Evolution of neurovirulent Zika virus.

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In 2015 Zika virus (ZIKV) became front page news following its association with foetal microcephaly and shortly afterwards was declared a Public Health Emergency by the WHO(1). However, ZIKV was not new, having first been isolated from the Zika forest in Uganda in 1947(2). Zika incited little interest compared to other related flaviviruses such as dengue, yellow fever and Japanese encephalitis as it was not thought to cause severe disease. Infections were largely sporadic, symptoms were usually mild and flu-like with self-limiting fever, rash and conjunctivitis(3). Around 80% of cases were asymptomatic and epidemic activity had not previously been described(4). In 2007 large scale explosive outbreaks of Zika infection were described in Micronesia and the virus spread across the Pacific reaching South America in 2015, where it rapidly spread through Brazil and onto neighbouring countries(4-6).

During these explosive outbreaks, more severe sequelae of Zika infection were recognised, including Guillain Barre syndrome, a polyneuropathy, which was first described in the French Polynesian outbreak and then a major cluster of microcephaly cases was recognised in North Eastern Brazil in late 2015(7, 8). Shortly afterwards ZIKV was isolated from the brain of a microcephalic foetus and the connection between ZIKV infection during pregnancy and a variety of foetal neurological abnormalities recognised(9). A great deal of research has been performed on ZIKV over the last two years, including the establishment of animal models of foetal infection. It is now clear that the virus can infect neural progenitor cells following transplacental transfer and lead to severe neurological damage to the foetus(9). Estimates of the risk of neurological damage to the foetus if the mother is infected during pregnancy vary from 1-20% and appear to be greatest when the mother is infected during the first trimester of pregnancy(10, 11).

Two big questions that remain about the recent ZIKV outbreak are why was it so explosive and how had the virus gained the ability to cause microcephaly? One possibility is that the virus has mutated to acquire neurovirulence and the ability to sustain epidemic activity. In this issue Yuan and colleagues look for such mutations by comparing three contemporary ZIKV strains isolated from patients infected in Samoa, Venezuela and Martinique in 2015/16, with an ancestral Asian ZIKV strain isolated from a patient in Cambodia in 2010(12). The three contemporary ZIKV strains were more neurovirulent when injected into one day old mouse brains compared to the ancestral strain (100% vs 16.7% mortality). In addition, the contemporary strains caused more severe microcephaly when injected into fetal brain and disturbed differentiation and proliferation of cultured neural progenitor cells.

Sequence comparison of the contemporary and ancestral strains revealed a number of changes had occurred and been maintained in the contemporary strains. To analyse for virulence determining mutations 7 amino acid substitutions were made individually into the

Cambodian ancestral virus strain and then tested in neonatal mouse brain; the S139N mutation showed the greatest neurovirulence and the importance of this change was further exemplified in the reciprocal experiment, where N139 was mutated to S in the contemporary Venezuelan strain, resulting in a reduction in neurovirulence.

The S139N mutation has occurred at residue 17 of the precursor membrane protein (prM) of ZIKV, which from inference from dengue virus structures, is likely to be surface exposed. prM closely associates with Envelope protein (E) in flaviviruses and is believed in part to act as a form of “chaperone”, preventing premature fusion of immature virions inside infected cells. In immature virions 180 copies of E associates with 180 copies of prM which are arranged into 60 heterohexameric (trimer) spikes. As the immature virion passes through the Golgi, low pH induces conformational change in E, from trimeric to dimeric conformation, potentially exposing the hydrophobic fusion loops of E protein on the virion surface. However, prM sits on top of the E-fusion loop preventing association with host cell membranes. In the Golgi prM is cleaved by Furin protease, which can then fall away when the virus exits the host cell, leaving mature virions lacking prM with E arranged in 90 head to tail dimers(13).

In dengue virus (DENV), prM cleavage is often incomplete leading to the production of a range of “partially mature” virus particles containing varying amounts of uncleaved prM. In DENV, the presence of prM alters the architecture of the virion surface and has implications for antibody binding, flexibility or “breathing” of the virus and infectivity; virions containing high levels of prM are non-infectious. It is not yet known how leaky the cleavage of prM is in ZIKV and therefore how much, if any, uncleaved prM is present on infectious virions, although it is thought that ZIKV virions are more rigid than DENV virions(14).

The S139N mutation in contemporary ZIKV strains is distant from the prM cleavage site, but it remains possible that it may influence cleavage or in some way alter virus stability/flexibility and these will need further study. prM has not been formally thought to be a viral receptor for cell adhesion and it is possible that the S139N mutation creates a novel receptor for neural progenitor cells, although this seems somewhat unlikely and even less so if the content of prM in ZIKV particles turns out to be low.

Another important question still remains unanswered; why has there still not been an explosive outbreak of ZIKV and associated microcephaly in South East Asia? ZIKV has been documented to be present in South-East Asia for a number of years, with small local outbreaks and occasional cases reported in returning travellers(15). However, because of the similarity of symptoms to DENV and the difficulty to distinguish ZIKV from DENV serologically, the true scale of infection in the region is not entirely clear and may have been underestimated. If the scale of ZIKV in SE Asia has been underestimated, it is possible that herd immunity has prevented explosive outbreaks and in the absence of such peaks in infection the association with microcephaly has not been unmasked as it has been in Brazil. Robust sero-diagnostics to distinguish previous DENV and ZIKV are sorely needed to further understand these issues.

The association between the S139N change and neurovirulence is tantalising, we are far from understanding the mechanism and it deserves further investigation. In the meantime, progress towards the design of ZIKV vaccines has progressed at pace with several promising candidates entering Phase II trials. As the incidence of new ZIKV infection has thankfully

dropped, it will be a challenge to design clinical trials to demonstrate efficacy following the initial immunogenicity studies.

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