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REVIEW

Flaviviruses are neurotropic, but how do they invade the CNS?

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Summary Flaviruses (FV) are RNA viruses carried by mosquitoes. Neurological signs including acute encephalitis, meningitis and acute flaccid paralysis develop in a small percentage of infected individuals; long term sequlae are, Parkinsonism, dystonias and cognitive changes. FV neuroinfection is neurotropic involving subcortical nuclei (substantia nigra and thalamus) anterior horn neurons and neocortex. Glycosylation of the FV E envelope protein is one determinant of neuroinvasion, increasing both axonal and trans-epithelial transportation. Neutralizing antibodies against the E and NS proteins prevents FV uptake into several cell types, including axons. CD8+ T cells are vital for clearance of WNF infected cells from the CNS, whereas TLR-3 and TLR-7 mediated anti-virus response through increased serum inflammatory cytokines to disrupt the BBB providing infected leucocytes and free virus access to the CNS (so called Trojan horse) Cellular virus attachment factors, promoting FV cell entry are widely distributed and include DC-SIGN (that detects complex carbohydrate molecules); Glycosamino glycans (GAG), Heparan sulphate(HSPG) Semaphorin 7A, Low Density Lipid receptors (LDLR); these are not FV specific virus entry receptors. The FV also crosses epithelial and endothelial barriers by disrupting Tight Junction complexes to increase BBB permeability. This review describes the multiple pathways responsible for the neuroinvasive properties of the Flaviviruses.

Introduction

West Nile Fever Virus (WNF), Japanese Encephalitis Virus (JEV), Dengue Virus (DV), Murray Valley Encephalitis Virus (MVE), St Louis encephalitis Virus (SLEV) and Hepatitis C (HCV) are members of the *Flaviviridae* family (FV). FV are enveloped, positive sense, single stranded RNA viruses (ssRNA) with significant neuroinvasive characteristics and are regarded as neurotropic viruses. Horses, pigs, birds (*Corvid* species) and dogs all provide a natural reservoir

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for the FV.[1–4] Whereas, humans are regarded as "dead end hosts" because they are infected accidentally when bitten by a Flavivirus (FV) carrying mosquito, usually from the *Culex* species (WNF, JEV)[3,4]; the Aedes aegypti and albopictus mosquitoes are responsible for DV infection.[5]

Of all the FV, WNF and JEV, are the two most commonly associated with neuroinfection. WNF is classified as linage I and 2 strains; strains I (North America) are neuro virulent, whereas, linage 2 are less virulent and found in Sub Saharan Africa. WNF infection is mainly asymptomatic, but in approximately 20% of cases there is a flu-like illness with pyrexia and joint pains. However, a small percentage of WNF infected individuals, (1 in 150)[2–4,6] develop meningoencephalitis 14 days after infection and in some an acute flaccid paralysis (AFP).[7,8] For JEV, the ratio of symptomatic to asymptomatic infection is varies between 1 in 25 to 1in 1000 cases, neuro infection is more frequent than WNF, with up to 60% individuals experiencing a seizure as well as AFP.[10–13] Comparable data regarding the frequency of neuroinfection in MEV and SLEV is not available, but DV infection (one of four serotypes DNEV1-4) is more likely to result in haemorrhagic shock than primary neuroinfection.[14–16] Recent reports have described extrahepatic neuroinfection with both DV and HCV, but neuropathological information is limited.[17,18]

Despite the evidence for FV being neurotropic little is known about the factors responsible for neuroinvasion.[9,10,19,20] Clinical and neuropathological findings, show the FV cross the blood brain barrier (BBB) and choroid plexus (CPLx)together with evidence for axonal transport from the periphery (skin bite) and through olfactory bulb neurons(systemic viremia) into the CNS.[21,22] The identification the virus entry receptors and the anti-virus intra cellular signalling pathways related to FV neuroinvasion will help to define potential therapeutic targets.[19] This review will describe the clinical, neuropathological and experimental information related to current understanding of neuroinvasion by the FV (Table 1).

The clinical consequences of Flaviviruses neuroinfection

WNF and JEV are responsible for acute encephalitis and meningitis are with cognitive impairment[23–27]; movement disorders such as AFP,[28] dystonia, Parkinsonism are reported in both WNF and JEV.[29–34] Initially, AFP was regarded clinically as Guillian-Barre syndrome (GBS) and electrophysiological examination was consistent with demyelination and involvement of anterior spinal horn cells. Autopsy examination confirmed these findings, but found anterior spinal horn neuronal loss.[30–35] Therefore, AFP is regarded as polio-like lower motor neuron disorder affecting the lower limbs, this has also been reported in MVF and SEV.[36–40] The involvement of the autonomic nervous system is common with FV infection with disorders of cardiac conduction and sphincter control.[33,34] Neurological involvement with DV is rare (6%) but includes quadriplegia, transverse meylitis, GBS syndrome and encephalopathy.[14,15,41] For HCV, about 6%of cases have cognitive impairment, vascular changes relating to endothelial infection, demyelination and inflammatory white matter changes. In up to 50% ofHCV infected individuals there is evidence of circulating cryoglobulins (cold deposited immunoglobulins) (CG) capable of causing vascular injury and a peripheral neuropathy[17,42–45]; there is some evidence the level of circulating CG corresponds to the level of impaired cognitive function.[44]

Neuroradiological findings in JEV, WNF, MEV and SLEV all show an increased MRI T2 weighted signal from the thalamus, brain stem and basal ganglia.[38,39,45,46] MRI signal changes in the anterior horn spinal cord are consistent with AFP in WNF, MEV and JEV.[2,23,46–50,53] Dystonias and Parkinsonism in SLEV, WNF and JEV was correlated with loss of neurons from the brain stem nuclei from each of these virus infections.[46–48] For HCV, the white matter and periventricular changes are attributed to small vessel disease and demyelination.[17]

Neuropathology of the FV demonstrates encephalitis and meningitis, but more specifically infection of neurons in the substantia nigra nucleus (SN), thalamus, cerebellum and cerebral cortex,[9,55] these findings mirror the neuroradiological findings and explain Parkinsonism, dystonias and AFP.[28] Immunohistochemical (ICH) evidence found WNF (Envelope and non-structural NS1 proteins) within neurons and glia.[56] In WNF infected astrocytes from autopsy tissue contained persistent virus infection for up to 114 days, providing an explanation for the persistence of neurological signs.[54] JEV virus antigen positive neurons and astrocytes are present in thalamus, hippocampus, SN and brain stem[49–52] whereas *in vitro*, JEV infects neurons, astrocytes and microglial.[57,58]

The neuropathology of HCV includes perivascular T cells and microglial nodules. ICH staining of HCV autopsy tissue found infected HVC in microglia and astrocytes, but unlike the other FV infections, neurons were not immunolabelled, however negative strand HCV RNA, was detected suggestive of HCV replicating within the CNS.[1,18] HCV infected microglia produce a range of proinflammatory cytokines Tumour necrosis factor (TNF) and interleukins (IL-1, IL-12 and IL-18) all capable of promoting tissue injury and altering BBB integrity.[42–45] The presence of inflammatory cytokines would also correspond to episodes of de meylination and recurrent transverse meylitis.[17,44]

The host immune response to Flavi virus infection and the risk of neuroinvasion

The host's innate and adaptive immune system response to systemic FV infection is vital in order to prevent neuroinvasion, a factor emphasized by increased risk of WNF in individuals with a compromised immune system. Although the risk of neuroinfection with systemic WNF infection is small, it is increased with age (20 fold increase over 50 years of age), male sex, diabetes mellitus, hypertension, systemic malignancies, trans fusion of WNF infected blood products and transplant recipients receiving organs from infected WNF donors.[6–8,20–22] WNF infection is uniformly fatal in Severe combined immunodeficiency involving both T and B cells and in humans with various immuno deficiency syndromes.[59,60] Similarly, for JEV, an intact immune system prevents neuroinvasion in both animal and human cases, but unlike WNF, neuroinfection with JEV declines in adolescents.[11]