

VLPs are unique when considering their advantages, including safety, ease of production and administration, lack of interference by an immunodominant vector backbone, concern of prior vector immunity, and the presentation of the relevant filovirus antigens in their native form.

#### Example 16

**[0153]** Our experiments have shown that small rodents and non-human primates VLP vaccine recipients require MARV or EBOV GP to be present for protection (Swenson, D. L., 2005, Vaccine 23, 3033-3042). MARV and EBOV VLPs, containing GP, stimulate dendritic cells and monocytes (Bosio C M, 2004, Virology 326, 280-287; Ye L, 2006, Virology 351, 260-270). They also generate high (greater than 1:10,000 titer) antibody responses in vaccinated and boosted mice (unpublished observation). Both GPs of EBOV and MARV contain GP1 and GP2 domains fused at a furin cleavage site; however, EBOV GP2, unlike MARV GP2, trimerizes on the surface of viral particles (Han Z, 2007, Virus Genes 34, 273-81). Neutralizing antibodies to MARV and EBOV GP are protective and CD8 T cell responses generated against epitopes to MARV and EBOV GP prevent disease in small rodent animal models. Since single agent filoviral VLP-based vaccine are efficacious, multi-agent filoviral VLP vaccines could additionally scale down vaccine production, deliver non-interfering immunity to MARV and EBOV, and eliminate pre-distribution testing of several vaccine lots. Importantly, there is so far no candidate vaccine targeting marburgviruses that protects simultaneously against an ebolavirus, or vice versa. More so, there is no vaccine candidate that confers protection to the diverse ebolavirus species such as ZEBOV and SEBOV.

**[0154]** We have generated chimeric filoviral glycoproteins (GP) by fusing MARV and EBOV GP1 and GP2 subunits. Each chimeric MARV/EBOV construct was co-expressed with EBOV VP40 to form VLPs. Guinea pigs were immunized with chimeric VLPs and challenged with MARV or EBOV. We discovered that guinea pigs vaccinated with VLPs expressing MARV GP1 and EBOV GP2 were fully protected when challenged with lethal MARV and EBOV. MARV and EBOV neutralization and complement fixing antibody titers were detected in vaccinated/protected animals. Overall, protective immunity to MARV and EBOV can be attained by vaccination with one of each virus' GP subunit.

**[0155]** The following Material and Methods were used in this example.

**[0156]** Making Chimeric GP1/GP2. Chimeric GP proteins were constructed by swapping the GP1 and GP2 subunits between EBOV strain Zaire and MARV strain Musoke. Two chimeras were made: EBOV-GP1 with MARV-GP2, and MARV-GP1 with EBOV-GP2. As controls, the GP1 and GP2 portions of EBOV and MBGV were also cloned with the same silent restriction site (PvuI), creating wild-type molecules EBOV-GP1 and GP2 and MBGV-GP1 and GP2 (FIG. 22). All VLPs were made in mammalian 293E cells grown in 50/50 Invitrogen Freestyle 293 medium (Invitrogen Cat# 12338) and HyClone HyO SFM4HEK293 medium (HyClone Cat# SH30521.02). 0.75 ug Ebola VP40, NP, MARVGP1/EBOVGP2, EBOVGP1/MARVGP2, EBOVGP1/2, or MARVGP1/2 DNA was used per mL of culture media. Following the addition of DNA to cells, a 5 to 1 ratio of polyethylenimine (1 mg/mL) to total DNA was added and incubated for 15 minutes at room temperature. The polyethylenimine-DNA mixture was then added to cell suspension, currently growing

in 125 mLs Freestyle medium, and incubated for 4 hours at 37° C. for transfection to occur. Following transfection 125 mLs of HyClone HEK293 medium was added to roller-bottles, and VLPs from supernatants were harvested 72 hours later. Ebola VLPs were purified by banding on sucrose gradients as previously described (Warfield et al., 2003, PNAS USA 100, 15889-15894). Purified VLPs were suspended at 2 mg/mL and analyzed by western blot and electron microscopy (EM).

**[0157]** Vaccination with VLPs. Hartley guinea pigs, weighing approximately 400 g (Charles River, Wilmington, Mass.), were housed at USAMRIID animal facilities while being immunized with VLPs. Each set of VLPs were mixed with titermax gold adjuvant (1:1) (Sigma St Louis, Mo.) until fully immersed. A dosage of 150 ug MARVGP1/EBOVGP2, EBOVGP1/MARVGP2, MARVGP1/2, EBOVGP1/2 VLPs, or saline in a volume of 0.5 cc was given intramuscularly to each guinea pig. All guinea pigs were boosted 14 days later with the same quantity of VLPs.

**[0158]** EBOV and MARV challenge. 30 days after receiving the second immunization all guinea pigs were placed in a biosafety level 4 containment suite and challenged with 1000 pfu guinea pig adapted EBOV Zaire Maying a or MARV Musoke via intraperitoneal route. Weights and clinical scores were monitored daily. Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adhered to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

**[0159]** ImmunoEM. VLP particles obtained by ultracentrifugation of the supernatants of 293T cells transfected with both MARVGP1/EBOVGP2 or EBOVGP1/MARVGP2 and VP40 were treated with 1:200 dilutions of mouse anti-EBOV GP1 and guinea pig anti-MARV GP2 or mouse anti-MARVGP1 and guinea pig anti-EBOVGP2 antibodies. Goat anti-mouse (15 nm) and anti-guinea pig (10 nm) immunogold labeled secondary antibodies (Ted Pella, Redding, Calif.) were added to each sample. Both samples were negatively stained with 2% aqueous uranyl-acetate (Electron Microscopy Sciences, Hatfield, Pa.) to reveal ultrastructure. The Joel 1011 transmission electron microscope was used to examine samples at 80 kv.

**[0160]** VLP Western Blots. VLPs were added to gradient (4-12%) Bis Tris gels and proteins were separated by SDS PAGE. Proteins were then transferred to PVDF membranes and probed with EBOV or MARV GP1 specific monoclonal antibodies. EBOV and MARV GP2 were detected with GP2 specific guinea pig polyclonal anti-sera. EBOV specific monoclonal antibodies were used to detect EBOV GP1 or GP2 on VLPs. All subsequent antibody additions were done using the ECF staining kit according to manufacture's instructions (Amersham Piscataway, N.J.). Standard MARV GP1/2 and EBOV GP1/2 VLPs were used as positive or negative controls depending on the primary antibody's specificity.

**[0161]** EBOV and MARV specific antibody titers. ELISAs were performed by coating PVC 96-well plates with inactivated EBOV strain Zaire or MARV strain Musoke antigen overnight at 4° C. Pre and Post-infection sera was diluted from 1:160 to 1:10240 and added to each plate in duplicate. Normal guinea pig sera from unvaccinated animals were used