ANTIVIRALS

Achilles heel of Ebola viral entry

Ebola virus (EboV) infection causes fatal haemorrhagic fever with mortality rates exceeding 75%. Owing to the lack of available vaccines or therapeutics, it presents a considerable unmet medical need. Now, two studies in *Nature* identify a potential 'Achilles heel' of EboV and related filoviruses. Using different approaches, both show that the cholesterol transporter protein Niemann–Pick C1 (NPC1) is a crucial host factor for infection, and describe inhibitors of NPC1 that are active in *in vitro* infection models of EboV.

The report by Carette *et al.* describes a genome-wide haploid genetic screen in human cells to identify the host factors required for EboV entry. By using a retroviral gene-trap vector, various candidate genes were found that, when disrupted by insertional mutagenesis, conferred resistance to infection with a recombinant vesicular stomatitis virus (VSV) construct, rVSV–GP-EboV, bearing the EboV glycoprotein (GP). Nearly all of the genes identified were involved in the architecture and trafficking of endo- and lysosomal compartments, including cathepsin B, the only known crucial host factor for EboV infection. The strongest 'hit' was NPC1. It was found that cells defective for NPC1 are resistant to EboV and the closely related Marburg virus, but remain susceptible to a range of unrelated viruses. Fibroblasts obtained from patients with Niemann–Pick disease, a neurovisceral disorder caused by the loss of NPC1, were also found to be resistant to rVSV–GP-EboV infection.

The authors found that the cholesterol synthesis inhibitor U18666A and the antidepressant imipramine, which both cause a cellular phenotype similar to NPC1 deficiency, potently protect from rVSV–GP-EboV infection *in vitro*. In U18666A-treated and NPC1-deficient cells, the fusion and uncoating of incoming viruses appeared to be arrested, indicating that late steps in filovirus entry are affected. Further analyses, including the infection of *NPC1*-mutant primary human fibroblasts with authentic EboV, all confirmed that NPC1 is crucial for EboV infection. Furthermore, in mouse models of lethal EboV infection, heterozygosity for NPC1 was shown to confer a large survival advantage.

The study by Côté *et al.* describes the screening of a library of small molecules for their ability to inhibit infection with VSV bearing the EboV GP. A benzylpiperazine adamantine diamide-derived compound was active in this assay, and further optimization resulted in a second compound with substantially increased potency. Analysis of the cellular effects of the two compounds indicated that they affect cholesterol uptake, and subsequent small interfering RNA knockdown experiments and immunohistochemistry identified NPC1 as a crucial conserved entry factor for EboV, as well as several other members of the Filoviridae family, including Marburg virus.

The authors also showed that EboV infectivity depended on the presence of NPC1, but not on its function as a cholesterol transporter. Previously they had shown that cleavage of the EboV GP by proteases such as cathepsin B removes heavily glycosylated domains in the GP1 subunit and exposes the amino-terminal domain of GP1. It has also been proposed that the binding of this domain by a host factor is essential for infection. The authors reasoned that NPC1 is this host factor, and found that their compounds directly interfere with the binding of cleaved GP1 to NPC1.

Taken together, these studies reveal new aspects of the molecular biology of viral entry, and hold promise for the development of antiviral compounds that are active against filoviruses such as EboV.

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ORIGINAL RESEARCH PAPERS Carette, J. E. *et al.* Ebola virus entry requires the cholesterol transporter Niemann–Pick C1. *Nature* 24 Aug 2011 (doi:10.1038/nature10348) | Côté, M. *et al.* Small molecule inhibitors reveal Niemann–Pick C1 is essential for Ebola virus infection. *Nature* 24 Aug 2011 (doi:10.1038/nature10380)