Maporal Viral Infection in the Syrian Golden Hamster: A Model of Hantavirus Pulmonary Syndrome

Mary Louise Milazzo, Eduardo J. Eyzaguirre, Claudia P. Molina, and Charles F. Fulhorst

Hantavirus pulmonary syndrome (HPS) is a severe and often fatal rodent-borne zoonosis. Maporal (MAP) virus is a newly discovered hantavirus that originally was isolated from an arboreal rice rat (Oecomys bicolor) captured in central Venezuela. The results of this study indicate that MAP virus in the Syrian golden hamster (Mesocricetus auratus) can cause a disease that is clinically and pathologically remarkably similar to HPS. The similarities include the time course of clinical disease, presence of virus-specific IgG at the onset of clinical disease, subacute pulmonary edema, rapid onset of diffuse alveolar edema in the absence of necrosis, hepatic-portal triaditis, mononuclear-cellular infiltrate in lung and heart, widespread distribution of hantaviral antigen in endothelial cells of the microvasculature of lung and other tissues, and variable lethality. These similarities suggest that the MAP virus–hamster system is a useful model for studies of the pathogenesis of HPS and for the evaluation of potential therapeutic agents.

Materials and Methods

Inoculation, husbandry, and sampling of animals. Twenty-two 4-week-old outbred female Syrian golden hamsters (Harlan Sprague Dawley) were each inoculated intramuscularly with 0.2 mL of a suspension that contained 3.1 log_{10} median cell-culture infectious doses (CCID_{50}) of the MAP virus prototype strain 97021050. The passage history and infectious titer of the stock virus were Vero E6+4 and 5.8 log_{10} CCID_{50}/0.2 mL, respectively, and the inoculum was prepared in 0.01 M PBS (pH 7.4). Each animal was inoculated at 1 site in the musculature of the right hind leg. The controls were 4 hamsters each injected with 0.2 mL of sterile PBS (pH 7.4), at 1 site in the musculature of the right hind leg.

The inoculated animals were housed in pairs in microisolator cages. All cages were kept inside a laminar-flow biosafety cabinet, and strict barrier care was practiced throughout the study, to obviate virus transmission between animals in different cages.

Two animals inoculated with virus were found dead in their cages on day 9 postinoculation (PI). The other inoculated animals and the 4 control animals were killed by intraperitoneal injection of a lethal dose (15 mg) of sodium pentobarbital: 2 inoculated...
animals were killed on each of days 7, 9, 11, 13, and 15 PI; 10 inoculated animals were killed on day 20 PI; and 1 control animal was killed on each of days 7, 11, 15, and 20 PI.

Cardiac blood, urine, oropharyngeal (OP) secretions, and the left kidney were collected from each animal and were stored at −80°C. The urine was collected by cystocentesis and was stored in 0.3 mL of PBS containing 10% vol/vol heat-inactivated (56°C for 30 min) fetal bovine serum (FBS). The OP secretions were collected with a sterile cotton swab wetted with PBS-FBS and then were expressed from the swab by agitation in a vial containing 0.3 mL of PBS-FBS.

**Assay for infectious virus.** Virus isolation was attempted on urine samples, OP secretions, and kidneys (prepared as 10% wt/vol crude homogenates in PBS-FBS), by cultivation in monolayer cultures of Vero E6 cells [4]. Hantaviral antigen in cultured cells was detected by an indirect fluorescent antibody test (IFAT). The primary antibody in that test was hamster IgG bound to cell-associated hantaviral antigen was detected by a goat anti–hamster IgG fluorescein isothiocyanate conjugate (Kirkegaard and Perry Laboratories).

**Assay for viral antigen.** An immunohistochemistry assay was used to test for hantaviral antigen in thin (4 μm) sections of the tissues dissected from the carcasses of (1) the 2 animals that died on day 9 PI; (2) the 2 animals killed on each of days 7, 9, 11, 13, 15, and 20 PI; and (3) the 4 control animals. The tissue sections were deparaffinized and then were treated with DAKO Target Retrieval Solution (DAKO) and incubated at 90°C for 30 min, to effect antigen retrieval. The primary antibody was a rabbit anti–AND virus immune serum. Nonspecific binding of

**Table 1.** Postinoculation (PI) results of laboratory tests on 22 hamsters inoculated with infectious Maporal virus.

<table>
<thead>
<tr>
<th>Source of virus</th>
<th>7 days</th>
<th>9 days</th>
<th>11 days</th>
<th>13 days</th>
<th>15 days</th>
<th>20 days</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throat swab</td>
<td>1/2</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
<td>8/9</td>
<td>17/19</td>
</tr>
<tr>
<td>Urine</td>
<td>0/2</td>
<td>1/3</td>
<td>1/2</td>
<td>2/2</td>
<td>2/2</td>
<td>5/10</td>
<td>9/21</td>
</tr>
<tr>
<td>Kidneya</td>
<td>2/2</td>
<td>4/4</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
<td>10/10</td>
<td>22/22</td>
</tr>
<tr>
<td>Antibody status</td>
<td>2/2</td>
<td>4/4</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
<td>10/10</td>
<td>22/22</td>
</tr>
<tr>
<td>Antibody titers, scoreb</td>
<td>1–2</td>
<td>2–3</td>
<td>2–5</td>
<td>4–5</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Histology (score)c</td>
<td>Lung</td>
<td>2/2 (1)</td>
<td>4/4 (2)</td>
<td>2/2 (1–2)</td>
<td>2/2 (2)</td>
<td>2/2 (2)</td>
<td>10/10 (1)</td>
</tr>
<tr>
<td>Heart</td>
<td>0/2</td>
<td>0/4</td>
<td>1/2 (1)</td>
<td>0/2</td>
<td>0/2</td>
<td>0/10</td>
<td>1/22</td>
</tr>
<tr>
<td>Liver</td>
<td>0/2</td>
<td>1/4 (1)</td>
<td>0/2</td>
<td>2/2 (1)</td>
<td>2/2 (1)</td>
<td>9/10 (0–2)</td>
<td>14/22</td>
</tr>
<tr>
<td>Kidney</td>
<td>0/2</td>
<td>1/4 (1)</td>
<td>0/2</td>
<td>1/2 (1)</td>
<td>1/2 (1)</td>
<td>6/10 (1)</td>
<td>9/22</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. positive/no. tested, unless otherwise indicated.

a Titers of infectious hantavirus in the kidneys of the 2 animals killed on day 7 PI, of 4 animals that died or were killed on day 9 PI, and of 4 animals killed on day 11 PI or day 20 PI were 4.8–5.3, 6.3–7.8, and 5.0–6.8 log10 median cell-culture infectious doses/0.1 g, respectively.

b 1, 80; 2, 320; 3, 1280; 4, 5120; 5, >20,480.

c Nos. in parentheses are median scores for inflammation: 1, mild inflammation; 2, moderate inflammation. Mild or moderate inflammation was found in the lungs of 1 animal each killed on day 11 PI. The liver of 1 animal killed on day 20 PI was unremarkable; mild inflammation was found in the liver of a second animal killed on day 20 PI; and moderate inflammation was found in the livers of the 8 other animals killed on day 20 PI.
survived through day 9 PI were lethargic, inappetant, and re-
turned moribund on the evening of day 9 PI and, at that time, 
died on the evening of day 9 PI. Two other animals rapidly 
became moribund on the evening of day 9 PI and, at that time, 
died on the evening of day 9 PI. Two animals were found 
active) lymphocytes and activated macrophages. Microscopic 
examination of representative sections of lung 
revealed a moderate, diffuse subacute interstitial pneu-
monitis, variable degrees of vascular congestion, diffuse alve-
olar edema, and focal hyaline membranes and fibrin deposits 
(figure 1). The inflammatory infiltrate in the pulmonary interstitial pneumonia appeared to be a mixture of small and large (re-
active) lymphocytes and activated macrophages. Microscopic 
examination of lung tissue from the other animals revealed a mild, 
diffuse subacute interstitial pneumonia in both animals killed 
on day 7 PI and in 1 animal killed on day 11 PI, as well as a

Table 2. Postinoculation (PI) results of immunohistochemistry assay for hantaviral antigen in tissues of 12 hamsters inoculated with infectious MaPoral virus.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>7 days</th>
<th>9 days</th>
<th>11 days</th>
<th>13 days</th>
<th>20 days</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>2/2 (1, 2)</td>
<td>4/4 (3)</td>
<td>2/2 (3)</td>
<td>2/2 (3)</td>
<td>2/2 (3)</td>
<td>12/12</td>
</tr>
<tr>
<td>Lung</td>
<td>2/2 (3)</td>
<td>4/4 (3)</td>
<td>2/2 (2, 3)</td>
<td>2/2 (2)</td>
<td>2/2 (2)</td>
<td>12/12</td>
</tr>
<tr>
<td>Heart</td>
<td>2/2 (1, 2)</td>
<td>4/4 (2, 3)</td>
<td>2/2 (2)</td>
<td>2/2 (2)</td>
<td>2/2 (2)</td>
<td>12/12</td>
</tr>
<tr>
<td>Spleen</td>
<td>2/2 (1, 2)</td>
<td>4/4 (1, 3)</td>
<td>2/2 (1, 2)</td>
<td>2/2 (1)</td>
<td>2/2 (1)</td>
<td>12/12</td>
</tr>
<tr>
<td>Lymph node</td>
<td>2/2 (1)</td>
<td>4/4 (1, 2)</td>
<td>2/2 (1)</td>
<td>2/2 (1)</td>
<td>2/2 (1)</td>
<td>12/12</td>
</tr>
<tr>
<td>Liver</td>
<td>2/2 (2)</td>
<td>4/4 (2)</td>
<td>2/2 (2)</td>
<td>2/2 (2)</td>
<td>2/2 (2)</td>
<td>12/12</td>
</tr>
<tr>
<td>Kidney</td>
<td>2/2 (2)</td>
<td>4/4 (2, 3)</td>
<td>2/2 (2)</td>
<td>2/2 (2)</td>
<td>2/2 (2)</td>
<td>12/12</td>
</tr>
</tbody>
</table>

NOTE. Data are no. positive/no. tested (median score for prevalence of antigen-positive cells in 10 randomly selected high-power [×400] microscope fields [see Materials and Methods]). Inflammation was not found in the brains, spleens, or lymph nodes of any of the experimental animals.

a Median scores were 1 and 2, for 1 animal each on day 7 or 11 PI or for 2 animals each on day 9 PI.

b Median scores were 2 and 3, for 1 animal each on day 7 or 11 PI or for 2 animals each on day 9 PI.

c Median scores were 1 and 3, for 1 animal each on day 7 or 11 PI or for 2 animals each on day 9 PI.

rabbit IgG was minimized by treating the tissue sections with a casein solution (Sigma Chemical) for 20 min before application of the rabbit immune serum. The secondary antibody was a biotinylated goat anti–rabbit IgG (Vector Laboratories). Goat antibody bound to rabbit anti–AND virus IgG was detected by the LSAB2 Streptavidin-Biotin System (DAKO). Endogenous peroxidase activity was minimized by treating the tissue sections with 3% hydrogen peroxide. Endogenous avidin was blocked by use of the DAKO Blocking Kit (DAKO) according to the manufacturer’s instructions. The chromogen and counterstain were diaminobenzidine and hematoxylin, respectively, and all steps in the staining process were done at room temperature, in an Autostainer Universal Staining System (DAKO). The negative controls included tissue sections of infected hamsters, stained with normal (nonimmune) rabbit serum, and sections of tissues from uninfected hamsters, stained with the rabbit anti–AND virus immune serum. The prevalence of antigen-positive cells in each of 10 randomly selected high-power (original magnification, ×400) fields was scored as follows: 1, ≥1% but <25%; 2, ≥25% but <50%; and 3, ≥50%. The median score for the 10 randomly 

Results

All 22 animals inoculated with MAP virus were clinically normal on days 1–7 PI and were lethargic, inappetant (uninterest-
ed in fresh vegetables), and reluctant to move on day 8 
PI and on the morning of day 9 PI. Two animals were found 
dead on the evening of day 9 PI. Two other animals rapidly 
became moribund on the evening of day 9 PI and, at that time, 
were killed by injection of a lethal dose of sodium pentobarbital.
Two animals were killed on each of days 11, 13, and 15 PI, 
and 10 animals were killed on day 20 PI. The 16 animals that 
survived through day 9 PI were lethargic, inappetant, and re-
luctant to move on days 10–12 PI. The 12 animals that survived 
through day 13 PI rapidly improved on day 13 or 14 PI and were clinically normal on day 15 PI.

The results of the tests for infectious hantavirus and antibody 
against MAP virus, as well as the results of the histological 
examination of tissues from the 22 inoculated animals, are sum-
marized in table 1. Each of the 4 control animals was clinically 
normal until killed, and results of the gross and microscopic examinations of those animals were unremarkable.

Infectious hantavirus was isolated from the kidneys of each of 
the 22 experimental animals. Virus also was isolated from 
the urine samples of 9 of 21 animals, from OP secretions of 1 
animal killed on day 7 PI, and from OP secretions of 16 of 17 
animals killed on or after day 9 PI (throat swabs or OP secre-
tions were not collected from the 2 animals that died on day 
9 PI). The titers of infectious virus in the kidneys of the 2 
animals killed on day 7 PI, of 4 animals that died or were killed 
on day 9 PI, and of 4 animals killed on day 11 or 20 PI were 
4.8–5.3, 6.3–7.8, and 5.0–6.8 log10 CCID50/0.1 g of tissue, re-
spectively. There was no obvious association between titer of 
infected virus in the kidneys and severity of pulmonary path-
ology or duration of infection.

Antibody (i.e., IgG) against MAP virus was detected in serum 
from each of the 22 experimental animals, and the antibody 
titers increased from day 7 through day 13 PI. Antibody reactive against MAP virus was not detected in either the blood samples 
collected from the 22 experimental animals prior to their in-
oculation with infectious MAP virus or the serum samples from 
the 4 control animals.

Gross abnormalities at necropsy were limited to the thoracic 
cavities of the 2 animals that died on day 9 PI and to the 8 
animals killed on day 9, 11, 13, or 15 PI. The most striking 
abnormalities were in the animals that died or were killed 
on day 9 PI, and they included reddened lungs, frothy tracheal 
fluid, and large volumes (2–6 mL) of clear, straw-colored pleural fluid. Microscopic examination of the pleural fluid from each 
animal revealed only an occasional mononuclear cell or eryth-
rocyte. Lungs of the animals killed on day 11, 13, or 15 PI were 
redden and failed to collapse when the thoracic cavity was 
opened. In addition, a scant amount of serosanguineous fluid 
was found in the pleural cavity of each of the 2 animals killed 
on day 11 PI.

Microscopic examination of representative sections of lung 
tissue from each of the 4 animals that died or were killed on 

day 9 PI revealed a moderate, diffuse subacutie interstitial pneu-
monitis, variable degrees of vascular congestion, diffuse alve-
olar edema, and focal hyaline membranes and fibrin deposits 
(figure 1A and 8). The inflammatory infiltrate in the pulmonary interstitium appeared to be a mixture of small and large (re-
active) lymphocytes and activated macrophages. Microscopic 
examination of lung tissue of the other animals revealed a mild, 
diffuse subacute interstitial pneumonia in both animals killed 
on day 7 PI and in 1 animal killed on day 11 PI, as well as a
Figure 1. High-power (original magnification, ×400) images of lung tissue stained with hematoxylin-eosin. A and B, Lung tissue of a hamster infected with Maporal virus and killed on day 9 postinoculation. Note the diffuse alveolar edema, interstitial pneumonitis, and alveolar fibrin deposit (arrow). C, Lung tissue of an infected hamster killed on day 20 postinoculation. Note the moderate interstitial pneumonitis and absence of alveolar edema. D, Lung tissue from a control (i.e., uninfected) hamster.

moderate, diffuse subacute interstitial pneumonitis in the other animal killed on day 11 PI and in all animals killed on or after day 13 PI (figure 1C). The cellular composition of the inflammatory infiltrate in the pulmonary interstitium of the animals killed on day 7, 11, 13, 15, or 20 PI appeared to be the same as that in the animals that died or were killed on day 9 PI—that is, a mixture of small and large lymphocytes and activated macrophages.

Other notable microscopic abnormalities in the inoculated animals were limited to the livers and kidneys. Subacute portal triaditis was found in 1 animal killed on day 9 PI (figure 2A) and in 13 of the 14 animals killed on or after day 13 PI. The intensity of inflammation in the livers of the 8 animals killed on day 20 PI was greater than that in the 4 animals killed on day 13 or 15 PI. Focal subacute interstitial nephritis was found in 1 animal killed on each of days 9, 11, 13, and 15 PI (figure 2B). The inflammatory infiltrate in the livers and kidneys of the affected animals was composed mostly of mononuclear cells.

The results of the immunohistochemistry assay for hantaviral antigen in the tissues of the 2 animals that died on day 9 PI and of the 2 animals killed on each of days 7, 9, 11, 13, and 20 PI are summarized in table 2. Hantaviral antigen was detected in endothelial cells of the microvasculature of brain, lung, heart, spleen, lymph node, liver, and kidney of each animal. The most intensive staining of antigen was in endothelial cells of the microvasculature of (1) lungs of the animals killed on day 7 PI; (2) brains, lungs, hearts, kidneys, and livers of the animals that died or were killed on day 9 PI (figure 3A–D); (3) brains and lungs of the animals killed on day 11 PI; and (4) brains of the animals killed on day 13 or 20 PI. Antigen also was detected in dendritic cells in the spleens and lymph nodes of the animals killed on or after day 7 PI, in small numbers of macrophages in lungs of the animals killed on day 9 PI, and in neurons, neuroglial cells, hepatocytes, and Kupffer’s cells of the animals killed on or after day 7 PI. Hantaviral antigen was not detected in salivary-gland, thymus, pancreas, adrenal-gland, ovary, or gastrointestinal-tract tissues of any of the 22 experimental animals. The apparent absence of hantaviral antigen in salivary gland suggests that the lungs were the source of infectious hantavirus in the OP secretions of the experimentally infected animals.

Discussion

HPS is characterized by 4 clinical phases: prodrome, pulmonary edema and shock, diuresis, and convalescence [6]. The
prodrome typically is 1–6 days in duration and consists of fever, malaise, myalgia, headache, and gastrointestinal disturbances. Most patients then develop (in the following order) pneumonitis, noncardiogenic pulmonary edema, hypotension, and shock. Death, when it occurs, usually comes 1–3 days after the onset of respiratory symptoms. Resolution of the pulmonary edema in nonfatal cases of HPS is rapid.

The results of the present study indicate that MAP virus in the Syrian golden hamster can cause a disease that, clinically and pathologically, is remarkably similar to HPS. The similar-
ities include the prolonged incubation period (compared with those of other acute viral diseases), time course of clinical disease, presence of virus-specific IgG at the onset of clinical disease, subacute pneumonitis, rapid onset of diffuse alveolar edema in the absence of necrosis, hepatic-portal triaditis, cellular composition of the inflammatory infiltrate in lung and liver, widespread distribution of hantaviral antigen in endothelial cells of the microvasculature of lung and other tissues, and variable lethality. These similarities suggest that the MAP virus–hamster system is a valid alternative to the AND virus–hamster system, both for studies of the pathogenesis of HPS (particularly the immunological events that precede or coincide with the onset of the life-threatening pulmonary edema) and for the evaluation of potential therapeutic agents. An advantage of the MAP virus–hamster system is that it can be studied at biosafety level 3, whereas work with infectious AND virus requires biosafety level 4.

The results of a recent study have suggested that production of monokines (tumor-necrosis factor [TNF]-α, interleukin (IL)–1, and IL-6) and lymphokines (interferon-γ, IL-2, IL-4, and TNF-β) in lung and spleen plays a significant role in the pathogenesis of the pulmonary edema in HPS [7]. Time-course studies of series of hamsters experimentally infected with MAP virus may provide insight into the important role that the various cytokines play in the pathogenesis of pulmonary edema in HPS.

Most deaths in hospitalized patients with HPS are the result of cardiac depression, rather than of hypoxia [8]. The hearts of patients with fatal HPS usually are grossly and microscopically unremarkable [2], suggesting that the cardiac depression is the result of a functional abnormality. The hearts of the hamsters killed on day 9, 11, or 13 PI were grossly and microscopically unremarkable. However, large amounts of hantaviral antigen were detected in endothelial cells in the myocardium of those animals (figure 3B). The apparent absence of inflammation in infected (i.e., antigen-positive) heart tissue suggests that the MAP virus–hamster system is a valid model for studies of the pathogenesis of cardiac depression in HPS.

The severity of clinical disease caused by a single hantavirus ranges from mild to fatal. The reasons for the variability are not well understood but probably include differences in inoculum dose, route of exposure, virus genetics, and human-host genetics. Each of the experimental animals in the present study was inoculated intramuscularly with 3.1 log10 CCID50 of MAP virus. Further work is needed to assess the effect that route of exposure and inoculum dose have on the time course and lethality of MAP viral infection in the Syrian golden hamster.

Humans usually become infected with hantaviruses by contact with either infected rodents or infectious rodent excreta or secreta. AND virus is the only hantavirus for which there is evidence for human-to-human virus transmission [9]. The recovery of infectious MAP virus from the OP secretions and urine of experimentally infected hamsters suggests both that hamsters infected with AND virus can shed infectious hantavirus and that hamsters, like humans, can initiate horizontal hantavirus transmission.

Acknowledgments

Pierre E. Rollin and Thomas G. Ksiazek (Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta) provided the rabbit anti–AND virus immune serum that was used in the immunohistochemistry assay. Robert B. Tesh (World Reference Center for Arboviruses, University of Texas Medical Branch, Galveston) provided the anti-hantavirus hyperimmune-mouse ascitic fluid. Linda Muehlberger (Histopathology Core Laboratory, University of Texas Medical Branch, Galveston) prepared the hamster tissues for histological examination.

References