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Simian hemorrhagic fever virus: Recent advances

Margo A. Brinton*, Han Di, Heather A. Vatter

Georgia State University, Atlanta, GA, USA

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ABSTRACT

The simian hemorrhagic fever virus (SHFV) genome differs from those of other members of the family Arteriviridae in encoding three papain-like one proteases (PLP1α, PLP1β and PLP1γ) at the 5′ end and two adjacent sets of four minor structural proteins at the 3′ end. The catalytic Cys and His residues and cleavage sites for each of the SHFV PLP1s were predicted and their functionality was tested in *in vitro* transcription/translation reactions done with wildtype or mutant polyprotein constructs. Mass spectrometry analyses of selected autoproteolytic products confirmed cleavage site locations. The catalytic Cys of PLP1α is unusual in being adjacent to an Ala instead of a Typ. PLP1γ cleaves at both downstream and upstream sites. Intermediate precursor and alternative cleavage products were detected in the *in vitro* transcription/translation reactions but only the three mature nsp1 proteins were detected in SHFV-infected MA104 cell lysates with SHFV nsp1 protein-specific antibodies. The duplicated sets of SHFV minor structural proteins were predicted to be functionally redundant. A stable, full-length, infectious SHFV-LVR cDNA clone was constructed and a set of mutant infectious clones was generated each with the start codon of one of the minor structural proteins mutated. All eight of the minor structural proteins were found to be required for production of infectious extracellular virus. SHFV causes a fatal hemorrhagic fever in macaques but asymptomatic, persistent infections in natural hosts such as baboons. SHFV infections were compared in macrophages and myeloid dendritic cells from baboons and macaques. Virus yields were higher from macaque cells than from baboon cells. Macrophage cultures from the two types of animals differed dramatically in the percentage of cells infected. In contrast, similar percentages of myeloid dendritic cells were infected but virus replication was efficient in the macaque cells but inefficient in the baboon cells. SHFV infection induced the production of pro-inflammatory cytokines, including IL-1β, IL-6, IL-12/23(p40), TNF-α and MIP-1α, in macaque cells but not baboon cells.

1. Introduction

SHFV is a member of the family Arteriviridae that also includes equine arteritis virus (EAV), lactate dehydrogenase-elevating virus (LDV), and porcine reproductive and respiratory syndrome virus (PRRSV) (Snijder and Kikkert, 2013). A related virus, wobbly possum disease virus (WPDV), was recently identified (Dunowska et al., 2012). Arterivirus genomes are positive-sense, single-stranded, polycistronic RNAs with a 5′ type I cap and a 3′ poly(A) tract (Snijder and Spaan, 2006). Among the arteriviruses, EAV has the shortest genome (12.7 kb) while SHFV has the longest (15.7 kb). The genome organization and replication strategies of arteriviruses are similar to those of coronaviruses. However, arterivirus genomes are about half the size of coronavirus genomes and the virions of the two virus groups differ in their structural protein compositions, capsid types (coronaviruses-helical and arteriviruses-spherical) and virion sizes.

Arteriviruses infect macrophages (MΦs) and dendritic cells (DCs) and have restricted host ranges; EAV infects horses and donkeys, PRRSV infects pigs, LDV infects mice and SHFV infects non-human primates (NHPs) (Snijder and Meulenberg, 1998). Disease symptoms associated with EAV and PRRSV infections include fever, respiratory disease, tissue necrosis and spontaneous abortions while LDV typically causes an asymptomatic, persistent infection (Snijder and Spaan, 2006). The majority of research on arteriviruses has focused on EAV and PRRSV due to the significant agricultural impact of the diseases they cause.

2. SHFV infections in NHPs

SHFV was first isolated in 1964 as the causative agent of hemorrhagic fever outbreaks associated with high mortality in macaque colonies in the United States and the USSR (Allen et al., 1968;

* Corresponding author. Tel.: +1 404 413 5388; fax: +1 404 413 5301.
E-mail address: mbrinton@gsu.edu (M.A. Brinton).

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Palmer et al., 1968; Tauraso et al., 1968; Shevtsova, 1969; Lapin and Shevtsova, 1971). African monkeys, such as baboons and African green, patas, guenons and colobus monkeys, are natural hosts of SHFV and typically develop asymptomatic, persistent infections with low level viremia (Bailey et al., 2014b; London, 1977; Lauck et al., 2011, 2013). SHFV-infected macaques develop fever, facial edema, anorexia, dehydration, depression and coagulation defects indicated by skin petechiae, retrobulbar hemorrhages and subcutaneous hematomas. Death occurs 7–13 days after infection (Allen et al., 1968; Palmer et al., 1968; London, 1977). The clinical signs and disease course closely resemble those induced in macaques by other hemorrhagic fever virus infections, such as Ebola Zaire, Marburg, and Lassa (Mahanty and Bray, 2004; Bray and Geisbert, 2005). The presence of persistently infected African NHPs in the NIH primate colony that had several SHFV outbreaks and the use of the same needle for tattooing or tuberculosis testing of multiple NHPs of African and Asian origin are thought to have resulted in inadvertent virus transmission from an African animal to macaques (Allen et al., 1968; Palmer et al., 1968; Tauraso et al., 1968). SHFV is typically transmitted between African NHPs during fighting but can spread efficiently among macaques by both direct and indirect contact (London, 1977; Renquist, 1990).

Based on an unpublished number of test animals, it was previously estimated that 1% of baboons, 1% of African green monkeys and 10% of patas monkeys are SHFV-positive (London, 1977). In a recent study, sera/plasma samples from 205 baboons from a US colony were tested for SHFV using RT-PCR assays targeted to two conserved sequence regions (Vatter et al., 2015). Of the wild-caught Olive baboons tested, 14.2% were positive for SHFV. However, because sera samples collected at the time of capture were not available, it is not known whether these animals were infected in the wild or after capture. Nineteen percent of the opportunistically sampled animals living in the colony were SHFV-positive. However, because most of the colony animals tested were males that were being treated for lacerations obtained during fighting, the percentage of SHFV-positive animals in the entire colony is expected to be lower. None of the SHFV positive baboons showed any signs of disease. Infections lasting >10 years were documented using serial archived sera samples and the viremia levels were consistently low (100–1000 PFU/ml).

3. SHFV isolates

The outcomes of experimental infections of patas monkeys with one of four previous SHFV isolates indicated differences in their biological properties (Gravell et al., 1986). LVR was isolated from a stump tail macaque that died of hemorrhagic fever disease (Tauraso et al., 1968), P-248 was isolated from a feral, persistently infected patas monkey, P-180 was isolated from a patas monkey that died from an SHFV infection, and P741 was isolated from a rhesus macaque that died after inoculation of SHFV from an asymptomatic persistently infected patas monkey. Infection of patas monkeys with two of the isolates (LVR and P-180) produced acute infections characterized by transient clinical signs of hemorrhagic disease with p-180 causing more severe disease that was only rarely fatal. Infection of patas monkeys with either P-248 or P741 produced asymptomatic, long-term persistent infections. Acutely infected animals produced high levels of anti-viral antibody while antibody titers in persistently infected animals were low. Lytic infections were induced by LVR in MA-104 cells, a rhesus macaque kidney cell line, as well as in both patas and rhesus peritoneal MΦs. P-180 could lytically infect both types of MΦs but not MA-104 cells while P-248 and P-741 could lytically infect only rhesus macaque MΦs. All four of these isolates efficiently induced acute fatal hemorrhagic fever disease in macaques. EAV and PRRSV can also efficiently infect MA104 cells. The MΦ-restricted protein sialoadhesion (CD169) mediates attachment and internalization by clathrin-mediated endocytosis of EAV and PRRSV (Van Breedam et al., 2010). CD163 was identified as an arterivirus co-receptor required for nucleocapsid release from endosomes and is also monocyte-macrophage lineage restricted (Welch and Calvert, 2010). MA104 cells express CD163 and CD151 but not sialoadhesin. It was recently reported that SHFV LVR enters MA104 cells by clathrin-mediated endocytosis and that treatment of MA104 cells with anti-CD163 antibody decreased SHFV replication (Cai et al., 2014).

Only the prototype SHFV isolate, LVR v42-0/M6941, survived from the earlier studies of SHFV (Tauraso et al., 1968). The species of African NHP from which LVR originated is not known for certain but patas monkeys housed in the same facility were subsequently shown to be persistently infected with SHFV and the source of the SHFV initiating later epizootics in macaques in the same facility (London, 1977). A complete sequence of the SHFV LVR genome was obtained in the early 1990s by sequencing “shot gun” clones using older methods (GenBank Accession number AF180391.1). During the construction of the first SHFV infectious clone, nucleotide differences were detected between the RT-PCR amplified cDNA fragments used to construct the full length clone and the GenBank sequence. The SHFV LVR genome RNA was also subjected to 454 sequencing and the same 18 nt differences compared to the GenBank sequence were detected by 454 genomic sequencing and individual fragment sequencing. The GenBank sequence was updated (GenBank Accession number AF180391.2).

Although inoculation of a low dose of SHFV LVR in sera or culture fluid by the intramuscular route consistently induced severe hemorrhagic disease in macaques in the 1960s (Palmer et al., 1968; Tauraso et al., 1968), no correlation between SHFV LVR dose (50–500,000 PFU) and hemorrhagic disease induction in macaques was observed in a 2011 study and the majority of the animals (12 of 16) that did not survive were found to have bacterial sepsis (Johnson et al., 2011b). The inoculum used in this study was generated by three freeze–thaw cycles of infected MA104 cells. Activation of cell RNA sensors by the large amounts of cell and viral [genomic and subgenomic (sg)] RNAs in these lysates may have induced a strong host antiviral response that contributed to the decreased efficiency of hemorrhagic disease induction observed. Also, defective SHFV genomes present in the LVR stock used may have reduced the efficiency of virus replication (Vatter et al., 2015).

SHFV isolates obtained from two SHFV-positive Olive baboons (B11661 and B11662) that were housed together only in the early 1990s and persistently infected >10 years were amplified by a single passage in primary rhesus macaque MΦs and then the genomes were 454 sequenced (Vatter et al., 2015). The two sequences obtained were very similar to each other, differing at 15 nt positions within the 15.7 kb genome. Surprisingly, these genome sequences were also very similar (differing at 7 or 10 unique positions, respectively) to that of a three times serially plaque-purified stock of SHFV LVR grown in MA104 cells. Four Japanese macaques injected intravenously with 100 PFU of B11661 developed severe hemorrhagic fever disease, characterized by high level viremia, the production of pro-inflammatory cytokines, elevation of tissue factor and coagulopathy, but showed no evidence of bacterial sepsis. Infected MΦs were detected in liver and spleen tissue sections using antibody to viral nonstructural proteins and the MΦ marker CD68. SHFV infection in macaques has been proposed as a BSL2 model for viral hemorrhagic fever disease (Johnson et al., 2011b).

Another recent study sequenced SHFV genomes obtained from wild Mikumi yellow baboons and captive Olive baboons in the same US primate facility used for the study described above and found them to be divergent (Bailey et al., 2014a,b). SHFV genome sequences obtained from additional wild species of African NHPs, red colobus and red-tailed guenons, have also been reported (Lauck et al., 2013; Bailey et al., 2014a,b). Two different strains