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ANIMAL MODELS FOR CCHFV AND BSL-2, BSL-3 SURROGATE MODELS

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Abstract

Crimean-Congo hemorrhagic fever virus (CCHFV) is an important tick-borne human pathogen endemic throughout Asia, Africa and Europe. The pathogenic mechanisms of CCHF are poorly understood, largely due to the dearth of animal models. However, several important animal models have been recently described, including novel murine models and a non-human primate model. This review, we examine the current knowledge of CCHF-mediated pathogenesis and describe how animal models are helping elucidate the molecular and cellular determinants of disease. This information should serve as a reference for those interested in CCHFV animal models and their utility for evaluation of medical countermeasures and in the study of pathogenesis.

Keywords: virus, zoonotic, surogate models

INTRODUCTION

In 1973, Crimean-Congo hemorrhagic fever virus (CCHFV) was identified as the singular causative agent of two separate illnesses, Congo fever (identified in 1956) and Crimean fever (identified in 1944). CCHFV is a member of the *Nairoviridae* family in the order *Bunyavirales*, a group of enveloped tri-segmented negative stranded RNA viruses. Despite having been originally identified in West Central Africa and the Crimea, today the virus is endemic throughout a wide geographical area that includes Africa, Asia and Europe. The presence of the virus in these regions is directly correlated with the presence of the main arthropod vector of CCHFV, *Hyalomma spp* ticks. While CCHFV is endemic in many areas, the expansion of the host-range of the ticks is allowing the virus to emerge in new areas [4].

CCHFV has a dichotomous relationship with animals and humans. While CCHFV infects a large number of wild and domesticated mammalian species, including bovines and ovines, and some avian species such as ostriches, the virus does not cause severe disease in these species. Instead, infections in these animals are predominantly asymptomatic, often resulting in a viremia that can last >5 days which helps maintain CCHFV in nature. In marked contrast, CCHFV infection in humans can lead to a severe, even life-threating, disease with key features that include coagulopathy, hepatic injury and neurological disorders. An in-depth understanding of CCHFV-mediated pathogenesis has been hampered by the lack of animal models. However, several murine and non-human primate models have recently been developed which will provide a means to investigate CCHFV pathogenesis, in addition to providing a platform to bridge medical countermeasure (MCM) development to humans.
One of the major problems to study this virus is the lack of BSL4 facilities.

Small Animal Models

CCHFV does not cause disease in immunocompetent adult rodents, including mice, rats, guinea pigs and hamsters. Until 2010, the only available models were neonatal mice and neonatal rats which were first used in 1967 by Chumakov and colleagues. However, Bereczky, S. et al. discovered that strain 129 mice lacking the type I interferon receptor A ($IFNAR^{-/-}$) were susceptible to CCHFV and produced a lethal/severe disease model. Subsequently, these

studies were repeated in C57BL/6 mice also lacking the type I interferon (IFN-I) receptor. Additionally, CCHFV produces severe disease in STAT-1$^{-/-}$ mice and mice lacking both the IFN-I receptor and IFN-gamma receptor (IFNAGR$^{-/-}$). These animals have deficiencies in both IFN-I and type II interferon (IFN-γ) signaling. We recently developed a novel murine system by exploiting an antibody against IFN-I receptor A (MAR1-5A3) that was previously shown to produce severe disease models with other unrelated viruses. This antibody produces a transient IFN-I blockade in mice and results in consistent lethal/severe CCHFV infection. The advantage to this model is it creates the same phenotype as an IFN-I receptor knockout animal in virtually any wild-type or transgenic mouse without the need for cross-breeding

The disease produced in the antibody-mediated IFN-I blockade model is essentially identical to the disease observed in genetic KO animals with similar mean times to death. In addition to conventional mouse systems, Spengler et al. developed a novel humanized mouse model by transferring human CD34^{+} stem cells into NOD-SCID-gamma (NSG)-SGM3 mice, which are extremely immunodeficient mice lacking mature T-cells, B-cells, and natural killer (NK) cells and have defects in cytokine signaling due to lack of the common gamma chain. Infection of these mice with CCHFV produces neurological disease. Below we describe how these murine systems are being used to evaluated CCHF pathogenic processes in addition to MCM development.

Non-Human Primate Models

The development of an NHP model that recapitulates human CCHF disease has been a challenging area of research. Earlier studies of CCHFV infection of African green monkeys, baboons, and patas monkeys were unsuccessful. Recently, a cynomolgus macaque severe disease model was described that establishes the first immunocompetent animal model for CCHF. NHPs were infected with the European human clinical isolate of CCHFV, strain Kosova Hoti, using an intravenous (IV) or combined IV and subcutaneous (SC) high dose (5 $\log_{10}$ $TCID_{50}$) exposure. The animals became viremic and developed a severe and sometimes fatal disease characterized by inflammatory immune responses, elevated liver enzymes, increased clotting times, thrombocytopenia, leukopenia and fever, which are all representative of human cases of CCHF. Histopathology demonstrated that CCHFV mainly targeted the liver and spleen where in situ hybridization identified viral RNA in the hepatocytes, Kupffer cells, and endothelial cells.
The development of the cynomolgus macaque model represents an important advancement in the field where an immunocompetent CCHF animal model is now available to study pathogenic disease mechanisms and evaluate candidate medical countermeasures. Adding further value is the ability to use two genetically unrelated strains, Hoti and Afg09-2990, which both produce disease in the NHP. This model should be further refined to determine reproducibility by evaluating variables such as virus strain/stock, dose, and genetic background of NHPs. Furthermore, the mechanism and impact of viral RNA persistence on the development of long-term sequela is an important area of future research in the NHP model.

Surrogate Models

Because CCHFV research requires BSL4 containment and many researchers do not have access to such facilities, several groups have developed surrogate nairovirus murine models. Hazara virus (HAZV) is a nairovirus isolated from the *Ixodes redikorzevi* tick and is a member of the CCHFV serogroup. Evidence to date indicates that HAZV is non-pathogenic in humans and can be manipulated in BSL2 environments. Dowall, et al. demonstrated that similar to CCHFV, HAZV is pathogenic in IFNAR$^{-/-}$ mice. HAZV infection in IFNAR$^{-/-}$ mice led to severe disease with a MTD of ~5 days depending on viral dose. Histopathological changes in the liver and spleen were detected and are analogous to that of CCHFV infection of mice. Recently a novel nairovirus called Tolfa virus (TFLV) was isolated from *Haemaphysalis flava* ticks and *Haemaphysalis fomsensis* ticks in Japan. TFLV is also in the CCHFV serogroup. Shimada, et al. found that this virus, though considered non-pathogenic in humans, produced severe disease in IFNAR$^{-/-}$ (A129 background) mice. Infection in these mice resulted in pathological effects in the intestinal tract and was lethal with a MTD of ~4–5 days. Liver involvement in TFLV murine infection was not specified in the published reports.

In addition to HAZV and TFLV, another nairovirus termed Dugbe virus (DUGV) has shown promise as a CCHFV surrogate. DUGV is a member of the Nairobi sheep disease virus serogroup.

Infection of mice either immunocompromised by cyclophosphamide treatment (within 48 h) or IFNAR$^{-/-}$ mice results in a lethal disease which included respiratory tract

involvement (lung edema) and also a neurological disease. Contrary to HAZV and TFLV, DUGV has been reported to occasionally cause human disease, particularly in children [101]. For this reason, study of DUGV requires BSL3 containment. Interestingly, one report suggested that a human isolate of DUGV (IbH11480), contrary to tick-isolates, could produce disease in immunocompetent mice. Despite DUGV not being in the same serogroup as HAZV and TFLV, the possibility that tick and human isolates have differing phenotypes in immunocompetent mice may allow for important insight into viral genetic factors influencing nairovirus pathogenesis. Overall, the use of BSL2 and BSL3 surrogates for CCHFV is promising and suggest that these viruses, in particular HAZV, should continue to be investigated as surrogate models for CCHFV pathogenesis.

CONCLUSIONS

There is an urgent need for not only rapid diagnostics to identify CCHF cases, but also MCMs that can mitigate disease, particularly in a post-exposure setting. The advent of new models for studying disease in rodents and NHPs lays the foundation for important advancements for CCHFV research. These systems will be critical in elucidating the complex host-pathogen dynamics leading to CCHFV-induced organ injury and severe disease.

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