PUUMALA VIRUS FULL-LENGTH M SEGMENT-BASED DNA VACCINES

This application claims priority from U.S. Provisional Application Ser. No. 60/901,414 filed Feb. 12, 2007. The entire contents of that provisional application are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

Currently, there are four known hantaviruses associated with hemorrhagic fever with renal syndrome (HFRS): Hantaan virus (HTNV), Dobrava-Belgrade virus (DOBV), Puumala virus (PUUV), and Seoul virus (SEOV). Because distinct hantaviruses are usually carried by only one principal rodent host species, their distribution is generally limited to the range of that host (reviewed in Schmaljohn and Hjelle, 1997, *Emerg. Infect. Dis.* 3, 95-104). HTNV, carried by *Apodemus agrarius*, is found in Asia; DOBV, carried by *Apodemus flavicollis*, and PUUV, carried by *Clethrionomys glareolus*, are found in Europe. SEOV is more widely disseminated than any other recognized hantaviruses because its host, the common urban rat (*Rattus norvegicus*), is found throughout the world.

Puumala virus (PUUV), genus *Hantavirus*, family Bunyaviridae, is responsible for the vast majority of hemorrhagic fever with renal syndrome (HFRS) cases in Scandinavia, Europe, and Western Russia. In addition to the HFRS associated hantaviruses, there are also several hantaviruses in North, Central, and South America (e.g. Andes virus [ANDV]) that cause a vascular-leak disease known as *hantavirus* pulmonary syndrome (HPS) (Jonsson, C. B., Hooper J, Mertz G. Treatment of *hantavirus* pulmonary syndrome. Antiviral Research 2007 Nov. 21 Epub ahead of print). Hantaviruses are carried by persistently infected rodents and transmission to humans occurs when persons are exposed to rodent secreta or excreta. This usually occurs when persons inhale aerosolized rodent excreta. There is no Food and Drug Administration (FDA) approved vaccine or specific drug to prevent or treat HFRS or HPS.

Viruses in the *Hantavirus genus* (family Bunyaviridae) are enveloped and contain a genome comprised of three single-stranded RNA segments designated large (L), medium (M), and small (S) based on size (reviewed in Schmaljohn, 1996, In *The Bunyaviridae Ed.* R. M. Elliott. New York, Plenum Press p. 63-90). The *hantavirus* L segment encodes the RNA dependent RNA polymerase, M encodes two envelope glycoproteins (G1 and G2, also known as G_n and G_c), and S encodes the nucleocapsid protein (N).

A number of inactivated HFRS vaccines derived from cell culture or rodent brain were developed and tested in Asia (Lee et al., 1990, *Arch. Virol*., Suppl. 1, 35-47; Song et al., 1992, Vaccine 10, 214-216; Lu et al., 1996, *J. Med. Virol*. 49, 333-335). Drawbacks of these traditional killed-virus vaccines include a requirement for appropriate containment for the growth and manipulation of virus, and the necessity to ensure complete inactivation of infectivity without destroying epitopes on the virion important for protective immunity. In order to overcome these drawbacks, vaccine approaches involving recombinant DNA technology were developed including: vaccinia-vectored vaccines (Schmaljohn et al. 1990, *J. Virol*. 64, 3162-3170; Schmaljohn et al. 1992, Vaccine 10, 10-13; Xu et al. 1992, *Am. Trop. Med. Hyg*. 47, 397-404), protein subunit vaccines expressed in bacteria or insect cells (Schmaljohn et al. 1990, supra; Yoshimatsu et al., 1993, *Arch. Virol*. 130, 365-376; Lundkvist et al., 1996, *Virology* 216, 397-406), and a hepatitis core antigen-based recombinant vaccine (Ulrich et al., 1998, *Vaccine* 16, 272-280). For a revew of *hantavirus* vaccine efforts see the review by Hooper and Li (Hooper and Li, 2001).

Vaccination with vaccinia recombinants expressing the M segment of either HTNV or SEOV elicited neutralizing antibodies and protected rodents against infection with both HTNV and SEOV, suggesting that an immune response to G1-G2 alone can confer protection (Schmaljohn et al. 1990, supra; Xu et al. 1992, supra; Chu et al. 1995, *J. Virol*. 69, 6417-6423). Similarly, vaccination with G1-G2 protein expressed in insect cells (baculovirus recombinant virus system) elicited neutralizing antibodies and protected hamsters from infection with HTNV (Schmaljohn et al. 1990, supra). In both the vaccinia and baculovirus systems, vaccination with G1-G2 provided more complete protection than G1 or G2 alone (Schmaljohn et al. 1990, supra). There are reports that candidate DNA vaccines comprised of around 500 nucleotide stretches of the Sin Nombre virus (SNV) M gene, or the full-length S gene, are immunogenic in mice (Bharadwaj, et al., 1999, Vaccine 17, 2836, 43) and conferred some protection against infection with SNV in a deer mouse infection model (Bharadwaj, et al., 2002, J. Gen. Virol. 83, 1745-1751). The protection was surmised to be cell-mediated because there was no convincing evidence that these constructs elicited a neutralizing, or otherwise protective, antibody response. There have been several publications reporting the successful use of plasmid DNA vaccines containing the full-length M gene of SEOV, HTNV, ANDV, including the following reports:

1. Hooper, J. W., K. I. Kamrud, F. Elgh, D. Custer, and C. S. Schmaljohn (1999). DNA vaccination with *hantavirus* M segment elicits neutralizing antibodies and protects against Seoul virus infection. Virology, 255:269-278.
2. Hooper, J. W., D. Custer, E. Thompson, and C. S. Schmaljohn (2001). DNA Vaccination with the Hantaan virus M gene protects hamsters against three of four HFRS hantaviruses and elicits a high-titer neutralizing antibody response in rhesus monkeys. Journal of Virology 75:8469-8477.
3. Custer, D. M., E. Thompson, C. S. Schmaljohn, T. G. Ksiazek, and J. W. Hooper (2003). Active and passive vaccination against *hantavirus* pulmonary syndrome using Andes virus M genome segment-based DNA vaccine. Journal of Virology 79:9894:9905.
4. Hooper, J. W., D. M. Custer, J. Smith, and Victoria Wahl-Jensen. Hantaan/Andes virus DNA vaccine elicits a broadly cross-reactive neutralizing antibody response in nonhuman primates (2006). Virology 347:208-216.

In all cases high titer neutralizing antibodies were detected in animals (including nonhuman primates) vaccinated with the full-length M gene DNA vaccines, and protection from infection was achieved in rodent models. Neutralizing antibody responses to G1-G2 in the aforementioned vaccine studies correlated with protection, suggesting that neutralizing antibodies not only play an important role in preventing *hantavirus* infection, but also might be sufficient to confer protection. Passive transfer of neutralizing monoclonal antibodies (MAbs) specific to either G1 or G2 protected hamsters against HTNV infection (Schmaljohn et al., 1990, supra; Arikawa et al., 1992, *J. Gen. Virol.* 70, 615-624), supporting the idea that neutralizing antibodies alone can confer protection. This is further supported by the finding that serum from nonhuman primates vaccinated using a gene gun with DNA vaccines containing the HTNV or ANDV full-length M genes protected hamsters from infection with HTNV or lethal disease caused by ANDV Custer, D. M., E. Thompson, C. S. Schmaljohn, T. G. Ksiazek, and J. W. Hooper (2003). Active and passive vaccination against *hantavirus* pulmonary syn-