

virus (Scheiffele et al., 1999, J. Biol. Chem. 274, 2038), measles virus (Manie et al., 2000, J. Virol. 74, 305), and human immunodeficiency virus (HIV) (Nguyen and Hildreth, 2000, J. Virol. 74, 3264; Rousso et al., 2000, Proc. Natl. Acad. Sci. U.S.A. 97, 13523) have been shown to localize to lipid rafts. These lipid platforms have also been implicated in the budding of HIV and influenza virus (Scheiffele et al., 1999, supra; Nguyen and Hildreth, 2000, supra). Therefore, rafts, as tightly regulated specialized domains, may represent a coordination site for the intimate interactions of viral proteins required for the assembly and budding process. While involvement of rafts in virus entry has been postulated (Dimitrov, D. S. 2000, Cell 101, 687), supporting data on this issue have been reported only for HIV infection of certain T cell lines (Manes et al., 2000, EMBO Rep. 1, 190).

[0007] Therefore, there exists a need in the art for elucidation of the factors that affect filovirus assembly and disassembly. There is also a need for an efficient in vitro method for generation of genome-free virus-like particles which are stable, and retain immunogenic properties, i.e., those which present conformational, and more particularly, neutralizing epitopes expressed on the surface of native, intact filovirus.

[0008] Further, there is a need for elucidating the method by which filoviruses enter and exit cells. Once the method is known, treatments and agents for disrupting attachment, fusion or entry of the virus, i.e. infection, can be ascertained.

SUMMARY OF THE INVENTION

[0009] The present invention satisfies the needs discussed above. Using a variety of biochemical and microscopic approaches, we demonstrate the compartmentalization of Ebola and Marburg viral proteins in lipid rafts during viral assembly and budding. Our findings also show that filovirus trafficking, i.e. the entry and exit of filoviruses into and out of cells, is dependent on functional rafts. This study, thus, provides a deeper understanding of the molecular mechanisms of filovirus pathogenicity at the cellular level, and suggests raft integrity and/or raft components as potential targets for therapeutic interventions. We also report, for the first time, the raft-dependent formation of Ebola-based and Marburg-based, genome-free, virus-like particles (VLPs), which resemble live virus in electron micrographs. Such VLPs, besides being a research tool, are useful as vaccines against filovirus infections, and as vehicles for the delivery to cells of a variety of antigens artificially targeted to the rafts.

[0010] Therefore, the present invention relates to filovirus virus-like particles (VLPs) and a method for generating genome-free Ebola or Marburg VLPs in a mammalian transfection system. This method generates VLPs that resemble native virus. The virus-like particles are useful for transferring into a cell a desired antigen or nucleic acid which would be contained in the internal space provided by the virus-like particles.

[0011] It is one object of the present invention to provide a method for generating genome-free filovirus virus-like particles (VLPs), specifically, Ebola and Marburg VLPs. The method includes expression of virus GP and VP40 in cells. The VLP of the present invention are more native in the filovirus-like morphology and more native in terms of the conformation of virus spikes.

[0012] It is another object of the present invention to provide VLP-containing compositions. The compositions con-

tain Ebola VLPs or Marburg VLPs or a combination of Ebola and Marburg VLPs for use as a vaccine, a delivery vehicle and in a diagnostic assay.

[0013] It is yet another object of the invention to provide a vaccine for inducing an immune response to a filovirus, namely Ebola or Marburg, said vaccine comprising Ebola VLP or Marburg VLP, respectively, or a combination of Ebola and Marburg VLPs.

[0014] It is another object of the invention to provide a method for encapsulating desired agents into filovirus VLP, e.g., therapeutic or diagnostic agents.

[0015] It is another object of the invention to provide filovirus VLPs, preferably Ebola VLPs or Marburg VLPs, which contain desired therapeutic or diagnostic agents contained therein, e.g. anticancer agents or antiviral agents.

[0016] It is still another object of the invention to provide a novel method for delivering a desired moiety, e.g. a nucleic acid to desired cells wherein the delivery vehicle for such moiety, comprises filovirus VLP.

[0017] It is another object of the invention to provide a diagnostic assay for the detection of Ebola or Marburg virus infection in a sample from a subject suspected of having such an infection. The method comprises detecting the presence or absence of a complex formed between anti-Ebola antibodies or anti-Marburg antibodies in the sample and Ebola VLPs or Marburg VLPs, respectively.

[0018] It is yet another object of the present invention to use noninfectious filovirus VLP in an in vitro assay for testing the efficacy of potential agents to inhibit or reduce filovirus entry into cells or budding from cells, i.e. infectivity.

[0019] It is another object of the invention to provide a method for identifying critical structural elements within filovirus proteins required for viral assembly and/or release. The method consists of detecting a change in VLP formation, assembly, or budding from a cell expressing filovirus mutant proteins as compared to a cell expressing wild type alleles of such mutations.

[0020] It is further an object of the invention to provide an immunological composition for the protection of mammals against Ebola or Marburg virus infection comprising Ebola or Marburg virus-like particles.

[0021] It is another object of the present invention to provide a method for evaluating effectiveness of an agent or chemical to block entry of filovirus into a cell, said agent or chemical able to alter the cell's lipid rafts, said method comprising introducing said agent or chemical to a cell and monitoring the effect of said agent or chemical by monitoring VLP entry or exit from a cell. Agents include chemicals, cellular agents or factors, and other viral agents.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] These and other features, aspects, and advantages of the present invention will become better understood with reference to the following description and appended claims, and accompanying drawings where:

[0023] FIGS. 1A, 1B, and 1C. Localization of filovirus glycoproteins in lipid rafts. 293T cells were transfected with Marburg GP (A), Ebo-GPwt, or Ebo-GP_{C670/672A} (B), or a control plasmid, rafts were prepared by ultracentrifugation and GP was detected by immunoblotting. GMI was detected by blotting with HRP-CTB in the corresponding fractions spotted on a nitrocellulose membrane, as a control for the quality of raft preparation. (C) 48 h after transfection of 293T cells with Ebola GP, a portion of cells were treated for 20