CLINICAL LABORATORY, VIROLOGIC, AND PATHOLOGIC CHANGES IN HAMSTERS EXPERIMENTALLY INFECTED WITH PIRITAL VIRUS (ARENAVIRIDAE): A RODENT MODEL OF LASSA FEVER

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Abstract. The clinical laboratory, virologic, and pathologic changes occurring in hamsters after infection with Pirital virus (Arenaviridae) are described. Pirital virus infection in the hamsters was characterized by high-titered viremia, leukocytosis, coagulopathy, pulmonary hemorrhage and edema, hepatocellular and splenic necrosis, and marked elevation of serum transaminase levels. All of the animals died within 9 days. The clinical and histopathological findings in the Pirital virus–infected hamsters were very similar to those reported in severe human cases of Lassa fever, suggesting that this new animal model could serve as a low-cost and relatively safe alternative for studying the pathogenesis and therapy of Lassa fever.

INTRODUCTION

Arenaviruses cause a spectrum of human illness ranging from a non-specific febrile syndrome to aseptic meningitis to fatal hemorrhagic fever. From a public health standpoint, Lassa fever (LF) is by far the most important arenavirus infection of humans. More than 200,000 cases of LF are estimated to occur annually in West Africa, with several thousand deaths. Among untreated hospitalized patients with LF, the case fatality is about 16%. One of the problems in the diagnosis of human arenaviral infections is that the onset of illness is insidious and usually begins with rather non-specific symptoms such as fever, weakness, malaise, headache, muscle and joint pains, and sore throat, which slowly worsen over a 6- or 7-day period; consequently, most patients are not hospitalized until more severe symptoms (neurologic signs, hemorrhagic manifestations, or shock) appear. For this reason, little is known about the early pathogenesis of most arenavirus infections in people.

Much of our knowledge of the early clinical and pathologic events that occur in arenaviral hemorrhagic fever (AHF) comes from studies of animal models of the various diseases in non-human primates or guinea pigs. We previously described a hamster model of AHF, using Pirital virus (PIRV), a New World arenavirus. Here, we report the clinical laboratory, virologic, and pathologic findings in hamsters during PIRV infection; our results indicate that the model has many of the characteristics of LF in humans.

MATERIALS AND METHODS

Viruses. The prototype strain of Pirital virus (VAV-488) was used to infect the hamsters. This virus was originally isolated from a cotton rat (Sigmodon albonti) collected in the Municipality of Guanarito, Portuguesa State, Venezuela, in 1994. Pirital virus is a member of phylogenetic lineage A of New World arenaviruses. The strain used in our experiments had been passaged three times in Vero cell cultures and twice in adult hamsters.

Animals. The animals used in this study were 5- to 6-week-old female Syrian golden hamsters (Mesocricetus auratus) obtained from Harlan, Sprague Dawley (Indianapolis, IN). Hamsters were cared for in accordance with guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, under an animal use protocol approved by the University of Texas Medical Branch. All work with infected animals and sera was carried out in biosafety level 3 facilities.

Experimental infection and sample collection. Before infection, five hamsters were bled by cardiac puncture to obtain serum and blood for baseline clinical laboratory studies. Two days later, 60 hamsters were inoculated intraperitoneally with ~10⁴ plaque-forming units (PFUs) of a stock of PIRV prepared from a homogenate of infected hamster liver. Beginning 24 hours after PIRV inoculation, three hamsters were killed each day for 8 consecutive days to obtain blood and tissue samples for examination. The animals were first exsanguinated by cardiac puncture under deep halothane anesthesia (Hydrocarbon Laboratories, River Edge, NJ). Whole blood was saved for subsequent use in hemologic, coagulation, and clinical chemistry studies and for virus assay, as described below. A necropsy was performed on each animal, and samples of the following tissues were collected: liver, spleen, pancreas, kidney, adrenal, lung, heart, lymph nodes, and intestine. Chunks of all of the above tissues were fixed in 10% neutral-buffered formalin for 36 hours and were transferred to 70% ethanol, before being processed for routine paraffin embedding. Several 4- to 5-μm sections were made and stained by the hematoxylin and eosin (H&E) method. Other unstained sections were used for immunohistochemistry and antigen detection, as described previously.

A portion of the fresh liver was also processed for ultrastructural studies as follows. Liver tissue was cut in a drop of fixative into pieces of ~1 mm³ and fixed in a mixture of 2.5% formaldehyde, 0.1% glutaraldehyde, 0.03% trinitrophenol, and 0.03% CaCl₂ in 0.05 mol/L cacodylate buffer. Postfixation was done in 1% OsO₄ in 0.1 mol/L cacodylate buffer and en bloc staining in 1% uranyl acetate in 0.1 mol/L maleate buffer. The sections were dehydrated in ethanol and embedded in Poly/Bed 812 (Polysciences, Warrington, PA). Ul-
### Table 1
Clinical chemistry values in hamsters experimentally infected with Pirital virus

<table>
<thead>
<tr>
<th>Clinical chemistry</th>
<th>Baseline (±SD)</th>
<th>D-1</th>
<th>D-2</th>
<th>D-3</th>
<th>D-4</th>
<th>D-5</th>
<th>D-6</th>
<th>D-7</th>
<th>D-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT U/L</td>
<td>98.2 ± 12.2</td>
<td>107* ± 19.0</td>
<td>88.9 ± 11.6</td>
<td>183.3 ± 25.1</td>
<td>320 ± 26.4</td>
<td>3373.3 ± 360.2</td>
<td>5996.7 ± 1349.2</td>
<td>7320.0 ± 789.2</td>
<td>4423.3 ± 2283.5</td>
</tr>
<tr>
<td>AST U/L</td>
<td>746 ± 9.3</td>
<td>61 ± 9.6</td>
<td>50.75 ± 3.6</td>
<td>200 ± 81.8</td>
<td>336.7 ± 70.2</td>
<td>5156.7 ± 239.6</td>
<td>7233.3 ± 1217.8</td>
<td>8751.7 ± 852.4</td>
<td>5493.3 ± 3331.8</td>
</tr>
<tr>
<td>T-bilirubin mg/dL</td>
<td>0.6 ± 0.2</td>
<td>2.3 ± 1.8</td>
<td>0.4 ± 0.14</td>
<td>1 ± 0.4</td>
<td>1.1 ± 0.8</td>
<td>4.7 ± 2.1</td>
<td>4.1 ± 1.4</td>
<td>3.0 ± 0.7</td>
<td>4.1 ± 2.0</td>
</tr>
<tr>
<td>Albumin g/dL</td>
<td>3.4 ± 0.3</td>
<td>4 ± 0.3</td>
<td>1.5 ± 0.0</td>
<td>3.0 ± 0.2</td>
<td>2.1 ± 0.8</td>
<td>3 ± 0.1</td>
<td>2.7 ± 0.1</td>
<td>2.3 ± 0.4</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>Potassium mmol/L</td>
<td>3.8 ± 0.1</td>
<td>3.8 ± 0.1</td>
<td>NA</td>
<td>3.1 ± 0.6</td>
<td>3.4 ± 0.2</td>
<td>4.2 ± 0.2</td>
<td>3.8 ± 0.9</td>
<td>3.5 ± 0.3</td>
<td>3.4 ± 0.4</td>
</tr>
<tr>
<td>BUN mg/dL</td>
<td>277 ± 14.5</td>
<td>19.7 ± 0.6</td>
<td>36.7 ± 5.7</td>
<td>18.7 ± 2.3</td>
<td>10 ± 5.2</td>
<td>163 ± 0.6</td>
<td>17.7 ± 2.5</td>
<td>44 ± 14.4</td>
<td>84.3 ± 46.1</td>
</tr>
<tr>
<td>Amylase U/L</td>
<td>1281.5 ± 84.7</td>
<td>832.3 ± 163.8</td>
<td>920 ± 182.9</td>
<td>715 ± 262.1</td>
<td>257.3 ± 411</td>
<td>904.3 ± 91.8</td>
<td>641 ± 135.1</td>
<td>533 ± 239.8</td>
<td>597.7 ± 106.3</td>
</tr>
</tbody>
</table>

* Each number shown in table represents the mean of values obtained on three hamsters.

NA, not available.

### Table 2
Hematologic findings in hamsters experimentally infected with Pirital virus

<table>
<thead>
<tr>
<th>Hematology</th>
<th>Day after infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Units</td>
<td>Baseline (±SD)</td>
</tr>
<tr>
<td>WBC K/µL</td>
<td>5.1 ± 0.5</td>
</tr>
<tr>
<td>NE K/µL</td>
<td>1.8 ± 0.7</td>
</tr>
<tr>
<td>LY K/µL</td>
<td>2.8 ± 0.8</td>
</tr>
<tr>
<td>MO K/µL</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>EO K/µL</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>BA K/µL</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>RBC M/µL</td>
<td>5.1 ± 0.9</td>
</tr>
<tr>
<td>Hb g/dL</td>
<td>11.1 ± 1.8</td>
</tr>
<tr>
<td>Hct %</td>
<td>41.5 ± 3.6</td>
</tr>
<tr>
<td>PLT K/µL</td>
<td>410.5 ± 130.1</td>
</tr>
</tbody>
</table>

* Each number shown in table represents the mean of values obtained on three hamsters.

K, 1,000; M, 1 million.
Thin sections were cut on a Reichert-Leica Ultracut S ultramicrotome and examined in a Philips 201 electron microscope at 60 kV.

Hematologic, biochemical, and coagulation studies. For coagulation studies, citrated blood was centrifuged at 2,500 g for 10 minutes at 4°C. After centrifugation, the plasma was transferred to clean tubes and analyzed on a Start-4 coagulation analyzer (Diagnostica Stago, Parsippany, NJ), and the following values were determined: prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen (FBG), and thrombin time (TT). Hematologic assays were performed directly on EDTA-treated whole blood, using a Hemavet 950 analyzer (Drew Scientific, Oxford, CT), which determined the total white blood cells (WBCs) and differential count, neutrophils (NE), monocytes (MO), lymphocytes (LY), basophils (BA), eosinophils (EO), red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT), and platelets (PLT). For clinical chemistry, whole blood without additives was allowed to clot at room temperature for several hours and was centrifuged for 5 minutes at 2,500 g. Serum was transferred to clean tubes and analyzed promptly on a Prochem-V clinical chemistry analyzer (Drew Scientific), according to the manufacturer’s instructions. The following biochemical parameters were determined: serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), serum amylase, blood urea nitrogen (BUN), and serum potassium (K).

Statistical analysis. In analyzing the clinical and virologic results, one-way ANOVA was used to analyze independent variables; a logarithmic transformation was applied to these variables before the analysis. The level of significance was established when $P \leq 0.05$. Turkey’s Honestly Significant Difference (HSD) test was used for post hoc analysis.

RESULTS

Clinical observations. For the first 5 days after infection (p.i.), the animals remained active and appeared normal; at necropsy, the organs also appeared grossly normal. However, by days 6 and 7 p.i., the infected animals had become lethargic.
and anorexic. Some of the hamsters had epistaxis and some showed hind limb paralysis. Petechiae were also evident in the abdomen at necropsy on days 7 and 8 p.i. All of the hamsters were dead by the ninth day. Post-mortem examination revealed focal pulmonary hemorrhage, splenomegaly, hepatomegaly, and intestinal hemorrhage.

**Blood chemistry.** The serum transaminases showed a marked increase on days 5 to 8 p.i., with a maximum of 7,300 U/L for ALT and 8,800 U/L for AST on day 7 (Table 1). An increasing trend was also observed in the total bilirubin (T-bilirubin) values, which also appeared elevated on days 5–8 p.i. BUN was increased on day 8 p.i; this may have been partially caused by dehydration, because at this time the hamsters appeared quite ill and had stopped feeding (Table 1). The potassium and amylase values did not show significant changes. The albumin values were lower on days 7 and 8 p.i. compared with the baseline.

**Coagulation studies.** PT, aPTT, and TT were significantly increased, with mean maximum values on days 7–8 p.i. of 223, 600, and 210 seconds, respectively (Figure 1). The FBG values did not change significantly (data not shown).

**Hematology.** The main hematologic changes were observed in the WBC counts (Table 2). On days 5–8 p.i., the WBC were elevated, with a mean of > 25,000 U/L on day 8. The primary component of the WBC population was neutrophils, almost 17,000 U/L on day 8; but a significant increase was also observed in the eosinophils and basophils on days 5–8.

The platelets showed a marked increase on days 5 and 6 p.i. (Table 2); but the red blood cell, hemoglobin, and hematocrit values did not change significantly.

**Viremia.** PIR V was detected in the blood (mean 10^{4.13} PFU/mL) on the first day (Figure 2); the level of viremia increased daily until day 5 p.i., when it reached a maximum of 10^{7.48} PFU/mL. It remained at this level for the next 3 days, until death of the animals.

**Histopathology.** In the first 2 days after infection with PIR V, a few foci of inflammatory cells appeared in the liver, surrounding dying hepatocytes (Figure 3A). Foamy macrophages increased in number in the white pulp of the spleen. In the lung sections, scattered neutrophils were seen in the interstitium.

As the infection progressed, many degenerating apoptotic hepatocytes were seen in the liver as acidophilic bodies. The distribution of mononuclear inflammatory foci and apoptotic hepatocytes were both centrilobular and portal, and scattered necrotic areas began to appear on days 3 and 4 p.i. (Figure...
Many neutrophils were visible in the airways and in the pulmonary blood vessels and extravascular interstitia. Focal hemorrhages were observed in the lung sections from day 4 p.i. onward. In the spleen, the mantle zone was reduced in thickness, and fibrin depositions appeared at the interface between the lymphoid follicles and the red pulp. Macrophages containing phagocytosed cellular debris were prominent in the white pulp (Figure 3C).

On days 5 and 6 p.i., the necrotic foci further increased in the liver. Inflammatory cells were observed, both in the portal tracts and around the terminal hepatic venules. In the lungs, foci of interstitial hemorrhage increased (Figure 3D). In the spleen and the mantle and marginal zones of the lymphoid follicles, most of the red pulp had disappeared and was replaced by areas of fibrinoid necrosis. Tangible body macrophages continued to increase in the white pulp (Figure 3E).

On days 7 and 8 p.i., the pathologic changes in the liver were very severe (Figure 3F and G). Pulmonary hemorrhage and edema were observed in all animals, and early hyaline membrane was seen in the lungs of several hamsters on day 8 p.i. (Figure 3H and I).

No significant changes were observed in the kidney, heart, or bowel of the animals examined.

**Immunohistochemistry.** Beginning on day 3 p.i., focal viral antigen staining was observed in the kidney tubules of a few animals, proximal to the glomeruli. In one of the animals, the capsule of the spleen also showed weak positive staining. In the liver, on day 4 p.i., focal staining was seen in hepatocytes around the portal tracts and the terminal hepatic venules (Figure 4A) and in the subcapsular area (Figure 4B). A few positive staining macrophages appeared in the spleen on day 5 p.i.

On day 6 p.i., viral antigen could be shown in the liver, spleen, kidney (tubular epithelium; Figure 4C and D), and intestine (epithelium; Figure 4E).

Scattered alveolar macrophages and pneumocytes stained positive in lung sections collected on day 7 and day 8 p.i. (Figure 4F).

**Transmission electron microscopy.** On day 6 p.i., many virions were visible in the liver sections of the infected hamsters (Figure 5A and B), as well as phagolysosomes and vesicles detaching from the endoplasmic reticulum (Figure 5C).

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![Figure 4](image_url)

**Figure 4.** Immunohistochemical staining for PIRV antigen in representative tissue sections of experimentally infected hamsters. (A) Liver, day 4 p.i.; magnification, ×1,000. (B) Liver, day 4 p.i.; ×1,000. (C) Kidney, day 6 p.i.; ×400. (D) Kidney, day 6 p.i.; ×400. (E) Intestine, day 6 p.i.; ×200. (F) Lung, day 7 p.i.; ×200.
PIRITAL VIRUS INFECTION IN HAMSTERS

DISCUSSION

Studies on the pathogenesis of LF have been limited because of the insidious nature of the early phases of the disease, its geographic occurrence, the paucity of good laboratory animal models, and the hazard of working with the etiologic agent. Lassa virus is classified as a biosafety level 4 agent, so it must be handled under maximum containment. PIRV, in contrast, is assigned to biosafety level 3. Results of this study indicate that the PIRV hamster model has many clinical and pathologic similarities to Lassa virus infection in humans, monkeys, and guinea pigs. Pirital virions (arrows) are observed in the hepatocytes and in the junctions between hepatocytes. Original magnification, \( \times 47,000 \) (A) and \( \times 62,700 \) (B). (C) Several vesicles separate from the endoplasmic reticulum of an infected cell (*), and a whole phagolysome is visible in the section (arrow). Original magnification, \( \times 14,100 \).

Another interesting finding in the PIRV-infected hamsters was the degree of pulmonary pathology. Pneumonitis was observed in the animals on the third or fourth day after infection. Later, pulmonary hemorrhage, edema, and hyaline membrane were present. This finding is very similar to the pathology observed with Lassa virus infection in guinea pigs. Guinea pigs dying of Lassa virus infection also develop interstitial pneumonitis, pulmonary edema, and hyaline membrane formation. Pneumonia, pulmonary edema, and adult respiratory distress syndrome also occur in some human patients with LF.

The two current animal models for experimental studies of LF are monkeys and guinea pigs. In comparing the histopathology of Lassa virus infection in humans, squirrel monkeys, and guinea pigs, Walker and others observed a disparity in the degree of pathology present in the liver and heart of the three species. Whereas humans develop hepatocellular necrosis, monkeys show only focal areas of hepatic necrosis with increased regenerative activity, and guinea pigs develop only foci of calcified hepatocytes. On the other hand, Lassa virus is myocardiotropic and myocardiopathic in guinea pigs and squirrel monkeys, but there are usually no observed myocardial lesions in humans. In this regard, the pathology seen with PIRV infection in hamsters is more like that observed with Lassa virus in humans.

Another point of interest was the marked elevation of aPTT values in the PIRV-infected hamsters on days 5, 7, and 8 (Figure 1). A similar finding was reported in a study of rhesus monkeys infected with Lassa virus. Lange and others reported that rhesus monkeys experimentally infected with Lassa virus developed elevated aPTT values during the terminal phase of their illness, but that FBG and PT levels remained near pre-infection (baseline) levels.

In summary, these studies indicate that hamsters infected with PIRV offer a realistic, low-cost, and relatively safe laboratory model for severe LF in humans. This model could provide new opportunities for studying the pathogenesis of LF and for evaluating potential therapeutics or vaccines.

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