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Pneumonitis in Syrian golden hamsters (*Mesocricetus auratus*) infected with Río Mamoré virus (family *Bunyaviridae*, genus *Hantavirus*)

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Abstract

Río Mamoré virus is an etiological agent of hantavirus pulmonary syndrome in South America. The purpose of this study was to determine whether Río Mamoré virus strain HTN-007 in Syrian golden hamsters is pathogenic. None of 37 adult hamsters infected by intramuscular injection of HTN-007, including 10 animals killed on Day 42 or 43 post-inoculation, exhibited any symptom of disease. Histological abnormalities included severe or moderately severe pneumonitis in 6 (46.2%) of the 13 animals killed on Day 7 or 10 post-inoculation. The primary target of infection in lung was the endothelium of the microvasculature. Collectively, these results indicate that Río Mamoré virus strain HTN-007 in adult Syrian golden hamsters can cause a nonlethal disease that is pathologically similar to hantavirus pulmonary syndrome.

Keywords

Hantavirus pulmonary syndrome; Maporal virus; Río Mamoré virus

1. Introduction

Hantavirus pulmonary syndrome (HPS) is a rodent-borne zoonosis caused by certain members of the virus family *Bunyaviridae*, genus *Hantavirus*. The viruses known to cause HPS include Sin Nombre virus (SNV) in Canada and the western United States (MacNeil et al., 2011; Stephen et al., 1994), Choclo virus (CHOV) in Panama (Vincent et al., 2000), Andes virus (ANDV) in Argentina and Chile (López et al., 1996; Toro et al., 1998), Laguna Negra virus (LANV) in Argentina and Paraguay (Johnson et al., 1997; Levis et al., 2004), and Río Mamoré virus (RIOMV) in French Guiana and Peru (Casapía et al., 2012; Matheus

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et al., 2012). Maporal virus (MAPV) is a South American hantavirus (Fulhorst et al., 2004) and phylogenetically closely related to ANDV (Richter et al., 2010). The human health significance of MAPV has not been rigorously investigated.

The severity of HPS ranges from mild febrile illness to fulminant, lethal pneumonitis. Virus genetics, inoculum dose, route of exposure, and human genetics likely affect the course and outcome of HPS (Koster and Levy, 2006; Vapalahti et al., 2001).

Severe HPS is characterized by diffuse pneumonitis, cardiac depression, and shock (Koster and Levy, 2006). The most striking gross abnormalities at autopsy are large volumes of frothy fluid in airways, severe pulmonary edema, and large volumes of pleural fluid (Zaki et al., 1995).

Previous studies revealed that MAPV strain HV 97021050 in hamsters is highly pathogenic (Milazzo et al., 2002), ANDV strain Chile-9717869 in hamsters is highly lethal (Hooper et al., 2001), and the pathology in hamsters infected with HV 97021050 and hamsters infected with Chile-9717869 is remarkably similar to the pathology in fatal HPS (Hooper et al., 2001; Milazzo et al., 2002). Hypothetically, hantaviruses phylogenetically closely related to MAPV and ANDV – in hamsters – can cause severe or fatal HPS-like disease.

The RIOMV strain HTN-007 was originally isolated from a small-eared pygmy rice rat (*Oligoryzomys microtis*) captured in Peru (Powers et al., 1999). The results of comprehensive analyses of genetic data indicated that RIOMV is phylogenetically more closely related to MAPV and ANDV than to SNV (Richter et al., 2010), which – in hamsters – appears to be highly infectious but not pathogenic (Hooper et al., 2001; Safronetz et al., 2013). The purpose of this study was to assess the pathogenicity of the RIOMV strain HTN-007 in Syrian golden hamsters (*Mesocricetus auratus*). We anticipated that HTN-007, like MAPV strain HV 97021050 and ANDV strain Chile-9717869, would cause severe and even highly lethal HPS-like disease.

2. Materials and Methods

2.1. Animals

Eight-week-old female Syrian golden hamsters were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN), housed in filter-bonneted microisolators on a 112-cage ventilated rack (Lab Products, Inc., Seaford, DE) inside a biosafety level 3 (BSL-3) laboratory, and acclimated to the BSL-3 laboratory environment for 6 days before they were inoculated with virus or injected with sterile 0.01-M phosphate-buffered saline, pH 7.4 (PBS). The care and use of the hamsters in the BSL-3 laboratory complied with the National Institutes of Health guidelines for the humane use of laboratory animals.

2.2. Viruses

Stocks of RIOMV strain HTN-007 and MAPV strain HV 97021050 were prepared from cultures of Vero E6 cells inoculated with twice plaque-purified virus. The passage history of the virus in the HTN-007 stock was Vero E6+9; the virus in the HV 97021050 stock was purified from virus isolated from lung from a moribund hamster in a previous study

(Milazzo et al., 2002); the passage history of the virus in the HV 97021050 stock was Vero E6+4, hamster lung+1, Vero E6+4; and the infectious titers of the RIOMV and MAPV stocks in monolayers of Vero E6 cells were $4.1 \log_{10}$ and $5.8 \log_{10}$ median infectious doses ($ID_{50-VeroE6}$)/0.2 mL, respectively.

2.3. Inoculation, husbandry and sampling of hamsters

Twenty-three animals each were injected with 0.2 mL of a $-1.0 \log_{10}$ dilution of the stock RIOMV, 14 animals each with 0.2 mL of a $-1.0 \log_{10}$ dilution of the $-1.0 \log_{10}$ dilution of the stock RIOMV, 4 animals each with 0.2 mL of a $-3.8 \log_{10}$ dilution of the stock MAPV, and 4 animals each with 0.2 mL of sterile PBS. The inocula that contained virus were prepared in sterile PBS; injection of virus suspension or sterile PBS was done at 1 site in the lumbar musculature, using a 27-ga X 5/8" hypodermic needle on a tuberculin syringe; back-titration of the inocula in monolayers of Vero E6 cells indicated that the virus-inoculated hamsters were injected with $3.3 \log_{10}$ plaque-forming units (PFU) of RIOMV (high dose), $2.3 \log_{10}$ PFU of RIOMV (low dose), or $2.1 \log_{10}$ PFU of MAPV. The 4 animals inoculated with MAPV were to provide an assurance that HV 97021050 could cause severe HPS-like disease in the hamsters used in this study.

The animals inoculated with RIOMV were housed by inoculum dose, with 2 or 3 hamsters per microisolator; the animals inoculated with MAPV were housed in a single microisolator; and the negative control (sterile PBS-injected) animals were housed in a single microisolator. Each animal was checked twice daily for symptoms of disease (e.g., lethargy, reluctance to move, inappetance, ruffled haircoat, tachypnea, dyspnea). Strict barrier care was followed throughout the study in order to obviate virus transmission between animals in different microisolators.

The animals inoculated with RIOMV were killed on Day 7, 10, 14, 21, 28, 42, or 43 post-inoculation (PI) (Table 1); the animals inoculated with MAPV were killed on Day 9 PI ($n = 2$) or died on Day 10 PI ($n = 2$); and the negative control animals were killed on Day 7 PI ($n = 2$) or Day 43 PI ($n = 2$). Euthanasia was by intraperitoneal (IP) injection of a lethal dose (150 mg/kg) of sodium pentobarbital.

Oropharyngeal secretions (OPsec), cardiac blood, lung, liver, spleen, kidney, and urine were collected from the animals inoculated with RIOMV; cardiac blood and lung were collected from the animals inoculated with MAPV and the negative control animals. The OPsec were collected on a sterile cotton swab and stored in 0.3 ml of sterile PBS containing 10% v/v heat-inactivated fetal bovine serum (FBS); urine was collected by cystocentesis, using a 27-ga X 5/8" hypodermic needle on a tuberculin syringe; the samples of blood, serum, OPsec, solid tissues, and urine were stored at -80°C ; and the carcasses were fixed by immersion in 10 volumes of 10% w/v neutral buffered formalin.

2.4. Antibody assays

Blood samples were tested for anti-hantavirus immunoglobulin G (IgG), using an indirect fluorescent antibody test (IFAT). The samples from the animals inoculated with RIOMV were tested for IgG to HTN-007, the samples from the animals inoculated with MAPV were tested for IgG to HV 97021050, and the samples from the negative control animals were

tested for IgG to HTN-007 and IgG to HV 97021050. Serial twofold dilutions (from 1:10 through 1:320) of each sample were tested against cell spots (Fulhorst et al., 1999) that contained either i) Vero E6 cells infected with HTN-007 mixed 1:1 with uninfected Vero E6 cells or ii) Vero E6 cells infected with HV 97021050 mixed 1:1 with uninfected Vero E6 cells; hamster IgG bound to antigen was revealed by using a goat anti-hamster IgG fluorescein isothiocyanate conjugate (Kirkegaard and Perry Laboratories, Gaithersburg, MD); and the titer of a positive sample was the reciprocal of the highest dilution that produced a specific pattern of fluorescence in the cytoplasm of approximately 50% of the cells in duplicate cell spots.

Serum samples from the animals inoculated with RIOMV and the negative control animals were tested for neutralizing antibody to HTN-007, using a plaque reduction neutralization test (PRNT) done in monolayers of Vero E6 cells in 6-well plates (each well 9.61 cm²). The virus challenge (approximately 100 PFU/0.2 mL) was prepared in sterile PBS containing 5% v/v guinea pig serum (Sigma-Aldrich®, St. Louis, MO, no. G9774); the serum samples were diluted 1:9 v:v in sterile PBS containing 0.75 g/100 mL bovine albumin (Sigma-Aldrich®, no. A4503), heat inactivated, and then mixed with an equal volume of the virus challenge; the serum-virus mixtures were incubated 1 h in a 37°C water bath; 0.2 mL of each serum-virus mixture was inoculated onto duplicate monolayers; adsorption was 1 h at 37°C in a humidified atmosphere containing 5% CO₂ in air; the first overlay was 3.0 mL of a semi-solid medium; the cultures were maintained at 37°C in a humidified atmosphere containing 5% CO₂ in air; the second overlay (applied on Day 8 PI) was 2.5 mL of a semi-solid medium and contained neutral red; plaques were counted each day, Days 9 through 12 PI; and a sample was considered positive if it neutralized 80% of the virus in the serum-virus mixture. The overlays were 1X Minimum Essential Medium Eagle with Earle's Balanced Salt Solution (Lonza, Walkersville, MD, no. 12-684F) containing 10% v/v heat-inactivated FBS, 0.01-M HEPES buffer, 0.15% w/v sodium bicarbonate, 1X L-glutamine (Mediatech, Inc., Manassas, VA, no. 25-005), 1X non-essential amino acids (Sigma-Aldrich®, no. M7145), 1X penicillin-streptomycin solution (Mediatech, Inc.), and either (first overlay) 0.6% w/v SeaKem® ME Agarose (Lonza, Rockland, MD, no. 50014) or (second overlay) 1% w/v Bacto™ Agar (Difco, Sparks, MD, no. 214010) and 0.033 g/100 mL of neutral red.

2.5. Virus assay

The fresh-frozen samples of blood, solid tissues, OPsec, and urine were tested for hantavirus by cultivation in monolayers of Vero E6 cells in culture flasks, each monolayer approximately 25 cm² (Milazzo et al., 2002). Cell spots (Fulhorst et al., 1999) prepared from the monolayers on Day 13 PI were tested for hantaviral antigen, using an IFAT in which the primary antibody was a hyperimmune mouse ascitic fluid raised against ANDV strain Chile-9717869. The titers of virus in the culture-positive lung samples were measured by plaque assay in monolayers of Vero E6 cells, using a modification of the PRNT described above.

2.6. Histopathology

The left lungs and samples of heart, liver, spleen, and kidney were dissected from the formalin-fixed carcasses, embedded in paraffin, and sectioned at 4 µm. The tissue slices

were deparaffinized, stained with hematoxylin and eosin, and then examined with a light microscope at X100 and X400. The extent of the inflammatory cell infiltrate in each section of lung, from apex to base, was assessed at X200 and then scored: 0 = no infiltrate, 1 = mild (occasional perivascular or interstitial aggregate of mononuclear inflammatory cells), 2 = moderately severe (perivascular and interstitial aggregates of mononuclear inflammatory cells in 25–30% of the section), 3 = severe (perivascular and interstitial aggregates of mononuclear inflammatory cells in 50% of the section).

2.7. Immunohistochemistry assay

Thin (4- μ m) sections of lung from the 6 animals inoculated with RIOMV and killed on Day 7 PI, 2 animals inoculated with MAPV and killed on Day 9 PI, and 2 negative control animals were tested for hantaviral antigen, using an immunohistochemistry assay (Milazzo et al., 2002). The primary antibody was immune serum from a rabbit inoculated with ANDV strain Chile-9717869; the secondary antibody was a biotinylated goat anti-rabbit IgG (Vector Laboratories, Inc., Burlingame, CA); goat antibody bound to rabbit IgG was detected by using the LSAB2 Streptavidin-Biotin System (DakoCytomation, Carpinteria, CA); and the chromogen and counterstain were diaminobenzidine and hematoxylin, respectively. Nonspecific binding of rabbit IgG was minimized by treating the tissue sections with 10% normal goat serum (Vector Laboratories, Inc.) for 20 min before application of the rabbit serum; endogenous biotin was blocked by using the DAKO Biotin Blocking Kit (DakoCytomation); endogenous peroxidase activity was minimized by treating the tissue sections with 3% hydrogen peroxide for 10 min immediately before heat-induced antigen retrieval; all steps in the staining process were done in an Autostainer Universal Staining System (DakoCytomation); and the negative controls included sections of lung from the MAPV-infected hamsters stained with normal (nonimmune) rabbit serum. The prevalence of antigen-positive cells in the entirety of an apex-to-base section of lung (X400) was scored: 0 = none, 1 = greater than or equal to 1% but less than 25%, 2 = greater than or equal to 25% but less than 50%, and 3 = greater than or equal to 50%.

3. Results

None of the 37 animals inoculated with RIOMV and none of the 4 negative control animals exhibited any symptom of illness. Examination of the carcasses revealed mild congestion in the lungs of 4 animals inoculated with RIOMV: 2 inoculated with the high dose inoculum ($3.3 \log_{10}$ PFU) and killed on Day 7 PI, 1 inoculated with the low dose inoculum ($2.3 \log_{10}$ PFU) and killed on Day 7 PI, and 1 inoculated with the low dose inoculum and killed on Day 10 PI. The internal organs and body cavities of the 33 other animals inoculated with RIOMV and 4 negative control animals were grossly unremarkable.

The 4 animals inoculated with MAPV appeared healthy until the morning of Day 9 PI. Two of these animals were killed late on Day 9 PI because they were in shock and moribund; the 2 other animals inoculated with MAPV were found dead in the morning of Day 10 PI. The gross abnormalities in the animals inoculated with MAPV were frothy fluid in large airways ($n = 2$), severely congested lungs ($n = 4$), and large volumes (2.1–6.0 mL) of straw-colored pleural fluid ($n = 3$).

Anti-hantavirus antibody (IgG) was found in 32 (86.5%) of the 37 animals inoculated with RIOMV (Table 1), all 4 animals inoculated with MAPV, and none of the 4 negative control animals. The IgG titers to RIOMV in the 22 antibody-positive animals inoculated with the high dose of HTN-007 ranged from 40 ($n = 2$) to 320 ($n = 10$), the IgG titers to RIOMV in the 10 antibody-positive animals inoculated with the low dose of HTN-007 ranged from 40 ($n = 1$) to 320 ($n = 9$), and the IgG titers to MAPV in the animals inoculated with HV 97021050 ranged from 40 ($n = 1$) to 160 ($n = 3$). Neutralizing antibody to RIOMV was found in 26 (70.3%) of the 37 animals inoculated with RIOMV (Table 1) and none of the negative control animals.

Hantavirus was isolated from 7 (77.8%) of 9 animals inoculated with the high dose of RIOMV and killed before Day 15 PI, 5 (45.5%) of 11 animals inoculated with the low dose of RIOMV and killed before Day 15 PI, none of 17 animals inoculated with RIOMV and killed after Day 15 PI, all 4 animals inoculated with MAPV, and none of the 4 negative control animals. The prevalence of culture-positive animals in the 23 hamsters inoculated with the high dose of RIOMV and killed before Day 15 PI was not statistically significantly different from the prevalence of culture-positive animals in the 14 hamsters inoculated with the low dose of RIOMV and killed before Day 15 PI (Fisher's exact test, two-sided, $p > 0.10$). The titers of hantavirus in lung from the 3 animals inoculated with the high dose of RIOMV and killed on Day 7 PI, 5 culture-positive animals inoculated with the low dose of RIOMV and killed on Day 7 or 10 PI, and 4 MAPV-inoculated animals ranged from 1.7 to 2.7, 1.7 to 2.4, and 5.7 to 6.3 $\log_{10}$ PFU/0.1 g, respectively.

All 23 hamsters inoculated with the high dose (3.3 $\log_{10}$ PFU) of HTN-007 and all 14 hamsters inoculated with the low dose (2.3 $\log_{10}$ PFU) of HTN-007 were positive for hantavirus and/or positive for anti-hantavirus antibody. Assuming that none of 14 hamsters inoculated with 1.3 $\log_{10}$ PFU of HTN-007 would become infected with hantavirus, 2.3 $\log_{10}$ PFU was equivalent to 0.5 $\log_{10}$ median hamster infectious doses ($ID_{50\text{-hamster}}$) of HTN-007 (Reed and Muench, 1938). Accordingly, the 23 hamsters inoculated with the high-dose inoculum each were injected with 1.5 $\log_{10}$ $ID_{50\text{-hamster}}$ of HTN-007.

Microscopic examination of lung revealed inflammation and edema in 2 animals inoculated with the high dose of RIOMV, inflammation in 12 (57.1%) of the 21 other animals inoculated with the high dose of RIOMV, inflammation and edema in 1 animal inoculated with the low dose of RIOMV, and inflammation in 9 (69.2%) of the 13 other animals inoculated with the low dose of RIOMV (Table 2). *RIOMV, high dose* – Severe pneumonitis, interstitial edema, and multiple foci of alveolar edema in 2 animals killed on Day 7 PI; moderately severe pneumonitis in 2 animals killed on Day 10 PI; mild pneumonitis in 10 other animals. *RIOMV, low dose* – Severe pneumonitis in 1 animal killed on Day 7 PI (Figure 1); severe pneumonitis, interstitial edema, and multiple foci of alveolar edema in 1 animal killed on Day 10 PI; mild pneumonitis in 8 other animals. There was a positive association between the success of virus isolation from lung and scores for extent of inflammation in lung from the 20 animals inoculated with RIOMV and killed on Day 7, 10, or 14 PI (Pearson's $r = 0.5971$ [$p < 0.01$]). Microscopic examination of lung from the animals inoculated with MAPV revealed severe inflammation and alveolar edema in 2 animals, severe inflammation and interstitial edema in 1 animal, and moderately severe

inflammation in 1 animal. The inflammatory infiltrate in the lungs of the affected animals, whether infected with RIOMV or MAPV, appeared to be composed mostly of lymphocytes. The pulmonary pathology in the RIOMV-infected animals with pulmonary edema and MAPV-infected animals was highly similar to the pulmonary pathology in MAPV-infected Syrian golden hamsters killed on Day 9 PI in a previous study (Milazzo et al., 2002).

Microscopic examination of liver revealed inflammatory cells in a small number of portal triads in 7 hamsters inoculated with the high dose of RIOMV: 3 killed on Day 7 PI, 2 (25.0%) of 8 killed on Day 10 or 14 PI, 2 (28.6%) of 7 killed on Day 21 or 28 PI. The inflammatory cells in the portal triads were mononuclear and appeared to be lymphocytes. The samples of heart, spleen, and kidney from the animals inoculated with the high dose of RIOMV were microscopically unremarkable; the samples of lung and other tissues from the negative control animals were microscopically unremarkable; and tissues other than lung from the animals inoculated with the low dose of RIOMV were not examined microscopically.

Hantaviral antigen was detected in 25–30% of the cells in lung from each of the 6 animals inoculated with RIOMV and killed on Day 7 PI. Hantaviral antigen also was detected in more than 50% of the cells in lung from the animals inoculated with MAPV and killed on Day 9 PI. The most intensive staining in the samples of lung from the RIOMV-inoculated animals and MAPV-inoculated animals was in the endothelium of small blood vessels.

4. Discussion

In a previous study (Hooper et al., 2001), as little as $1.3 \log_{10}$ PFU ($0.8 \log_{10} \text{ID}_{50}\text{-hamster}$) of ANDV strain Chile-9717869 in adult Syrian golden hamsters was highly lethal. Yet none of the hamsters inoculated with $3.3 \log_{10}$ PFU ($1.5 \log_{10} \text{ID}_{50}$) or $2.3 \log_{10}$ PFU ($0.5 \log_{10} \text{ID}_{50}$) of HTN-007 in this study exhibited any sign of illness. Perhaps a dose of HTN-007 substantively greater than $3.3 \log_{10}$ PFU would be highly lethal; however, numerous attempts to grow HTN-007 in cultures of Vero E6 cells to $4.1 \log_{10} \text{ID}_{50}\text{-VeroE6}$ (the titer of the stock HTN-007 in this study) were unsuccessful.

The endothelium in small blood vessels, in particular – pulmonary capillaries, is the primary target of infection in fatal HPS cases (Zaki et al., 1995) and in hamsters infected with ANDV, CHOV, MAPV, or SNV (Eyzaguirre et al., 2008; Hooper et al., 2001; Milazzo et al., 2002; Safronetz et al., 2013). The results of the immunohistochemistry assay in this study suggest that the endothelium in capillaries is the primary target of infection in the lungs of hamsters infected with HTN-007.

Microscopic abnormalities in lung from fatal HPS cases and lung from hamsters infected with ANDV or MAPV include vascular congestion, subacute interstitial pneumonitis, and interstitial and alveolar edema (Hooper et al., 2001; Milazzo et al., 2002; Zaki et al., 1995). The results of this study indicate that HTN-007 in hamsters can cause a transient pneumonitis, which – in some animals – is accompanied by interstitial or alveolar edema. Hypothetically, HTN-007 is not highly pathogenic in hamsters because it grows slowly in lung and/or other hamster tissues.

Presently, there are no post-exposure preventatives or curative therapeutics for HPS; however, the results of several studies done in hamsters (Custer et al., 2003; Hooper et al., 2008) suggested that neutralizing antibody administered before the onset of clinical disease can prevent HPS caused by ANDV. Conceptually, RIOMV in hamsters could be used to investigate the potential effect(s) of antibody to ANDV on the course and outcome of HPS caused by RIOMV.

The OPsec (throat swabs) and urine samples from the animals inoculated with HTN-007 were tested for hantavirus to assess whether secretions or excretions from infected hamsters pose a high risk of infection to laboratorians working at BSL-3 and following BSL-3 safety procedures (Chosewood and Wilson, 2009). The failure to isolate hantavirus from the samples of OPsec and urine suggest that work with HTN-007 in nine-week-old hamsters can be done safely at BSL-3.

In a previous study (Milazzo et al., 2002), MAPV strain HV 97021050 (passage history: Vero E6+4) killed 2 Syrian golden hamsters and sickened 20 (90.9%) of 22 other four-week-old Syrian golden hamsters infected by intramuscular injection of $3.1 \log_{10} \text{ID}_{50-\text{VeroE6}}$ of virus. The results of this study suggest that $2.1 \log_{10} \text{PFU}$ of plaque-purified HV 97021050 (passage history: Vero E6+4, hamster lung+1, Vero E6+4) in nine-week-old Syrian golden hamsters is more lethal than $3.1 \log_{10} \text{ID}_{50-\text{VeroE6}}$ of HV 97021050 (passage history: Vero E6+4) in four-week-old Syrian golden hamsters (Fisher's exact test, $p < 0.001$). Hypothetically, the lethality of MAPV can be significantly affected by inoculum dose, passage history of the virus, and/or age of the hamster at the onset of infection. Perhaps HTN-007 isolated from lung from a hamster with severe pneumonitis would be highly pathogenic in nine-week-old hamsters.

The results of a previous study (Wahl-Jensen et al., 2007) suggested that hamsters do not shed ANDV; however, in unpublished work (M. L. Milazzo), hantavirus was isolated from throat swabs and/or samples of urine from 9 (29.0%) of 31 adult Syrian golden hamsters killed 3–14 days after inoculation with ANDV strain Chile-9717869. Given that MAPV is phylogenetically closely related to ANDV, it seems prudent to assess the magnitude and duration of virus shedding in MAPV-infected hamsters at biosafety level 4 (BSL-4) before further work with MAPV-infected hamsters at BSL-3.

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- Rio Mamoré virus in Syrian golden hamsters is pathogenic but usually not lethal.
- The pathology in hamsters during the acute phase of infection includes diffuse pneumonitis and pulmonary edema.
- Rio Mamoré virus in hamsters may prove useful in the assessment of candidate therapies for hantavirus pulmonary syndrome.

Figure 1.

Thin (4- μ m) sections of lung (original magnification, X100) from 1A) a hamster infected with Rio Mamoré virus strain HTN-007 and 1B) an uninfected hamster. Note the diffuse interstitial pneumonitis and perivascular cuffing by lymphocytes in the infected hamster. No morphological evidence of inflammation was found in the section of lung from the uninfected hamster. The infected hamster was killed 7 days after intramuscular injection of $2.3 \log_{10}$ plaque-forming units of HTN-007, the uninfected hamster was killed 43 days after intramuscular injection of sterile phosphate-buffered saline, the sections of lung were stained with hematoxylin and eosin, and the sizes of the images were reduced 60% to fit into the figure.

Table 1

Results of assays for hantavirus, anti-hantavirus antibody (IgG), and neutralizing antibody in 37 hamsters inoculated with Rio Mamoré virus, by inoculum dose and number of days post-inoculation

Dose, 3.3 log ₁₀ PFU ¹	Number of days post-inoculation ²						Total
	7	10	14	21	28	43	
Hantavirus ³							
Blood	0/3	0/4	0/4	0/4	0/3	0/5	0/23
Lung	3/3	0/4	0/4	0/4	0/3	0/5	3/23
Liver	2/3	0/4	0/4	0/4	0/3	0/5	2/23
Spleen	2/3	1/4	1/4	0/4	0/3	0/5	4/23
Kidney	2/3	0/4	0/4	0/4	0/3	0/5	2/23
Total	3/3	1/4	1/4	0/4	0/3	0/5	5/23
Antibody (IgG) ⁴	2/3 (40)	4/4 (160)	4/4 (320)	4/4 (320)	3/3 (160)	5/5 (160)	22/23
Neutralizing antibody	0/3	3/4	3/4	4/4	3/3	5/5	18/23

Dose, 2.3 log ₁₀ PFU ¹	Number of days post-inoculation ²						Total
	7	10	14	21	28	42	
Hantavirus ³							
Blood	2/3	0/3	0/3	--	--	0/5	2/14
Lung	3/3	2/3	0/3	--	--	0/5	5/14
Liver	3/3	0/3	0/3	--	--	0/5	3/14
Spleen	1/3	2/3	1/3	--	--	0/5	4/14
Kidney	3/3	0/3	1/3	--	--	0/5	4/14
Total	3/3	2/3	2/3	--	--	0/5	7/14
Antibody (IgG) ⁴	0/3	2/3 (40)	3/3 (320)	--	--	5/5 (320)	10/14
Neutralizing antibody	0/3	1/3	2/3	--	--	5/5	8/14

¹ PFU, plaque-forming units.

² Number of hamsters positive/number of hamsters tested.

³ The tests for hantavirus in the oropharyngeal secretions and urine samples from all 37 hamsters were negative.

⁴ The numbers in parentheses are the medians of the IgG titers to Rio Mamoré virus in the antibody-positive animals.

Table 2

Prevalence of pneumonitis and pulmonary edema in 37 hamsters inoculated with Rio Mamoré virus, by inoculum dose and number of days post-inoculation

	Number of days post-inoculation ¹						Total
	7	10	14	21	28	42 or 43	
Dose, 3.3 log ₁₀ PFU ²							
Inflammation	3/3	3/4	2/4	2/4	2/3	2/5	14/23
Edema	2/3	0/4	0/3	0/4	0/3	0/5	2/23
Dose, 2.3 log ₁₀ PFU ²							
Inflammation	3/3	3/3	2/3	--	--	2/5	10/14
Edema	0/3	1/3	0/3	--	--	0/5	1/14

¹ Number of hamsters with pneumonitis or pulmonary edema/number of hamsters examined. The pneumonitis in 3 animals killed on Day 7 post-inoculation (PI) and 3 animals killed on Day 10 PI was severe or moderately severe; the pneumonitis in the 18 other animals with microscopic evidence of inflammation in lung, including 3 animals killed on Day 7 PI and 3 animals killed on Day 10 PI, was mild.

² PFU, plaque-forming units.