

FILOVIRUS FUSION PROTEINS AND THEIR USES

FIELD OF THE INVENTION

[0001] This invention relates to the use of Filovirus glycoprotein fusion proteins for the prevention and diagnosis of Filovirus infection.

BACKGROUND OF THE INVENTION

[0002] Ebola virus (EBOV) and Marburgvirus (MARV) are members of the Filoviridae, a family of viruses classified as “Category A” bioterrorism agents that cause severe hemorrhagic fever in humans and nonhuman primates with high morbidity and mortality rates up to 90% (Sanchez et al., *Filoviridae: Marburg and Ebola viruses*, p. 1409-1448. In D. M. Knipe, P. M. Howley, D. E. Griffin, M. A. Martin, R. A. Lamb, B. Roizman, and S. E. Straus (ed.), *Fields Virology*, 5th ed. Philadelphia, Pa.: Lippincott Williams & Wilkins, 2007). After a short incubation period of 4 to 10 days, Filovirus-infected individuals develop an abrupt onset of symptoms that include fever, chills, malaise, and myalgia that are common to many other viral infections. MARV is antigenically stable and exists in only one species, whereas EBOV is more variable and has five species. The Bundibugyo EBOV emerged recently in late 2007 outbreak in Uganda (Towner et al., *PLoS Pathog* 2008 November; 4(11):e1000212), and is more related to the Ivory Coast than to the Zaire, Sudan, or Reston EBOV species. Zaire EBOV (ZEBOV) is typically associated with the highest lethality. The increased number of outbreaks in Africa and the recent EBOV outbreak in pigs (Normile *Science* 2009 Jan. 23; 323(5913):451), which raised concerns that livestock could transmit the deadly disease to humans, highlighted the urgency for the development of vaccines and rapid diagnostic tests to contain outbreaks. Vaccines based on the Filovirus glycoprotein (GP) are in preclinical and clinical evaluation, and currently there are no therapeutic agents to treat Filovirus infection. Since licensing of safe and effective Filovirus vaccine could take several more years, diagnosis and quarantine of infected individuals is currently the main tool to limit outbreaks.

[0003] Several Filovirus vaccine candidates are currently being developed, including recombinant adenovirus expressing the EBOV GP (Sullivan et al., *PLoS Med* 2006 June; 3(6):e177; Sullivan et al., *Nature* 2003 Aug. 7; 424(6949):681-4; and Sullivan et al., *Nature* 2000 Nov. 30; 408(6812):605-9), recombinant parainfluenza virus (Bukreyev et al., *J Virol* 2007 June; 81(12):6379-88), recombinant Venezuelan equine encephalitis virus (Pushko et al., *Vaccine* 2000 Aug. 15; 19(1):142-53), recombinant replication-competent (Feldmann et al., *PLoS Pathog* 2007 January; 3(1):e2 and Jones et al., *Nat Med* 2005 July; 11(7):786-90) and—deficient (Halfmann et al., *J Virol* 2009 April; 83(8):3810-5) vesicular stomatitis virus, and virus-like particles carrying the Filovirus GP (Warfield et al., *Proc Natl Acad Sci USA* 2003 Dec. 23; 100(26):15889-94 and Warfield et al., *J Infect Dis* 2007 Nov. 15; 196 Suppl 2:S430-7). Initial studies using baculovirus-expressed Filovirus GP showed partial protection, which could be attributed to the nature of the glycosylation and processing of GP in insect cells (Mellquist-Riemenschneider et al., *Virus Res* 2003 April; 92(2):187-93).

[0004] Despite advances in this regard, a need exists to develop new vaccines against Filovirus infections. The present invention addresses these and other needs.

BRIEF SUMMARY OF THE INVENTION

[0005] This invention provides fusion proteins comprising a Filovirus glycoprotein segment and an immunoglobulin polypeptide segment. In the typical embodiment, the Filovirus glycoprotein segment is an extracellular domain, for example, from an Ebola virus, particularly, the Zaire Ebola virus, Maying a strain. The immunoglobulin polypeptide segment can be an immunoglobulin heavy chain constant domain polypeptide from IgG1 (e.g., an Fc fragment). In some embodiments, the fusion protein may further comprise a linker between the Filovirus glycoprotein segment and the immunoglobulin polypeptide segment. An exemplary fusion protein of the invention is encoded by a nucleic acid sequence as shown in SEQ ID NO:1 without a linker and SEQ ID NO:3 with a FLAG tag linker.

[0006] The invention further provides immunogenic compositions comprising the fusion protein of the invention. The immunogenic composition may further comprise an adjuvant.

[0007] Also provided are nucleic acid vectors comprising a nucleic acid sequence encoding a fusion protein of the invention. An exemplary nucleic acid sequence is shown in SEQ ID NO: 1.

[0008] The invention further provides methods of inducing a protective immune response against Filovirus infection in a patient. The methods comprise administering to the patient an immunologically effective amount of the immunogenic composition of the invention. The immunogenic composition can comprise the fusion proteins of the invention or nucleic acid molecules encoding them. The composition can be administered by any of a number of routes. In some embodiments, the composition is administered intramuscularly.

[0009] The invention also provides methods of detecting an immune response against Filovirus in a patient. The methods include contacting a biological sample from the patient with the fusion protein of the invention and detecting either a humoral or a cellular immune response. Antibodies can be detected using ELISA, chemiluminescence assays, or fluorescence assays. A cellular immune response can be detected by detecting IFN- γ , TNF- α , or other cytokines and cell activation and proliferation assays.

[0010] Yet another aspect of the invention are methods of detecting ant-Filovirus antibodies (e.g., neutralizing antibodies) in a biological sample from a patient immunized with Filovirus glycoprotein Fc fusion proteins or other immunogens. Such methods comprise contacting the biological sample with a recombinant Vesicular Stomatitis Virus (VSV) expressing a Filovirus GP (e.g., a Zaire Ebola virus, Maying a strain) and assessing residual infectivity in a cell susceptible to Filovirus infection. The cells used in the assay can be Vero E6 cells. Alternatively the recombinant VSV particles can be immobilized on a solid support and anti-Filovirus antibodies can be detected using standard methods, such as ELISA, chemiluminescence assays, or fluorescence assays.

DEFINITIONS

[0011] The terms “adjuvant” and “immune stimulant” are used interchangeably herein, and are defined as one or more substances that cause stimulation of the immune system. In this context, an adjuvant is used to enhance an immune response to the fusion proteins of the invention.

[0012] The terms “amino-terminal” (or “N-terminal”) and “carboxyl-terminal” (or “C-terminal”) are used herein to