

The Effect of Different Glycoprotein Coats on Alphavirus-Replicon Particle Immunogenicity

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Abstract

We have used a propagation-defective, single cycle, RNA replicon vector system derived from an attenuated strain of Venezuelan equine encephalitis (VEE) virus, to produce virus-like replicon particles (VRP) packaged with a number of different VEE-derived glycoprotein (GP) coats. The GP coat is responsible for the cellular tropism noted for VRP and it is believed that different VEE GP coats may have different cellular tropisms. Because wild type VEE has a known dendritic cell tropism it is possible that VRP, expressing a gene of interest (GOI), packaged with different GP coats may have a different cellular tropism and induce different levels of GOI specific immune responses. We prepared VRP packaged with GP coats derived from wild type VEE (V3000), from GP coats from two attenuated VEE infectious clones (V3014 & V3042) and from the GP coat from the current VEE IND vaccine, TC-83. The genome equivalent (GE) to infectious unit (IU) ratio was determined for each VRP preparation. The VRP packaged with the different VEE GP coats demonstrated widely varying GE/IU ratios. To control for this, mice were immunized based on GE doses. Analysis of the humoral and cellular responses to the expressed GOI indicated that no significant differences were noted for any group of VRP regardless of GP coat. These data suggest that the GP coats studied do not impart to VRP different immunogenicity *in-vivo*.

Introduction

The AlphaVax Platform System is based on an attenuated strain of Venezuelan equine encephalitis (VEE) virus that is genetically re-engineered as a vaccine vector. Alphavaccines are being developed for a number of infectious disease and tumor vaccine targets. The system is based on the replication machinery of VEE virus (Figure 1) and consists of a replicon RNA vector and helper nucleic acid(s) (Figure 2). Because the replicon RNA does not contain the structural genes for VEE, it is a single-cycle, propagation-defective RNA and replicates only within the cell into which it is introduced.

Figure 1. Organization & Replication of the Alphavirus Genome

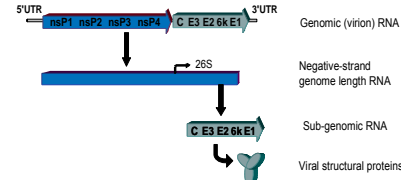


Figure 1. Organization and replication of the alphavirus genome. Following the introduction of positive strand RNA into the cell cytoplasm, the nonstructural proteins are translated and form a replication complex. This replication complex utilizes the genomic RNA as template for the generation of negative strand (antigenomic) RNA. The negative strand RNA serves as a template for replication of additional genomic RNA, and for synthesis of a subgenomic messenger RNA (26S mRNA) which directs the synthesis of the VEE structural proteins, capsid (C) protein and the two envelope glycoproteins E1 and E2.

Figure 2. Alphavaccine Production

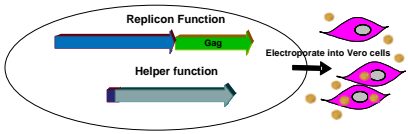
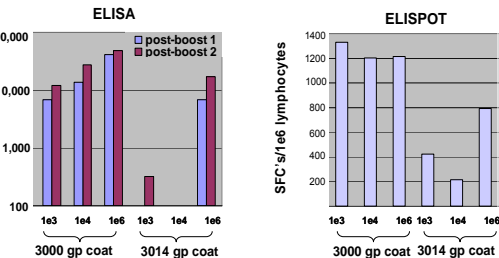


Figure 2. Packaging of the replicon RNA into VRP is accomplished by the co-transfection of the replicon and helper nucleic acid (encoding the capsid and GP) into Vero cells. The structural proteins efficiently package the replicon RNA into VRP. Alphavaccines are harvested and purified at 18-24 hours post-electroporation.

The GP found in the envelop of VRP are believed to play an important role in tissue tropism. The VRP tissue tropism may in turn affect the type or magnitude of immune response elicited to the antigen that is expressed from the replicon vector. Previously we have noted that VRP packaged with different GP coats induce quantitatively different immune responses and an example is shown in Figure 3. In this study, mice were immunized based on VRP titers determined in a Vero cell-specific infectious unit assay. The VRP that were used expressed the HN/Gag gene and the Gag specific ELISA and ELISPOT results are shown. The results suggest that VRP packaged with the V3000 GP coat are more immunogenic than VRP packaged with the V3014 GP coat.

Figure 3. Apparent VRP Immunogenicity Imparted by Different GP Coats



Question

We were interested in determining whether different VEE GP coats may impart higher immunogenicity to VRP expressing a gene of interest. There are a number of well characterized amino acid mutations in the VEE GP that impart an attenuating phenotype to live VEE virus and a few are shown in Figure 4. Because it is possible for different GP coats to have different cellular tropisms it is also possible that different GP coats may alter particle to infectious unit ratios to the VRP packaged with them. In order to control for this possibility BALB/c mice were immunized based on genome equivalents (GE) rather than Vero cell-specific infectious units (IU). Using this approach we could determine whether the immune response that was detected in immunized mice was due to the presence of a population of VRP that were not scored in the infectivity assay (on Vero cells) but are ineffective *in vivo*.

Figure 4. Summary of Amino Acid Differences Between Different VEE GP Coats

	E3	E2	6K	E1
	E2_120	E2_209	E2_239	E2_323
	E1_81	E1_253	E1_272	
V3000	T	E	I	G
V3014	T	K	N	G
TC83	R	E	N	E
V3042	T	E	I	G

Figure 5. Production of Gag VRP Packaged with Various GP Coats

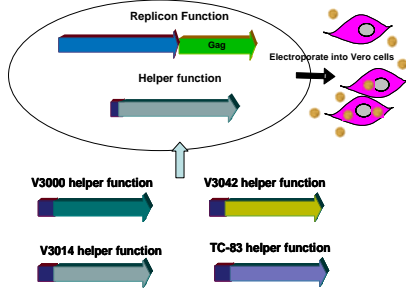


Figure 5. Replicon RNA was packaged into VRP by co-transfection of the Gag replicon RNA and helper nucleic acid (capsid and GP) into Vero cells. The GP helper function was changed during each production run to generate VRP with the different GP coats.

Results

Table 1. Comparison of Infectious units (Vero cell) and Genome Equivalents (Q-PCR)

Construct	IU Titer (Vero)	GE Titer (Q PCR)	GE/IU
3014	4.90E+09	2.22E+11	45
TC83	3.00E+10	6.92E+11	23
V3000	3.90E+08	1.40E+12	3590
V3042	5.30E+08	6.60E+11	1245

Amplimers specific for the nsP2 gene were used to quantify genome equivalents present in VRP vaccines

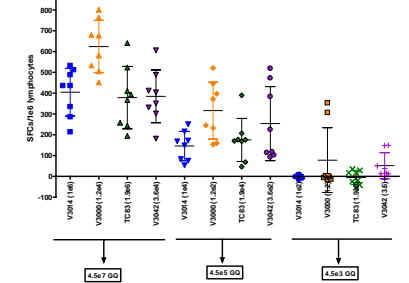
Table 2. Mouse Immunogenicity Study With Immunizing Dose Based on Genome Equivalents

Group #	GP Coat	GE Dose	IU Dose	# animals
1	3014	4.50E+07	1.00E+06	24
2	3000	4.50E+07	1.20E+04	24
3	TC-83	4.50E+07	1.90E+04	24
4	3042	4.50E+07	3.60E+04	24
5	3014	4.50E+05	1.00E+04	24
6	3000	4.50E+05	1.20E+02	24
7	TC-83	4.50E+05	1.90E+04	24
8	3042	4.50E+05	3.60E+02	24
9	3014	4.50E+03	1.00E+02	24
10	3000	4.50E+03	1.2	24
11	TC-83	4.50E+03	1.90E+02	24
12	3042	4.50E+03	3.6	24
Total Animals =				288

Three GE VRP doses were tested to increase the possibility of detecting differences in immunogenicity between GP coats. Mice were immunized at 0, 3 and 6 weeks. Gag-specific Antibody and T cell responses were monitored 1 week after each immunization.

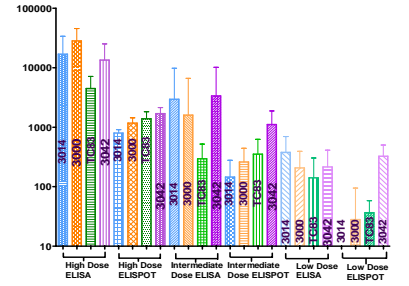
Eight animals from each dosage and GP coat group were sacrificed and splenocytes collected 1 week after the priming dose. Gag-specific INF γ ELISPOT analysis was carried out on individual spleens and the results are shown in Figure 6. The mean (+/- 1 SD) and individual spot forming cells (SFC) per 1×10^6 splenocytes are represented. No significant difference in INF γ ELISPOT response was noted within a dosage group although there was a reduction in SFC detected between dosage groups.

Figure 6. GP Coat Study ELISPOT 1 Week Post Prime



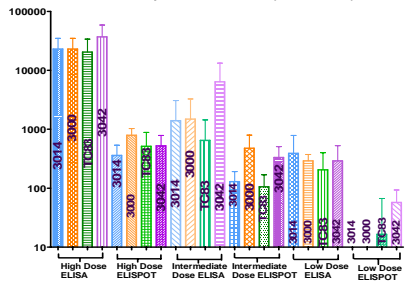
Eight animals from each dosage and GP coat group were sacrificed and splenocytes collected 1 week after the first VRP boost. Gag-specific INF γ ELISPOT and ELISA analysis was carried out and the results are shown in Figure 7. The mean (+1 SD) Gag-specific ELISA and INF γ ELISPOT responses are depicted.

Figure 7. GP Coat Study ELISA & ELISPOT (Post Boost 1)



Eight animals from each dosage and GP coat group were sacrificed and splenocytes collected 1 week after the second VRP boost. Gag-specific INF γ ELISPOT and ELISA analysis was carried out and the results are shown in Figure 8. The mean (+1 SD) Gag-specific ELISA and INF γ ELISPOT responses are depicted.

Figure 8. GP Coat Study ELISA & ELISPOT (Post Boost 2)



Summary

- Previous immunogenicity studies with VRP suggested a link between immunogenicity and the GP coat used to package the VRP
 - Immunizing dose in these studies was normalized on infectious titer (in cells)
- We have shown that particle to infectious unit ratios (GE/IU) vary between VRP packaged with different VEE GP coats
 - V3000>V3042>V3014>TC-83
- If VRP dose is normalized based on genome equivalents no significant differences in immunogenicity are noted in mice
 - Humoral or Cellular