Virus Research 160 (2011) 108–119

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Puumala virus infection in Syrian hamsters (*Mesocricetus auratus*) resembling hantavirus infection in natural rodent hosts

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ARTICLE INFO

Article history:
Received 23 April 2011
Received in revised form 28 May 2011
Accepted 28 May 2011
Available online 6 June 2011

Keywords:
Hantavirus
Puumala virus
Animal model
Persistent infection
Hamster

ABSTRACT

The mechanism of hantavirus persistent infection in natural hosts is poorly understood due to a lack of laboratory animal models. Herein, we report that Syrian hamsters (*Mesocricetus auratus*) infected with Puumala virus (PUUV) at 4 weeks old show persistent infection without clinical symptoms for more than 2 months. IgG and IgM antibodies against the viral nucleocapsid protein and neutralizing antibody were first detectable at 14 days postinoculation (dpi) and maintained through 70 dpi. Viral RNA was first detected from 3 dpi in lungs and blood clots, and was detected in all tissues tested at 7 dpi. The viral RNA persisted for at least 70 days in the lungs, kidney, spleen, heart, and brain. The highest level of RNA copies was observed at 14 dpi in the lungs. Slight inflammatory reactions were observed in the lungs, adrenal glands, and brain. Immunohistochemical analysis revealed that PUUV antigen persisted until 56 dpi in the kidneys and adrenal glands. Infected hamsters showed no body weight loss or clinical signs. These results indicate that PUUV infection in hamsters is quite similar to the hantavirus infection of natural host rodents.

1. Introduction

Hantaviruses, which belong to the family *Bunyaviridae*, are distributed worldwide and carried by a variety of rodent and insectivore species (Jonsson et al., 2010). Hantaviruses possess a trisegmented, negative-stranded RNA genome consisting of small (S), medium (M), and large (L) segments (Plyusnin et al., 1996), which encode nucleocapsid proteins (N), envelope glycoproteins (Gn and Gc), and RNA-dependent RNA polymerase, respectively (Plyusnin et al., 1996). Hantaviruses cause two human diseases: hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS) (Jonsson et al., 2010). The clinical symptoms of HFRS are characterized by fever, renal failure, and hemorrhage with capillary leakage (Muranyi et al., 2005). HFRS occurs mainly in Asia and Europe, with 150,000–200,000 cases annually (Jonsson et al., 2010; Muranyi et al., 2005), and its case fatality rate is 0.1–15% (Kanerva et al., 1998; Muranyi et al., 2005). Hantaan virus (HTNV), Seoul virus (SEOV), Amur virus (AMRV), Dobrava-Belgrade virus (DOBV), Saaremaa virus (SAAV), and Puumala virus (PUUV) have been identified as etiologic agents of HFRS, and they are each carried by a specific rodent species: striped field mouse, *Apodemus agrarius*; Norway rat, *Rattus norvegicus*; Korean field mouse, *Apodemus peninsulae*; yellow necked mouse, *Apodemus flavicollis*; striped field mouse, *Apodemus agrarius*; and bank vole, *Myodes glareolus*, respectively (Jonsson et al., 2010). HCPS is characterized by fever and severe cardiopulmonary dysfunction (Muranyi et al., 2005). Sin Nombre virus (SNV), Laguna Negra virus (LNV), and Andes virus (ANDV) are considered to be the major pathogens of HCPS (Bi et al., 2008; Jonsson et al., 2010). The case fatality rate of HCPS is as much as 50% (Muranyi et al., 2005).

In contrast to human infections, hantaviruses are generally believed to infect natural hosts persistently and are nonpathogenic to their reservoir hosts (Gavrilovskaya et al., 1990; Yanagihara et al., 1985). Although an immune response to the virus is induced, the natural hosts harbor viral RNA and antigens, especially in the lungs, and maintain the virus for over a year after infection (Gavrilovskaya et al., 1990; Lee et al., 1981; Yanagihara et al., 1985). The virus is shed in rodent excreta, such as urine, feces, and saliva, which are believed to represent the major source of hantavirus infection in humans by inhalation (Lee et al., 1981; Vapalahti et al., 2003; Yanagihara et al., 1985).

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doi:10.1016/j.virusres.2011.05.021

The mechanisms for persistent infection of hantaviruses in their natural hosts remain unclear. One factor that impedes the clarification of these mechanisms is a lack of animal models. To date, no laboratory animal model is persistently infected with hantavirus that shows no clinical signs as natural hosts. Some researchers have used colonized wild rodents for analyzing the kinetics of hantavirus in natural hosts (Botten et al., 2000; Gavrilovskaya et al., 1990; Hardestam et al., 2008; Lee et al., 1981; Yanagihara et al., 1985), but four major problems exist associated with the use of wild rodents species in laboratory experiments. First, wild rodents should be handled and bred with specialized technical skill. Second, wild rodents have a diverse genetic background. Third, microbiological control is difficult in wild rodents because they are potential carriers of various microbes. And fourth, few research tools are available to analyze wild rodents. Therefore, developing animal models of hantavirus persistent infection is necessary using common laboratory animals.

In this study, we report that Syrian hamsters infected at 4 weeks old with PUUV, which is the etiologic agent of HFRS, showed persistent infection. Despite a high level of antibodies against PUUV, the animals harbored high levels of viral RNA in the acute phase of infection and maintained the virus in lungs for 70 days postinoculation (dpi). Viral RNA and antigens were also detected in some organs, but the hamsters showed no signs of illness. These data suggest that the Syrian hamster could be a suitable animal model resembling hantavirus persistent infection in the natural host.

2. Materials and methods

2.1. Cells and virus

The Sotkamo strain of PUUV was propagated in Vero E6 cells. Cells were cultivated in Eagle's minimum essential medium (EMEM) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a 5% CO_2 incubator. Following incubation for 14 days, the culture fluid from the cell monolayer was collected as the virus stock and stored at −80 °C until use.

2.2. Animals

Four-week-old (subadult) and 8-week-old (adult) male Syrian hamsters (SLC Inc., Hamamatsu, Japan) were inoculated subcutaneously with 3,300 focus-forming units (FFU) or EMEM as control. Hamsters were observed daily and their body weight was measured. 2–3 animals from 4-week-old group were killed on 3, 7, 14, 28, 42, 55, or 70 dpi, and 3 to 5 animals from 8-week old group were killed on 3, 7, 14, 28, 42, 56, or 70 dpi. Their internal organs (brain, heart, lung, liver, spleen, and kidney) and sera were collected and stored at −80 °C until analysis. All animal experiments were performed according to the guidelines of animal experimentation at the School of Veterinary Medicine, Hokkaido University, and carried out at a biosafety level 3 animal facility.

2.3. Antibody

The mouse monoclonal antibody E5/G6 against HTNV N and cross-reactive with PUUV N was obtained by immunization with HTNV-infected cell lysate (Yoshimatsu et al., 1996).

2.4. Indirect immunofluorescent antibody test (IFA)

The sera were tested for antibodies to PUUV using an IFA method. Vero E6 cells were infected with Puumala virus Sotkamo strain and cultured for 21 days. The infected cells were collected by trypsinization and spotted onto 24-well slides. After incubation for 4 h in a CO_2 incubator, the cells were fixed with cold acetone for 20 min and air-dried. The slides were stored at −40 °C until use. Hamster sera were diluted serially by twofold (starting at 1:16) with phosphate-buffered saline (PBS) and spotted onto the slide. After incubation at 37 °C for 1 h, the slides were washed three times with PBS. Alexa Fluor® 488 conjugated anti-hamster IgG (Invitrogen, Carlsbad, CA, USA) diluted 1:1000 in PBS was spotted on the slides and incubated at 37 °C for 1 h. The IFA titers of each serum were determined as the reciprocal of the maximum dilution of serum that yielded scattered granular fluorescence in the cytoplasm.

2.5. IgG detection enzyme-linked immunosorbent assay (IgG-ELISA)

Recombinant N (rN) of Hokkaido virus (Kariwa et al., 1995) was expressed as a fusion protein with N-utilization substance A (NusA) by cloning into the pET-43.1c(+) vector (Novagen, San Diego, CA, USA) and purified using the ProBond™ purification system (Invitrogen). Then 96-well EIA/RIA plates (Corning Inc., Corning, NY, USA) were coated overnight at 4 °C with 50 μl per well of the rN or the NusA diluted in PBS at a concentration of 1.6 μg/ml. The coated plates were blocked with 200 μl per well of 3% bovine serum albumin (BSA) in PBS at 37 °C for 1 h, followed by washing three times with PBS containing 0.5% Tween 20 (PBST); then 50 μl of the serum samples diluted to 1:200 in PBST were added to the plates. Each serum sample was reacted with the rN and the NusA protein. After 1 h of incubation at 37 °C, the plates were washed three times with PBST. The plates were then incubated with 50 μl of peroxidase conjugated anti-hamster IgG diluted to 1:1,000 in PBST at 37 °C for 1 h. After washing, 100 μl of *o*-phenylenediamine substrate with hydrogen peroxide was added to each well, and the plates were incubated at 37 °C for 30 min. The absorbance was then measured at 450 nm and the value for each sample in the well with NusA was subtracted from that of the corresponding well containing rN.

2.6. Preparation of antigens for IgM detection ELISA (IgM-ELISA) and N detection ELISA (N-ELISA)

Monolayers of Vero E6 cells were infected with the Sotkamo strain of PUUV and incubated for 14 days. After centrifugation at 100 × *g* for 2 min, the cells were resuspended in lysis buffer (0.01 M Tris–HCl, 2% Triton X-100, 0.15 M NaCl, 0.6 M KCl, 5 mM EDTA) to 2×10^7 cells/ml. The lysates were centrifuged at 16,000 × *g* for 15 min. The supernatants were collected and used for IgM-detection ELISA.

Lungs were mixed with lysis buffer and homogenized by shaking with a zirconium bead at 30 times/s for 3 min using a mixer mill (MM300; Retsch, Haan, Germany). The homogenates were kept on ice for 30 min and centrifuged at 6000 × *g* for 10 min. Supernatants were collected and used for viral antigen detection.

2.7. IgM-ELISA

Plates were coated with 50 μl of rabbit IgG against hamster IgM μ chain (diluted to 25 μg/ml in PBS; Rockland Immunochemicals, Gilbertsville, PA, USA) at 4 °C overnight. The plates were blocked with 200 μl of 3% BSA in PBS at 37 °C for 1 h. The plates were washed three times with PBST, and 50 μl of each serum sample diluted to 1:100 was added and incubated at 37 °C for 1 h. After washing, 50 μl of each cell lysate diluted to 1:10 with PBST was added to each well. The plates were then incubated at 37 °C for 1 h and washed. The biotinylated monoclonal antibody E5/G6 diluted to 1 μg/ml with PBST was added and incubated at 37 °C for 1 h. The plates were then washed, and 50 μl of peroxidase conjugated NeutrAvidin™ (Pierce Biotechnology, Inc., Rockford, IL, USA) diluted to 1 μg/ml with PBST