

**Recombinant Baculovirus Derived
HIV-1 Virus-Like Particles Elicit
Potent Neutralizing Antibody
Responses**

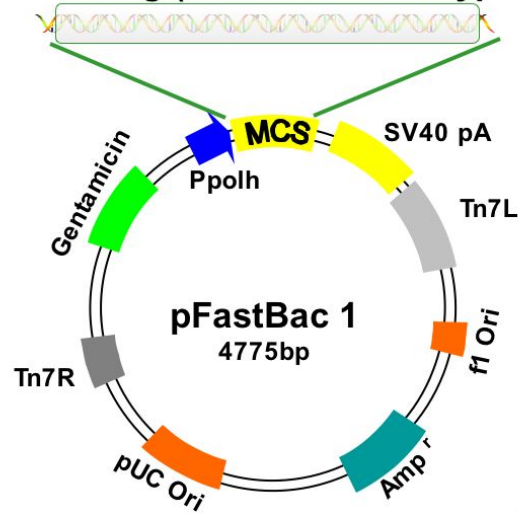
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University of Alabama
at Birmingham**

Introduction and Rationale

- Virus-like particles (VLPs) represent an attractive vaccine platform for HIV-1, but have been limited by the amount of envelope glycoprotein (Env) that can be delivered.
- Using chimeric constructs in which the trans-membrane and cytoplasmic domains of the HIV-1 *env* gene were replaced with those of other viruses (influenza virus hemagglutinin, mouse mammary tumor virus envelope, baculovirus gp64 protein), we recently increased the Env content of recombinant baculovirus (rBV) derived HIV-1 Gag/Env VLPs by more than 10-fold.
- Here, we investigated the potential of these improved VLP preparations to elicit neutralizing antibody responses in guinea pigs.

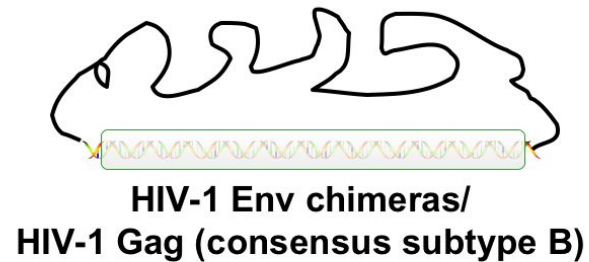
Generation of HIV-1 Gag/Env VLPs Using a Recombinant Baculovirus Expression System

HIV-1 Env chimeras/
HIV-1 Gag (consensus subtype B)



Recombination with
DH10Bac E. coli

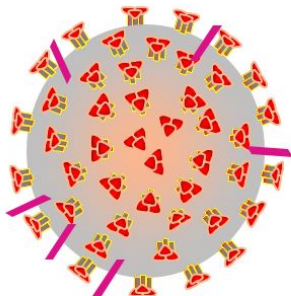
Bacmid



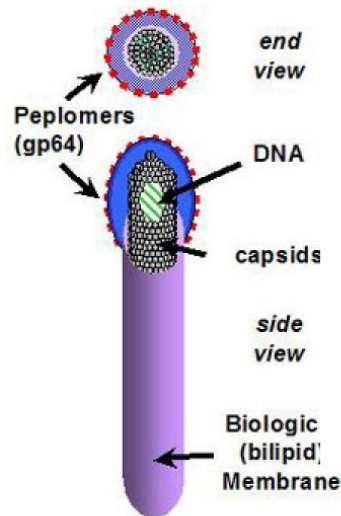
Transfection



Sf9 (insect) cells



HIV-1 Gag/Env VLP



Baculovirus

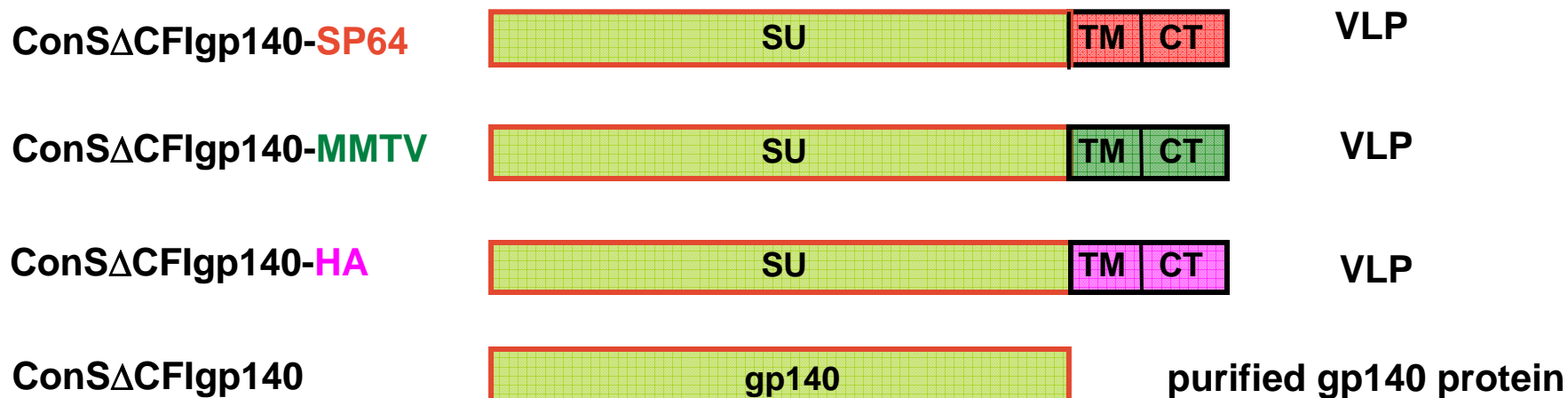
Novavax, Inc

Choice of Immunogens

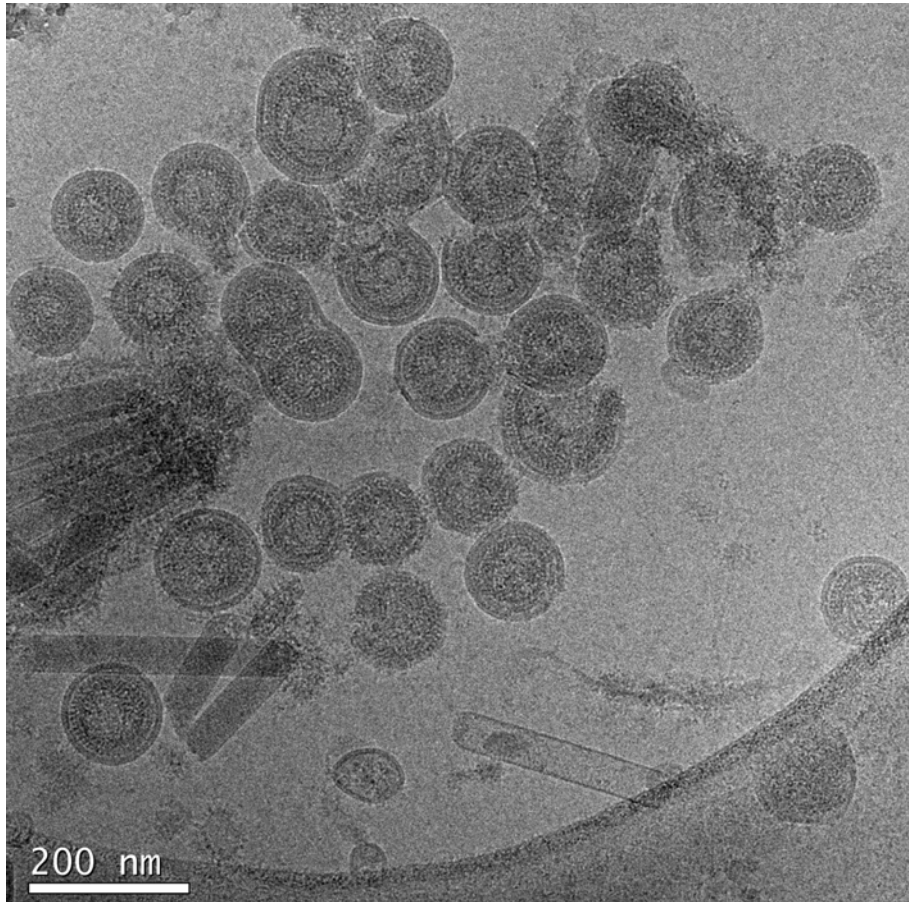
- For Gag: subtype B consensus *gag*.
- For Env: ConS Δ CFI *env* (group M consensus *env* gene lacking the gp120/gp41 cleavage site [C], the fusion domain [F], and an immunodominant [I] region in gp41).
- The ConS Δ CFI *env* was selected because
 - soluble ConS Δ CFI gp140 protein elicits high titer cross-subtype Nabs and self-assembles into trimers (Liao et al., 2006).
 - cleavage mutation prevents gp120 shedding.
 - chimeric ConS Δ CFI Env proteins (with HA, MMTV and SP64 TM and CT domains) are efficiently packaged into rBV derived VLPs (Wang et al., in press. P13-34).
 - chimeric ConS Δ CFI Env proteins are properly folded, bind CD4 and mAbs, and undergo conformational changes following CD4 binding (Wang et al., in press).

Purpose of the Study

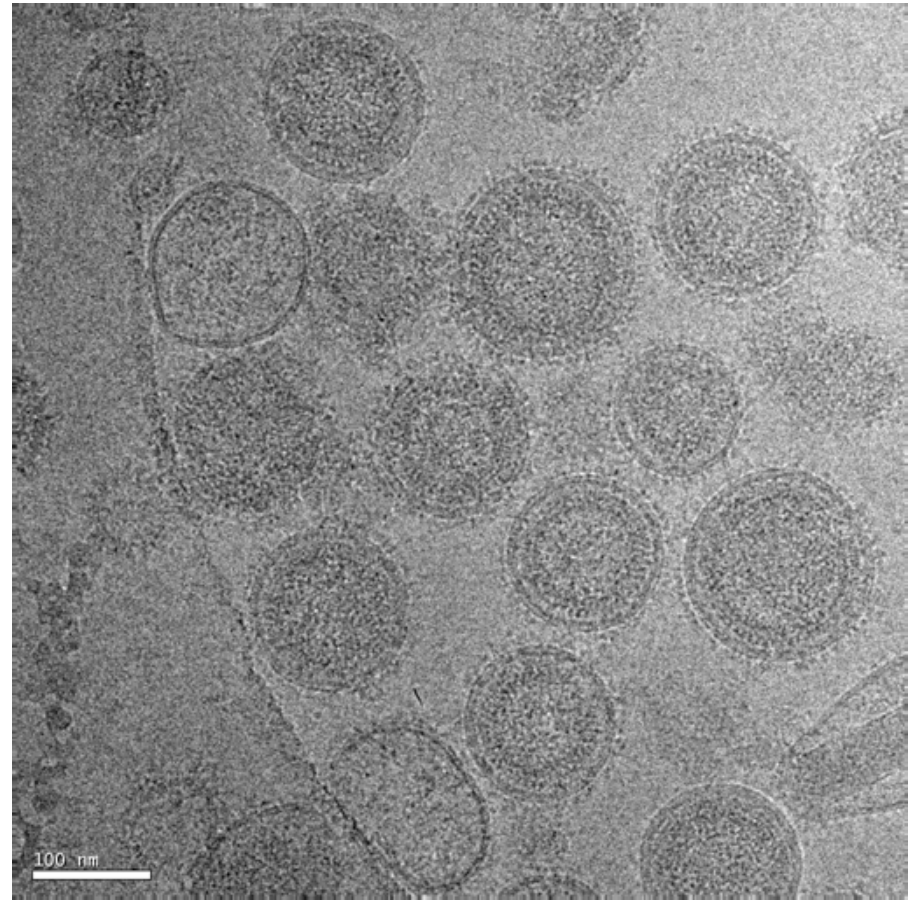
- Evaluate three different types of rBV derived Gag/Env VLP preparations, along with the cognate (soluble) gp140 protein, for their ability to induce neutralizing antibodies in guinea pigs.



Morphology of rBV Derived VLPs

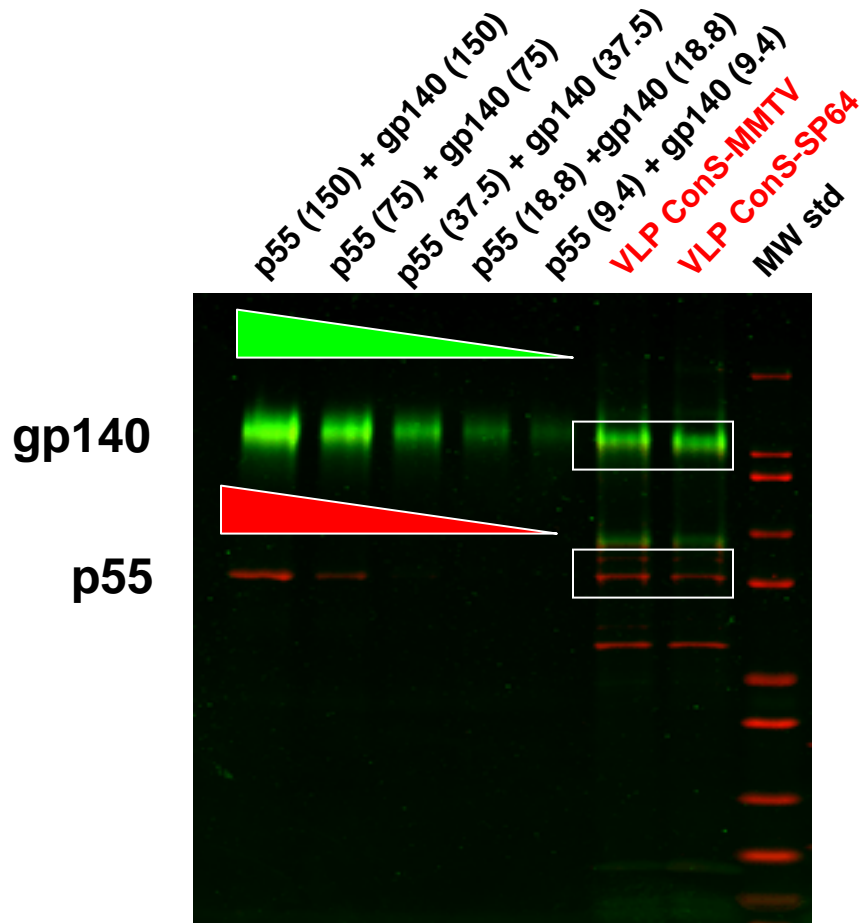


Env-minus VLPs

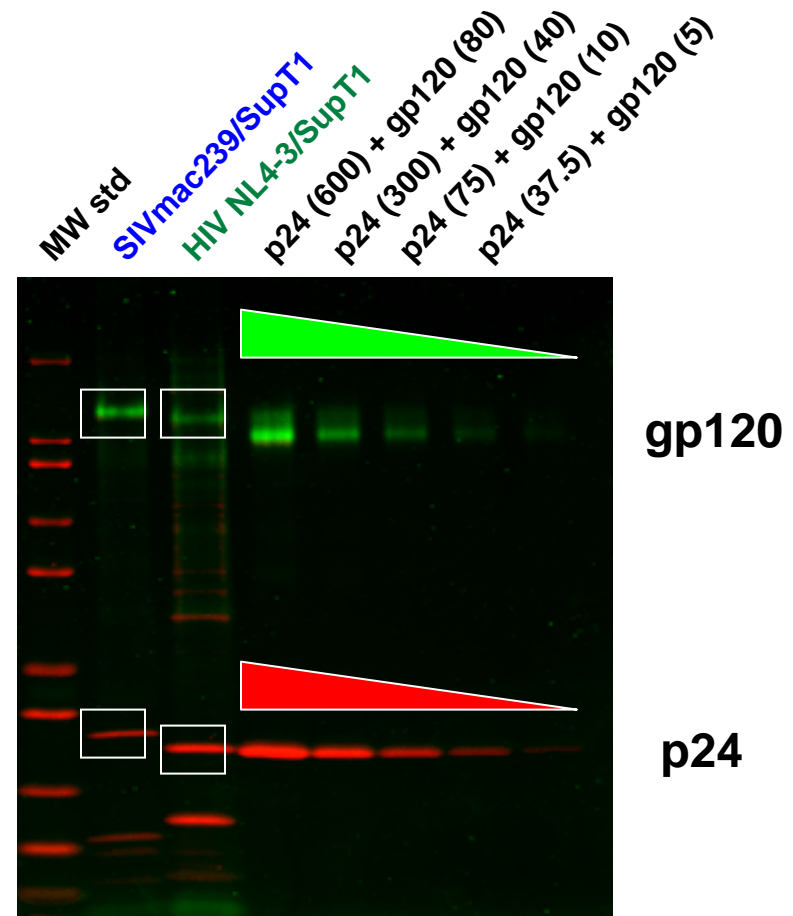


ConS-HA/Gag VLPs

Envelope Content of rBV Derived VLPs

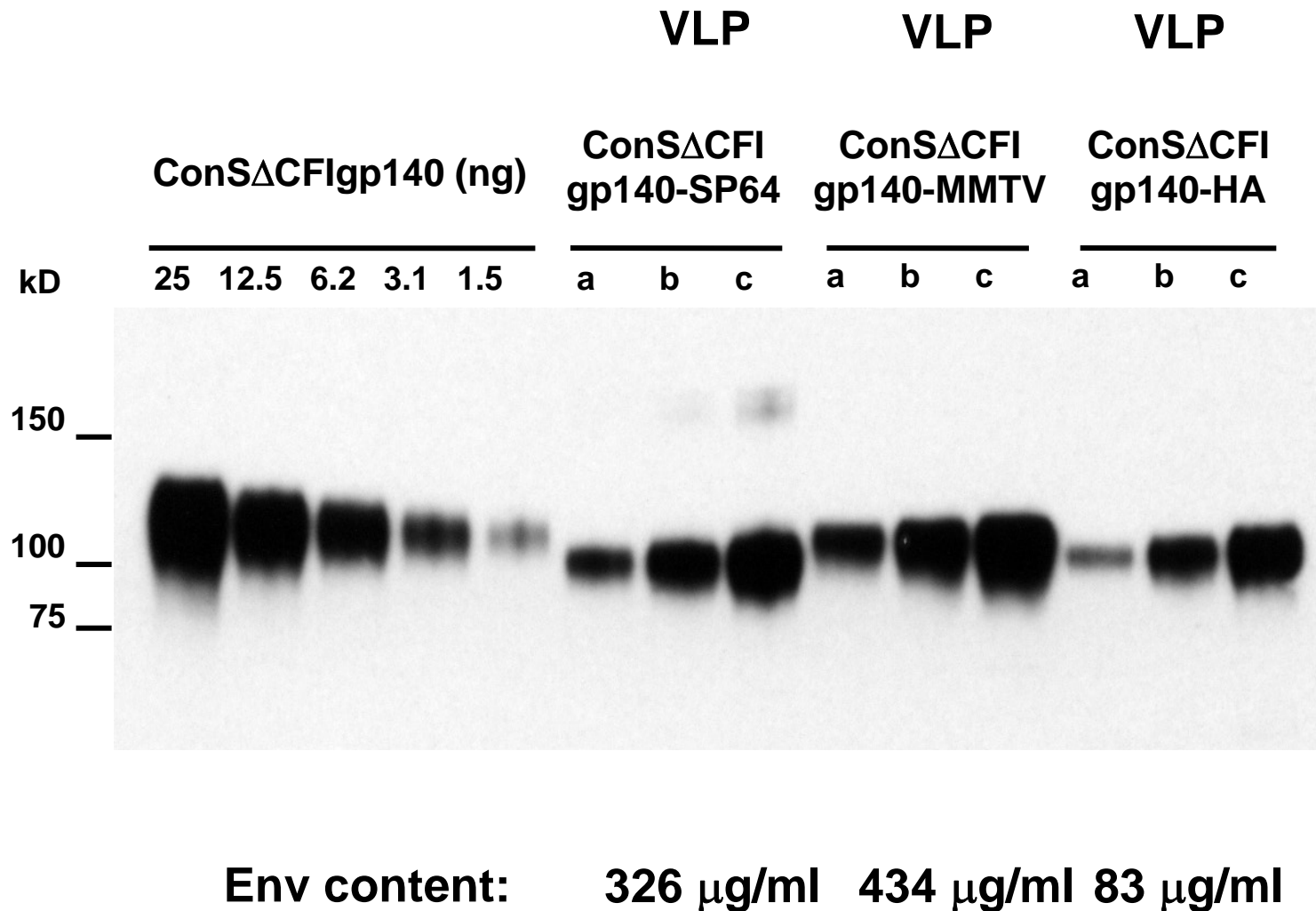


Molar ratio
 Gag/Env: **3.4 3.2**



8.4 57.0

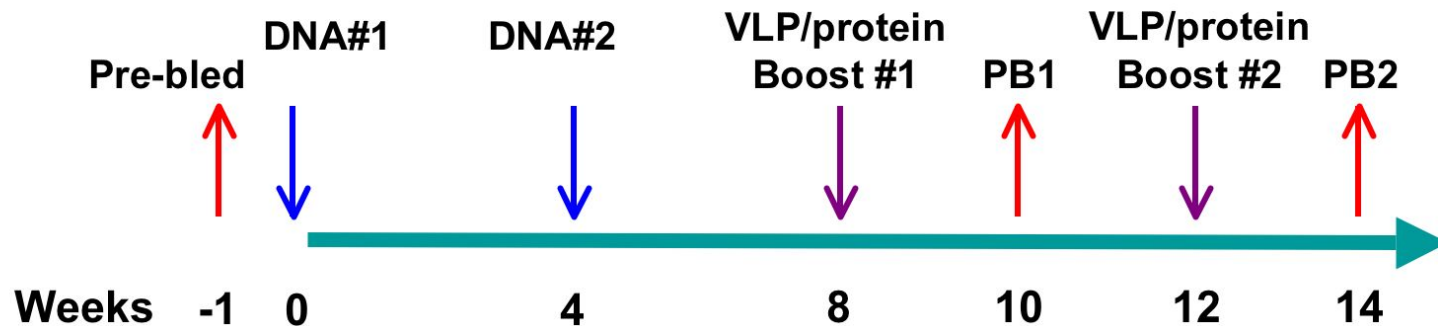
Characterization of VLPs used for Guinea Pig Immunization



Immunization Schedule



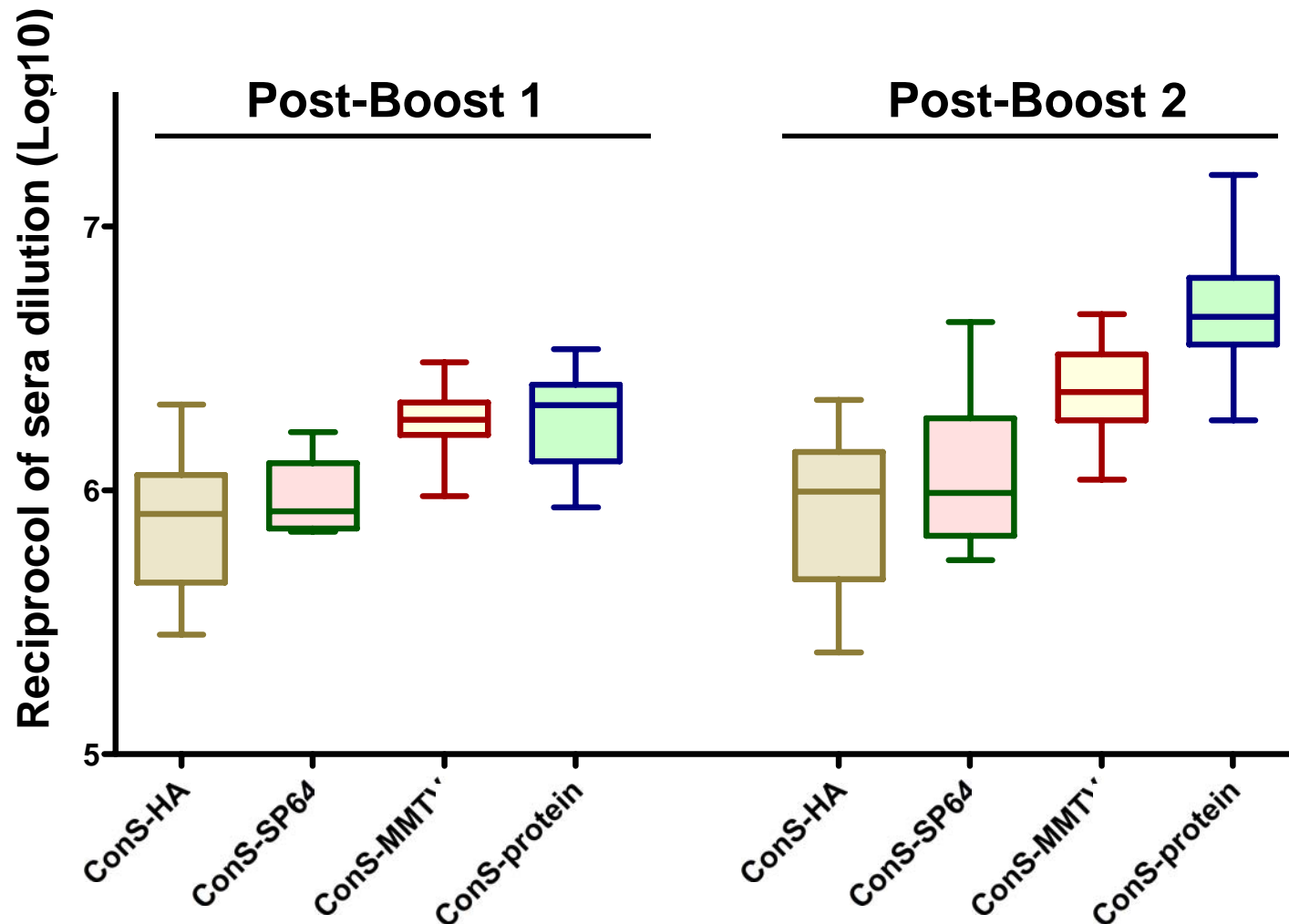
Group	Guinea pig	DNA prime	VLP Boost
1	8	CMVR-ConSΔCFI gp145 (400μg)	ConSΔCFI gp140-HA (100 μg Env)
2	8	CMVR-ConSΔCFI gp145 (400μg)	ConSΔCFI gp140-SP64 (100 μg Env)
3	8	CMVR-ConSΔCFI gp145 (400μg)	ConSΔCFI gp140-MMTV (100 μg Env)
4	8	CMVR-ConSΔCFI gp145 (400μg)	ConSΔCFI gp140 Protein (100 μg Env)



VLPs: no adjuvant

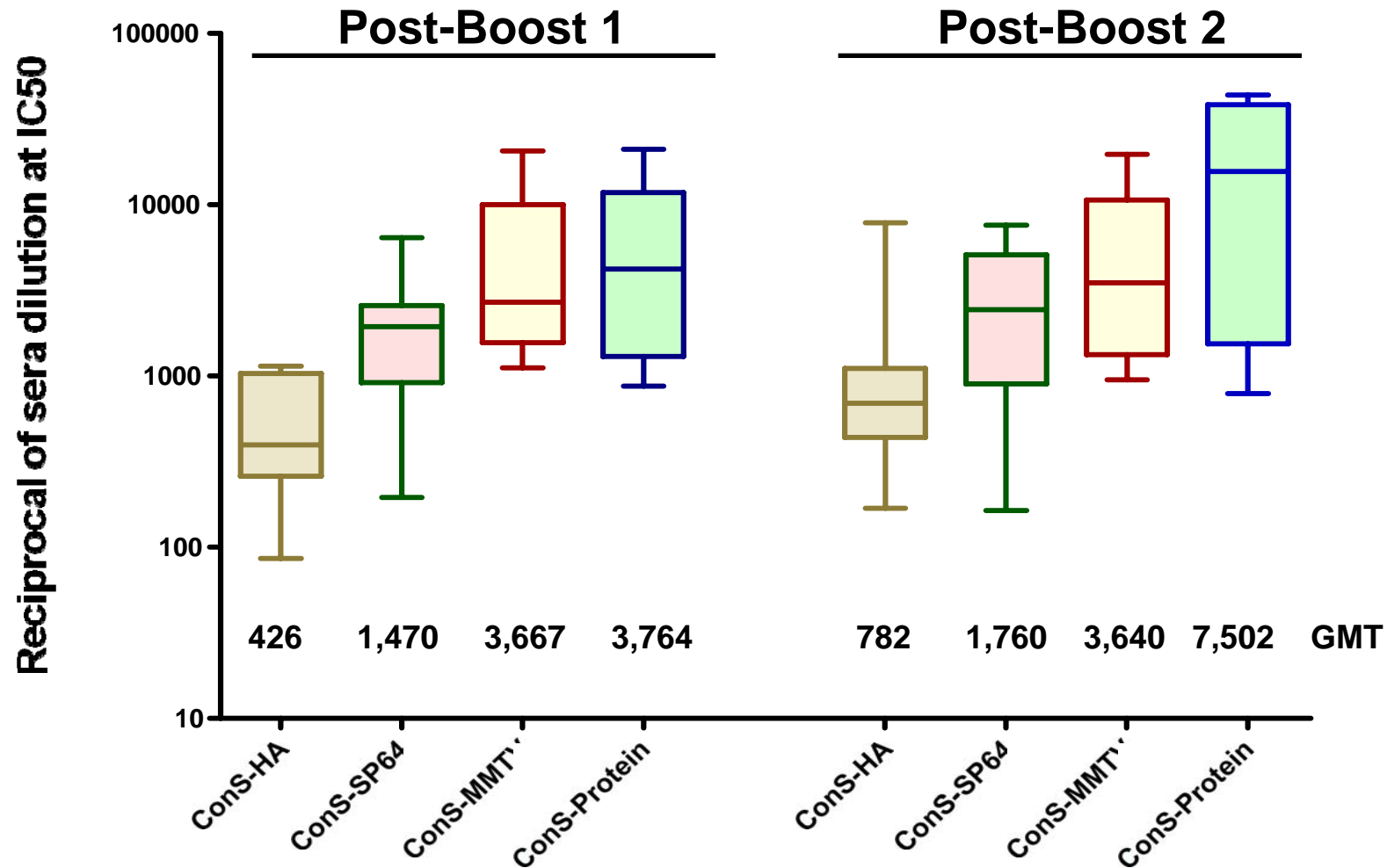
Protein: emulsigen (15% total volume) / CpG (50 μg)

Endpoint gp120 Binding Titers



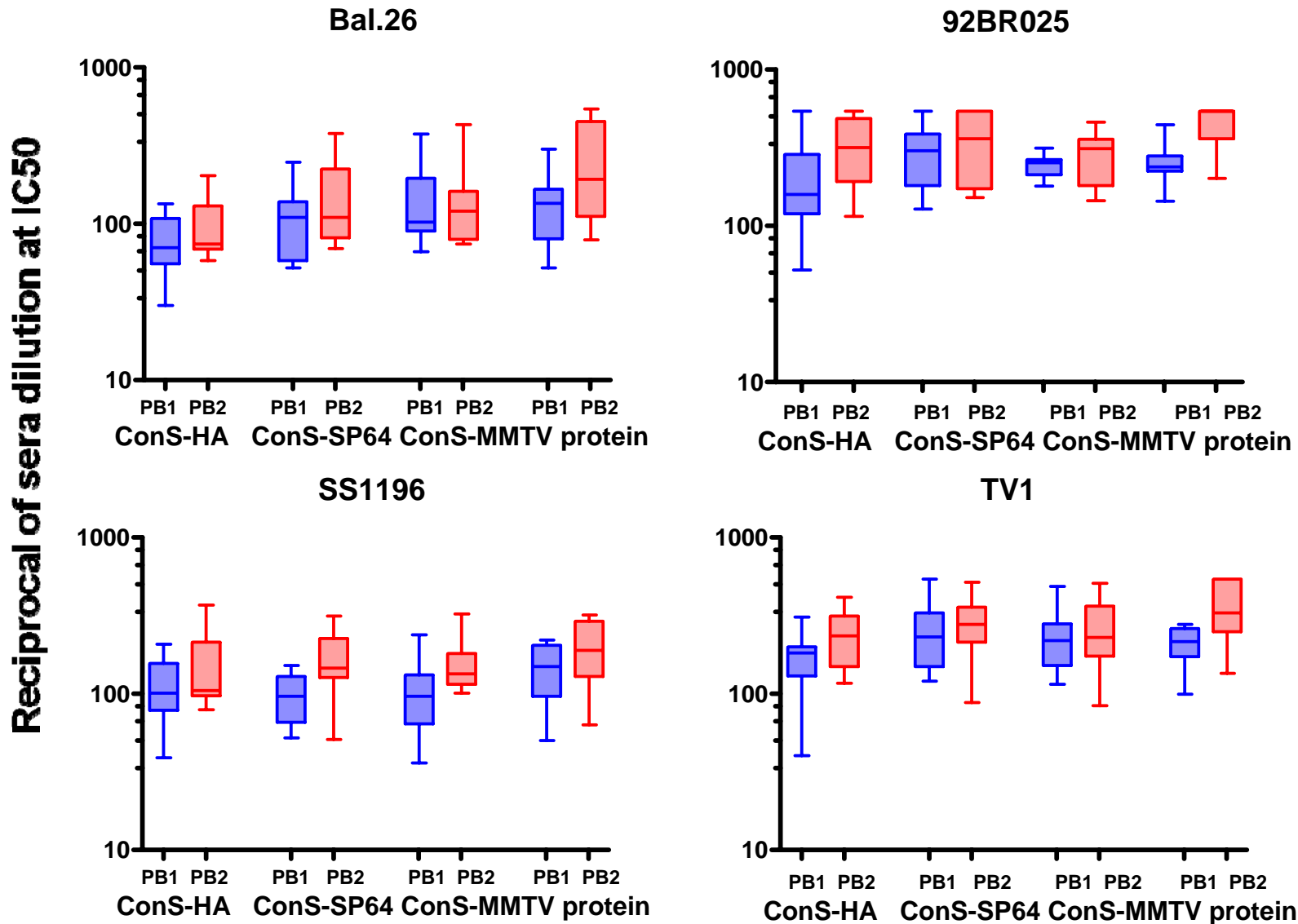
VLP preparations are immunogenic in guinea pigs and elicit high titer gp120 binding antibodies, with geometric mean titers of $> 10^6$.

Serum Neutralizing Activity to SF162



VLP preparations elicit high titer neutralizing antibodies to SF162, with geometric mean IC₅₀ values of > 1: 3,000.

Serum Neutralizing Activity to Tier 1 Viruses



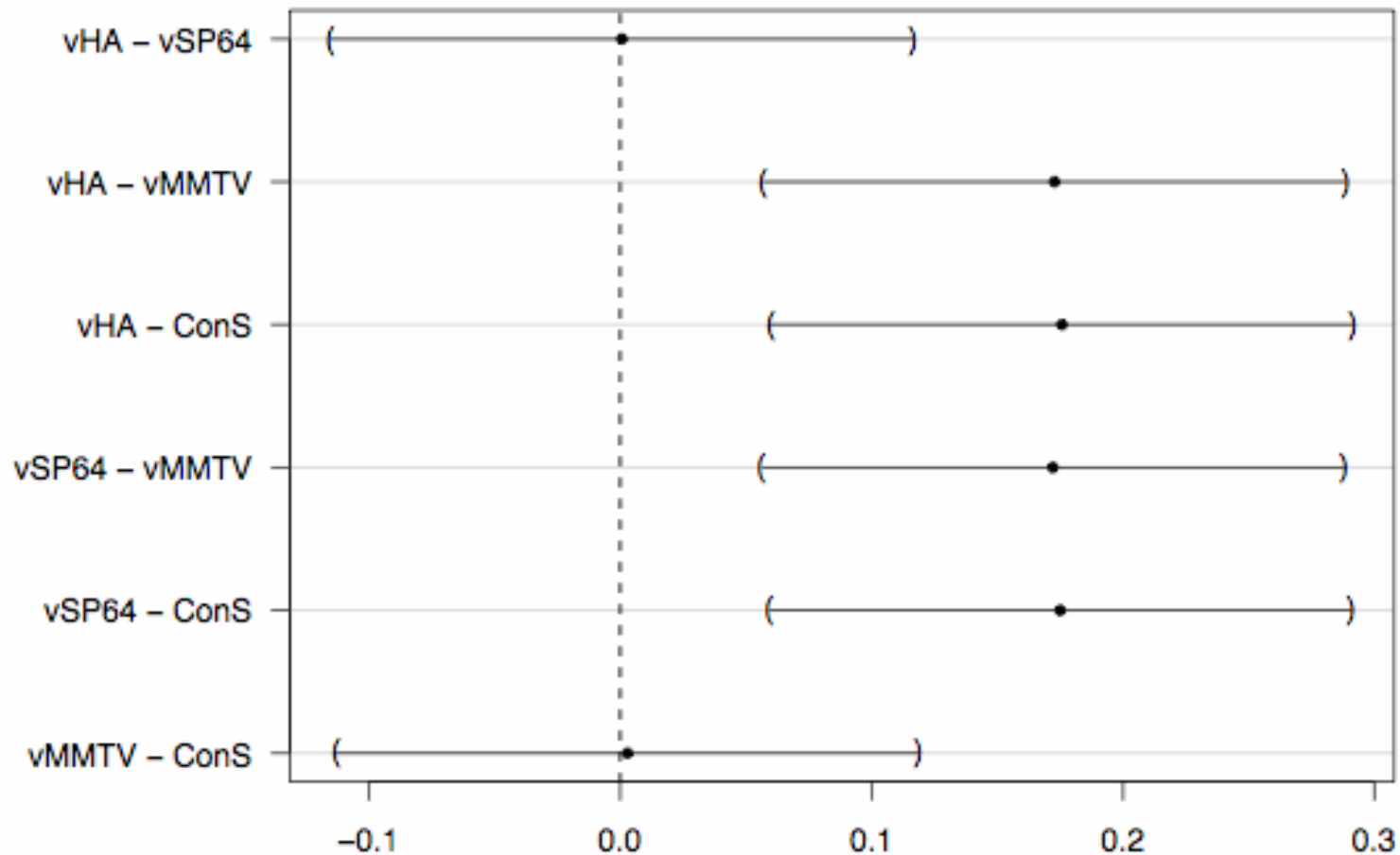
VLP preparations elicit neutralizing antibodies to additional tier 1 viruses, with geometric mean IC₅₀ titers ranging from 71 (Bal) to 311 (92BR025).

Serum Neutralizing Activity to Tier 2 Viruses

	GP	QH0692		PVO		TRO		AC10		THRO4156		CAAN5342		SC422661		ZM214M		ZM53M		CAP45		DU422		DU156	
		PB1	PB2	PB1	PB2	PB1	PB2	PB1	PB2	PB1	PB2	PB1	PB2	PB1	PB2	PB1	PB2	PB1	PB2	PB1	PB2	PB1	PB2	PB1	PB2
ConS-HA	1	0.39	0.41	0.44	0.39	0.35	0.38	0.25	0.34	0.28	0.31	0.42	0.33	0.39	0.36	0.31	0.29	0.30	0.20	0.33	0.33	0.31	0.28	0.34	0.38
	2	0.59	0.65	0.60	0.60	0.38	0.46	0.37	0.45	0.37	0.40	0.36	0.41	0.38	0.48	0.35	0.40	0.37	0.34	0.45	0.53	0.50	0.56	0.34	0.43
	3	0.28	0.41	0.38	0.40	0.17	0.25	0.12	0.19	0.29	0.30	0.30	0.37	0.41	0.45	0.23	0.43	0.04	0.37	0.00	0.27	0.04	0.36	0.33	0.33
	4	0.42	0.42	0.40	0.35	0.45	0.45	0.24	0.25	0.38	0.27	0.44	0.35	0.43	0.39	0.38	0.38	0.28	0.18	0.38	0.27	0.33	0.31	0.36	0.45
	5	0.61	0.50	0.75	0.52	0.50	0.16	0.56	0.23	0.50	0.34	0.53	0.36	0.57	0.45	0.65	0.33	0.62	0.25	0.60	0.32	0.71	0.26	0.68	0.25
	6	0.31	0.21	0.38	0.28	0.36	0.03	0.52	0.07	0.29	0.21	0.28	0.24	0.30	0.20	0.36	0.00	0.15	0.00	0.29	0.09	0.25	0.13	0.41	0.22
	7	0.24	0.39	0.39	0.45	0.42	0.41	0.41	0.26	0.16	0.18	0.23	0.23	0.36	0.37	0.33	0.33	0.07	0.13	0.18	0.16	0.22	0.34	0.36	0.30
	8	0.00	0.23	0.28	0.42	0.02	0.16	0.17	0.24	0.16	0.12	0.22	0.39	0.10	0.37	0.00	0.19	0.00	0.18	0.03	0.22	0.04	0.33	0.23	0.31
ConS-SP64	9	0.00	0.22	0.00	0.04	0.00	0.00	0.00	0.00	0.13	0.13	0.00	0.07	0.09	0.13	0.00	0.01	0.00	0.06	0.00	0.12	0.00	0.03	0.00	0.08
	10	0.19	0.42	0.15	0.39	0.21	0.45	0.04	0.26	0.27	0.44	0.20	0.41	0.22	0.32	0.24	0.28	0.10	0.29	0.09	0.23	0.03	0.27	0.13	0.34
	11	0.49	0.48	0.47	0.42	0.19	0.06	0.17	0.08	0.18	0.31	0.42	0.41	0.33	0.29	0.22	0.14	0.15	0.18	0.35	0.35	0.34	0.34	0.27	0.25
	12	0.44	0.57	0.49	0.68	0.33	0.56	0.22	0.54	0.27	0.41	0.31	0.50	0.45	0.60	0.28	0.52	0.29	0.49	0.21	0.35	0.26	0.55	0.28	0.62
	13	0.53	0.63	0.41	0.57	0.39	0.47	0.32	0.39	0.53	0.52	0.32	0.46	0.35	0.46	0.34	0.46	0.37	0.48	0.43	0.44	0.27	0.42	0.26	0.41
	14	0.56	0.46	0.45	0.45	0.40	0.46	0.35	0.34	0.30	0.27	0.44	0.26	0.58	0.36	0.50	0.34	0.66	0.51	0.39	0.36	0.41	0.33	0.42	0.25
	15	0.39	0.22	0.35	0.23	0.25	0.18	0.14	0.07	0.15	0.08	0.11	0.09	0.18	0.11	0.15	0.01	0.07	0.00	0.25	0.13	0.28	0.09	0.11	0.01
	16	0.32	0.44	0.22	0.35	0.28	0.31	0.22	0.19	0.31	0.35	0.20	0.27	0.29	0.29	0.23	0.33	0.12	0.03	0.15	0.28	0.10	0.17	0.20	0.10
ConS-MMTV	17	0.36	0.76	0.28	0.81	0.19	0.66	0.27	0.76	0.29	0.58	0.31	0.63	0.27	0.72	0.26	0.72	0.00	0.61	0.29	0.74	0.12	0.75	0.24	0.75
	18	0.21	0.46	0.00	0.21	0.10	0.03	0.00	0.03	0.29	0.25	0.19	0.23	0.25	0.34	0.15	0.17	0.08	0.09	0.07	0.34	0.00	0.29	0.14	0.21
	19	0.40	0.51	0.23	0.40	0.24	0.12	0.28	0.19	0.35	0.22	0.33	0.27	0.38	0.33	0.21	0.24	0.17	0.11	0.24	0.39	0.12	0.30	0.25	0.21
	20	0.08	0.06	0.13	0.01	0.14	0.18	0.14	0.12	0.20	0.07	0.00	0.00	0.16	0.18	0.18	0.12	0.00	0.00	0.01	0.00	0.00	0.00	0.17	0.12
	21	0.42	0.37	0.42	0.39	0.41	0.35	0.43	0.30	0.51	0.46	0.33	0.28	0.48	0.37	0.39	0.36	0.32	0.20	0.34	0.31	0.32	0.35	0.41	0.31
	22	0.45	0.80	0.41	0.82	0.18	0.67	0.26	0.74	0.43	0.66	0.36	0.70	0.41	0.76	0.33	0.78	0.20	0.56	0.35	0.69	0.30	0.74	0.21	0.70
	23	0.61	0.66	0.57	0.62	0.39	0.51	0.51	0.56	0.40	0.42	0.47	0.53	0.22	0.14	0.45	0.47	0.41	0.34	0.56	0.61	0.49	0.52	0.34	0.39
	24	0.14	0.34	0.17	0.25	0.00	0.04	0.05	0.00	0.15	0.19	0.12	0.23	0.27	0.35	0.13	0.15	0.00	0.27	0.15	0.19	0.21	0.21	0.32	0.21
ConS protein	25	0.39	0.53	0.27	0.48	0.28	0.37	0.22	0.49	0.40	0.52	0.18	0.35	0.21	0.51	0.25	0.48	0.07	0.43	0.30	0.41	0.19	0.53	0.13	0.36
	26	0.54	0.66	0.53	0.65	0.50	0.63	0.50	0.57	0.63	0.67	0.46	0.52	0.44	0.57	0.50	0.64	0.33	0.51	0.46	0.60	0.41	0.55	0.41	0.53
	27	0.33	0.40	0.32	0.47	0.27	0.26	0.25	0.35	0.24	0.25	0.50	0.38	0.36	0.42	0.34	0.39	0.23	0.24	0.29	0.33	0.23	0.35	0.27	0.29
	28	0.29	0.69	0.22	0.68	0.21	0.70	0.00	0.47	0.23	0.62	0.24	0.60	0.21	0.59	0.25	0.54	0.05	0.41	0.28	0.64	0.09	0.59	0.08	0.49
	29	0.40	0.46	0.41	0.41	0.35	0.30	0.44	0.38	0.00	0.29	0.03	0.49	0.06	0.51	0.36	0.39	0.18	0.35	0.37	0.37	0.31	0.39	0.13	0.56
	30	0.00	0.28	0.00	0.25	0.00	0.17	0.00	0.26	0.09	0.26	0.00	0.22	0.16	0.39	0.05	0.31	0.00	0.17	0.00	0.18	0.00	0.24	0.00	0.28
	31	0.52	0.45	0.54	0.41	0.46	0.28	0.52	0.32	0.56	0.41	0.46	0.35	0.56	0.48	0.47	0.34	0.30	0.21	0.44	0.27	0.48	0.28	0.43	0.37
	32	0.27	0.46	0.19	0.32	0.15	0.25	0.02	0.14	0.24	0.42	0.25	0.33	0.36	0.48	0.19	0.40	0.09	0.31	0.16	0.33	0.02	0.32	0.09	0.26

A select number of VLP (and ConS protein) immunized animals develop low titer neutralizing antibodies (1:20 serum dilution) to tier 2 viruses.

Relative Potency of VLP and Protein Immunogens



The MMTV chimera is more potent than the HA and SP64 chimeras at inducing neutralizing antibodies to tier 2 viruses; the potencies of MMTV VLPs and ConS gp140 protein are equivalent.

Mark Muldoon

Results

- rBV derived HIV-1 VLPs containing chimeric Envs are similar to HIV-1 virions in morphology and size, but incorporate considerably more envelope glycoprotein, with Gag/Env molar ratios as low as ~3.2.
- The Env content of purified VLP preparations usually exceeds 200 $\mu\text{g/ml}$.
- rBV derived HIV-1 VLPs elicit high titer gp120 binding ($>10^6$) as well as cross-clade neutralizing antibodies against tier 1 viruses (SF162, Bal, SS1196, TV1 and 92BR025) in guinea pigs, with geometric mean (IC_{50}) titers ranging from 71 (Bal) to 3,667 (SF162).
- HIV-1 VLPs also elicit neutralizing antibodies to tier 2 viruses. Although this response is only modest (observed in only a subset of animals at a 1:20 serum dilution), is similar in breadth and magnitude to that of animals immunized with a potent (soluble) oligomeric gp140 protein.
- Of the three types of VLP preparations analyzed, the MMTV chimera is significantly more potent at inducing neutralizing antibodies to tier 2 viruses than the HA and SP64 chimeras.

Conclusions

- When primed with DNA, non-adjuvanted HIV-1 Gag/Env VLPs are at least as potent as CpG adjuvanted gp140 oligomeric Env protein with respect to eliciting neutralizing antibody responses to both tier 1 and tier 2 viruses.
- rBV derived HIV-1 VLPs thus represent a new attractive delivery platform that should be further evaluated as a potential component of future AIDS vaccines.

Collaborators

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