Basic Biology of Filovirus Persistence and its impact on filovirus re-emergence
• EBOV discovered in 1976.
• Only latest outbreak resulted in enough EVD survivors to study EVD sequelae.
• The presence of EBOV RNA and, rarely, infectious virus during convalescence had been documented previously as early as 1976.
• EBOV genomic RNA was detected in seminal fluid of survivors of a 1995 EVD outbreak with successful EBOV isolation from a single sample.
• In addition to scarce human data, no indication of EBOV persistence was collected in the almost universally lethal EVD animal models used for medical countermeasure development.
• 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.

Martini et al., 1968. Klin Wochenschr
Bausch et al., 2007. J Infect Dis
Rodriguez et al., 1995. J Infect Dis
Rowe et al., 1999. J Infect Dis
Other filoviruses:

- The first report of sex transmission of MARV from an MVD survivor to his wife in 1967.
- MARV was isolated from the aqueous fluid of the eye of a 1975 MVD survivor with uveitis.
- MVD recurred in a previous MVD survivor in 1991.
- SUDV was isolated from seminal fluid of an EVD survivor in 1977 and from seminal fluid and breast milk of two survivors in 2000.
March 24, 2015

Exposure Concerns Grow in Liberia After Diagnosis of First Ebola Case in Weeks

By SHERI FINK  MARCH 24, 2015

Worries have widened in recent days over the number of people in Liberia who may have been exposed to the country’s first Ebola case in more than two weeks, a street vendor who lived in a one-bathroom house shared with 52 others in a Monrovia suburb and who had sold food at a school where more than 1,900 students are enrolled.

The patient, identified as Ruth Tugbah, 44, had been in contact with a range of people, including her children and a pastor who had sought to comfort her, after she developed a fever and was contagious, aid workers said Tuesday.

Ms. Tugbah received a diagnosis of Ebola on Friday, ending a short-lived period of optimism that Liberia would be the first of three afflicted West African countries to emerge from the worst epidemic of the deadly virus in history.
Molecular Evidence of Sexual Transmission of Ebola Virus

Timeline
Potential for additional mechanisms to generate flare-ups
Reduced rate of evolution observed in flare-ups

Ebola carriers? Why the virus keeps coming back  April 29, 2016

We might now know why Ebola keeps popping up in West Africa  May 1, 2016

Ebola se cache dans le corps des malades pour survivre  May 18, 2016
Impact in Vaccines and Therapeutics

BRIEF REPORT

Ebola Virus Transmission Initiated by Relapse of Systemic Ebola Virus Disease


Table 1. Diagnostic Test Results in Samples Obtained during the First and Second Episodes of EVD in the Patient in 2019.5

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Date Sample Collected</th>
<th>Sample Type</th>
<th>Glycoprotein Ct Value</th>
<th>Nucleoprotein Ct Value</th>
<th>Glycoprotein IgG EC50 Titre</th>
<th>Virus Sequenced?</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA844194</td>
<td>June 13</td>
<td>Serum</td>
<td>32.5</td>
<td>29.9</td>
<td>Negative</td>
<td>Yes</td>
</tr>
<tr>
<td>MA84437</td>
<td>June 18</td>
<td>Serum</td>
<td>41.7</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>MA8454</td>
<td>June 20</td>
<td>Serum</td>
<td>41.3</td>
<td>19.2</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>MA84524</td>
<td>June 21</td>
<td>Serum</td>
<td>38.3</td>
<td>38.5</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>MA84694</td>
<td>June 25</td>
<td>Serum</td>
<td>38.8</td>
<td>38.8</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>MA84796</td>
<td>June 27</td>
<td>Serum</td>
<td>Negative</td>
<td>1:478.79</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Sample 414 MA84707</td>
<td>June 29</td>
<td>Serum</td>
<td>Negative</td>
<td>1:478.79</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Unknown</td>
<td>Aug. 27</td>
<td>Serum</td>
<td>Negative</td>
<td>Negative</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Sample 417 MA121309</td>
<td>Dec. 3</td>
<td>Serum</td>
<td>33.3</td>
<td>30.1</td>
<td>1:16,009</td>
<td>Yes</td>
</tr>
<tr>
<td>Sample 417 MA121309</td>
<td>Dec. 5</td>
<td>Oral swab</td>
<td>28.7</td>
<td>24.8</td>
<td>--</td>
<td>Yes</td>
</tr>
</tbody>
</table>

5 The glycoprotein and nucleoprotein targets of Ebola virus RNA were detected with the use of GeneXpert diagnostic quantitative reverse-transcriptase-polymerase chain reaction assays (Cepheid, Sunnyvale, Calif.) and are reported as cycle-threshold (Ct) values. Glycoprotein binding titers were assessed with the use of an enzyme-linked immunosorbent assay (Alpha Diagnostic International) with a readout for the anti-glycoprotein IgG (EC50, the concentration at which there is a 50% decrease in antigen binding). EVD denotes Ebola virus disease.

*Samples identified were assigned only to the samples described in this article.
**Samples from which full viral genomes were determined are indicated.

Figure 1. Timeline of the First and Second Episodes of EVD in the Patient, from December 2018 through December 2019.

ETU denotes Ebola treatment unit, EVD Ebola virus disease, MEURI Monitored Emergency Use of Unregistered and Investigational Interventions, RT-PCR reverse transcriptase–polymerase chain reaction, and rSV-ZEBOV recombinant vesicular stomatitis virus–based vaccine expressing a ZEBOV glycoprotein.
Impact in Spillover?

Article

Resurgence of Ebola virus in 2021 in Guinea suggests a new paradigm for outbreaks

https://doi.org/10.1038/s41596-021-03901-0

Received: 6 April 2021
Accepted: 11 August 2021
Published online: 15 September 2021

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Ebola virus persistence as a new focus in clinical research
Katie Caviness¹, Jens H Kuhn² and Gustavo Palacios¹

[Diagram showing viral replication and persistence pathways]
Ebola Virus Defective Interfering Particles and Persistent Infection

Philippe Calain, Martha C. Monroe, and Stuart T. Nichol

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Received March 1, 1999, returned to author for revision May 7, 1999; accepted July 20, 1999

Ebola virus (Zaire subtype) is associated with high mortality disease outbreaks that commonly involve human-to-human transmission. Surviving patients can show evidence of prolonged virus persistence. The potential for Ebola virus to generate defective interfering (DI) particles and establish persistent infections in tissue culture was investigated. It was found that serial undiluted virus passages quickly resulted in production of an evolving population of virus minireplicons possessing both deletion and copyback type DI genome rearrangements. The serial undiluted virus passage resulted in the establishment of virus persistently infected cell lines. Following one or two crises, these cells were stably maintained for several months with continuous shedding of infectious virus. An analysis of the estimated genome lengths of a selected set of the Ebola virus minireplicons and standard loneliness revealed no obvious genome length rule, such as "the rule of six" found for the phylogenetically related Paramyxoviridae paramyxoviruses. Minimal promoters for Ebola virus replication were found to be contained within 156 and 177 nucleotide regions of the genomic and antigenomic RNA 3' termini, respectively, based on the length of authentic termini retained in the naturally occurring minireplicons analyzed. In addition, using UV-irradiated preparations of virus released from persistently infected cells, it was demonstrated that Ebola virus DI particles could potentially be used as natural minireplicons to assay standard virus support functions.

Historical In Vitro Models of Persistence
Sierra Leone Ebola Virus Persistence Study

- Aim: To investigate the persistence of Ebola virus in body fluids in a cohort of EVD survivors
- Pilot study: cohort of 100 men
- Specimens: semen
- Joint study between CDC, Sierra Leone Ministry of Health and Sanitation, Sierra Leone Armed Forces, and WHO.

Semen-acquired viral sequences ("SAVS")
Active Ebola Virus Replication and Heterogeneous Evolutionary Rates in EVD Survivors

Highlights
- During persistence, EBOV exhibits heterogeneous evolutionary rates
- Active EBOV transcription and replication occurs during persistence
- RNA hyper-editing observed during viral persistence
- No evidence for significant selective pressure during persistence
Identification and pathological characterization of persistent asymptomatic Ebola virus infection in rhesus monkeys

**Cell Host & Microbe**
Persistent Marburg Virus Infection in the Testes of Nonhuman Primate Survivors

Ebola virus persistence and disease recrudescence in the brains of antibody-treated nonhuman primate survivors
Treatment of Junin Virus-Infected Guinea Pigs With Immune Serum: Development of Late Neurological Disease

Richard H. Kenyon, David E. Green, Gerald A. Eddy, and Clarence J. Peters
United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland

Guinea pigs infected with Argentine hemorrhagic fever virus (Junin) were treated with pooled, homologous convalescent sera. Use of 15,000 or 5,000 therapeutic units of immune sera prevented all signs of illness when administered within 24 hr of infection. We could also prevent illness and death in infected guinea pigs as late as 6 days after infection if we used more antisera (30,000 therapeutic units/kg). In some treatment groups, surviving animals developed a late neurological syndrome with prominent rear-limb paralysis. Treated animals typically expressed...
Conclusions

• The frequency of EBOV persistent infections in primates is more frequent than previously expected.

• Most survivors show a reduced rate of evolution during persistent infection.

• There is evidence of active replication in both human and NHPs.

• Filovirus persistence is also common in Marburg infections.
Thanks!

Questions?