Supporting Information

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SI Materials and Methods

Biosafety and Containment. All work with infected animals and potentially infectious materials derived from them was conducted in the Biosafety Level 4 (BSL4) facility at the Rocky Mountain Laboratories (RML), Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health. Sample inactivation and removal were performed according to standard operating protocols approved by the local Institutional Biosafety Committee.

Virus Preparation. Wild-type (deer mouse-only–passaged) Sin Nombre virus (SNV) strain 77734 (DM-SNV) was propagated in deer mice supplied from an in-house breeding colony at RML that is routinely tested for prominent murine pathogens including ectromelia (mouse pox), mouse rotavirus (epizootic diarrhea of infant mice), lymphocytic choriomeningitis virus, *Mycoplasma* pulmonis, mouse adenoviruses 1 and 2, mouse hepatitis virus, mouse parvovirus, minute virus of mice, pneumonia virus of mice, murine norovirus, polyomavirus, reovirus 3, Theiler's murine encephalomyelitis virus, and Sendai virus. Deer mice (pathogen-free according to testing described above) were infected with a 10% (wt/vol) tissue homogenate containing SNV by i.m. injection into the hind-leg musculature (1). On day postinfection (dpi) 10, mice were euthanized, and lungs were collected and mechanically homogenized. Pooled aliquots were frozen immediately after small samples were collected for RT-PCR analysis. A tissue culture isolate of the same SNV strain (VA-SNV) was propagated in Vero cells according to standard practice. Supernatants were collected at 10 dpi and were concentrated by ultracentrifugation to remove tissue culture-derived mediators such as IFN (2). Aliquots were frozen immediately, and subsamples were titered on Vero cells using a focus assay as previously described (3). Because of the requirement for adapting SNV to in vitro cell culture to achieve a reliable infection, the infectious titers of the two inocula could not be standardized. The challenge dose was 6×10^6 focus-forming units (FFU) for VA-SNV. The DM-SNV inoculum was standardized to the VA-SNV based on quantitative real-time RT-PCR analysis of S genomic segment copies.

Animal Infection. Seventeen rhesus macaques (*Macaca mulatta*) weighing 3.3–6.7 kg were assigned randomly among three groups and were exposed to lung homogenates (10% wt/vol) from naive deer mice ($n = 3$,) or from deer mice infected with DM-SNV ($n = 10$), or VA-SNV ($n = 4$). Animals were infected using an established protocol of simultaneous installation by intratracheal (4 mL), intranasal (0.5 mL per nostril), intraocular (0.5 mL per eye), and oral (1 mL) routes. The challenge dose for VA-SNV was 6×10^6 FFU. Because the deer mouse-only–passaged SNV had not been adapted previously to growth in cell culture, it was not possible to obtain accurate infectious titers using in vitro methods. Therefore, the inoculum for nonhuman primates (NHPs) receiving DM-SNV was adjusted to an approximate challenge dose of 6×10^6 based on the cycle threshold values obtained through quantitative real-time RT-PCR. Mock-infected NHPs received an equivalent amount of uninfected deer mouse lung homogenates.

Clinical Score and Sample Collection. After challenge, animals were monitored twice daily and scored for clinical signs of disease (respiration, posture, fever, feces/urine, food intake, recumbence, attitude, and skin turgor) (4). On days −7, 0, 1, 3, 6, 9, 12, 16, 20, 24, 30, 36, and 42 animals were examined under anesthesia (i.m. injection of ketamine at 10–15 mg/kg), and chest radiographs, pulse rate, blood pressure, temperature, and respiration rate were taken. Blood samples were collected for analysis of blood chemistries, coagulation parameters, differential blood count, virology, and cytokine profiles and oral, nasal, and rectal swabs were collected in 1 mL DMEM for detection of viral RNA. Animals were euthanized when respiratory distress indicated terminal disease according to an approved endpoint scoring sheet (4). Complete necropsies were performed with collection of clinical specimens from nasal mucosa, oral mucosa, conjunctiva, tonsils, salivary gland, trachea, bronchi, right and left lung (upper, middle, and caudal lobes), lymph nodes (bronchial, cervical, axillary, inguinal, mesenteric) heart, liver, spleen, pancreas, jejunum, transverse colon, kidney, adrenal gland, testes/ovary, femoral bone marrow, urinary bladder, cervical spinal cord, and brain (stem, frontal, cerebellum).

Hematology, Serum Biochemistry, and Coagulation Parameters. Total WBC count, lymphocyte, platelet, reticulocyte, and RBC counts, hemoglobin, hematocrit values, mean cell volume, mean platelet volume, mean corpuscular volume, and mean corpuscular hemoglobin concentrations were measured in EDTA blood with the HemaVet 950FS+ hematology analyzer (Drew Scientific). Serum samples were tested for concentrations of glucose, blood urea nitrogen, creatinine, uric acid, calcium, albumin, total protein, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, gamma-glutamyltransferase, amylase, and cholesterol using a Piccolo Blood Analyzer (Abaxis). Citrate plasma was tested for coagulation parameters, including activated partial thromboplastin time (aPTT), prothrombin time, and fibrinogen concentration on a STart4 instrument using the PTT Automate, STA Neoplastine CI Plus, and Fibri-Prest Automate kits, respectively (all from Diagnostica Stago). D-dimer concentrations were determined in plasma samples using the Asserachrom D-Di ELISA (Diagnostica Stago).

Histopathology, Immunohistochemistry, and in Situ Hybridization. Tissues were inactivated and fixed with 10% formalin and processed according to standard methodologies and BSL4 standard operating procedures. Thin (5-μm) sections were stained with H&E using standard procedures or with phosphotungstic acid-hematoxylin using a commercially available kit according to the manufacturer's specifications (American MasterTech). Tissues for immunohistochemistry (IHC) and in situ hybridization (ISH) were stained on a Discovery XT automated instrument (Ventana Medical Systems) using polyclonal immune sera generated against SNV N (5) or probes specific for the SNV N gene (6) (nucleotides 151–1194; Advanced Cell Diagnostics, Inc.) as described (7). To confirm the presence of viral antigen in endothelial cells, a subset of lung specimens was costained with a monoclonal antibody targeting the N antigen (clone 5F1/F7; Austral Biologicals; 1:1,000 dilution) and polyclonal rabbit anti-CD31 antibodies (ab28364; Abcam; 1:50 dilution) with Alexa-Fluor 594 (anti-mouse) and 488 (anti-rabbit) secondary antibodies (Life Technologies, A11005 and A11034, respectively, each at 1:200 dilution). Slides were coverslipped with ProLong Gold antifade reagent with DAPI stain (P36931; Life Technologies). Slides were blinded and evaluated and scored by a veterinary pathologist.

To quantify cytokine-producing cells in tissues, specimens were stained with anti-CD3 (2GV6; 790–4341; Ventana; used undiluted), IL-4 (ab9622; Abcam; 1:100 dilution), TNF (ab1793; Abcam; 1:50

dilution), and INF-γ (ab9657; Abcam; 1:100 dilution) antibodies as outlined above. Lung sections (upper, middle, and lower right lobe) and heart samples from all animals and kidney, spleen, and liver samples from a subset of animals were stained. Sections were scanned with an Aperio ScanScope XT (Aperio Technologies, Inc.) and analyzed using the ImageScope Positive Pixel Count algorithm (version 9.1). For each tissue, ~25 mm^2 were evaluated at 2× magnification. The default parameters of the Positive Pixel Count (hue of 0.1 and width of 0.5) detected antigen adequately.

Polychromatic Flow Cytometric Analysis of Peripheral Blood Mononuclear Cells. Cryopreserved peripheral blood mononuclear cells were thawed and plated at 10^6 cells per well of 96-well round-bottom plates in DMEM supplemented with 10% FCS and antibiotics and were rested overnight at 37 °C, 5% CO_2. The following day cells were washed in PBS and resuspended in Live/Dead fixable Yellow dye (Life Technologies) according to the manufacturer's recommendation before antibody labeling. For surface staining, properly titrated antibodies were added to cells in PBS/0.6 mM EDTA/2% FBS for 30 min at 4 °C. Cells then were washed and fixed in 4% paraformaldehyde (PFA). For staining of intracellular antigens, cells were washed in BD Fixation/Permeabilization buffer, followed by incubation in 1× BD Permeabilization/Wash buffer. Titrated antibodies were added to cells in 1× BD Permeabilization/Wash buffer for 30 min at 4 °C. Cells then were washed and fixed in 4% PFA overnight before removal from high containment. Antibodies used were CD3-PE-Cy7 (SP34-2), CD8α-APC (SK1), Ki67-Alexa-Fluor700 (B56), CD69-FITC (L78), TNF-PerCP-Cy5.5 (Mab11), IFNγ-FITC (B27), Granzyme B-Alexa-Fluor700 (GB11), CD28-PE (CD28.2), CD95-FITC (DX2), CD14-Alexa-Fluor700 (M5E2) (BD), and CD4-eFluor450 (OKT4) (eBioscience). For stimulation experiments, rested cells were treated with phorbol12-myristate13-acetate (25 ng/mL) and ionomycin (250 ng/mL) for 6 h, with the addition of brefeldin A (10 μg/mL) for the last 4.5 h before antibody staining. Polychromatic analysis was performed using a LSR II flow cytometer equipped with FACS DiVA software (BD), and data were analyzed using FlowJo software (TreeStar).

Next-Generation Sequencing. DM-SNV was concentrated via ultracentrifugation of serum collected from a terminally ill NHP. RNA for DM-SNV and VA-SNV was extracted using RNeasy extraction kits (Qiagen). Viral cDNA synthesis, random PCR amplification, and generation of library pools followed previously described methods (8). For DM-SNV, a second set of cDNAs was synthesized using the Ovation RNA-Seq System V2 and the manufacturer's protocol following the Agencourt RNAclean XP purification method (Nugen Technologies). The cDNA was fragmented using the Covaris S2 adaptive focused acoustics instrument (Covaris) with the following settings: duty 10%, intensity 5, 200 cycles per burst, for 180 s. Libraries were prepared using the Ovation Ultralow Library System and the manufacturer's protocol (Nugen). VA-SNV was sequenced on the Genome Sequencer FLX generating 146,458 reads for an overall coverage of ~5,000-fold. DM-SNV was sequenced on the HiSEq. 2000 using standard reagents according to the manufacturer's instructions for paired-end libraries (Illumina).

The genomic sequence for VA-SNV was assembled de novo using the GS De Novo Assembler v2.6 (454 Life Sciences) and CLC Genomics workbench 4.0 (CLC Bio). Translated BLAST (blastx) was performed to weed out nonviral contaminants, and assembly was performed using Sequencher v5.0 (Gene Codes). Assembled contigs then were refined by mapping the 454 reads using GS Reference Mapper v2.6 (454 Life Sciences). DM-SNV reads were reference mapped to VA-SNV using Bowtie2 (v2.1.0) (9) at 122-fold coverage. SAMtools (10) variant call pipeline was used to determine variance and generate the consensus sequence.

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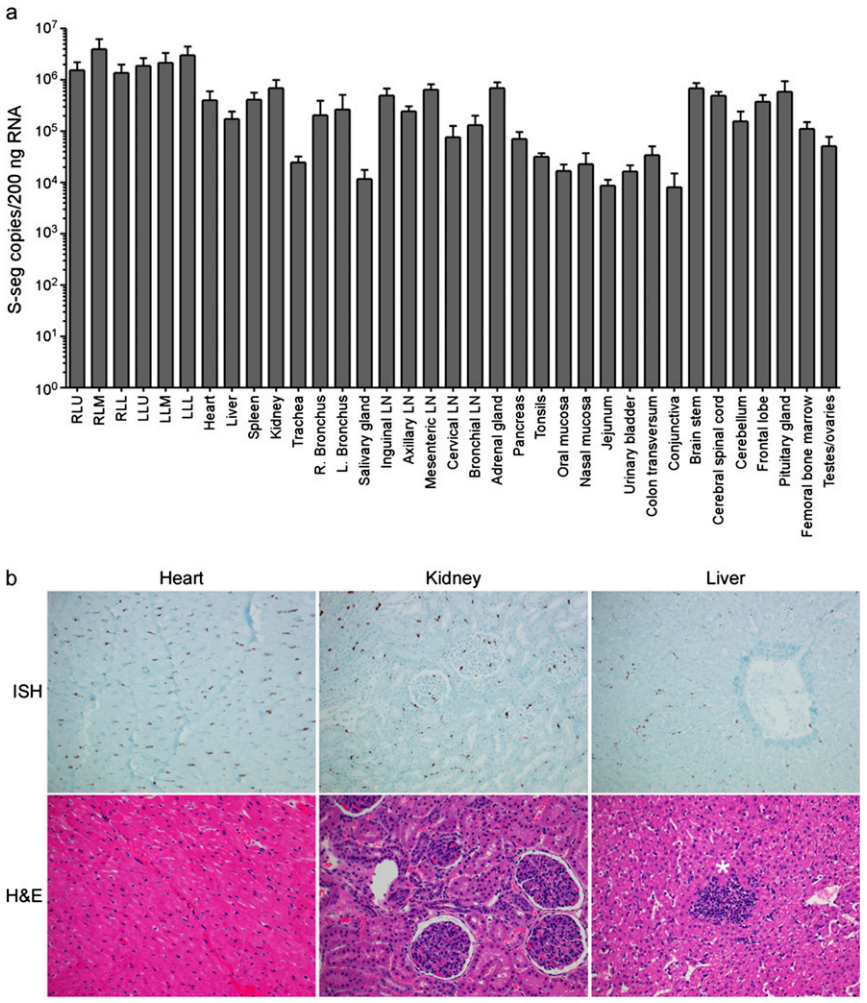

Fig. S1. Deer mouse-propagated SNV establishes a systemic infection in macaques. (*A*) Quantitative RT-PCR analysis. Inoculation of rhesus macaques with DM-SNV resulted in a systemic infection with viral RNA detected in all organs analyzed. Shown are the average RNA titers from organs collected from seven macaques that developed HPS. Error bars represent the SEM. (*B*) Histopathology. ISH for N RNA confirmed the presence of viral RNA in endothelial cells of peripheral organs. Despite widespread replication, no discernable pathological changes were noted outside of the lungs in most animals that developed HPS. The exception was multifocal hepatic coagulative necrosis with acute inflammation (indicated by an asterisk), which was noted in liver samples from two animals. Shown are heart, kidney, and liver samples collected from a representative animal that developed HPS at 18 dpi and stained with H&E or tested for the presence of viral RNA by ISH.

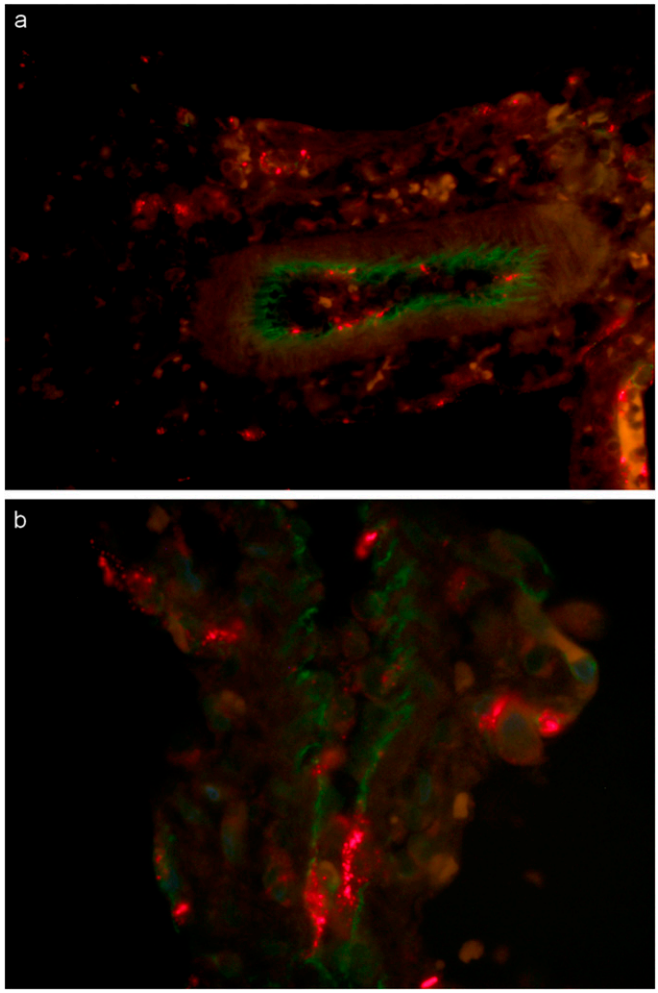

Fig. S2. Immunofluorescence staining for endothelial cell markers and hantaviral antigen in lungs. A subset of lung specimens from macaques infected with DM-SNV that developed HPS was costained for an endothelial cell marker (CD31 antigen, green) and hantavirus N antigen (red). The colocalization of hantaviral antigen and CD31 supports the notion that SNV replicates in pulmonary endothelial cells. (Magnification: *A*, 400×; *B*, 1,000×.)

Table S1. Summary of SNV infection in rhesus macaques

Inoculum group	NHP ID no.	Age, y	Sex	Weight, kg	Outcome (time of euthanasia)	Anti-hantavirus titer* (IgG) at euthanasia
Mock-infected	Mock 1	2.5	F	3.5	No signs of disease (42 dpi)	Negative
	Mock 2	3.3	F	3.8	No signs of disease (21 dpi)†	Negative.
	Mock 3	2.3	M	3.3	No signs of disease (21 dpi)†	Negative
DM-SNV	DM-SNV 1	4.5	M	5.2	Developed HPS (15 dpi)	100
	DM-SNV 2	4	M	5.4	No signs of disease (22 dpi)†	400
	DM-SNV 3	3	M	3.7	Developed HPS (18 dpi)	Negative.
	DM-SNV 4	2.5	M	4	No signs of disease (42 dpi)	Negative.
	DM-SNV 5	2.5	M	4	Developed HPS (18 dpi)	800
	DM-SNV 6	2.5	M	4.2	Developed HPS (22 dpi)	≥12,800
	DM-SNV 7	3	M	4.2	Developed HPS (18 dpi)	800
	DM-SNV 8	2.5	M	3.8	Developed HPS (20 dpi)	400
	DM-SNV 9	2.5	M	3.8	No signs of disease (42 dpi)	≥12,800
	DM-SNV 10	4	F	3.6	Developed HPS (16 dpi)	100
VA-SNV	VA-SNV 1	2.3	M	3.3	Mild signs of disease (21 dpi)†	≥12,800
	VA-SNV 2	2.3	M	3.3	No signs of disease (21 dpi)†	3,200
	VA-SNV 3	3.3	F	5.3	No signs of disease (42 dpi)	≥12,800
	VA-SNV 4	2.3	M	3.3	No signs of disease (42 dpi)	≥12,800

*Serological analysis of terminal serum samples was accomplished using standard ELISA methodologies with a bacterially expressed recombinant SNV nucleocapsid protein used as antigen.
†Euthanized to assess extent of virus replication.

Table S2. Summary of hematological, biochemical, and coagulation parameters in macaques that developed HPS

	Prechallenge	At time of euthanasia
Hematological parameters		
WBC ($\times 10^3$/μL)	5.68 (3.5–16.96)	12.22 (8.38–20.34)
Neutrophils ($\times 10^3$/μL)	1.88 (1.39–4.35)	4.80 (2.37–15.24)
Lymphocytes ($\times 10^3$/μL)	3.80 (1.47–11.28)	4.06 (1.01–6.42)
Monocytes ($\times 10^3$/μL)	0.21 (0.03–0.71)	0.35 (0.18–1.97)
Eosinophils ($\times 10^3$/μL)	0.17 (0.12–0.61)	0.65 (0.07–2.38)
Basophils ($\times 10^3$/μL)	0 (0–0.01)	0.02 (0–0.09)
RBC, M/μL	6.01 (4.85–6.97)	6.36 (5.01–8.12)
Hemoglobin, g/dL	12.4 (11.7–14.5)	13 (10.5–18.6)
Hematocrit, %	39.9 (31.3–45.5)	42.4 (31.3–54.7)
Mean corpuscular volume, fL	66.6 (64.6–71.6)	66.1 (62.4–69.2)
Mean corpuscular hemoglobin, pg	21.2 (20.4–24.1)	22.1 (19.6–25.7)
Mean corpuscular hemoglobin concentration, g/dL	31.2 (30.8–37.4)	33.2 (30.7–39.2)
RBC distribution width, %	15 (14.2–16.3)	15.3 (14.2–16.7)
Platelets ($\times 10^3$/μL)	374 (260-479)	201 (82–332)
Mean platelet volume, fL	11.3 (9.5–16.7)	12.6 (11.2–16)
Serum biochemistries		
Glucose, mg/dL	68 (56–83)	92 (51–148)
Blood urea nitrogen, mg/dL	20 (17–22)	24 (15–86)
Creatinine, mg/dL	0.7 (0.5–0.9)	0.9 (0.8–6.2)
Calcium, mg/dL	9.7 (9.2–10)	8.9 (8.6–9.6)
Albumin, g/dL	3.4 (3.1–3.6)	2.6 (2.1–3.1)
Total protein, g/dL	6.8 (6.2–8)	5.9 (5–7.2)
Alanine aminotransferase, U/L	32 (24–43)	38 (30–213)
Aspartate aminotransferase, U/L	44 (30–61)	92 (49–378)
Alkaline phosphatase, U/L	408 (309–682)	246 (197–364)
Total bilirubin, mg/dL	0.5 (0.3–0.6)	0.5 (0.5–0.6)
Gamma-glutamyltransferase, U/L	83 (66–137)	54 (36–99)
Amylase, U/L	331 (241–454)	186 (145–417)
Coagulation parameters		
Activated partial thromboplastin time, s	26.80 (22.9–34.8)	51.3 (33.5–60.8)
Prothrombin time, s	14.9 (12.4–15.9)	18.6 (14.4–20.5)
Fibrinogen	213 (160–264.7)	235.7 (184.7–330)
D-dimers	Negative	Negative

Shown are median values and range from seven macaques that developed respiratory distress indicative of HPS following infection with DM-SNV. Shaded areas indicate parameters which demonstrated changes throughout the course of disease. fL, femptoliter.