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Pathophysiological mechanisms of Flavivirus infection of the central nervous system

Mécanismes physiopathologiques de l'infection du système nerveux central par les flavivirus

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Abstract

Flaviviruses are important human pathogens. Transmitted by the bite of infected mosquitoes, Flaviviruses such as West Nile and Japanese encephalitis may reach the central nervous system where they can elicit severe diseases. Their ability to cross the blood-brain-barrier is still poorly understood. The newly emerging Zika Flavivirus on the other hand very rarely reaches the brain of adults, but can infect neural progenitors in the developing central nervous system of fetuses, eliciting devastating congenital malformations including microcephaly. This short review focuses on selected aspects of West Nile, Japanese encephalitis and Zika virus pathophysiological features such as neuroinvasion and neurovirulence, and highlights what we know about some possible mechanisms involved in Flaviviral neuropathogenesis.

Keywords: Flavivirus; Neuropathogenesis; Neuroinvasion; Neurovirulence

Résumé

Les Flavivirus sont d'importants pathogènes humains. Transmis par la piqûre de moustiques infectés, les flavivirus tels le virus West Nile et de l'encéphalite japonaise sont capables d'atteindre le système nerveux central au sein duquel ils peuvent provoquer des maladies graves. Leur capacité à traverser la barrière hématoencéphalique est encore mal comprise. En revanche, le nouveau flavivirus émergent Zika accède rarement le cerveau des personnes adultes, alors qu'il est capable d'infecter les progéniteurs neuronaux dans le système nerveux central en développement du fœtus, où il suscite de terribles malformations congénitales incluant la microcéphalie. Cette courte revue est centrée sur quelques aspects de la physiopathologie des virus West Nile, de l'encéphalite japonaise et Zika tels la neuroinvasion et la neurovirulence, et met en lumière ce que nous savons de quelques mécanismes possibles mis en jeu lors de la neuropathogenèse induite par ces virus.

Mots clés : Flavivirus ; Neuropathogenèse ; Neuroinvasion ; Neurovirulence

Viruses that cause natural infections of the central nervous system (CNS) must be able to enter the CNS (aka neuroinvasion) and to infect and propagate in the CNS (aka neurotropism). Virus-induced perturbation of neuronal homeostasis is neurovirulence *stricto sensu*. Neuroinvasiveness, neurotropism and neurovirulence are all players of viral neuropathogenesis. Neurovirulence *in vitro* is usually tested in cell culture (for example, viral infection of neuronal or glial cells), more rarely in complex systems, with co-culture of different cell types (for example, neurons and astrocytes, or neurons, astrocytes and microglia). Mixed cell cultures might provide a better understanding of virus/host tissue interactions, but add complexity in the analyses. *In vivo*, neurovirulence is tested by direct injection in the animal brain (intracranial). If a neurovirulent virus is injected peripherally, it must have the capacity to cross the Blood Brain Barrier or BBB (*i.e.* neuroinvasiveness) in order to reach the CNS. Cerebral endothelial cells constitute the main anatom-

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ical basis of the BBB. There is a dynamic interaction between the brain endothelium and other neighboring cells (such as astrocytes, pericytes, perivascular microglia) and neurons. One of the physiological functions of the BBB is to protect the CNS from exogenous toxic injuries, such as those produced by viruses.

Flaviviruses (*Flaviviridae* family) include viruses that elicit CNS diseases in infected hosts, such as West Nile virus (WNV), Japanese encephalitis virus (JEV) and the newly emerging Zika virus (ZIKV) (Fig. 1). Flaviviruses are arthropod-borne, single-stranded positive-sense RNA viruses, that cause infections in humans with a spectrum of clinical syndromes ranging from mild fever to hemorrhagic and encephalitic manifestations, and infrequently, congenital malformations [1]. While neuroinvasive/neurotropic flaviviruses such as WNV and JEV are responsible for post-natal encephalitis, they are rarely linked to congenital brain malformations such as microcephaly, contrary to ZIKV [2–4].

WNV is transmitted by the bite of infected *Culex* mosquitoes and includes several lineages, with lineage 1 viruses being the most neurovirulent. Although a majority of WNV infection is asymptomatic, 20% of infected people develop flu-like symptoms. Among them, one in 150 individuals may develop meningoencephalitis or acute flaccid paralysis [5]. For JEV, also transmitted by *Culex* mosquito species, several genotypes are described with mainly genotypes 3 and 1 circulating. Symptomatic cases are even rarer than for WNV infection, but for those individuals with symptoms, up to 60% may experience paralysis and seizures [6]. ZIKV that recently emerged in French Polynesia and in the Western hemisphere is transmitted by *Aedes* mosquito species. Originally described as a self-limiting flu-like febrile disease, ZIKV infection has more recently been associated with more severe diseases, including Guillain-Barré syndrome [7,8]. While rarely causing meningitis and encephalitis in adults, ZIKV has been associated with congenital malformations including microcephaly and fetal death [9].

Flaviviruses have a genome of about 11 kilobases encapsidated in the C capsid protein (nucleocapsid). The viral particle is enveloped in a bilayer membrane originating from the cellular endoplasmic reticulum, in which the viral E envelope and M membrane proteins are anchored. The viral genome includes a single open reading frame coding for a polyprotein framed by untranslated regions at the 5′ and 3′ ends (5′ and 3′UTR). The polyprotein is processed into the structural C, E and M proteins, and the non-structural NS1, NS2A and B, NS3, NS4A and B and NS5 proteins by cellular and viral protease cleavage. The structural proteins make up the viral particles, while the non-structural proteins have multiple functions during the viral life cycle, mainly for genome replication and transcription, as well as host immune evasion [1].

The molecular bases of neuroinvasion and neurovirulence are still a mystery for most Flaviviruses (Fig. 2).

Neuroinvasion of JEV may be preceded and facilitated by a cytokine-mediated increased permeability of the BBB [10] possibly through a subversion of host endothelial cell apoptosis [11]. For WNV, the BBB disruption may rely on hematogenous entry, while a possible transneural entry through retrograde axonal transport has also been proposed (see [12] for a review). Molecules maintaining BBB integrity such as claudins and occludins show reduce levels both *in vitro* and *in vivo* during WNV infection that may lead to BBB disruption, especially in the presence of pro-inflammatory cytokines [12]. However, *in vitro* studies not validated *in vivo*, as well as the use of rodent models for pathogenesis investigations that do not recapitulate pathogenesis in natural hosts impair understanding of WNV, and more generally neuroinvasive Flaviviruses, infection mechanisms. In any case, as a result of BBB breakdown, viruses may access the CNS more easily, either by passive diffusion or via infected leukocytes trafficking, although neither of these mechanisms has been supported by direct proof. In any case, an intact immune system is vital to prevent neuroinvasion by WNV and JEV, as exemplify by complete fatality of WNV infection in immunoincompetent humans, or in animal models of JEV [13,14].

Neuroinvasion does not seem to be a major feature of ZIKV virus infection in adults with very rare cases of meningoencephalitis described [15], while an increase in peripheral nervous system syndromes incidence such as Guillain-Barré syndrome have been observed [8]. Interestingly, ZIKV invasion of the CNS seems to occur in animals that have high viremia, suggesting that ZIKV intrusion in the CNS may be the result of passive spill over of virus from the periphery [16]. Moreover, ZIKV may enter the developing brain of foetuses injuring neural progenitor cells [4], while other neurovirulent Flaviviruses such as JEV and WNV are not linked to congenital malformations [7].

Neurovirulence mechanisms for WNV and JEV are still poorly understood. The causes for WNV neuronal injury are unclear, even though infection involves neuronophagia and neuronal loss, with several brain regions affected (cerebral cortex, hippocampus, cerebellum, brainstem and spinal cord). Apoptotic cell death of the WNV-infected neurons by both caspase-3 dependent and independent mechanisms has been described. WNV may also infect glial cells as well as astrocytes, the latter possibly contributing to the death of neurons through release of neurotoxic molecules [17]. JEV viral antigen has been found in neurons of the cerebral cortex, thalamus, and brain stem. The hippocampus seems to be a privileged target with a particular tropism for developing neurons [18]. In a macaque model, neuronal apoptotic death was observed, along with the release of cytokines that initiate activation of microglia. Subsequent apoptotic death of infected and uninfected neurons originated from the release of pro-inflammatory and apoptotic mediators by microglial cells as well as astrocytes, through a bystander effect [19].

Although developing neurons are one target of JEV that may be a factor for the severity of JEV infections in children, congenital WNV or JEV infection leading to brain developmental disorders are rarely observed [2,3]. Perhaps one possible reason is that JEV or WNV do not easily cross the placental barrier in pregnant mothers before reaching the developing brain of the foetus. While JEV seems to be able to do this in a mouse model of JEV infection, it does not seem to happen with WNV in pregnant women [2,3]. ZIKV, on the other hand, is able to cross the placental barrier as infection of placental stromal macrophages,