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Ebola virus persistence as a new focus in clinical research

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Ebola virus (EBOV) causes severe acute human disease with high lethality. Viremia is typical during the acute disease phase. However, EBOV RNA can remain detectable in immune-privileged tissues for prolonged periods of time after clearance from the blood, suggesting EBOV may persist during convalescence and thereafter. Eliminating persistent EBOV is important to ensure full recovery of survivors and decrease the risk of outbreak re-ignition caused by EBOV spread from apparently healthy survivors to naive contacts. Here, we review prior evidence of EBOV persistence and explore the tools needed for the development of model systems to understand persistence.

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Introduction

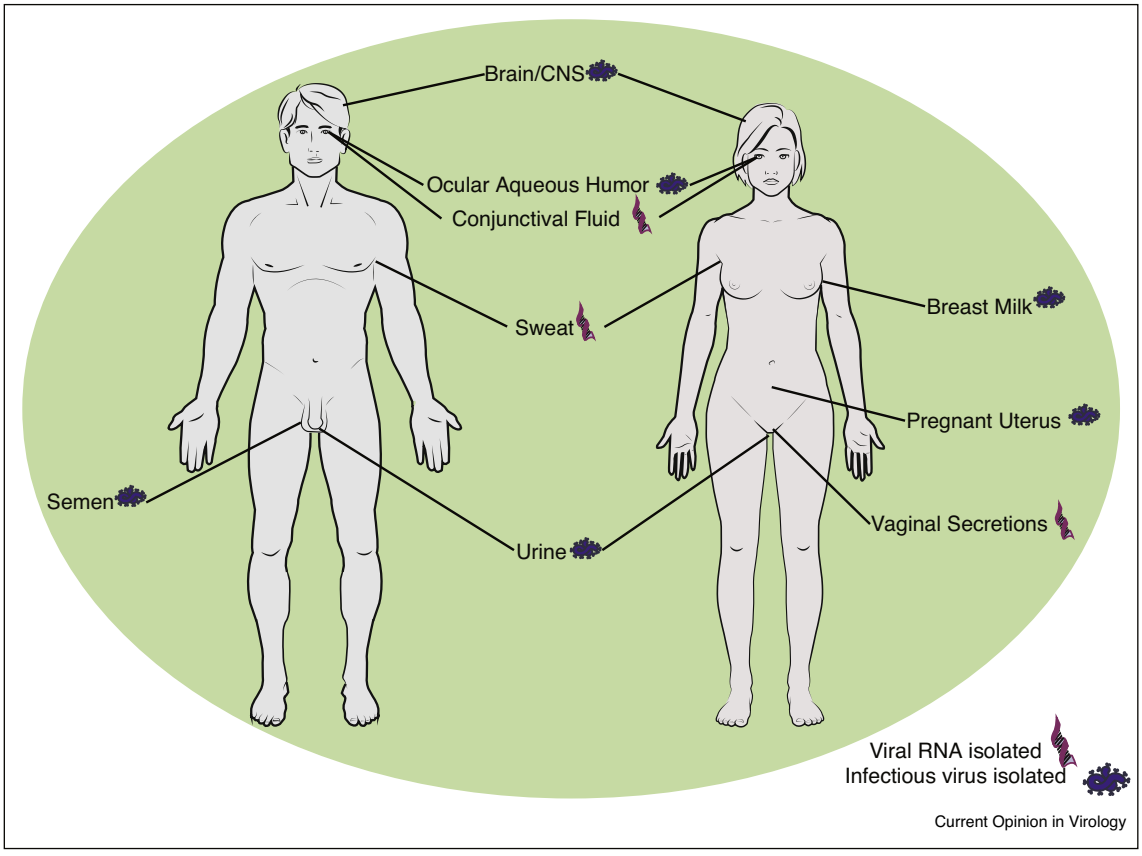
Ebola virus (EBOV) was first discovered in 1976 during an outbreak of what is now referred to as Ebola virus disease (EVD) [1]. In late 2013, a large EVD outbreak commenced in Western Africa, ultimately resulting in more than 28 000 human infections and 11 000 deaths. Evaluating the numerous EVD survivors has become a unique opportunity to study EVD sequelae.

Before the Western African EVD outbreak, studies of survivors revealed the presence of EBOV RNA and, rarely, infectious virus during convalescence [2]. EBOV RNA was found in immune-privileged sites or fluids, including semen, as early as 1995 [3,4]. However, due to the high lethality of EVD and the small and sporadic nature of previous outbreaks, limited attention was given to these studies. In addition to scarce human data, there has been no indication of EBOV persistence in the almost universally lethal EVD animal models used for medical countermeasure development. The Western African EVD outbreak included individual cases of EBOV (most likely sexual) transmission from apparently healthy EVD survivors in areas previously declared EBOV-free [5–7,8•,9,10,11•,12•], thus providing evidence of EBOV persistence. Identifying the immune-privileged sites that harbor EBOV and the molecular mechanisms governing persistence within and transmission from people are essential for improved containment of future outbreaks.

Ebola virus persistence in humans

In recovering patients, EBOV RNA can remain in breast milk, sweat, urine, vaginal secretions, ocular aqueous humor, conjunctival fluid, and semen. Infectious EBOV has been recovered from breast milk, urine, ocular aqueous humor, and semen (Figure 1) [13]. Commonly reported sequelae of EVD survivors include arthralgia, hearing loss, and uveitis [14,15]. Neurological complications include late-onset encephalitis and meningitis [15–17] with EBOV RNA or EBOV spilling over from the cerebrospinal fluid (CSF) into the bloodstream in one case during convalescence [17]. The effects of EVD during pregnancy are just beginning to be addressed. Thus, the risks of persistent EBOV to the developing fetus conceived before acute EVD, let alone those conceived during convalescence, remain unclear. Acute EBOV infection in pregnant women is associated with higher lethality compared to non-pregnant EVD patients, and fetal and neonatal lethality is virtually 100% [18,19]. The very few pregnant women who survived EVD delivered stillborn fetuses during convalescence with high concentrations of EBOV RNA detected in the placenta and associated tissues [20–22]. This finding may be important as these tissues are among the most immune-regulated sites in the body that function to protect the fetus from infections while also avoiding the generation of an anti-fetus immune response. In a case study of 70 female EVD survivors who conceived after recovery from EVD, adverse outcomes occurred in ≈28% of pregnancies [23]; however, the significance of these data are unclear due to reporting issues of adverse outcomes in uninfected women. Although these studies together may be suggestive of placental EBOV persistence, hard evidence is lacking.

Figure 1



Sources of Ebola virus shedding in patients surviving Ebola virus disease.

EBOV persistence in the male reproductive tract may enable virus transmission from apparently healthy EVD survivors. EBOV RNA was detected in 100% of semen samples taken 2–3 months after acute EVD onset, in 65% of samples taken 4–6 months after onset, and 26% of samples taken 7–9 months after onset [5,10,24]. Sexual EBOV transmission has not only been recorded, but also implicated in the initiation of entirely new EBOV transmission chains [5–7,8•,9,10,11•,12•]. EBOV genomic sequence analysis was consistent with male-to-female transmission in two separate events, 199 and 470 days after EVD onset [7,11•]. Likewise, outbreak flare-ups in allegedly EVD-free areas have been linked to EBOV reemergence from persistently infected survivors [8•].

Many aspects of EBOV persistence remain unknown. The development of *in vitro* and *in vivo* models are needed to identify persistently infected cell types, characterize the host immune response to persistence, and define molecular mechanisms governing persistence. These models are important to understand the contribution of EBOV persistence to the size and spread of EVD outbreaks.

Models of persistence

In vitro models

Cell-culture models for EBOV persistence have only been established using grivet kidney epithelial (Vero) cells, laboratory mouse fibroblasts (NIH 3T3) and macrophages (RAW264.7), and Brazilian free-tailed bat fibroblasts (Tb 1 Lu) [25,26]. After 7–10 virus passages, viral progeny titers decreased and cytopathic effects diminished [25]. This phenotypic change is likely due to the appearance of defective interfering particles (DIPs, Figure 2), which contain truncated EBOV genomes that rely on, but also compete with, wild-type EBOV for replication. Both deletion and copy-back (or snap-back) EBOV DIPs of various lengths have been described, with shorter length copy-back DIPs being more prevalent in later cell passages [25]. One could hypothesize that only a few individual cells of an immune-privileged site become infected with EBOV, resulting in DIPs suppressing overall viral replication while promoting persistence.

Interestingly, treatment of persistently infected mouse macrophages and bat fibroblasts with phorbol-12-myristate-13-acetate (PMA) increased EBOV protein synthesis.