Virology 474 (2015) 186–198

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

A simian hemorrhagic fever virus isolate from persistently infected baboons efficiently induces hemorrhagic fever disease in Japanese macaques

Heather A. Vatter [a], Eric F. Donaldson [b], Jeremy Huynh [b], Stephanie Rawlings [c], Minsha Manoharan [c], Alfred Legasse [d], Shannon Planer [d], Mary F. Dickerson [e], Anne D. Lewis [e], Lois M.A. Colgin [e], Michael K. Axthelm [c,d], Jerilyn K. Pecotte [f], Ralph S. Baric [b], Scott W. Wong [c,d], Margo A. Brinton [a,*]

[a] Department of Biology, Georgia State University, Atlanta, GA 30302, United States
[b] Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, United States
[c] Vaccine and Gene Therapy Institute, Oregon Health & Science University, Beaverton, OR 97006, United States
[d] Division of Pathobiology and Immunology, Oregon National Primate Research Center, Beaverton, OR 97006, United States
[e] Division of Comparative Medicine, Oregon National Primate Research Center, Beaverton, OR 97006, United States
[f] Southwest National Primate Research Center, Texas Biomedical Research Institute, San Antonio, TX 78227, United States

ARTICLE INFO

Article history:
Received 16 September 2014
Returned to author for revisions
19 September 2014
Accepted 13 October 2014
Available online 19 November 2014

Keywords:
Simian hemorrhagic fever virus
Persistent infection
Baboon
Japanese macaque
Hemorrhagic fever disease
Coagulopathy

ABSTRACT

Simian hemorrhagic fever virus is an arterivirus that naturally infects species of African nonhuman primates causing acute or persistent asymptomatic infections. Although it was previously estimated that 1% of baboons are SHFV-positive, more than 10% of wild-caught and captive-bred baboons tested were SHFV positive and the infections persisted for more than 10 years with detectable virus in the blood (100–1000 genomes/ml). The sequences of two baboon SHFV isolates that were amplified by a single passage in primary macaque macrophages had a high degree of identity to each other as well as to the genome of SHFV-LVR, a laboratory strain isolated in the 1960s. Infection of Japanese macaques with 100 PFU of a baboon isolate consistently produced high level viremia, pro-inflammatory cytokines, elevated tissue factor levels and clinical signs indicating coagulation defects. The baboon virus isolate provides a reliable BSL2 model of viral hemorrhagic fever disease in macaques.

Introduction

Simian hemorrhagic fever virus (SHFV) was first isolated in 1964 and shown to be the causative agent of fatal hemorrhagic fever outbreaks in macaque colonies in the United States and the USSR (Allen et al., 1968; Palmer et al., 1968; Tauraso et al., 1968; Shevtsova, 1969; Lapin and Shevtsova, 1971). Mortality approached 100% by 2 weeks after infection (Tauraso et al., 1968; Gravell et al., 1980). The clinical signs of an SHFV infection in macaques closely resemble those of other hemorrhagic fever viruses such as Ebola, Marburg and Lassa viruses (Mahanty and Bray, 2004; Bray, 2005). Viral hemorrhagic fever disease is characterized by the release of pro-inflammatory cytokines from infected macrophages (MΦs) and dendritic cells (DCs) that induces tissue factor production and subsequent disseminated intravascular coagulopathy (Geisbert et al., 2003b; Bray, 2005; Levi et al., 2006). Previous SHFV outbreaks in primate facilities are thought to have been initiated by inadvertent blood-to-blood transmission from a persistently infected African nonhuman primate (NHP) to a macaque (London, 1977). In the case of an outbreak at NIH, virus was likely transmitted by the use of the same needle for tattooing or tuberculosis testing of multiple NHPs of African and Asian origin (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).

SHFV is a member of the Family *Arteriviridae*, which also includes equine arteritis virus (EAV), porcine reproductive and respiratory syndrome virus (PRRSV), and lactate dehydrogenase-elevating virus (LDV), in the order *Nidovirales* (Snijder and Kikkert, 2013). A related virus, wobbly possum virus, was recently identified (Dunowska et al., 2012; Snijder and Kikkert, 2013). Arteriviruses typically have restricted

* Correspondence to: Department of Biology, Georgia State University, P.O. Box 4010, Atlanta, GA 30303, United States. Fax: +1 404 413 5301.
E-mail address: mbrinton@gsu.edu (M.A. Brinton).

http://dx.doi.org/10.1016/j.virol.2014.10.018
0042-6822/

cell tropisms and host ranges; MΦs and DCs are infected by EAV in horses and donkeys, by PRRSV in pigs, by LDV in mice and by SHFV in several species of African NHPs and macaques but not in chimpanzees or humans (Snijder and Meulenberg, 1998). EAV and PRRSV infections can cause diseases in susceptible host species characterized by fever, anorexia, tissue necrosis, inflammation of the respiratory tract and reproductive failure, such as spontaneous abortions or delivery of weak offspring (Snijder and Kikkert, 2013). In mice, LDV typically causes lifelong, asymptomatic, persistent infections that are characterized by increased serum levels of lactate dehydrogenase (Brinton and Plagemann, 1983; Snijder and Kikkert, 2013). Due to the significant agricultural impact of diseases caused by EAV and PRRSV, the majority of research on arteriviruses has been focused on these two viruses.

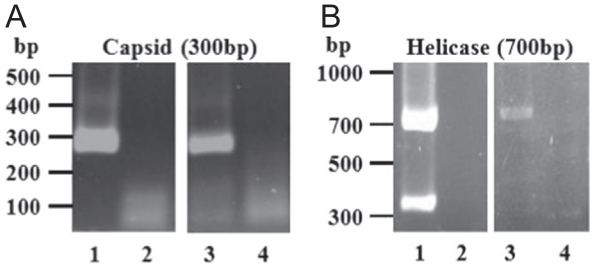
Only a single SHFV isolate, LVR v42-0/M6941, obtained from a stump-tailed macaque that died of SHF during the Bethesda 1964 SHFV epizootic (Tauraso et al., 1968), survived from earlier studies of SHFV and was available from the American Type Culture Collection (ATCC). Although the origin of this virus is not known for certain, patas monkeys (*Erythrocebus patas*) housed in the same facility were later found to be persistently infected with SHFV and to be the source of the SHFV infecting new rhesus macaques in subsequent epizootics in the same facility (London, 1977). Although SHFV LVR efficiently induced SHF disease in macaques in the 1960s (Tauraso et al., 1968), in a recent study, no correlation was observed between virus dose (ranging from 50 to 500,000 PFU/ml) and efficiency of induction of fatal hemorrhagic fever disease in macaques (Johnson et al., 2011). Bacterial sepsis was detected in 75% (12 of 16) of the SHFV-infected animals that did not survive suggesting that this played a major role in mortality. Genome sequences for a number of divergent strains of SHFV have recently been reported (Lauck et al., 2011; 2013). However, viruses containing these genomes have not yet been biologically characterized.

In the present study, a survey for SHFV in wild caught and captive baboons was done using two RT-PCR assays targeting conserved regions of the SHFV genome. The data indicated that more than 10% of both populations were SHFV-infected compared to the previous estimate of 1% (London, 1977). Data from serial archival samples used for testing indicated that animals remained infected for at least 10 years. Persistently infected baboons had very low levels of viremia. SHFV isolates from two persistently infected baboons (> 10 years) were amplified once in primary macaque MΦs and subsequently sequenced. The genome sequences of these isolates were very similar both to each other and to that of SHFV LVR, an isolate from the 1960s from a macaque that may have been infected with virus originating from a patas monkey. Intravenous injection of Japanese macaques (*Macaca fuscata*) with 100 PFU of one of the baboon SHFV isolates resulted in consistent induction of severe hemorrhagic fever disease, characterized by high level viremia, the production of pro-inflammatory cytokines, elevation of tissue factor and coagulopathy, in each of four infected animals. Cells co-expressing viral nonstructural proteins and the MΦ marker CD68 were detected in liver and spleen. Sepsis was not identified in any of the macaques. SHFV infection in macaques has been proposed as a BSL2 model for viral hemorrhagic fever disease (Johnson et al., 2011). Using the new baboon isolate of SHFV and Japanese macaques, we demonstrated consistent disease induction in the absence of bacterial infection with a low dose virus inoculum.

Results

Survey of wild-caught and current baboons at the Southwest National Primate Research Center

Because we required PBMCs from SHFV-negative baboons for studies comparing MØ and DC responses to experimental



C

Baboon ID	Capture/Birthdate	Sampling Date	Nucleocapsid	Helicase
B11661	4/1/1990	12/1/2000	+	+
		9/28/2007	+	+
		1/19/2010	+	+
B11662	1/1/1992	11/4/1999	+	+
		7/29/2003	+	+
		8/11/2005	+	+
		1/19/2010	+	+
B19927	2/10/2004	4/7/2008	+	+
		4/21/2010	+	+
		10/19/2010	+	+
B25360	9/5/2004	10/20/2008	+	+
		4/21/2010	+	+
		10/19/2010	+	+

Fig. 1. Detection of SHFV RNA by RT-PCR in baboon sera. RNA isolated from 100 μl of baboon serum was analyzed by one-step RT-PCR. Pairs of primers were designed to amplify (A) nucleocapsid or (B) helicase regions of the SHFV genome. RNA extracted from an aliquot of a stock pool of SHFV-LVR containing 10^7 PFU/ml of virus was used as the positive control and nuclease-free water was used as the negative control. PCR products were separated on 1% agarose gels. Lane 1, SHFV-LVR RNA; lane 2, water; lane 3, SHFV-positive baboon serum; and lane 4, SHFV-negative baboon serum. The results shown are representative of the results obtained for sera from a total of 33 SHFV-positive and 166 SHFV-negative baboons. (C) Assay of SHFV RNA in multiple archived samples from the same animal. RNA extracted from 100 μl of an archived serum sample was tested for SHFV using both RT-PCR assays.