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SHORT COMMUNICATION

Ebola Virus Localization in the Macaque Reproductive Tract during Acute Ebola Virus Disease

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Sexual transmission of Ebola virus (EBOV) has been demonstrated more than a year after recovery from the acute phase of Ebola virus disease (EVD). The mechanisms underlying EBOV persistence and sexual transmission are not currently understood. Using the acute macaque model of EVD, we hypothesized EBOV would infect the reproductive tissues and sought to localize the infection in these tissues using immunohistochemistry and transmission electron microscopy. In four female and eight male macaques that succumbed to EVD between 6 and 9 days after EBOV challenge, we demonstrate widespread EBOV infection of the interstitial tissues and endothelium in the ovary, uterus, testis, seminal vesicle, epididymis, and prostate gland, with minimal associated tissue immune response or organ pathology. Given the widespread involvement of EBOV in the reproductive tracts of both male and female macaques, it is reasonable to surmise that our understanding of the mechanisms underlying sexual transmission of EVD and persistence of EBOV in immune-privileged sites would be facilitated by the development of a nonhuman primate model in which the macaques survived past the acute stage into convalescence. *(Am J Pathol 2018, 188: 550–558; https://doi.org/10.1016/j.ajpath.2017.11.004)*

Sexual transmission of filoviruses has been suspected for as long as these viruses have been recognized as human pathogens. During the first recorded outbreak of Marburg virus disease in 1967, sexual transmission from an infected man to a woman was presumed but never proved.[1] Ebola virus (EBOV) was detected in the semen of a researcher infected while handling diagnostic samples from the first recorded EBOV outbreak in 1976.[2] The 2014 to 2016 EBOV outbreak in West Africa again raised the question of sexual transmission of filoviruses and the potential for asymptomatic infections and EBOV persistence in immune-privileged sites.[3–6] Findings from >17,000 survivors of the outbreak in West Africa suggest sexual transmission is rare. To date, only two recorded incidences of sexual transmission of EBOV from that outbreak have been documented.[7,8] However, the implications of EBOV transmission by this route are significant given an Ebola virus disease (EVD) mortality rate of >40%.[9,10] We hypothesized that EBOV would infect the reproductive tissues in the macaque model of acute EVD. The purpose of this study was to identify and localize the virus within the reproductive tissues in the macaque model, typically acutely fatal between 6 and 10 days after i.m. challenge. Using this EVD model, we demonstrate EBOV infection of the

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Table 1 Signalment, EBOV in Peripheral Blood, and EBOV Distribution in the Reproductive Tract of Female and Male Macaques

Animal ID	Macaque species	Age, months	Necropsy after inoculation, days	EBOV vRNA, copy/mL (log10)	EBOV IHC results						TEM results
					Ovary	Uterus	Testis	Prostate gland	Seminal vesicle	Epididymis	
1	Cynomolgus	46	7	10.6	Stromal/vascular	Stromal/vascular					Stromal/EC
2	Cynomolgus	53	8	9.96	Stromal/vascular	Stromal/vascular					Stromal
3	Cynomolgus	53	7	10.1	Stromal/vascular	Stromal/vascular					Stromal/EC
4	Rhesus	35	7	10.4	Stromal/vascular	Stromal/vascular					Stromal
5	Cynomolgus	48	6	10.3			Stromal/vascular	Stromal/vascular	Stromal/vascular	Stromal/vascular	Stromal/EC
6	Cynomolgus	44	8	10			Stromal/vascular	Stromal/vascular	Stromal/vascular	Stromal/vascular	Stromal/EC
7	Rhesus	44	9	8.83			Stromal/vascular	Stromal/vascular	Stromal/vascular	NP	Stromal/EC
8	Rhesus	48	7	9.78			Stromal/vascular	Stromal/vascular	Stromal/vascular	NP	Stromal/EC
9	Rhesus	58	8	9.95			Stromal/vascular	Stromal/vascular	Stromal/vascular	Stromal/vascular	Stromal/EC
10	Rhesus	56	7	10.2			Stromal/vascular	Stromal/vascular	Stromal/vascular	Stromal/vascular	Stromal/EC
11	Rhesus	35	7	10.1			Stromal/vascular	Stromal/vascular	Stromal/vascular	Stromal/vascular	Stromal/EC
12	Rhesus	38	6	10.6			Stromal/vascular	Stromal/vascular	Stromal/vascular	Stromal/vascular	NP

EBOV, Ebola virus; EC, endothelial cell; ID, identification; IHC, immunohistochemistry; NP, not performed; TEM, transmission electron microscopy; vRNA, viral RNA.

interstitial or supporting connective tissues of the male and female reproductive organs, with minimal associated tissue immune response or organ pathology.

Twelve macaques, seven rhesus (*Macaca mulatta*; one female and six males) and five cynomolgus (*Macaca fascicularis*; three females and two males), ranging in age from 35 to 58 months, were challenged with 1000 plaque-forming units i.m. of EBOV Makona C05.[11] Macaques were euthanized after meeting end point criteria between 6 and 9 days after EBOV challenge. Each macaque underwent a complete postmortem examination when predetermined end point criteria[12] for EVD were reached. Reproductive tissues were collected from all macaques for routine histology, immunohistochemistry (IHC) for EBOV viral protein 40 (VP40) matrix protein and/or glycoprotein (GP), and transmission electron microscopy examination.[13] Immunohistochemistry for EBOV VP40 and/or GP was positive in the reproductive organs of all 12 macaques. Transmission electron microscopy of the reproductive tissues was performed in 11 of 12 macaques that succumbed to EVD, and widespread EBOV infection of the stromal connective tissues, including endothelial cells, was observed in all macaques examined. A summation of findings can be found in Table 1.

Materials and Methods

Animal Ethics Statement

This study was performed in strict adherence to the *Guide for the Care and Use of Laboratory Animals*[14] of the NIH, the Office of Animal Welfare, and the US Department of Agriculture. All work was approved by the US National Institute of Allergy and Infectious Diseases, Division of Clinical Research Animal Care and Use Committee, and performed at the US National Institute of Allergy and Infectious Diseases Research Facilities. Procedures were performed after animals had been anesthetized by trained personnel under the supervision of veterinary staff. Food and water were available ad libitum.

Animals

This study includes 12 macaques that served as controls in three separate studies that were designed to evaluate the efficacy of a therapeutic agent intended to protect against the development of EVD. The first two experiments are described by Johnson et al.[15] The seven rhesus macaques (*M. mulatta*) and five cynomolgus macaques (*M. fascicularis*) ranged in age from 35 to 58 months, with a