac251 and SIVsmE660 trimeric envelopes in rabbits and nonhuman primates in a DNA prime/protein boost vaccine regimen. In the macaques, envelope proteins were delivered using a novel polysaccharide adjuvant, ADVAX, which has been shown to enhance adaptive immune responses without inducing reactogenicity.

Methods

Native Blue and Tris Acetate (reducing) Gel analysis of SIVmac251 gp140 and SIVsmE660 gp145 Trimers

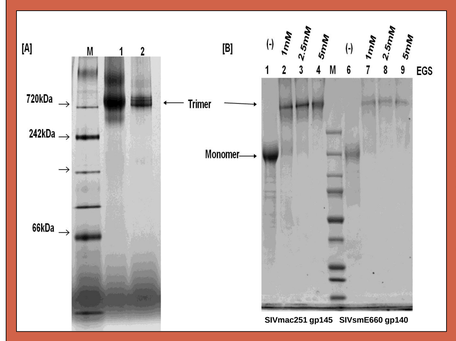


Figure 1: [A] Purified SIVmac251 and SIVEsm660 envelope proteins were analyzed on Native Blue (4-6% Bis-Tris gel). Bands corresponding to the high molecular weight trimeric forms are shown in Lane 1 for SIVmac251 gp140 and in Lane 2 for SIVsmE660 gp145. **[B]** Trimeric envelopes were cross linked with increasing concentrations of EGS and analyzed by reducing gel.

Binding Characteristics of SIVmac251 gp140 and SIVsmE660 gp145 Trimers to rhesus and human sCD4

SIV Envelope proteins	Human sCD4			Rhesus sCD4		
	k_{on} (1/Ms)	k_{dis} (1/s)	K_D (nM)	k_{on} (1/Ms)	k_{dis} (1/s)	K_D (nM)
SIVmac251gp140	6.42×10^4	2.49×10^{-4}	3.9	1.91×10^4	1.46×10^{-4}	7.63
SIVsmE660 gp145	6.60×10^3	3.13×10^{-5}	4.7	6.04×10^3	1.18×10^{-4}	19.65
SIVsmE660rgp120	2.59×10^4	5.68×10^{-4}	22.0			
SIVmac251rgp120	1.89×10^4	9.50×10^{-4}	50.4			
SIVmac251native gp120	2.95×10^4	4.75×10^{-4}	16.1			

Table 1: Binding affinity constants (Kd) of SIVmac251 and SIVsmE660 gp145 trimers and gp120 monomers to human and rhesus sCD4 as measured by biolayer interferometry (k_{on}, on-rate constant; k_{off} ,off rate constant; K_D, equilibrium dissociation constant).

Plasmid DNAs: Codon optimized *env* genes, lacking the native signal peptide, were cloned in frame with the tissue plasminogen activator signal peptide in a mammalian expression vector. Truncations were generated by site directed mutagenesis using the QuickChange Site Directed Mutagenesis kit (Stratagene). The gp120-gp41 cleavage sites sites were abolished by substitution of an arginine for a serine residue at R527S and potential secondary site R516S in SIVmac251 and R533S in SIVsmE660 and a stop codon was introduced before the gp41 transmembrane domain (TM).

SIV Envelope Oligomeric Proteins: The gp145 proteins were purified from stably transfected 293H (Invitrogen) culture supernatant using lectin affinity chromatography followed by Q-Sepharose chromatography to enrich the trimers.

ELISAs: Serum antibody titers against trimeric SIVmac251 gp140 and SIVsmE660 gp145 were measured by endpoint ELISA. Reactivity to linear epitopes of SIVmac251 gp145 was examined by PepScan ELISA using overlapping peptides. Specific antibody concentrations were determined by selecting sample dilutions that fell within the upper and lower bounds of the standard curve of purified antigen-specific serum antibodies. Antibody standard was purified from pooled immune sera over an affinity column of SIVmac251 gp145 coupled to CN-Br. Concentrations were calculated using a single-point interpolation without slope correction. Specific antibody Isotyping was performed using isotype-specific antibodies for rhesus from the Nonhuman Primate Reagent Resource and SIGMA.

Neutralization Assays: Neutralization assays were performed in TZM-bl cells in Dr. Montefiori's laboratory at Duke University.

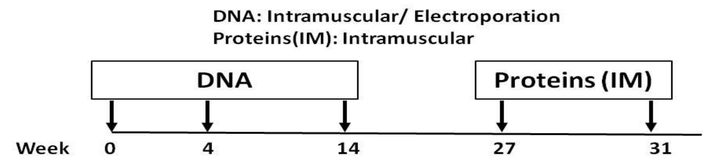
Affinity Measurements: SIV envelope trimer proteins immobilized to amine reactive (AR2G) biosensors were used to measure affinity to rhesus and human sCD4 by BLI using an Octet RED96 (Fortebio) instrument. To measure relative serum avidity, SIV envelope trimer proteins immobilized to streptavidin (SA) biosensors were dipped into wells containing 2-fold serially diluted immune sera (starting at 1:50). Sensors will then be dipped into wells containing 1 x kinetics buffer to measure the dissociation rate. Results were analyzed using Data Analysis 9.0 Software (ForteBio) to calculate kinetic parameters including (k_{on}, k_{off}, K_D) using a global fit 1:1 model.

B- and T-cell ELISpot: Assays were performed using kits from Mabtech and according to manufacture's protocol.

Viral Load Assay: SIVmac251 RNA in plasma of challenged macaques was quantitated by nucleic acid sequence-based amplification (NASBA) assay.

Results

Rabbit Study



Vaccination Protocol:
To test the immunogenicity of a prime boost vaccination regimen using trimeric SIV envelopes, four New Zealand White (NZW) rabbits were electroporated with plasmid DNA encoding the Env gp145 of SIVmac251 and gp140 of SIVsmE660 at week 0, 4 and 14. On week 27 and 31, rabbits were immunized with a mixture of the trimeric homologous proteins formulated in Adjuplex adjuvant (Sigma) and injected simultaneously via intramuscular, intradermal and subcutaneous routes. Serial bleeds were collected 2 weeks post each immunization.

Anti SIV Env gp140 Antibody Titers and Neutralization in Rabbit Sera Following gp145 Immunization

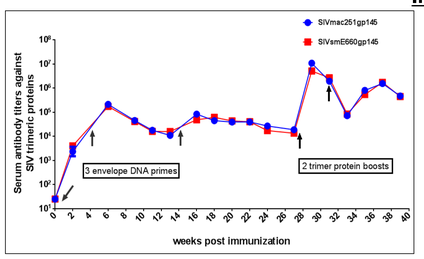


Figure 2: Serum antibody binding titers to SIVmac251gp140 and SIVsmE660gp140

ANIMAL ID	BLD WEEK	ID50 in TZM-bl Cells [1]				
		Tier 2			Tier 1	
		SIVmac 251.30	SIVmac251/ M766	SIVsmE660-BR/CG7V.IR	SIVmac251.6	SIVsmE660-BR/CG7G.IR1
C2471	0	<20	<20	<20	<20	63
	16	<20	29	<20	nd	nd
	29	34	35	<20	nd	nd
	35	57	146	27	12882	77916
C2472	0	<20	<20	<20	<20	<20
	16	<20	<20	<20	nd	nd
	29	35	36	<20	nd	nd
	35	98	69	26	81901	133790
C2473	0	<20	<20	<20	<20	<20
	16	<20	44	<20	nd	nd
	29	137	57	26	nd	nd
	35	79	133	41	31204	94878
C2474	0	<20	<20	<20	<20	<20
	16	<20	39	<20	nd	nd
	29	nd	nd	nd	nd	nd
	35	98	472	37	14859	78935

nd= not determined 40- 100 100- 1000 >1000

Table 2: Neutralizing antibody responses

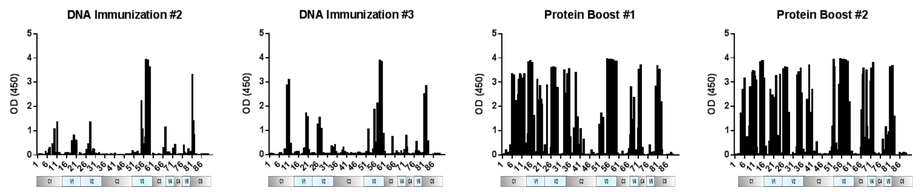
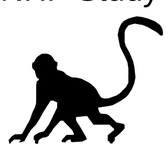
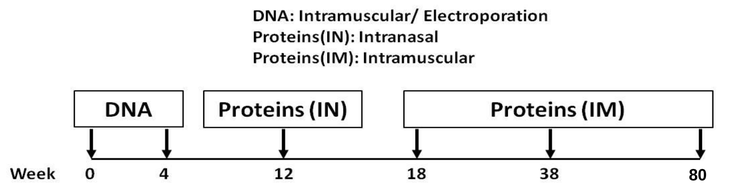


Figure 3: Serum reactivity to 15-mer overlapping peptides of SIVmac251gp160 in immunized rabbits C2467 and C2472 .

NHP Study





Vaccination and Challenge Protocol:
To evaluate the immunogenicity of our prime boost vaccination regimen formulated with Advax, four Chinese rhesus macaques were electroporated with plasmid DNA encoding the Env gp145 of SIVmac251 and SIVsmE660 at week 0 and 4. On week 12, macaques were immunized with a mixture of the trimeric homologous proteins intranasally in Advax-M (25 µg/animal) and then intramuscularly on week 18, 38 and 80 with Advax-1 (5mg/animal) adjuvant. On week 82 immunized animals were challenged intravaginally with 2000 TCID50 of a previously titrated SIVmac251 stock once weekly for four weeks. Advax is novel patented delta inulin polysaccharide preparation that has shown to enhance B cell and T cell responses with negligible reactogenicity.

Anti SIV Env gp140 Antibody Titers and Neutralization in Macaque Sera Following gp145 Immunization

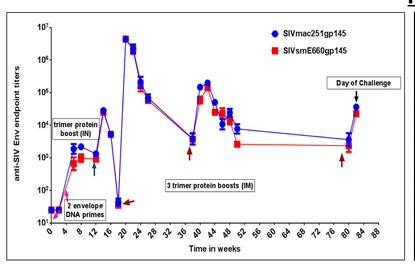


Figure 4: Antibody binding titers to SIVmac251 gp145 and SIVsmE660 gp145

ANIMAL ID	BLD WEEK	ID50 in TZM-bl Cells [1]				
		Tier 2			Tier 1	
		SIVmac 251.30	SIVmac251/M766	SIVsmE660-BR/CG7V.IR	SIVmac251.6	SIVsmE660-BR/CG7G.IR1
M907	0	<20	29	<20	<20	39
	20	<20	71	<20	nd	nd
	42	<20	35	41	18301	27138
M908	0	<20	<20	<20	<20	<20
	20	<20	52	<20	nd	nd
	42	<20	106	141	5051	20072
M909	0	<20	<20	<20	<20	<20
	20	<20	157	32	nd	nd
	42	33	313	305	8080	4542
M910	0	<20	23	<20	<20	<20
	20	36	125	48	nd	nd
	42	<20	85	234	12515	11245

nd= not determined 40- 100 100- 1000 >1000

Table 3: Neutralizing antibody responses

PepScan Binding Antibody Analysis of Macaque Sera to 15mer Peptides of SIVmac251 gp140.

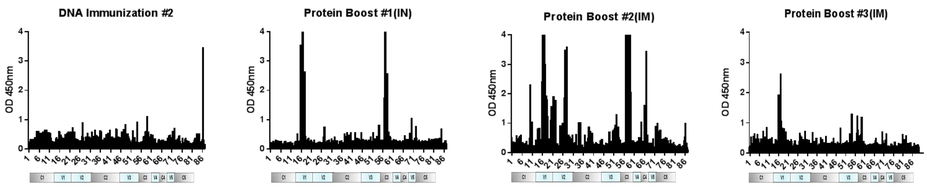


Figure 5. Serum reactivity to 15-mers with 11 amino acid overlapping peptides of SIVmac251 gp140 in immunized macaques.

Results

Relative Serum Avidity of Immunized Sera to SIVmac251 gp140 and SIVsmE660 gp145

Time	k_{on} (1/Ms)	k_{dis} (1/s)	K_D (nM)
Protein Boost #1(IM)	2.31E+04	8.49E-05	3.67E-09
Protein Boost #2(IM)	8.12E+04	2.17E-04	2.67E-09
Protein Boost #3(IM)	7.18E+05	6.84E-03	9.53E-09
Day of Challenge	6.14E+04	1.42E-05	2.31E-10

Table 4: Label free, real time binding analysis of the immunized sera to SIVmac251 gp145 for animal M909.

Quantitation of Specific IgG and Isotyping of Immune Sera to SIVmac251 gp140 and SIVsmE660 gp145

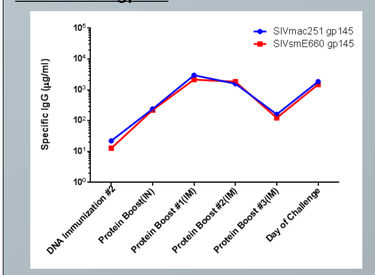


Figure 6: Quantitation of serum IgG antibodies specific for SIVmac251 and SIVsmE660 proteins.

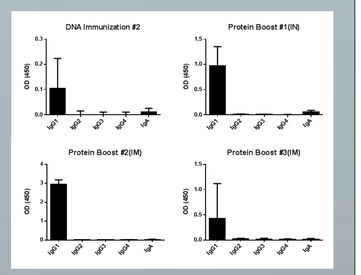


Figure 7: Antibody isotype profile in serum samples from immunized animals.

Generation of B- and T-cell responses during and after DNA prime-protein boost with Advax adjuvants

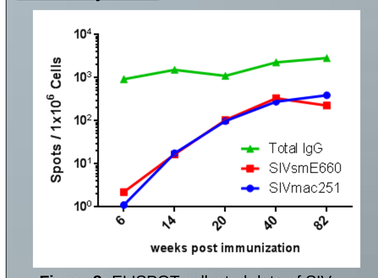


Figure 8: ELISPOT collected data of SIV envelope-specific B cells/10^6 PMBCs.

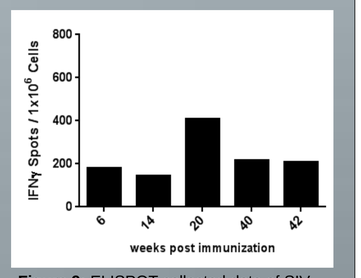


Figure 9: ELISPOT collected data of SIV envelope-specific T cells/10^6 PMBCs.

Intravaginal Challenge of macaques and assessment of SIV_{mac251} infection

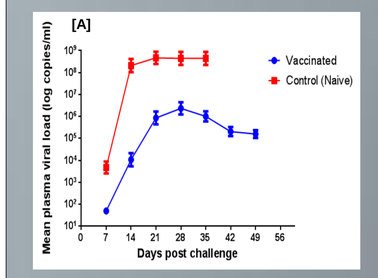


Figure 10: **[A]** The mean (+/- SEM) plasma viral load of the combined group of infected vaccinees (n = 3) and the controls (n = 5) monitored over 7 weeks post infection. **[B]** Kaplan–Meier survival curves after four low dose SIVmac251 inoculations.

Conclusions

1. Purified SIVmac251 gp140 and SIVsmE660 gp145 proteins expressed in 293H cells had trimeric conformation and high affinity binding to CD4.
2. Immunization with oligomeric SIVmac251 gp140 and SIVsmE666 gp145 was highly immunogenic in rabbits and generated a strong response to the V2 and V3 domains of SIV gp120. The rabbit sera displayed strong neutralizing against Tier 1 sensitive viruses and moderate neutralization against Tier 2 isolates.
3. Immunization of macaques with oligomeric SIVmac251 gp140 and SIVsmE660 gp145 envelopes using a DNA prime/protein boost vaccine regimen with inulin-based adjuvant, elicited robust and persistent anti Env antibody response. Antibody titers peaked following DNA electroporation and 1 protein boost but specific IgG concentration was highest following second protein boost.
4. PepScan analysis of rhesus sera demonstrated the presence of highly reactive antibodies to C1,V1,V2 ,V3 and V4 domains following Env protein boost.
5. Rhesus immune sera had moderate neutralizing activity against a few Tier 2 molecular clones of SIV isolates
6. The immune macaque sera antibodies elicited by the prime/boost immunization regimen showed an increasing improvement in binding affinity to SIVgp145 trimeric proteins over time.
7. The envelope-specific antibody response was primarily of the IgG1 isotype throughout, with low levels of IgA noted following DNA and intranasal protein boost.
8. Prime boost vaccine strategy generated persistent level of antigen-specific B and T cells as revealed in ELISPOT assays.
9. Delayed acquisition of viral infection in the immunized animals was noted following low dose vaginal challenge with SIVmac251 compared to untreated controls.
10. These results suggest that trimeric SIV Env delivered as a DNA prime/protein boost vaccine elicits broad high affinity antibody response capable of neutralizing SIV isolates of varying level of neutralizing sensitivity.