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 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 pneumonia, rapid onset of diffuse alveolar edema in the absence of necrosis, hepatic-portal triaditis, mononuclear-cellular 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 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 hyperimmune-mouse ascitic fluid prepared against Sin Nombre virus and several other hantaviruses.

The titers of infectious hantavirus in the kidneys of the 2 animals that died on day 9 PI and of the 2 animals killed on each of days 7, 9, 11, and 20 PI were determined by cultivation in monolayer cultures of Vero E6 cells [4]. In brief, serial 10-fold dilutions of a 10% wt/vol crude homogenate of kidney were prepared in PBS, and 50 μl of each dilution was inoculated onto each of 4 Vero E6 cell monolayer cultures in 24-well plastic plates (each well 1.78 cm²). Cells scraped from the monolayers on day 13 PI were tested for cell-associated hantaviral antigen, by the IFAT described above. The infectious titer of each kidney was calculated by a method described elsewhere [5].

**Antibody assay.** Serial 4-fold dilutions (range, 1:20–1:20,480) of sera from the blood samples collected at necropsy were tested for IgG reactive against MAP virus, by IFAT. The test antigen was a mixture of MAP virus–infected Vero E6 cells and uninfected Vero E6 cells. Hamster IgG bound to cell-associated hantaviral antigen was detected by a goat anti–hamster IgG fluorescein isothiocyanate conjugate (Kirkegaard and Perry Laboratories).

**Histopathology.** Brain, heart, lung, thymus, spleen, lymph node, kidney, urinary bladder, salivary gland, liver, pancreas, esophagus, stomach, duodenum, jejunum, ileum, colon, adrenal gland, and an ovary were dissected from each formalin-fixed carcass and were embedded in paraffin. Thin (4 μm) sections of the various tissues were stained with hematoxylin-eosin and then were examined by light microscopy. The intensity of inflammation in each of 10 randomly selected high-power (original magnification, ×400) fields in each tissue section was scored as follows: 1, mild (only occasional perivascular and interstitial mononuclear cells); 2, moderate (widely distributed patchy perivascular and interstitial aggregates of mononuclear cells); and 3, severe (diffuse mononuclear infiltrate). The median score for the 10 randomly selected high-power fields was used for analysis.

**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. Non-specific binding of

<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>
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<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>
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<tr>
<td>Urine</td>
<td>0/2</td>
<td>1/3</td>
<td>1/2</td>
<td>2/2</td>
<td>0/2</td>
<td>5/10</td>
<td>9/21</td>
</tr>
<tr>
<td>Kidney</td>
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<td>4/4</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
<td>10/10</td>
<td>22/22</td>
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<tr>
<td>Antibody status</td>
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<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, score</td>
<td>1–2</td>
<td>2–3</td>
<td>2–5</td>
<td>4–5</td>
<td>4–5</td>
<td>5–5</td>
<td>5–5</td>
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</tbody>
</table>

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

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Postinoculation (PI) results of laboratory tests on 22 hamsters inoculated with infectious Maporal virus.
but survived through day 9 PI were lethargic, inappetant, 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 reluctant 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 summarized 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 secretions 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, respectively. There was no obvious association between titer of infectious virus in the kidneys and severity of pulmonary pathology 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 inoculation 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 erythrocyte. Lungs of the animals killed on day 11, 13, or 15 PI were reddened 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 subacutely interstitial pneumonitis, variable degrees of vascular congestion, diffuse alveolar edema, and focal hyaline membranes and fibrin deposits (figure 1A and B). The inflammatory infiltrate in the pulmonary interstitium appeared to be a mixture of small and large (reactive) lymphocytes and activated macrophages. Microscopic examination of lung tissue of the other animals revealed a mild, diffuse subacute interstitial pneumonitis 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>
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<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>
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<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, 3)</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 2, for 1 animal each on day 7 or 11 PI or for 2 animals each on day 9 PI.
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 hantavirus 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 (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-

Figure 2.  A. Liver tissue of an infected hamster killed on day 20 postinoculation, stained with hematoxylin-eosin (original magnification, ×400). Note the intense mononuclear-cell infiltrate adjacent to a portal vein. B. Kidney tissue of an infected hamster killed on day 20 postinoculation, stained with hematoxylin-eosin (original magnification, ×400). Note the interstitial mononuclear-cell infiltrate.

Figure 3.  Hantaviral antigen in tissues of an infected hamster killed on day 9 postinoculation (original magnification, ×400). The primary antibody and chromogen used in the immunohistochemistry assay were a rabbit anti–Andes virus immune serum and diaminobenzidine, respectively. A. Lung tissue with a large number of antigen-positive endothelial cells. B. Cardiac tissue with hantaviral antigen within interstitial endothelial cells. Note the absence of inflammation. C. Kidney tissue with hantaviral antigen within glomerular and interstitial endothelial cells. D. Liver tissue with hantaviral antigen within endothelial cells and hepatocytes.
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 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. 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 log_{10} CCID_{50} 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

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References