

EXTRANEOUS DNA SEQUENCE THAT FACILITATES HANTAVIRUS GENE EXPRESSION

This is a continuation-in-part application of U.S. Ser. No. 09/491,974 (now abandoned) filed on Jan. 27, 2000 which claims priority from U.S. Provisional Application Ser. No. 60/117,680 filed Jan. 29, 1999. This application also claims benefit from an earlier filed Provisional Application Ser. No. 60/367,128 filed on Mar. 22, 2002 and Provisional Application Ser. No. 60/398,985 filed on Jul. 26, 2002.

INTRODUCTION

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 hantavirus because its host, the common urban rat (*Rattus norvegicus*), is found throughout the world.

New-World hantaviruses have been associated with outbreaks of a highly lethal disease, hantavirus pulmonary syndrome (HPS), in the Americas (reviewed in Schmaljohn and Hjelle, 1997, *Emerg. Infect. Dis.* 3, 95–104). The disease is characterized by fever and vascular leakage resulting in non-cardiogenic pulmonary edema followed by shock. Case-fatality for HPS caused by the most prevalent North American and South American hantaviruses, Sin Nombre virus (SNV) and Andes virus (ANDV), respectively is 30–50%.

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), 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. 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. J. 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).

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). Neutralizing antibody responses to G1-G2 in the aforementioned vaccine studies correlated with protection, suggesting that neutralizing antibodies play an important role in preventing hantavirus infection. 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.

The N protein also plays a role in protecting against hantavirus infection. Vaccination with N expressed in bacteria, insect cells, or as chimeric hepatitis B virus (HBV) core particles protected rodents from hantavirus infection (Schmaljohn et al., 1990, supra; Yoshimatsu et al. 1993, supra; Lundkvist et al., 1996, supra; Ulrich et al., 1998, supra). Vaccination with vaccinia recombinants expressing the S segment were less conclusive. A construct expressing the HTNV S segment did not protect hamsters from HTNV infection, possibly due to low N expression levels (Schmaljohn et al. 1990, supra); and a construct expressing the S segment of SEOV protected three of four gerbils from SEOV infection (Xu et al. 1992, supra).

Similarly, basic research towards a gene-based vaccine that protects against HPS has been ongoing since the isolation of the first HPS-associated hantavirus in the mid 1990s. There are reports that candidate DNA vaccines comprised of around 500 nucleotide stretches of the 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.

Therefore, it remains unclear whether or not G1 alone, G2 alone, or fragments of the glycoproteins can elicit neutralizing antibody and protect against infection. Vaccination with recombinant baculovirus-infected cell lysates containing G1 or G2 alone, and recombinant vaccinia viruses expressing G1 or G2 alone, failed to elicit neutralizing antibody, and exhibited incomplete protection in a hamster infection model (Schmaljohn et al., 1990). Even though these vaccinia vaccines showed some potential, recombinant vaccinia virus vaccines and vaccinia-based vaccines present disadvantages including the potential for disseminated infection, especially in immunocompromised individuals, since the vaccines consist of live virus. Also, vaccination with these viruses can result in a lesion (pock) that contains infectious virus. Virus from these lesions can be inadvertently spread to other sites (e.g., eyes) or to other individuals. In addition, vaccinia-vectored vaccine are poorly immunogenic in persons previously vaccinated with smallpox vaccine (McClain et al., 2000, *J. Med. Virol.* 60, 77–85). Other drawbacks of vaccinia-based vaccines include discomfort due to swollen lymphnodes and scarring at the site of inoculation.