A Syrian Golden Hamster Model Recapitulating Ebola Hemorrhagic Fever

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Ebola hemorrhagic fever (EHF) is a severe viral infection for which no effective treatment or vaccine is currently available. While the nonhuman primate (NHP) model is used for final evaluation of experimental vaccines and therapeutic efficacy, rodent models have been widely used in ebolavirus research because of their convenience. However, the validity of rodent models has been questioned given their low predictive value for efficacy testing of vaccines and therapeutics, a result of the inconsistent manifestation of coagulopathy seen in EHF. Here, we describe a lethal Syrian hamster model of EHF using mouse-adapted Ebola virus. Infected hamsters displayed most clinical hallmarks of EHF, including severe coagulopathy and uncontrolled host immune responses. Thus, the hamster seems to be superior to the existing rodent models, offering a better tool for understanding the critical processes in pathogenesis and providing a new model for evaluating prophylactic and postexposure interventions prior to testing in NHPs.

Keywords. ebolavirus; Ebola hemorrhagic fever; animal model; pathogenesis; coagulopathy.

Zaire ebolavirus (ZEBOV) has been responsible for multiple Ebola hemorrhagic fever (EHF) outbreaks in Africa with high case-fatality rates [1]. There are no licensed vaccines or effective therapeutics presently available. Currently, 3 immunocompetent animal models have been developed for studying ZEBOV pathogenesis: different nonhuman primate (NHP) species, the guinea pig, and the mouse [2]. The NHP model is used for final evaluation of efficacy for vaccine and postexposure treatment, since, when infected with wild-type (WT) ZEBOV, NHPs exhibit the hallmark pathological and clinical features of EHF seen in humans with almost 100% lethality [3–5]. Conversely, the lethal rodent disease models depend on infection with rodent-adapted variants since WT-ZEBOV causes no apparent illness in immunocompetent rodents [6, 7]. Because of their ease of handling and the availability of research tools, mice are relevant and convenient animal models widely used to study multiple aspects of pathogenicity and host immune responses [8–11], as well as for in vivo prescreening of antivirals [12–14]. However, the mouse model does not consistently predict the efficacy of candidate vaccines or postexposure therapies in NHPs [15, 16]. This is perhaps because ZEBOV causes only limited and inconsistent coagulation abnormalities and hemorrhagic manifestations in rodents. In particular, disseminated intravascular coagulation (DIC) is often found in end-stage disease in humans and NHPs [1–5] but is absent in mice as assessed by the lack of fibrin thrombi in
spleen and visceral vasculature [16] and the minimal degree of change in coagulation parameters [17] (Supplementary Figure 1). The guinea pig model has a slightly better predictive value and shows a certain degree of coagulopathy upon EBOV infection but not to the extent of NHPs [17] (Supplementary Figure 1). Moreover, the lack of available reagents and tools, such as quantitative reverse-transcription polymerase chain reaction (qRT-PCR) and enzyme-linked immunosorbent assay (ELISA) for cytokine profiling, makes the guinea pig model less favorable. Therefore, it would be helpful to develop a small rodent model that better recapitulates EHF in humans, allowing for more relevant pathogenesis studies and high-throughput screening of prophylactic and postexposure treatments prior to their testing in NHPs.

Syrian golden hamsters (Mesocricetus auratus) have been broadly used as animal models for human infectious diseases, particularly for studying highly pathogenic RNA viruses [18]. Here, we have developed and characterized a novel lethal Syrian hamster model of EHF based on infection with mouse-adapted (MA) ZEBOV that manifests many of the clinical and pathological findings observed in ZEBOV-infected NHPs and humans, including a DIC-like syndrome. To determine the mechanisms of pathogenesis in this model, we characterized and compared host responses induced as a result of lethal versus nonlethal infection.

**MATERIALS AND METHODS**

**Cells, Viruses, and Infectivity Titration**

Vero E6 cells were maintained in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum. WT-ZEBOV (strain Mayinga 1976) and MA-ZEBOV [6] were propagated in Vero E6 cells. Virus infectivity titers (focus-forming units, FFU) were determined by counting the number of infected cell foci using an indirect immunofluorescent antibody assay, as previously described [11].

**Hamster Studies**

Syrian hamsters (male, 4–6 weeks) were obtained from Harlan Laboratories Inc. All animals were housed in microisolator cages and allowed to acclimatize prior to use in experiments. To examine the virulence of ZEBOVs, groups of animals were inoculated via the intraperitoneal route at 2 different sites with a total of 1000 FFU of either MA-ZEBOV (n = 14) or WT-ZEBOV (n = 8) in a total volume of 0.4 mL DMEM. For the pathogenesis study, 3–8 infected hamsters were anesthetized per time point (on days 1–6 and 8 postinfection) via inhalational isoflurane, bled, and exsanguinated via cardiac puncture. Collected organ samples were processed for virus infectivity assays, qRT-PCR, and pathological analyses. Mock controls were inoculated by the same route and with the same volume of DMEM.

**Hematology and Coagulation Parameter Assays**

Hematological parameters were determined from EDTA blood with the HemaVet 950FS+ laser-based hematology analyzer (Drew Scientific). Blood samples for coagulation parameters were collected into 1.8-mL citrate vacutainers. Plasma was separated by centrifugation and analyzed for coagulation parameters on the STart4 instrument (Diagnostica Stago).

**Histopathology and Immunohistochemistry**

Ten percent neutral-buffered, formalin-fixed tissues were processed and embedded in paraffin according to standard procedures. Thin sections (5 μm) were cut and stained with hematoxylin and eosin. For the detection of viral antigen and caspase-3, a rabbit polyclonal anti-VP40 antibody [11] and Anti-ACTIVE Caspase-3 pAb (Promega) were used as primary antibodies, respectively, and biotinylated goat antirabbit (BioGenex) secondary antibody and the DAB Map kit (Ventana Medical Systems) were used for visualization. For in situ terminal deoxynucleotidyl transferase–mediated dUTP nick-end label (TUNEL) staining, slides were pretreated with the enzyme Protease 2 (Ventana Medical Systems) before addition of the TUNEL reaction mixture (Roche). Sections were then incubated with an anti–digoxin-biotin conjugate (Sigma) and stained with diamobenzidine (DAB) using the DAB Map detection kit followed by hematoxylin counterstaining.

**Transcriptional Profiling of Host Responses by qRT-PCR**

A quantitative real-time RT-PCR assay for detection of hamster immunological-related gene transcripts was performed as described previously [18]. All experimental gene probes were labeled with 5′ 6FAM dye and quenched by 3′ BlackBerry Quencher (BBQ), while all internal control gene probes were labeled with Yakima Yellow and quenched by BBQ (TIB MOLBIOL). The data were analyzed using the ΔΔCT method [19]. In brief, the CT value for each test gene in both infected and mock-treated hamsters was first normalized to the 2 housekeeping gene (β-actin and RPL18) CT (ΔCT) and then compared to the averaged normalized CT value from all mock-treated (calibrator) hamsters to determine the ΔΔCT. The final value is displayed as the relative fold increase between the infected and mock-treated hamsters. To ensure the accuracy of the assay, we chose 2 housekeeping genes, β-actin and RPL18, and compared the results.

**Coagulation Parameters in ZEBOV-Infected Rhesus Macaques**

Three healthy, filovirus-seronegative, male rhesus macaques, designated subjects 1, 2, and 3, and weighing 10.8 kg, 12.6 kg, and 10.4 kg, respectively, were inoculated in the caudal thigh with 1 mL of virus stock containing 1000 FFU of WT-ZEBOV (strain Mayinga). Animals were monitored daily through clinical scoring (nonanesthetized) and examination (anesthetized). Venous blood was collected and analyzed using coagulation...
assays described above, with pre-bleeds collected 3 days prior to and at time of infection. All animals were euthanized when clinical signs indicated terminal disease according to an approved endpoint-scoring sheet. These experiments were performed under an animal study protocol approved by the RML Institutional Animal Care and Use Committee (IACUC).

Statistical Analyses
For descriptive means, results from coagulation parameters and hematology, virus growth, pathological scores, and qRT-PCR were expressed as mean values ± standard error of mean. Statistical analyses of data in Figures 1, 3–5, and 7 were calculated using the Student t test (2-tailed distribution, 2 sample equal variance). P values of <.05 were considered to indicate statistical significance.

Biosafety and Animal Ethics Statements
All work with ZEBOV was carried out in the biosafety level 4 (BSL-4) facilities at Rocky Mountain Laboratories (RML), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), and sample inactivation/removal was performed according to standard operating protocols approved by the local Institutional Biosafety Committee. All animal experiments were performed at RML in compliance with the guidelines of the NIAID/RML IACUC and were approved by the IACUC and performed following the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) by certified staff in an AAALAC-approved facility (RML).

RESULTS

Virulence of MA-ZEBOV and Course of Illness in Syrian Golden Hamsters
To develop a lethal ZEBOV infection model, 6-week-old hamsters were infected intraperitoneally with either 1000 FFU of WT-ZEBOV (strain Mayinga) or MA-ZEBOV, a lethal variant of WT-ZEBOV [6]. All animals infected with MA-ZEBOV started to show signs of disease (ruffled fur, decreased activity) at 3 days postinfection and succumbed to disease within the following 24 to 48 hours (4–5 days postinfection). In contrast, infection with WT-ZEBOV did not cause any apparent illness in hamsters (Figure 1A). Notably, MA-ZEBOV failed to produce lethal disease in hamsters when inoculated subcutaneously with doses ranging from 10 to 10⁵ FFU per animal (data not shown).

Evaluation of Coagulation Parameters
To evaluate the suitability of this animal as an EHF model, we investigated multiple coagulation parameters. MA-ZEBOV–infected hamsters showed significantly prolonged prothrombin time, activated partial thromboplastin time, and thrombin time values during late stages of infection, suggesting severe coagulopathy in fatally infected animals. Conversely, WT-ZEBOV–infected hamsters did not show significant changes from those of mock-infected animals (Figure 1B–D). Levels of fibrinogen in MA-ZEBOV–infected hamsters were increased at 3 days postinfection, peaked on 4 days postinfection, and suddenly dropped below normal levels prior to death (4 or 5 days postinfection) (Figure 1E). WT-ZEBOV–infected animals also showed increased but lower levels of fibrinogen peaking on day 5 postinfection and returning to normal by day 8 post-infection (Figure 1E). In MA-ZEBOV–infected animals, decreased protein C (PROC) activity was observed in plasma at 2 days postinfection. By 3 days postinfection, PROC activity had decreased to <40% of the levels in mock-infected animals, whereas WT-ZEBOV–infected animals showed increased levels of PROC activity that returned to normal levels by 8 days postinfection (Figure 1F). Thrombocytopenia was also observed in MA-ZEBOV–infected hamsters at the terminal stage of infection (Figure 1G). For comparative purposes, we also monitored coagulation parameters in rhesus macaques infected with WT-ZEBOV, and, notably, the course of coagulopathy was remarkably similar between hamsters and macaques (Figure 1H–M). These results clearly indicated that the hamster EHF model displays clinical features similar to those observed in humans and NHPs [1–5].

Tissue/Cell Tropism and Dissemination
The growth characteristics of MA-ZEBOV and WT-ZEBOV in hamsters were compared by examining viral titers in several organs. As expected, MA-ZEBOV replicated systemically to high titers in all tested organs (2- to 5-log difference), whereas WT-ZEBOV replicated poorly in hamster tissues (Figure 2A–D).

Immunohistochemistry highlighted the increased and more rapid replication of MA-ZEBOV compared to WT-ZEBOV in the main target organs, including the mesenteric lymph node (MLN), spleen, liver, and adrenal gland (Figure 2E–H, Supplementary Figure 2A–C). In MLN, target cells were morphologically identified as cells of the mononuclear phagocytic system (MPS), for example, macrophages and dendritic cells (DCs) (Figure 2E). In the spleen, viral antigen was mainly found in macrophages and marginal reticular–like cells (MRCs) in the red pulp and marginal zone. In the red pulp zone, macrophages and DC-like cells were positive for viral antigens, whereas no antigens were detected in lymphocytes (Figure 2F). In the liver, Kupffer cells were the first target cells and viral antigen was detected from 1 day postinfection in both MA- and WT-ZEBOV–infected animals (Supplementary Figure 2C). In MA-ZEBOV–infected animals, infection progressed from Kupffer cells to hepatocytes (Figure 2G) starting at 2 days postinfection; only a few antigen-positive hepatocytes were detected in
Figure 1. Virulence of mouse-adapted (MA) and wild-type (WT) Zaire ebolavirus (ZEBOV) in Syrian golden hamsters and coagulation parameters in hamsters infected with MA-ZEBOV or WT-ZEBOV and in rhesus macaques infected with ZEBOV. A, Kaplan-Meier survival curve. Groups of hamsters were inoculated intraperitoneally with 1000 focus-forming units (FFU) of MA-ZEBOV (n = 14) or WT-ZEBOV (n = 8). During the course of infection, weight loss was not apparent and hamsters maintained their weight throughout the study within 5% of baseline values (data not shown). There was no evidence of petechial rash or bleeding, both important clinical signs of Ebola hemorrhagic fever in nonhuman primates. Following infection of hamsters with 1000 FFU of either MA-ZEBOV or WT-ZEBOV, blood samples were collected and analyzed for (B) prothrombin time (PT), (C) activated partial thromboplastin time (aPTT), (D) thrombin time (TT), (E) fibrinogen concentration (FIB), (F) protein C activity (PROC), and (G) platelet count (PLT) using the STart4 instrument using the PTT Automate, STA Neoplastine CI plus, STA Thrombin, Fibri-Prest automate, and STA Staclot protein C kits, respectively (all from Diagnostica Stago) for coagulation parameters or HemaVet 950FS+ for platelet count. Changes in platelet count were indicated as percentage of change from values in mock-treated animals (100%). Solid circle/line, open square/line, and dashed line indicate values from MA-ZEBOV–infected, WT-ZEBOV–infected, and mock control hamsters, respectively. The number of animals at each time points for the infected groups ranged from 3 to 8 and for mock-infected controls from 3 to 7 (depending on the numbers of animals left at each time point). The day 5 postinfection (pi) values for hamsters infected with MA-ZEBOV also include values derived from moribund-terminal stage animals collected on day 4–4.5 pi, which is shown as 5/T on the x-axis. Graphs show the mean ± SEM for each measurement. *P < .05 and **P < .005 compared with mock-infected controls; *P < .05 and **P < .005 compared with WT-ZEBOV–infected animals. Three healthy, filovirus-seronegative male rhesus macaques designated as subjects 1, 2, and 3, weighing 10.8 kg, 12.6 kg, and 10.4 kg, respectively, were inoculated in the caudal thigh with 1 mL of virus stock containing 1000 FFU of WT-ZEBOV. Clinical examination and sample (plasma) collections were performed daily until animals were euthanized. H, PT; I, aPTT; J, TT; K, FIB; L, PROC (y-axis describes values as percentage of normal human protein C activity); M, PLT, as described above. Open circles, solid squares, and solid triangles indicate subjects 1, 2, and 3, respectively.
Figure 2. Growth characteristics of mouse-adapted (MA) and wild-type (WT) Zaire ebolavirus (ZEBOV) in hamsters. On days 1, 2, 3, 4, and 5 postinfection (pi), selected organs were collected from 3 infected animals per group, with the exception of the day 5 pi sample for MA-ZEBOV–infected animals where n = 1 due to the progression of infection. Virus infectivity titers in (A) spleen, (B) liver, (C) blood, and (D) kidney, heart, lung, and brain were determined in Vero E6 cells using a focus-forming assay. Virus titers of WT-ZEBOV in lung, heart, and brain were under the detection limit (50 focus-forming units (FFU)/mL or gram) of the focus-forming assay used for the titration. Solid circle/line and open square/line indicate values from MA-ZEBOV–infected and WT-ZEBOV–infected hamsters, respectively. Graphs show the mean ± SEM for each measurement. Viral antigen was detected using a rabbit polyclonal anti-VP40 antibody. Level of viral replication in tested organs (including viremia levels) in MA-ZEBOV–infected hamsters were comparable to the titers obtained in ZEBOV nonhuman primate models [3, 4]. The presence of viral antigen in MA-ZEBOV– and WT-ZEBOV–infected hamsters was compared among (E) mesenteric lymph nodes (MLNs), (F) spleen, (G) liver, and (H) adrenal gland. E, Major target cells in the MLN of MA-ZEBOV–infected hamsters (on day 3 pi). Macrophages (histiocytes) as well as dendritic cell (DC)–like cells were mainly positive for viral antigens. F, Major target cells in the spleen of MA-ZEBOV–infected hamsters (on day 3 pi). Viral antigens were mainly detected in macrophages in the red pulp. In the marginal zone, antigen-positive macrophages and marginal reticular-like cells (MRCs) were detected. In the white pulp zone, DC-like cells and MRCs were positive for viral antigens, whereas no antigen was detected in lymphocytes mainly consisting of T cells and B cells (in the B-cell follicle). G, Major target cells in the liver of MA-ZEBOV–infected animals (on day 3 pi). Kupffer cells were mainly positive for viral antigens at early stage of infection, while infection was expanded to include hepatocytes at later stages of infection. H, Antigen detection in the adrenal glands of hamsters infected with MA-ZEBOV (day 5 pi). In MA-ZEBOV–infected animals, viral antigens were detected at regions of the cortex (including zona glomerulosa and zona fasciculata) and medulla (containing chromaffin cells). There were only a limited number of antigen-positive cells detected in the adrenal glands of WT-ZEBOV–infected animals (data not shown).
Figure 3. Pathological changes in mesenteric lymph nodes (MLNs). A, Comparison of histopathological change scores in mouse-adapted (MA) and wild-type (WT) Zaire ebolavirus (ZEBOV)–infected animals. Histopathological scores were determined and compared between MA-ZEBOV–infected (n = 6 on day 1, 2, and 3 postinfection [pi]; n = 5 on day 4 pi; n = 1 on day 5 pi) and WT-ZEBOV–infected (n = 6 at all time points) using the following scoring system: 0 = no pathological changes; 1 = minimally increased numbers of inflammatory cells; 2 = moderately increased numbers of inflammatory cells and localized cellular depletion/necrosis; 3 = severe increase in the numbers of inflammatory cells and expanded cellular depletion/necrosis with disappearance of tissue compartments. *P < .05 and **P < .005 between MA- and WT-ZEBOV–infected animals. B–D, Hematoxylin-eosin staining of MLN from hamsters infected with MA-ZEBOV. B, Focal mild lymphocyte depletion was noted on day 3 pi. C, Depletion of cortical and paracortical lymphocytes, lymphadenitis, and variable lymphoblastic hyperplasia (black arrow) was noted in the infected animals on day 4 pi. D, Extensive lymphocytolysis and sinus hemorrhage were noted on day 5 pi. E–J, Detection of active caspase-3 and in situ terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining in MLN as markers for apoptosis. Detection of active caspase-3 for (E) MA-ZEBOV–infected animals on day 4 pi. F, WT-ZEBOV–infected animals on day 4 pi. G, Mock-infected control animals. H, In situ TUNEL for MA-ZEBOV–infected animals on day 4 pi. I, WT-ZEBOV–infected animal on day 4 pi. J, Mock-infected control animals. Apoptotic cells were morphologically identified as macrophages, dendritic cells, and lymphocytes.
WT-ZEBOV–infected hamsters (Supplementary Figure 2C). Additionally, ultrastructural analysis by electron microscopy confirmed viral replication in the liver (Supplementary Figure 3).

**Histopathologic Findings in Major Target Organs**

Despite the systemic viral replication, histopathological changes induced by MA-ZEBOV infection, including inflammatory cell infiltrations, cellular necrosis, and apoptosis, were
mainly noted in the lymphoid organs and liver. These pathological changes were similar to those seen in NHPs and other rodent models [4, 6, 7, 20]. In contrast, WT-ZEBOV infection in hamsters induced a limited inflammatory response and caused only minimal tissue damage (Figures 3A, 4A and 5A, and Supplementary Figure 2D–F).
In the MLN, MA-ZEBOV infection induced inflammatory lesions represented by histocytosis and neutrophilia at early stages of infection as well as lymphocyte depletion and extensive lymphocytolysis (Figure 3B–D). In situ TUNEL staining and detection of active caspase-3 in the MLN suggested that lymphocyte depletion and tissue destruction were due to apoptosis and possibly necrosis (Figure 3D, E, and H). Similar to mock-infected animals, WT-ZEBOV produced less prominent pathology, presented as only mild to moderate inflammatory lesions associated with a minimal number of apoptotic cells (Figure 3F, G, I, J, and Supplementary Figure 2D).

MA-ZEBOV infection caused splenitis throughout infection, resulting in severe diffuse necrosis associated with lymphocyte depletion and tissue destruction in the spleen (Figure 4B–D). Moreover, numerous apoptotic cells, mostly lymphocytes and MPS cells, were observed at late times postinfection (Figure 4E and 4H). In contrast, no lymphocyte depletion or necrotic or apoptotic lesions were observed in WT-ZEBOV–infected or mock-infected hamsters (Figure 4F, G, I, J, and Supplementary Figure 2E).

Acute hepatitis was noted in all hamsters infected with MA-ZEBOV on day 3 postinfection. Eosinophilic intracytoplasmic inclusion bodies (ICIBs) were observed in hepatocytes of MA-ZEBOV–infected animals (Figure 5B), confirming earlier electron microscopic data (Supplementary Figure 3B). On day 4 postinfection, MA-ZEBOV–infected hamsters exhibited disseminated, subacute hepatitis associated with hemorrhagic lesions (Figure 5C), and by day 5 postinfection,

![Figure 6. Cytokine and chemokine gene expression profiles in hamsters infected with mouse-adapted (MA) or wild-type (WT) Zaire ebolavirus (ZEBOV). A, Mesenteric lymph node (MLN). B, Spleen. C, Liver. D, Blood. Heat maps demonstrate the average responses with respect to gene induction as a fold increase over uninfected controls. Values for day 5 postinfection (pi) from hamsters infected with MA-ZEBOV also include values derived from moribund/terminal stage animals on day 4–4.5 pi, which are shown as 5/T days pi. The number of animals for these assays was 3–8 for MA-ZEBOV infection and 2–6 for WT-ZEBOV and was dependent on the availability of animals at each time point. The number of mock-infected animals for the assay was 3–7. The data were analyzed using the ΔΔCT method [19]. The final value is displayed as the relative fold increase between the infected and mock-treated hamsters. To assure the accuracy of the assay, we chose 2 housekeeping genes, β-actin and ribosomal protein L18 (RPL18), and compared the results. There was no apparent difference in the results of assay runs using either β-actin or RPL18 as the housekeeping gene, and the data presented in the figure were calculated relative to β-actin.](https://academic.oup.com/jid/article-abstract/207/2/306/2192360)
Figure 7. Type I interferon responses in hamsters infected with mouse-adapted (MA) or wild-type (WT) Zaire ebolavirus (ZEBOV). STAT1, Mx2, and PKR gene expression in mesenteric lymph node (MLN), spleen, liver, and blood. Solid circle/line, open square/line, and dashed lines indicate values for MA-ZEBOV–infected, WT-ZEBOV–infected, and mock control hamsters, respectively. Data are indicated as the relative fold increase between the infected/treated and mock treated hamsters. The CT value for each test gene in both infected and mock treated hamsters was first normalized to the β-actin and ribosomal protein L18 (RPL18) CT (ΔCT) and then compared to the averaged normalized CT value from all mock-treated (calibrator) hamsters to determine the ΔΔCT. The final value is displayed as the relative fold increase between the infected and mock treated hamsters and plotted on the graph. The number of animals for these assays was 3 at each time point. All values on day 5 postinfection (pi) from hamsters infected with MA-ZEBOV also include values derived from moribund/terminal stage animals on day 4–4.5 pi, which is shown as 5/T on the x-axis. Graphs show the mean ± SEM for each measurement. *P < .05 and **P < .005 from mock-infected controls; +P < .05 and ++P < .005 from WT-ZEBOV–infected animals.
pressed (Figure 6). Furthermore, fibrin deposits in liver sinuses, a hallmark of ZEBOV infection in macaques and humans, were detected by electron microscopy (Supplementary Figure 3E). In contrast, WT-ZEBOV–infected hamsters showed only random, mild, subacute hepatitis through the course of infection (Supplementary Figure 2F). Similar to the lymphoid organs, an extensive number of apoptotic Kupffer cells and hepatocytes were detected only in MA-ZEBOV–infected animals (Figure 5E–F).

**Profiling Cytokine and Chemokine Gene Expression**

The expression of 10 cytokine/chemokine genes in the MLN, spleen, liver, and blood of infected hamsters was monitored by our established quantitative real-time RT-PCR assay [18]. Overall, MA-ZEBOV infection induced significant activation of proinflammatory and anti-inflammatory responses, whereas WT-ZEBOV induced only weak, temporary activation of cytokine/chemokine genes in all tested organs, except for IP-10 at early stages of infection (Figure 6). Surprisingly, in MLN, only interleukin 1β (IL-1β), interleukin 6 (IL-6), and IP-10 were upregulated (Figure 6A) despite extensive viral replication. In the spleen, MA-ZEBOV infection induced significantly higher levels of expression of IL-1β, IL-6, and interleukin 10 (IL-10) throughout infection. In terminal MA-ZEBOV–infected animals, all selected cytokine/chemokine genes were highly expressed, suggesting an uncontrolled immune response in the spleen of animals at the terminal stage of infection (Figure 6B). Proinflammatory cytokines/chemokines including IL-1β, IL-6, tumor necrosis factor alpha (TNF-α), interleukin 12p35 (IL-12p35), and IP-10 were highly expressed in the liver of MA-ZEBOV–infected animals. At the terminal stage of infection, expression of interleukin 2, IL-12p35, interferon (IFN) γ, interleukin 4, and IL-10 was significantly suppressed, whereas IL-1β, IL-6, and transforming growth factor β were highly expressed (Figure 6C). In the blood of terminal MA-ZEBOV–infected animals, we observed intense expression of all analyzed genes (Figure 6D), similar to what is seen in the serum/plasma of NHPs infected with WT-ZEBOV [3, 4].

**Evasion of the Type I IFN Response**

The ability to counteract type I IFN responses and IFN–induced antiviral responses by viral proteins has been suggested to be a key determinant of ZEBOV virulence [21]. To determine whether the suppression of type I IFN responses by MA-ZEBOV infection is critical to pathogenesis in hamsters, we monitored STAT1, Mx2, and PKR gene expression as markers of the type I IFN response in multiple organs. WT-ZEBOV induced levels of gene expression significantly higher than MA-ZEBOV at early stages of infection (1 and 2 days postinfection) in all organs examined (Figure 7). Moreover, STAT1 and Mx2 expression levels were significantly higher at early and midstages of infection in target organs of WT-ZEBOV–infected animals. On the other hand, PKR expression was higher in the liver of MA-ZEBOV–infected animals (Figure 7). These results indicated that suppression of type I IFN by MA-ZEBOV is critical for ZEBOV pathogenesis in the hamster model.

**DISCUSSION**

In the present study, we have developed a novel lethal hamster model that closely reflects EHF in humans and NHPs. Importantly, this is the only small-animal model of EHF that exhibits severe coagulopathy, a clinical symptom that is critical for an EHF animal model to be valid and one that has made the macaque the gold standard [2–5, 16]. A decline in PROC coagulation inhibitor activity was associated with prolonged coagulation time and hypofibrinogemia in the terminal stage of disease. This coagulopathy was preceded by an initial abnormal increase in fibrinogen levels, representing the acute phase response to infection, which indicated severe dysfunction in coagulation and resembled the DIC observed in other VHFs, including EHF and sepsis patients [3, 5, 22–30]. Both the consumption of fibrinogen, represented by fibrin deposition in tissues, and the rapid increase in D-dimer plasma levels are hallmark features of EHF in cynomolgus macaques and humans [5, 30]. Similarly, fibrin deposition in liver sinuses, another hallmark feature, was detected in MA-ZEBOV–infected hamsters by electron microscopy, but we were unable to demonstrate increased plasma D-dimer levels in this model (most likely a result of technical problems in detection using a commercially available ELISA kit). PROC plays an important regulatory role in the anti-inflammatory, anti-apoptotic, and cytoprotective responses and thus helps to control the severe inflammatory responses and coagulation leading to hypercoagulation during sepsis [31, 32]. Thus, decreased production and consumption of PROC in ZEBOV-infected hamsters and NHPs is potentially one of the driving forces for severe disease progression. This concept is supported by the positive effect seen in postexposure treatment of ZEBOV-infected rhesus macaques with recombinant human activated PROC, which attenuates the coagulation impairment and hinders disease progression [33]. It will be interesting to employ the hamster model to validate the efficacy of drugs used to control coagulopathy, such as recombinant activated PROC.

Extensive apoptosis in major target organs was the most prominent histopathological change observed in MA-ZEBOV–infected hamsters. As observed in the NHP model and humans [34–36], bystander apoptosis of lymphocytes without evidence for viral replication may be the principal mechanism behind the depletion of lymphocytes. Although a recent study in mice suggested that lymphocyte apoptosis is
not essential for lethal ZEBOV infection [8], apoptosis is clearly prominent in MA-ZEBOV–infected but not WT-ZEBOV–infected hamsters, suggesting that, at least in hamsters, apoptosis contributes significantly to lethal outcome. In addition, because PROC and fibrinogen are synthesized in the liver [24], severe hepatocellular degeneration, massive apoptosis, and necrosis with inflammation most likely result in decreased production of PROC and fibrinogen [24, 31, 32], which may explain the severe coagulopathy seen in MA-ZEBOV–infected hamsters. In fact, liver dysfunction has also been implicated in the coagulation defects seen during severe sepsis [23, 24, 26, 29].

The absence of a type I IFN response at the early stage of infection in MA-ZEBOV–infected hamsters likely enhances viral replication in target organs and contributes to lethal disease. In contrast, replication of WT-ZEBOV in hamsters might be suppressed by the strong induction of type-I IFN genes in target organs. A significant role for type I IFN responses in ZEBOV pathogenesis was also reported for the mouse model [37, 38]. To date, 2 viral proteins, VP35 and VP24, have been shown to inhibit RIG-I–mediated IFN activation and Jak/STAT type I IFN signaling, respectively [21, 39, 40]. Our study suggests that VP35 of WT-ZEBOV might not be able to significantly antagonize the type I IFN response in hamsters, as indicated by the massive upregulation of type I IFN–related genes in infected animals. On the other hand, we have previously demonstrated that specific amino acid changes acquired in NP and VP24 during mouse adaptation of ZEBOV represent the minimum/critical requirement for the acquisition of virulence in the mouse model [11]. Moreover, it has also been suggested that VP24 is a key determinant of virulence in guinea pigs [41]. Together, this implies that VP24 might be a species-specific virulence determinant. Indeed, we observed significantly higher expression of Mx2—a marker for type I IFN signaling—in hamsters infected with WT-ZEBOV than in those with MA-ZEBOV, implying that that VP24 might play a significant role for virulence in hamsters. Whether VP24 truly represents a virulence determinant in hamsters is the subject of future research.

Notably, suppression of the type I IFN response and appearance of coagulation abnormalities were followed at late stages of disease by an uncontrolled systemic inflammatory response, which is thought to be a significant factor for EHF disease progression in humans and NHPs [42–45]. This aberrant host response might be triggered by organ-specific activation of IL-6 and TNF-α, both of which are associated with disease severity and fatal outcome in VHF cases including EHF [42–47]. Our data therefore suggest that simultaneous modulation of host innate immunity and coagulation by ZEBOV at early stages of infection is a key trigger for the systemic pathogenic process in EHF. Interestingly, hamsters infected with WT-ZEBOV exhibited a downregulation of the proinflammatory response, despite showing a strong induction of type I IFN genes in target organs. Induction of the type I IFN response simultaneously initiates a negative feedback loop that serves to tightly regulate the innate immune response to pathogens. Indeed, such negative regulation of the Toll-like receptor–mediated induction of proinflammatory cytokines prevents endotoxemia in mice [48], and a similar mechanism may explain the reduction in proinflammatory cytokines observed here.

The present data suggest that our newly developed hamster EHF model is superior to the current ZEBOV rodent models and may one day replace those models as an alternative for pathogenesis studies and efficacy testing of vaccines and therapeutics. Although the predictive value of the hamster model remains to be shown, numerous research tools are now available—including hamster lymphocyte–specific monoclonal antibodies and qRT-PCR assays [18, 49, 50]—that will facilitate the use of this animal model in future research on Ebola virus pathogenesis.

### Supplementary Data

**Supplementary materials** are available at The Journal of Infectious Diseases online (http://www.oxfordjournals.org/our_journals/jid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

### Notes

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### References


34. Reed DS, Hensley LE, Geisbert JB, Jahrling PB, Geisbert TW. Depletion of peripheral blood T lymphocytes and NK cells during the course of Ebola hemorrhagic fever in cynomolgus macaques. Viral Immunol 2004; 17:390–400.


