GENERATION OF VIRUS-LIKE PARTICLES AND USE AS PANFILOVIRUS VACCINE

[0001] This application is a continuation-in-part of U.S. application Ser. No. 12/590,368 filed on Nov. 6, 2009, which is a divisional of U.S. application Ser. No. 11/105,031 filed on Apr. 13, 2005, now U.S. Pat. No. 7,682,618, issued Mar. 23, 2010, which is a continuation in part of application Ser. No. 10/289,839, filed on Nov. 7, 2002, now abandoned, which claims benefit of priority under 35 U.S.C. 119 (e) from U.S. Application Ser. No. 60,338,936 filed on Nov. 7, 2001, Provisional application 60/562,800 and 60/562,801 filed on Apr. 13, 2004, now expired, all of which are herein incorporated by reference in their entirety.

INTRODUCTION

[0002] The filoviruses Ebola (EBOV) and Marburg (MBGV) are two of the most pathogenic viruses in humans and non-human primates (Feldman and Klenk, 1996, Adv. Virus Res. 47, 1), which cause a severe hemorrhagic fever (Johnson et al., 1997, Lancet 1, no. 8011, P. 569). The mortality rates associated with infections of Ebola or Marburg virus are up to 90% (Feldman and Klenk, 1996, supra; Johnson et al., 1997, supra). Although natural outbreaks have been geographically restricted so far, limited knowledge of the mechanisms of pathogenicity, potential of aerosol transmission (Jaax et al., 1995, Lancet 346, no. 8991-8992, 1669), unknown natural reservoir, and lack of immunological and pharmacological therapeutic measures, pose a challenge to classification of the public health threat of Marburg and Ebola viruses.

[0003] Currently, there are no vaccines or therapeutics available to prevent or treat filovirus infections. Classical, subunit, DNA, and vector-based vaccine strategies have been tested for protective efficacy against filovirus challenge in rodents and nonhuman primates (reviewed in Hevey et al., 1997, Virology 239, 206-16; Hevey et al., 2001, Vaccine 20, 586-93). Several vaccine candidates, including DNA, liposome-encapsulated inactivated virus, Venezuelan equine encephalitis virus replication-deficient particles (VRP) expressing filovirus proteins, have been used with varying degree of success in the mouse and guinea pig models of filovirus infection (Hevey et al., 1997, supra; Hevey et al., 1998, Virology 251, 28-37; Pushko et al., 2000, Vaccine 19, 142-153; Rao et al., 2002, J. Virol. 76, 9176-85; Vanderzanden et al., 1998, Virology 246, 134-144; Wilson et al., 2001, Virology 286, 384-90; Wilson and Hart, 2001, J. Virol. 75, 2660-4). For protection against MARV infection, a VRP vaccine encoding MARV GP was completely efficacious in both guinea pigs and nonhuman primates (Hevey et al, 1998, supra; Hevey et al., 2001, supra). Additionally, vaccinating guinea pigs or nonhuman primates with a DNA vaccine encoding GP or purified GP is only partially protective against MARV challenge (Hevey et al., 1997, supra; Hevey et al., 2001, supra; Riemenschneider et al., 2003, Vaccine 21, 4071-80). Administration of DNA vaccine encoding GP followed by $>10^{10}$ plaque-forming units (pfu) of a replication-defective, adenovirus-vectored vaccine expressing GP or the adenovirus vaccine alone expressing GP and nucleoprotein (NP) protects nonhuman primates against EBOV challenge (Nabel, G. J., 2003, Virus Res. 92, 213-17; Sullivan et al., 2003, Nature 424, 681-4; Sullivan et al., 2000, Nature 408, 605-9). Collectively, these efforts indicate that protection against lethal filovirus infection is attainable. Unfortunately, questions remain about many of the vaccine strategies used thus far, including acceptable vaccine doses, safety considerations, the impact of prior immunity to the vaccine vector, and the ability of these vaccine strategies to cross-protect against multiple strains of EBOV and MARV (Hart, M. K., 2003, Vaccine research efforts for filoviruses. International Journal for Parasitology 33, 583-595; Hevey et al., 2001, supra; Hevey et al., 2001, supra; Yang et al., 2003, J. Virol. 77, 799-803). Therefore, alternate approaches to filovirus vaccines are still needed.

[0004] Efforts to develop therapeutics against Ebola and Marburg have been hampered, in part, by poor understanding of the process of filovirus entry and budding at the molecular level. Understanding the nature of interactions between filoviruses and the host, both at the cellular and organism levels, is essential for successful development of efficacious prophylactic and therapeutic measures.

[0005] Both entry and release of enveloped virus particles are dependent on an intimate interaction with components of the cellular membranes. While the plasma membrane was initially envisioned as a fluid, randomly arranged lipid bilayer with incorporated proteins, recent advances demonstrate that this important cellular barrier is more sophisticated and dynamic than portrayed by the original simplistic models. Cholesterol-enriched regions in the lipid bilayer have been recently defined that adopt a physical state referred to as liquid-ordered phase displaying reduced fluidity and the ability for lateral and rotational mobility (Simons and Ikonen, 1997, Nature 387, 569; Brown and London 1998, Annu. Rev. Cell Dev. Biol. 14, 111). These low density, detergent-insoluble microdomains, known as lipid rafts, accommodate a selective set of molecules such as gangliosides, glycosphingolipids, glycosylphosphatidylinositol (GPI) anchored proteins, and signaling proteins such as Src family kinases, T and B cell receptors, and phospholipase C (Simons and Ikonen, 1997, supra; Brown and London 2000, J. Biol. Chem 275, 17221; Simons and Toomre, 2000, Nature Rev. 1, 31; Aman and Ravichandran, 2000, Cur. Biol. 10, 393, Xavier et al., 1998, Immunity 8, 723). By virtue of these unique biochemical and physical properties, lipid rafts function as specialized membrane compartments for channeling certain external stimuli into specific downstream pathways (Cheng et al., 2001, Semin. Immunol. 13, 107; Janes et al., 2000, Semin. Immunol. 12, 23), act as platforms in cell-cell interactions (Viola et al., 1999, Science 283, 680; Moran and Miceli, 1998, Immunity 9, 787), and have also been implicated in membrane trafficking (Brown and London, 1998, supra; Verkade and Simons, 1997, Histochem. Cell Biol. 108, 211). Lipid rafts are believed to perform such diverse functions by providing a specialized microenvironment in which the relevant molecules for the initiation of the specific biological processes are partitioned and concentrated (Brown and London, 2000, supra). Such compartmentalization may help the signals achieve the required threshold at the physiological concentrations of the stimuli. Partitioning in lipid rafts can also be perceived as a measure to perform functions in a more specific and efficient manner while keeping distinct pathways spatially separated.

[0006] Several lines of evidence suggest a role for cholesterol-enriched lipid rafts in host-pathogen interactions. Cholesterol has been shown to play a critical role for the entry of mycobacterium into macrophages (Gatfield and pieters, 2000, Science 288, 1647). Multiple components of influenza