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VIRAL PATHOGENESIS

Unlocking Ebola persistence

The 2013–2016 West African Ebola virus outbreak evidenced that the virus can persist in survivors long-term, leading to sequelae and risks of new transmission chains. Ebola virus has now been shown to behave similarly in rhesus macaques, enabling their use to study persistence and intervention strategies.

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From its initial discovery in the Democratic Republic of the Congo in 1976 until 2013, Ebola virus (EBOV) disease (EVD) was considered a neglected tropical infection of low public health impact. This changed as a result of the massive outbreak in West Africa from 2013 to 2016 that decimated the public health infrastructure in Guinea, Sierra Leone and Liberia, and resulted in 28,646 infections and 11,323 deaths[1]. The unprecedented number of EVD cases and survivors revealed numerous shortcomings in our previous understanding of EBOV physiopathology. A major breakthrough was the realization that EBOV can hide, potentially for months, in human immune-privileged sites and subsequently reappear, bringing risk of new chains of transmission. In this issue of *Nature Microbiology*, Zeng *et al.*[2] report that rhesus macaques can serve to study EBOV persistence and develop interventions to alleviate its consequences in humans.

A virus that enters an immune-privileged site, such as the central nervous system (CNS), eye or testis, may continue to reside there largely unchecked by the host immune defences. For EBOV, this means that it can inflict further damage and even re-emerge in the systemic circulation of a convalescent individual months after initial clearance[3], and can be involved in a new chain of transmission[4], including through sexual contact[5,6]. The lack of well-documented clinical evolution of EVD in humans may explain why 35 years of documentation from smaller-scale outbreaks failed to identify these immune-privileged sites and their importance. Experimental infections in animal species capable of modelling EVD also failed to reveal the involvement of these immune-privileged sites, probably due to low frequency of clinical manifestations. Of note, the large majority of experimental animal protocols followed were too short to detect phenotypes occurring months after infection, as models were developed to be fully lethal in less than ten days. In fact, only one commentary has communicated rare anecdotal observations suggesting that EBOV could induce neurological symptoms in non-human primates. At the time, the frequency of these events was estimated to be in the range of 1–2% of infections[7].

The large studies of survivors of the West African Ebola outbreak were critical to advance our understanding of human EVD[8]. However, these observations were retrospective, as the studies used samples collected from individuals after they had survived acute EBOV infection. A drawback of these types of studies is that the efficacy of possible intervention strategies in preventing or reversing the symptoms of EBOV persistence in immune-privileged sites cannot be specifically tested as one would, for example, in an animal model of EVD. The study by Zeng and co-workers provides the first experimental evidence that rhesus macaques, a well-characterized model of EVD progression, can also recapitulate EBOV physiopathology in the eyes, testes and CNS, similar to what has been described

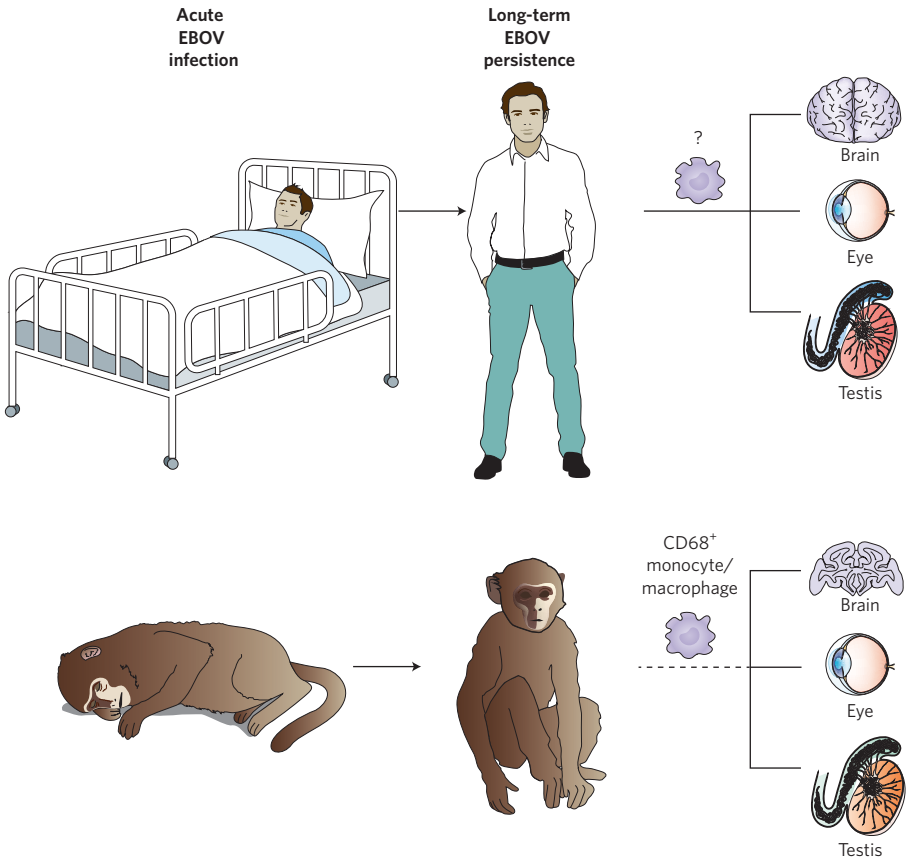

Figure 1 | Ebola persistence can be explored in rhesus macaques. Survivors of EBOV infection that are thought to be cured of the disease can carry the virus long-term in tissues such as the eyes, brain and testes, where subsequent reactivation can cause EVD sequelae and new transmissions. EBOV is believed to persist within $CD68^+$ monocytes/macrophages in rhesus macaques, causing similar pathophysiology and opening new paths to understanding and treating EBOV persistence.

in humans. Importantly, the study of these tissues, all retrieved from archived samples, unlocked some of the mechanisms that could be behind the less common symptoms or phenotypes observed in the eyes, brain or testes of infected individuals. Of note, the fact that this study and its findings were generated from archived samples is an exemplary success of following the principles of the 3Rs — reduction, refinement and replacement — which guide humane animal research. It also speaks strongly of the benefits of archiving samples.

Zeng and colleagues performed *in situ* hybridization on a collection of 112 archived eye, testicle, brain, lymph node, liver and spleen samples from rhesus monkeys that survived experimental EBOV infection to day 43 post-exposure, either in the absence of or, more commonly, after treatment with a medical countermeasure. Only 11 of these samples (9.8%) had detectable RNA in the eye (9 of 11), testes (1 of 11) and brain (1 of 11). Subsequently, the authors examined survivor eye sections by immunofluorescence using antibodies against EBOV glycoprotein $(GP)_{1,2}$ and CD68, a monocyte/macrophage marker. Interestingly, in this one survivor, most anti-EBOV-$GP_{1,2}$-positive cells were also positive for CD68, suggesting that these cells may be targets for EBOV persistence (Fig. 1). Interestingly, $CD68^+$ microglia/macrophages have also been linked to entry into the CNS by Hendra virus, a member of the genus *Henipavirus*, which can cause severe encephalitis and other CNS pathologies[9].

The low frequency of Ebola-infected immune-privileged sites in rhesus monkeys, as reported by Zeng *et al.*, may prove to be comparable to the frequency seen in humans. This data could further support the accuracy with which rhesus macaques can recapitulate EVD as documented in humans. On the other hand, the results also suggest that more work is required to develop macaques as a useful model to study the role of immune-privileged sites in EVD. If rhesus macaques are to be used to study the roles of the CNS, eye and testis in EVD or to develop interventions, vaccines or treatments, the model will need to produce a high percentage of animals with the desired phenotype. The current frequency of animals harbouring EBOV in immune-privileged areas is an impediment to developing reasonable and ethical animal protocols in rhesus macaques. Nevertheless, currently available experimental treatments and vaccines will promote long-term survival of animals and increase the possibility of developing conditions where a higher proportion of animals could show a specific phenotype, such as EBOV in the CNS.

The available experimental vaccines and treatments against EBOV will need to be evaluated for their ability to protect immune-privileged sites and, more importantly, to ensure that they do not enable EBOV access to or amplification in these sites. The importance of this type of work is underscored by the resurgence of EBOV in the CNS and systemic circulation in a nurse nine months after discharge from the Royal Free Hospital in London, UK[3]. Considering her peak viral loads, it is reasonable to assume that this patient would have died without intensive supportive care and the experimental therapies she received (convalescent plasma, brincidofovir and ZMAb monoclonal antibody therapy). Although unsubstantiated by observations in humans or animals, one cannot rule out that intensive care and/or experimental therapies could have promoted access of EBOV to the CNS. One hypothesis is that this patient's exceptionally high viraemia (more than 10^8 infectious particles per ml of blood) favoured entry of EBOV into immune-privileged sites, which are otherwise difficult to access (for example, with the blood–brain barrier protecting the CNS). This patient became the first well-documented clinical case of EBOV circulating systemically and in the CNS, months after viral clearance and clinical recovery, and because she survived conditions leading to death in over 99% of cases. An animal model would allow the identification of conditions that could promote or protect immune-privileged sites from EBOV invasion and clinical consequences. While there are at least two additional reports documenting the resurgence of EVD in West Africa from EBOV hiding in the CNS, it is a rare event considering the 11,323 survivors. Notably, the identification of EBOV persistence in macaques provides the opportunity to test how novel therapeutic approaches affect persistence and to improve clinical management of humans with EVD. ❐

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Competing interests

The authors declare no competing financial interests.