

## IMMUNOGENIC COMPOSITIONS AND VACCINES FOR EBOLA

### BACKGROUND OF THE INVENTION

This application is a continuation-in-part of U.S. utility application Ser. No. 10/696,633, filed Oct. 29, 2003 (now U.S. Pat. No. 6,984,504, issued Jan. 10, 2006), which is a continuation application of U.S. utility application Ser. No. 09/337,946, filed Jun. 22, 1999 (now abandoned), which claims priority from U.S. provisional application 60/091,403 (filed Jun. 29, 1998). This application is also a continuation-in-part of U.S. utility application Ser. No. 10/384,976, filed Mar. 10, 2003 (now U.S. Pat. No. 7,267,823, issued Sep. 11, 2007), which is a continuation-in-part application of U.S. utility application Ser. No. 09/337,946, filed Jun. 22, 1999 (now abandoned), which claims priority from U.S. provisional application 60/091,403 (filed Jun. 29, 1998). The entire contents of these applications are incorporated herein by reference.

Ebola viruses, members of the family Filoviridae, are associated with outbreaks of highly lethal hemorrhagic fever in humans and nonhuman primates. The natural reservoir of the virus is unknown and there currently are no available vaccines or effective therapeutic treatments for filovirus infections. The genome of Ebola virus consists of a single strand of negative sense RNA that is approximately 19 kb in length. This RNA contains seven sequentially arranged genes that produce 8 mRNAs upon infection (FIG. 1). Ebola virions, like virions of other filoviruses, contain seven proteins: a surface glycoprotein (GP), a nucleoprotein (NP), four virion structural proteins (VP40, VP35, VP30, and VP24), and an RNA-dependent RNA polymerase (L) (Feldmann et al. (1992) *Virus Res.* 24, 1-19; Sanchez et al., (1993) *Virus Res.* 29, 215-240; reviewed in Peters et al. (1996) *In Fields Virology*, Third ed. pp. 1161-1176. Fields, B. N., Knipe, D. M., Howley, P. M., et al. eds. Lippincott-Raven Publishers, Philadelphia). The glycoprotein of Ebola virus is unusual in that it is encoded in two open reading frames. Transcriptional editing is needed to express the transmembrane form that is incorporated into the virion (Sanchez et al. (1996) *Proc. Natl. Acad. Sci. USA* 93, 3602-3607; Volchkov et al. (1995) *Virology* 214, 421-430). The unedited form produces a nonstructural secreted glycoprotein (sGP) that is synthesized in large amounts early during the course of infection. Little is known about the biological functions of these proteins and it is not known which antigens significantly contribute to protection and should therefore be used to induce an immune response.

Recent studies using rodent models to evaluate subunit vaccines for Ebola virus infection using recombinant vaccinia virus encoding Ebola virus GP (Gilligan et al., (1997) *In Vaccines* 97, pp. 87-92. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.), or naked DNA constructs expressing either GP or sGP (Xu et al. (1998) *Nature Med.* 4, 37-42) have demonstrated the protective efficacy of Ebola virus GP in guinea pigs. (All documents cited herein supra and infra are hereby incorporated in their entirety by reference thereto.) Additionally, Ebola virus NP and GP genes expressed from naked DNA vaccines (Vanderzanden et al., (1998) *Virology* 246, 134-144) have elicited protective immunity in BALB/c mice. There has been one study that showed protection in nonhuman primates with a high dose DNA prime/high dose adenovirus boost and a 6 pfu challenge. However, this study provides limited benefit for humans or non-human primates because such high dosing is unlikely to be given to humans due to high inherent risks and other factors.

VRPs are one platform currently being evaluated for the filoviruses. VRP constructs expressing six Ebola virus proteins (GP, nucleoprotein, VP24, VP30, VP35 and VP40) are efficacious in mice. VRP expressing GP induced protective antibody responses and were used to generate protective monoclonal antibodies (Wilson et al. *Science* 2000.). In contrast, the other proteins induce protective cytotoxic T cell responses. The involvement of cytotoxic T cells in protection presents an immunologic issue (major histocompatibility complex restriction of the presentation of epitopes) not present for pathogens cleared by antibodies.

Non-human primate studies using VRPs to protect against filovirus challenge have had different results. The successful protection of vaccinated cynomolgus macaques against Marburg Musoke virus was the first published success of any filovirus vaccine approach. In one published study, a vaccination schedule ( $1 \times 10^7$  ffu on days 0, 28 and 56) using the VRP expressing Ebola Zaire GP induced rather modest antibody titers and the macaques did not survive challenge with 1000 pfu.

So there still exists a need for a human vaccine which is efficacious for protection from Ebola virus infection.

### SUMMARY OF THE INVENTION

The present invention satisfies the need discussed above. The present invention relates to a method and composition for use in inducing an immune response which is protective against infection with Ebola virus.

The inventors have induced protection against Ebola infection in mammals using virus replicon particles (VRPs) expressing the Ebola GP, NP, VP24, VP30, VP35 or VP40 genes. These VRPs and some uses are described in U.S. utility patent application Ser. No. 09/337,946 (filed Jun. 22, 1999), the entire contents of which are hereby incorporated by reference.

One embodiment of the present invention entails a DNA fragment encoding each of the Ebola Zaire 1976 GP, NP, VP24, VP30, VP35, and VP40 virion proteins (SEQUENCE ID NOS. 1-7).

Another embodiment provides the DNA fragments of Ebola virion proteins in a recombinant vector. When the vector is an expression vector, the Ebola virion proteins GP, NP, VP24, VP30, VP35, and VP40 are produced. It is preferred that the vector is an alphavirus replicon vector, especially a replicon vector that has the ability to produce the desired protein or peptide in a manner that induces protective B and T cells in vivo in mammals. Any alphavirus vector may be effective, including but not limited to the Venezuelan Equine Encephalitis (VEE) virus, eastern equine encephalitis, western equine encephalitis, Semliki forest and Sindbis. For instance, in a preferred embodiment the VEE replicon vector comprises a VEE virus replicon and a DNA fragment encoding any of the Ebola Zaire 1976 (Maying a isolate) GP, NP, VP24, VP30, VP35, or VP40 proteins. In another preferred embodiment, the VEE replicon vector comprises a VEE virus replicon and a DNA fragment encoding any of the amino acid sequences set forth in SEQ ID NOS:24-53. The construct can be used as a nucleic acid vaccine or for the production of self replicating RNA. To that end, a self replicating RNA of this invention can comprise the VEE virus replicon and any of the Ebola Zaire 1976 (Maying a isolate) RNAs encoding the GP, NP, VP24, VP30, VP35, and VP40 proteins described above, or the amino acid sequences set forth in SEQ ID NOS:24-53. The RNA can be used as a vaccine for protection from Ebola infection. When the RNA is packaged, a VEE virus replicon particle is produced.